SYNTHESIS OF GLYCOCONJUGATES AFFECTING CELL ADHESION

by

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ABBREVIATIONS

CAN	ceric ammonium nitrate
CMP-Neu5Ac	cytidine monophosphate sialic acid
CRD	carbohydrate recognition domain
DAST	diethylaminosulphur trifluoride
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMF	dimethylformamide
DMTSB	dimethyl(methylthio)sulphonium tetrafluoroborate
DMTST	dimethyl(methylthio)sulphonium triflate
FAB MS	fast atom bombardment mass spectrometry
HEV	high endothelial venules
HMPA	hexamethylphosphoric triamide
IDCP	iodonium dicollidine perchlorate
IDCT	iodonium dicollidine triflate
KI-8110	1-(5'-*O*-[methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate]-2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-5-fluorouracil
LeX	Lewis X
MST	methylsulphenyl triflate
NIS	*N*-iodosuccinimide
PST	phenylsulphenyl triflate
SLeA	sialyl Lewis A
SLeX	sialyl Lewis X
THF	tetrahydrofuran
TMSOTf	trimethylsilyl triflate

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PUBLICATIONS

Full papers

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J. Carbohydr. Chem., **15** 383-398

"Synthesis of sialyl Lewis X analogues 2" (1997)
G. Dekany, K. Wright, P. Ward and I. Toth
J. Carbohydr. Chem., **16** 11-24

"An economical synthesis of Lewis X and sialyl Lewis X and their α-galactosyl analogues" (1997)
G. Dekany, K. Wright and I. Toth
J. Carbohydr. Chem., in press

Poster presentations

"A novel silver salt promoter for the formation of α-sialosides"
K. Wright, G. Dekany and I. Toth
Abstract BP 175, XVIII International Carbohydrate Symposium,
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"Polymeric lipoamino acid based *C*-glycosides for drug and peptide delivery"
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Abstract P378, 24th Symposium of the European Peptide Society,
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G. Dekany, R. Falconer, B. Drouillat, K. Wright and I. Toth

Abstract P376, 24th Symposium of the European Peptide Society,
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Abstract A119, 9th European Carbohydrate Symposium
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ABSTRACT

In recent years, it has been recognised that carbohydrates play an important role in specific cell interactions.

The formation of metastases from a primary tumour is a major cause of treatment failure and mortality in cancer patients. A sialic acid - nucleoside conjugate was previously found to reduce the metastatic potential of some carcinoma cell lines. Analogues of this compound, with modifications in the nucleoside or sialic acid moiety were synthesised by silver ion promoted sialylation of the corresponding nucleosides.

The tetrasaccharide sialyl Lewis X and trisaccharide Lewis X epitopes have been the subject of intense research interest in recent years because of their involvement in leucocyte migration mediated by selectins in inflammatory processes. Analogues with increased selectin binding activity over the naturally occurring structures have been widely sought. This research requires the use of the natural compounds as references. A gram-scale synthesis of the trisaccharide and tetrasaccharide was developed using relatively cheap processes. Methyl-thioglycosides were used as donors to construct the desired compounds in a stepwise manner in dimethyl(methylthio)sulphonium triflate promoted glycosidations. A disaccharide benzyl 3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside was a key structure in these syntheses and was synthesised in large scale. Difficulties were encountered in the formation of a β-D-galactoside of this structure, which were overcome by the use of donors with benzyl-type protection. α-Sialylation of a trisaccharide acceptor afforded the anticipated tetrasaccharide and also a structure formed by intramolecular transesterification.

Thio-linked oligosaccharides are of interest because of their increased resistance to enzymatic cleavage and hydrolysis over the natural *O*-linked structures. The synthesis of thio-linked analogues of the Lewis X and Lewis A trisaccharides were attempted using displacement of triflate by thiols to form *S*-glycosides.

1 BIOLOGICAL INTRODUCTION

1.1 CARBOHYDRATE DEPENDENT CELL ADHESION

1.1.1 Carbohydrate Recognition

Carbohydrates form a major component of the outer surface of animal cells. The type of carbohydrates displayed can be restricted to certain cell lines, and can vary throughout the stages of cell differentiation[1]. Their unique monomer structure, with multiple sites for linking or derivatisation, allows for a great diversity of oligomeric forms, and thus possibilities for selective recognition[2].

The oligosaccharides displayed on a cell surface are linked through *O*- or *N*-glycosidic linkages to proteins (the glycoproteins) or lipids (the glycolipids). These compounds are known to play vital roles in cell- cell recognition, and adhesion between cells or between cells and the extracellular matrix[3]. They can act as cell surface antigens and as receptors for micro-organisms, proteins and hormones[4,5,6].

1.1.2 The lectins

Lectins (proteins that recognise carbohydrate ligands) have been known since the discovery of plant lectins in the 1950's. Animal lectins have been discovered since; they can be divided into five currently recognised families (Table 1)[7]. Each family shares characteristic structural features, often in their carbohydrate recognition domain (CRD). The carbohydrate ligands recognised by the members of a family can be varied, as with the C-type and I-type lectins, or constrained to a single type in the cases of the galectins and P-type lectins.

The mechanisms for sugar recognition by the diverse lectins share some key features[8]. Binding sites for oligosaccharides are formed at shallow pockets on the protein surface. Binding of single sugars to lectins is of relatively low affinity, but can be increased by "clustering" of several binding sites together on the protein surface[9]. Clustering of lectin molecules, or repeated CRD motifs allow multiple receptor-ligand interactions to strengthen binding, and the arrangement of the binding sites allows differential binding to displayed oligosaccharides. Selectivity of carbohydrate recognition is achieved through hydrogen bonding to sugar hydroxyl groups and by van der Waals interactions between the protein and a hydrophobic face of the sugar.

Table 1 : The animal lectins

Family	Structural motif	Carbohydrate ligand
C-type	Conserved CRD	Varied (mannose, fucose, galactose)
I-type	Immunoglobulin-like CRD	Varied
Galectins	Conserved CRD	β-galactosides
Pentraxins	Pentameric	Galactose, sulphated/ phosphorylated sugars
P-type	Similar CRD	mannose-6-phosphate glycoproteins

The C-type lectins carbohydrate-binding properties are dependent on the presence of Ca^{2+} ions and a preserved CRD which has 14 invariable and 18 highly conserved amino acids[10]. They recognise a variety of carbohydrate ligands, including galactose, mannose, fucose and glucosamine. The binding affinity of the CRD for carbohydrate ligands is rather low. The asialoglycoprotein receptor of hepatocytes, endocytosis receptors, IgE Fc receptor and a pulmonary surfactant are all C-type lectins with known biological roles.

Recently a new group of C-type lectins were identified and named 'selectins'[11]. They have been implicated in two important pathological processes - inflammation and metastasis.

1.1.3 Leukocyte recruitment in the inflammatory response

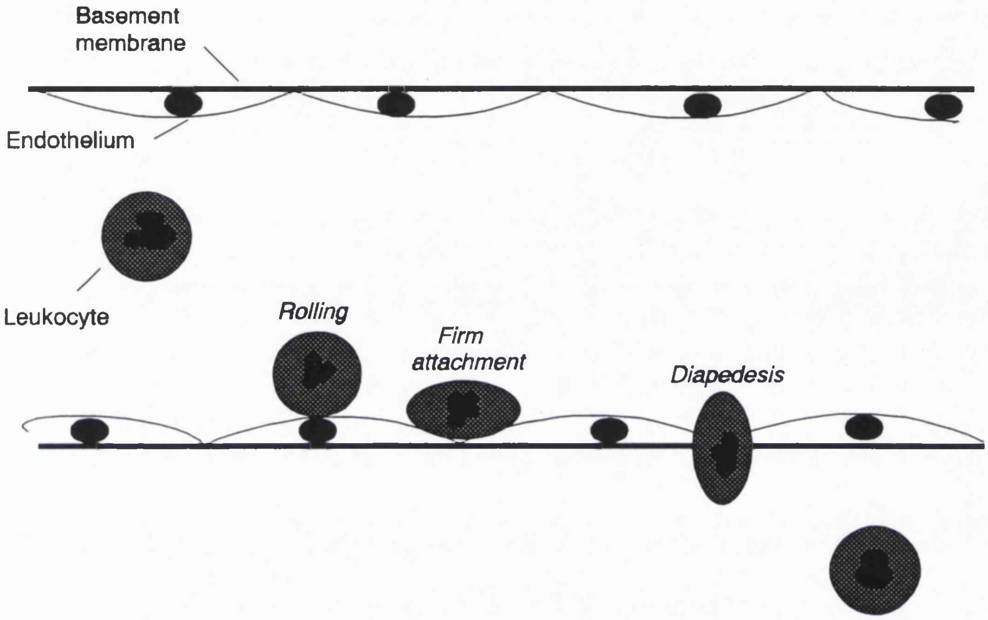


Figure 1.1 Leukocyte infiltration of tissues

In the inflammatory response to tissue damage, leukocytes are attracted to the site of damage by chemotactic factors, eg. leukotriene B_4 and platelet activating factor. They adhere to the endothelial cells of capillaries before crossing the cell wall by diapedesis. Once in the tissue bed, they migrate to the site of damage and engulf dead cells and micro-organisms (Fig. 1.1)[12].

Adhesion to the endothelial cell wall is the critical first event in this inflammatory cascade. In the postcapillary venule (the most common site of leukocyte migration) blood cells are flowing at the rate of approximately 2 mm/s[13]. Leukocytes have to be slowed and then stopped at the site of damage. This is achieved by specific cell- cell adhesion processes between multiple sets of receptor - ligand pairs on endothelial cells and leukocytes[14].

1.1.4 The three stage process of leukocyte adhesion

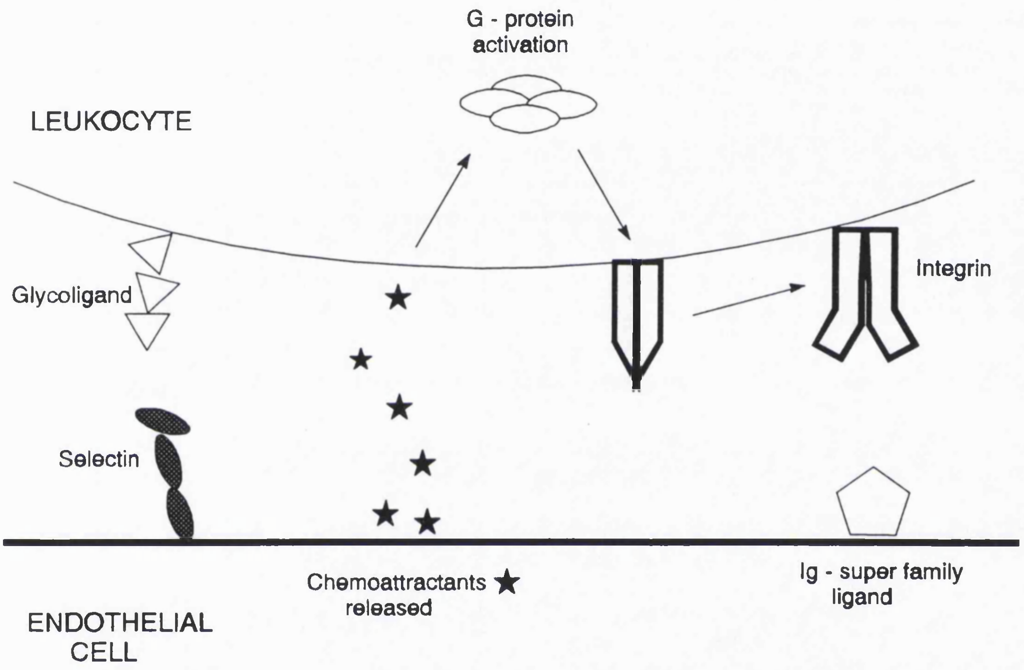


Figure 1.2 The three stage process of leukocyte adhesion

The proposed mechanism of the adhesion is represented in Fig. 1.2[15,16]. The adhesion takes place in three stages. In the first stage, the selectins bind to carbohydrate ligands on opposing cells with a relatively low affinity, allowing leukocytes to roll along the endothelial wall. The leukocytes are exposed to chemoattractants displayed on or released from endothelial cells in the second stage. The chemoattractants can bind to G-protein coupled receptors on the leukocyte. Signal transduction by G-proteins activates the adhesiveness of leukocyte integrins. The integrins are then able to bind firmly to immunoglobulin superfamily ligands on the endothelial cell surface in the third stage, resulting in arrest of the rolling leukocyte. The leukocyte is finally able to cross the endothelial cell layer and enter the underlying tissue.

1.1.5 The selectins

There are three known members of the selectin family, E-selectin (endothelial leukocyte adhesion molecule 1, ELAM-1), P-selectin (platelet activation dependent granule external membrane protein, PADGEM) and L-selectin (MEL-14 antigen).

Analysis of the amino acid sequence of these lectins revealed that they share similar structural motifs[17]. At their N-termini they have sequences related to the carbohydrate recognition domain of the other C-lectins, followed by an epidermal growth factor like domain, then a variable number of repeated units with homology to complement binding proteins.

E-selectin is expressed on endothelial cell surfaces following their exposure to inflammatory cytokines eg. tumour necrosis factor, interleukin-1β, bacterial endotoxin[18]. Cytokine stimulation was found to activate the transcription factor NF-κB, inducing E-selectin mRNA and expression[19].

In vitro studies have shown E-selectin expression is maximal 2-8 hours after cytokine activation, declining to no expression after 24 hours. *In vivo* expression is seen for longer periods, and has been observed in many inflammatory states.

P-selectin is stored in the α-granules of platelets and in the Weibel-Palade bodies of endothelial cells[20,21]. It is brought to the cell surface by the action of thrombogenic agents (eg. thrombin, histamine)[22]. Transcription of P-selectin can also be initiated by the exposure of endothelial cells to inflammatory cytokines[23]. Platelet expression of P-selectin allows their adherence to leukocytes to form thrombi[24]

L-selectin is expressed by lymphocytes on their surface and plays an important part in their recirculation between the blood and lymph. It interacts with ligands displayed on postcapillary high endothelial venules (HEV) in peripheral lymph nodes[25,26].

1.1.6 Carbohydrate ligands for the selectins

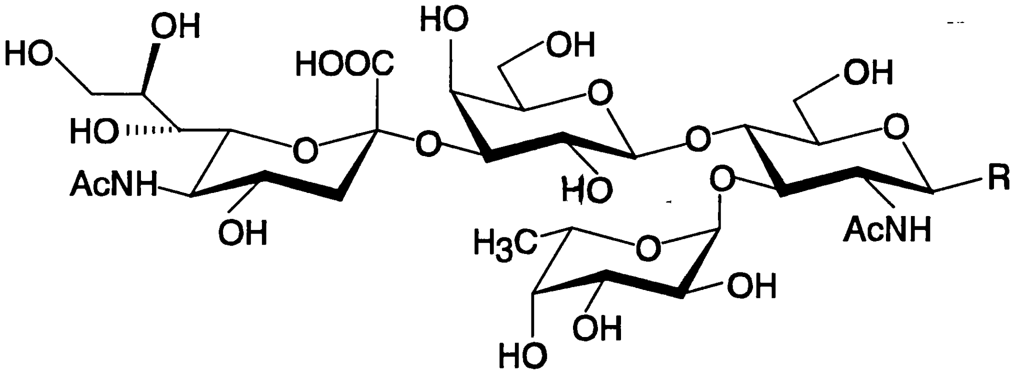


Figure 1.3 Sialyl Lewis X

Rosen and Stoolman provided the first evidence that the ligands for the selectins were carbohydrates[27]. They demonstrated that the adhesion of lymphocytes to HEV could be blocked by anionically charged sugars eg. mannose-6-phosphate, polyphosphomannon ester and fucoidin. In addition, sialidase treatment of HEV prevented subsequent binding of lymphocytes[26]. The N-terminal lectin domain of each of the selectins was also highly suggestive of carbohydrates being their ligands.

Reports of the glycolipids and glycoproteins found on neutrophils were surveyed to identify possible selectin ligands[28]. It was noticed that unusual terminal carbohydrate antigens were present - Lewis X (LeX) and sialyl Lewis X (SLeX) (Fig. 1.3). Chinese hamster ovary cells that expressed LeX, or SLeX, or neither of these groups were tested for adhesion to cytokine treated endothelium. Only the cells that expressed SLeX could adhere.

Antibodies against SLeX were able to block cell adhesion mediated by both E- and P-selectin[29].

In a separate study, glycolipids were extracted from human leukocytes, fractionated and tested for adhesion to E-selectin cDNA transfectants[30]. Fractions that supported adhesion were further fractionated and tested to give a single active fraction. This fraction contained five components, three of which were adhesive. Their structure was determined by monosaccharide content analysis, enzyme treatment and FAB MS. They each had a terminal sialic acid residue, fucose linked $\alpha 1 \rightarrow 3$ to *N*-acetyl glucosamine, and a repeated lactosamine core linked to a ceramide. The two non-adhesive components lacked fucose. The requirement for fucose in binding was further demonstrated by transfecting cells with cDNA for a fucosyl transferase, which could add fucose to the glucosamine residue of a lactosamine core. Transfected cells were able to bind to E-selectin while control cells could not.

Eventually, it was demonstrated that SLeX is recognised by all the selectins with differing affinities, and this appears to be the minimal oligosaccharide epitope recognised by the selectins[31]. Other ligands for the selectins have been identified: sialyl Lewis A (SLeA), Lewis X, sulphated Lewis X and Lewis A, sulfatide and a fraction of heparin have all shown binding activity[32-36]. L-selectin (and to some extent P-selectin) seems to have a greater affinity for SLeX which is modified with sulphate at either Gal-6 or GlcNAc-6[37,38].

It has been suggested that the natural selectin ligands present carbohydrate as a multivalent array, as the measured affinity for monomeric oligosaccharides is low. However, molecular modelling of the lectin domain of the selectins suggests only a single carbohydrate binding site. It has been suggested that the selectins 'cluster' on the cell surface, and interact with a similarly arranged group of ligands[39]. Synthetic multimeric forms of SLeX, and SLeX incorporated into liposomes, block interactions with E-selectin more effectively than the monomeric structure[40,41].

1.1.7 The selectins and metastatic disease

The selectins may play a similar role in the metastasis of some cancers as they do in leukocyte migration, slowing cells at the endothelium to allow invasion into the tissue bed[42,43]. The selectin ligands SLeX and SLeA are overly expressed in some cancer cell types eg. colon, breast, prostate cancer and some leukaemias[44-51]. In colon and prostate cancer the level of expression of SLeX has been shown to correlate with the prognosis for patients[52]. E-selectin can mediate binding between activated endothelium and human colon cancer cells, and this interaction is stronger with highly metastatic cell lines than with cells of lesser metastatic ability[43]. P-selectin may have a permissive role in metastasis. Platelet interactions with tumour cells are known to assist in their spread, perhaps by forming aggregates which protect the cells from the immune system[53].

1.1.8 Selectins in inflammatory disease

The adhesion of leukocytes to endothelial cells has been shown to be a preliminary step in both acute and chronic inflammatory diseases[54,55].

E-selectin was shown to be expressed in cutaneous inflammatory conditions, including contact dermatitis, atopic dermatitis and psoriasis[56]. Tissue samples from psoriatic skin showed neutrophils concentrated around E-selectin expressing cells and could be seen between cells, suggesting diapedesis.

Biopsies of inflamed airway tissues showed the presence of E-selectin, and this selectin was found to mediate antigen-induced acute airway inflammation[57].

Synovial tissue from patients with rheumatoid arthritis and osteoarthritis showed E-selectin expression. This finding demonstrated the involvement of selectins in chronic as well as acute inflammation[58]. P-selectin could also be implicated in the recruitment of leukocytes into joints in rheumatoid arthritis[59].

Neutrophil infiltration into myocardial tissue subjected to ischaemia and subsequent reperfusion play a major role in myocardial reperfusion injury. All three of the selectins have been implicated in the early stages of reperfusion injury[60]. It was also demonstrated that the selectin ligand SLeX was rapidly but temporarily expressed by vascular endothelial cells and cardiac myocytes which underwent hypoxia and reoxygenation[61].

1.1.9 Sialyl Lewis X and Lewis X expression

The sialyl Lewis X and Lewis X antigens are expressed in a variety of human tissues. Lewis X was found on cell surface glycoconjugates in the gastro-intestinal tract, lung, pancreas and kidney, and also in tumours originating from these organ systems. The level of expression in normal and malignant tissue was often found to differ. The level of expression of LeX by bladder carcinoma cells was reported to correlate with disease progression and metastatic spread[62]. Similar findings were made in studies of gastric carcinomas and rectal adenocarcinomas, where high levels of LeX expression were correlated with poor prognoses[63,64]. Human lens tissue was found to express LeX containing glycoconjugates, and increases in expression were associated with cataract formation[65].

Human pathogens may also express Lewis X. The blood fluke *Schistosoma mansoni* was found to excrete large quantities of glycoconjugates containing the LeX trisaccharide, causing its host to generate antibodies to the trisaccharide. These antibodies caused lysis of host cells displaying the trisaccharide[66]. The outer membrane of the bacteria *Helicobacter pylori*, which has been implicated in the formation of duodenal ulcers, was found to display LeX structures, and it was proposed that this offers some protection from the host immune system[67].

Sialyl Lewis X is found on cell surface glycoconjugates in the intestines, pancreas, kidney, on the endothelium of peripheral lymph nodes, and on neutrophils, monocytes and some T-lymphocytes. It is more strongly associated with expression in carcinomas than LeX[68]. Blast cells from patients with acute lymphocytic and acute myelocytic leukaemia were found to strongly express SLeX and could bind to monoclonal antibodies to this antigen. It has been suggested that this interaction could be used to remove blast cells from bone marrow used in autologous transplants[50,51]. Serum SLeX levels were elevated in patients with hepatic disease (cirrhosis, chronic hepatitis and carcinoma)[69] or fibrosing lung disease[70] and could be indicative of disease activity or response to treatment.

1.1.10 Selectin ligands and drug discovery

Since their role in inflammatory pathways was discovered, the selectins have become of interest to researchers seeking novel anti-inflammatory drugs. The tetrasaccharide SLeX has been shown to have anti-inflammatory effects *in vivo*[71,72]. When injected as the free tetramer or incorporated in liposomes, it showed cardioprotective effects in cat and dog models of myocardial reperfusion injury[73,74]. It was effective in reducing tissue damage after ischaemia-reperfusion injury to rabbit ear[75]. Sulphated Lewis X has also shown *in vivo* activity[71].

While these compounds appear to be non-toxic, their relatively low affinities and short half-lives have prompted many to look for analogues of the natural compounds with improved activity. This search has met with some success, and may eventually lead to clinically available drugs which are able to block the interaction between the selectins and their carbohydrate ligands.

Glycotargeting of drugs is based on the conjugation of a pharmacologically active agent with a carbohydrate carrier which could be recognised by a lectin, allowing delivery of the agent to a specific site[76]. The carrier could be a glycoprotein, a glycosylated polymer or a liposome. This approach has been used to selectively deliver anti-viral or anti-tumour agents and DNA to liver cells with lactosaminated carriers, and doxorubicin or methotrexate to macrophages with a mannosylated carrier. It could be possible to use this approach to target anti-inflammatory drugs to sites of selectin expression. Multimeric and liposomal forms of SLeX have been synthesised[40,41]. The anti-adhesive tripeptide Arg-Gly-Asp (RGD) has been conjugated with SLeX, and this substance proved to be a very potent inhibitor of P-selectin mediated adhesion[77].

1.2 SIALOGLYCOCONJUGATES IN METASTATIC DISEASE

1.2.1 The metastatic cascade

The formation of metastases from a primary tumour is a major cause of treatment failure in cancer patients. While the primary tumour may be surgically removed, if metastatic cells have entered the general circulation they can move around the body to colonise other organs or tissues. If secondary tumours form in essential organs, they can destroy their ability to function, and lead to death. Where metastatic spread is suspected, damaging radiotherapy or chemotherapy are the only treatment options available.

The events that take place during the spread of tumour cells are termed the metastatic cascade (Fig. 1.4)[78]. These events comprise the detachment of cells from the primary tumour mass, movement into the blood or lymphatic circulation, arrest at a distant site and invasion of new tissues. The cells must also escape immune surveillance. The ability to complete these steps is conferred by multiple genetic mutations in a cell line. In general, these mutations are separate from those which allow growth transformation.

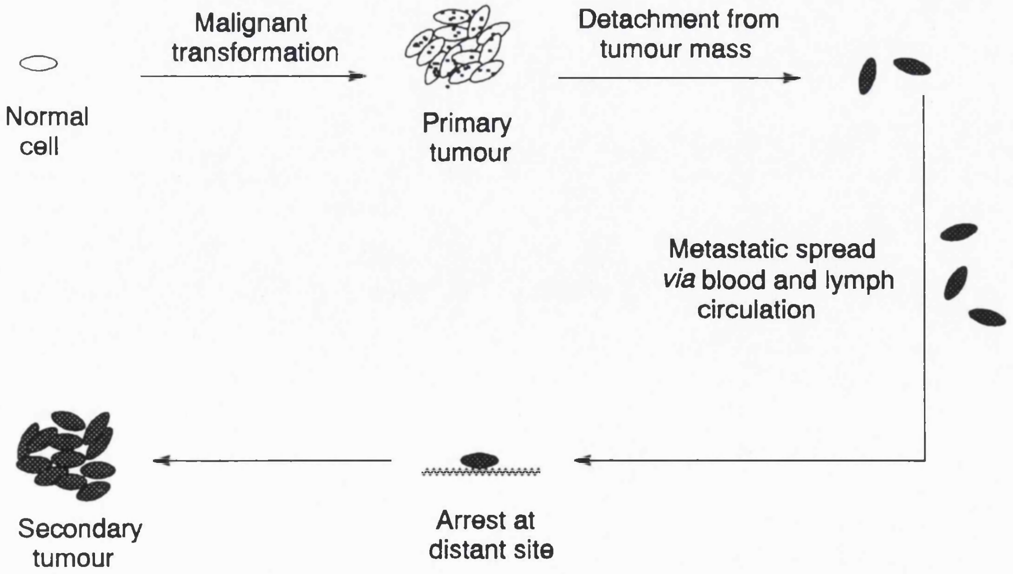


Figure 1.4 The metastatic cascade

To metastasize successfully, tumour cells must interact with other cells in their immediate environment. Changes in adhesive behaviour are important in several stages of the metastatic cascade. Separation from the primary tumour requires a decrease in adhesion to other tumour cells in the tumour mass. Once in the circulatory system, arrest at a new site can be mediated through specific adhesive interactions between tumour cell surface molecules and receptors on endothelial cells or the basement membrane.

Alterations in the tumour cell surface allow the changes in its interactions with surrounding cells. The majority of human cancers were found to exhibit "aberrant glycosylation"[79], with unusual glycoproteins and glycolipids present on the cell surface. Treatment of malignant cells with trypsin (to remove cell surface glycoproteins) or with tunicamycin (an inhibitor of protein glycosylation) reduced the metastatic ability of these cells.

1.2.2 Cell surface sialoglycoconjugates of metastatic cells

The observation that sialidase treatment of cancer cells caused substantial decreases in oncogenicity[80] led to detailed investigations of the sialoglycoconjugates present on such cells. Quantitative and qualitative changes in cell surface sialoglycoproteins and gangliosides (sialic acid conjugated glycosphingolipids) were found in cells that had undergone malignant transformation[81]. These differences in sialylation were considered to contribute to the altered behaviour of cancer cells, such as uncontrolled growth, altered cell adhesion, immunological resistance, invasiveness and metastatic spread.

The nature of the changes in cell surface sialoglycoconjugates varied between cell lines. The carbohydrate chains of both sialoglycolipids and sialoglycoproteins were found to be attenuated in many malignant cell lines[82,83]. The genetic causes for these changes in surface sialylation of metastatic cells are not yet understood.

Highly metastatic cancer cells (potentially the most dangerous to their host) were found to contain elevated levels of sialic acid[84,85]. Specifically, increases in sialylated oligosaccharide chains *N*-linked to Asn residues or *O*-linked to Ser/Thr residues of glycopeptides were shown to correlate with tumour progression and the metastatic potential of cells[86]. The glycosyltransferases required for the synthesis of these structures are present at higher levels in such cells[87]. High levels of sialyl Tn antigen (Neu5Acα2→6GalNAcα→R) on colorectal cancer cells was found to be predictive of poor prognoses[88].

As metastatic cells possess biological features distinctive from normal cells, there is a possibility that drug treatment could be targeted at them to combat metastatic spread. The increased levels of sialic acid in metastatic cells offer such a target.

Sialic acid is incorporated into oligosaccharide chains by the action of sialyltransferases, which utilise the sugar nucleotide cytidine monophosphate sialic acid (CMP- Neu5Ac). The sialyltransferases are concentrated in the Golgi bodies of cells[89,90], and are also localised at cell surfaces[91,92]. CMP-Neu5Ac must be actively transported first into cells from the extracellular fluid, and then from the cytoplasm into the Golgi body[93]. Inhibition of sialyltransferases or the CMP-Neu5Ac transport mechanism would decrease the formation of sialylated oligosaccharides.

1.2.3 The biological effects of KI-8110 on mouse lymphocytes

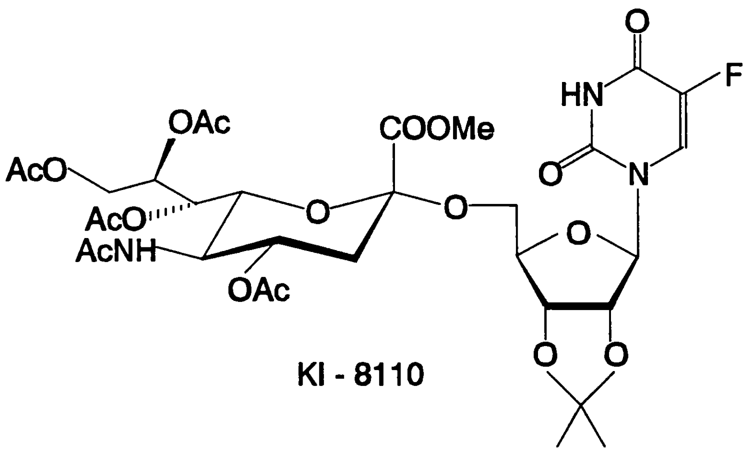


Figure 1.5 KI-8110

Kijima *et al.* reported the biological effects of a protected disaccharide nucleoside KI-8110 (1-[5'-*O*-{methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate}2',3'-*O*-isopropylidene-β-D-ribofuranosyl]-5-fluoro-uracil), (Fig.1.5). This compound was initially tested for its effects on the immunological functions of mouse lymphocytes[94]. It caused suppression of *in vitro* and *in vivo* primary antibody response to sheep red blood cells. The authors suggested that KI-8110 could induce suppressor T cells, but offered no explanation of the mechanism involved.

1.2.4 Inhibition of incorporation of sialic acid into glycoconjugates by KI-8110

The ability of KI-8110 to influence incorporation of sialic acid into glycoconjugates on the surface of mouse lymphocytes was examined[95]. Mixtures containing whole cells or cell homogenate were incubated with CMP-[^{14}C]Neu5Ac in the presence or absence of KI-8110. Incorporation of radio-labelled sialic acid into glycoproteins and glycolipids was significantly decreased (by 20-30 %) in the presence of KI-8110. Exogenous substrates for sialic acid conjugation were added to incubation mixtures to test the specificity of inhibition. Incorporation of radio-labelled sialic acid into desialylated glycoproteins having Ser/Thr-*O*-linked carbohydrate chains was inhibited by KI-8110, whereas incorporation into a glycoprotein carrying only Asn-*N*-linked chains was unaffected. When 4-nitrophenyl-galactoside was added to the incubation mixture, KI-8110 inhibited the formation of α2→3 linked sialic acid disaccharides, but not α2→6 linked products.

From these results it was concluded that KI-8110 was an inhibitor of sialyltransferases that incorporate sialic acid into *O*-linked oligosaccharide chains via an α2→3 link.

1.2.5 Inhibition of formation of pulmonary metastases by KI-8110

The effects of KI-8110 on the formation of metastases from mouse colon adenocarcinoma cell lines were investigated[96].

Cultures of tumour cells were incubated with CMP-[^{14}C]Neu5Ac in the presence or absence of KI-8110. Incorporation of radio-labelled sialic acid into tumour cell glycoconjugates was inhibited in the presence of KI-8110. An analogue of KI-8110 where the sialic acid residue was β-linked was also tested, and gave similar results.

Mice were injected with adenocarcinoma cells to provide a model of metastasis to the lungs. Metastasis to the lung was evaluated three weeks after tumour cell injection on the basis of lung weight and the number of pulmonary surface nodules. Mice received either intravenous treatment with KI-8110, or with its β-analogue, or no treatment. The treated mice showed increased survival time and fewer metastatic colonies in their lungs than untreated mice. The α- and β- forms of KI-8110 were found to be equally effective. When treated mice were injected with tumour cells that were incubated in the presence of KI-8110 for 24 hours prior to injection, a greater anti-metastatic effect was observed.

KI-8110 was not cytotoxic or cytostatic to the adenocarcinoma cells *in vitro.* It was suggested that it exerted anti-metastatic effects by causing modification of the tumour cell surface sialic acid glycoconjugates.

1.2.6 Inhibition of formation of hepatic metastases by KI-8110

Wagner *et al.* tested the effects of KI-8110 on the metastasis of human colorectal cancer cells following intrasplenic injection into mice[97]. Tumour cells were cultured with or without KI-8110 for 24 hours prior to injection. The cells that were treated had a lower neuraminidase-releasable sialic acid content than non-treated cells. Both treated and untreated cells were able to grow locally in the spleen. Hepatic colonies formed in 50 % of the mice which were injected with untreated cells, but no liver metastases formed in the mice which received pre-treated cells

Harvey *et al.* conducted a similar study on the metastasis of five human colon cancer cell lines to the liver of mice[98]. Pre-treatment of the cancer cells with KI-8110 decreased the incidence of metastatic growth in the liver following intrasplenic injection.

1.2.7 The effects of KI-8110 on platelet aggregation by tumour cells

Platelet aggregation by tumour cells may be important for their arrest at distant sites[99]. The effects of KI-8110 treatment on the platelet aggregation ability of mouse adenocarcinoma cells was investigated[100]. Untreated tumour cells could induce irreversible platelet aggregation, but this activity was reduced after incubation with KI-8110 for 24 hours. Neuraminidase treatment of these cells also reduced platelet aggregation, suggesting cell surface sialic acid residues were at least partly responsible for this activity. While KI-8110 had no effects on cell growth under growth-factor free conditions, it inhibited platelet-derived growth factor (PDGF) - dependent growth of the mouse adenocarcinoma cells.

1.2.8 The mechanism of inhibition of sialic acid incorporation by KI-8110

Harvey and Thomas made further investigations into the mechanism of inhibition of sialic acid incorporation by KI-8110[101]. Human colorectal cancer cells and human liver cells were used to measure the incorporation of CMP-[^{14}C]Neu5Ac into sialoglycoconjugates. The sialyltransferase activity of cell homogenates were assayed in the presence and absence of KI-8110. They found no decrease in incorporation of radio-labelled sialic acid in the presence of KI-8110 when membranes in the homogenates were disrupted by addition of detergent. From this result they concluded that KI-8110 was not acting as a competitive inhibitor of sialyltransferases.

When the assay was repeated in the absence of detergent, KI-8110 was found to inhibit the incorporation of the radio-labelled sialic acid by approximately 50 % in both the cancer cells and the liver cells. It was suggested that this result indicated that KI-8110 was acting as an inhibitor of CMP-Neu5Ac transport across membranes into Golgi vesicles. This suggestion is not inconsistent with the findings of Kijima-Suda *et al.* previously reported[94-96], as their assays were performed either with whole live cells, or with homogenates in the absence of detergents.

1.2.9 The effect of KI-8110 on tumour cell adhesion to extracellular matrix proteins

Sawada *et al.* investigated the effects of modifying cell surface sialoglycoconjugates and the adhesion of pancreatic cancer cells to extracellular matrix proteins[102]. Cells were either incubated with KI-8110, incubated with the glycosylation inhibitor tunicamycin, or treated with neuraminidase. When low concentrations of KI-8110 or neuraminidase were used, an increase in adhesiveness to extracellular proteins (laminin, fibronectin and collagen) was observed, while at higher concentrations there was a decrease in adhesiveness. The glycosylation inhibitor tunicamycin produced mixed effects on adhesiveness. An explanation of the biphasic effect observed with sialic acid removal could be that at low levels, this removal reduces a physical barrier between the cells and the matrix. When greater amounts of sialic acid were removed, specific adhesive interactions were affected.

2 CHEMISTRY

2.1 *O*-GLYCOSYLATIONS

The recent recognition of the biological roles played by oligosaccharides has led to increased efforts to synthesise these compounds for biomedical applications. The controlled formation of *O*-glycosidic bonds is the foremost requirement for these syntheses.

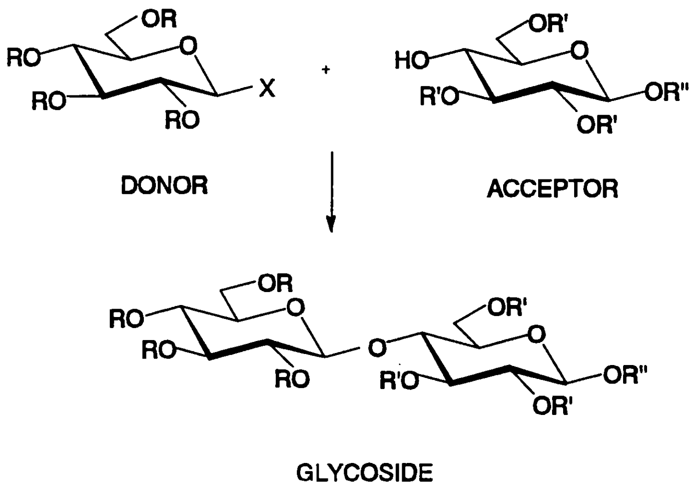


Figure 2.1

In *O*-glycosylation reactions, a polyfunctional sugar (the glycone) is conjugated to an alcohol (the aglycone) *via* an acetal or ketal linkage to form a glycoside (Fig. 2.1)[103].

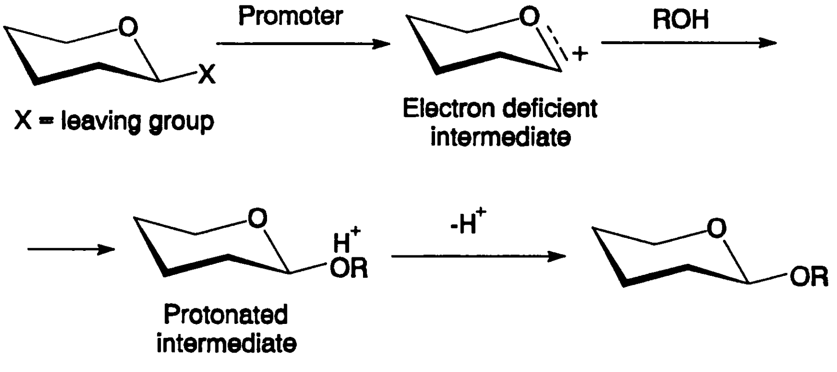


Figure 2.2

Most glycosylation methods involve the activation of a sugar donor to form an electron deficient reactive intermediate, followed by nucleophilic attack by an oxygen atom of a sugar acceptor. The protonated intermediate formed undergoes proton abstraction to give a glycoside (Fig. 2.2)[104].

Stereocontrol of glycosylation reactions, to form selectively either the α or β anomers, is important to avoid difficult separations and loss of material, especially in oligosaccharide synthesis. Stereoselectivity has been found to depend on stereoelectronic effects (the anomeric and reverse anomeric effects), neighbouring group participation and the choice of catalyst. The acceptor can also influence stereoselectivity, particularly if it has an unreactive, weakly nucleophilic hydroxyl.

Regioselectivity has been generally achieved by protection of the hydroxyl groups of the glycosylating sugar (glycosyl donor) with the introduction of a leaving group group at its anomeric centre, with protection of the glycosyl acceptor (the alcohol component) at all positions except that which will take part in the reaction. Such protection patterns may require long reaction paths to achieve.

The type of protecting group (R, R', R'') employed has been found to exert considerable effects on the reactivity of both the anomeric centre of the donor and the hydroxyl group of the acceptor. Changing the type or pattern of protection could exert a decisive influence on the outcome of glycosylation reactions. The reactivity of sugars was also affected by conformational and steric factors. Reactivity tends to decrease with an increase in size of either reactant.

Because so many factors can affect the outcome of a glycosylation reaction, no universally successful conditions have been found for *O*-glycoside synthesis.

2.1.1 Stereoelectronic effects

2.1.1a The anomeric effect

Studies on cyclohexane ring systems have shown that substituents favoured equatorial positions over axial positions. It was proposed that there are van der Waal's repulsions between axial substituents and axial protons. In the pyranose ring of a sugar, substituents were also found to favour equatorial orientations, with one exception. Electronegative substituents at a carbon atom adjacent to the ring oxygen were found to preferentially occupy the axial position, and this was termed the anomeric effect[105].

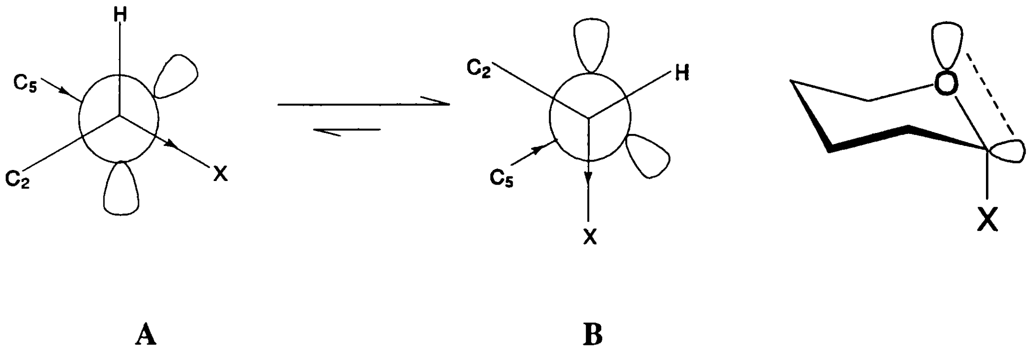


Figure 2.3

Carbohydrates with an electronegative group X at the anomeric position (where X = *O*-alkyl, *O*-acyl, halide etc.) prefer conformations where X is axial (conformation B) (Fig 2.3). The effect increases with the increasing electronegativity of X, and decreases in polar solvents. It was proposed that this effect is due to the interaction between the axial lone pairs of the ring oxygen and the anti-bonding σ^*-orbitals of the C-R bond[106].

2.1.1b The reverse anomeric effect

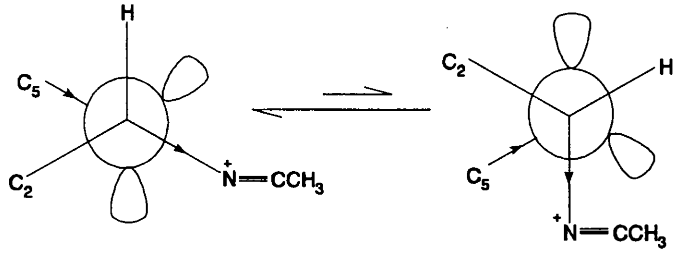


Figure 2.4

Groups attached to the anomeric carbon which reverse the dipole of that bond show a reverse anomeric effect.

For example, a nitrilium-nitrile group at the anomeric position having a positive charge associated with it would favour the equatorial position (Fig 2.4)[107].

2.1.1c Neighbouring group participation

Acyl type protective groups at C-2 of a sugar donor are able to 'participate' in glycosylation reactions to affect their outcome.

When the leaving group is removed, both the axial and equatorial donors are able to react via an oxocarbenium ion to give a more stable acetoxonium ion form (Fig. 2.5)[108]. Nucleophilic ring opening with an alcohol at C-1 will, for steric reasons, give only the 1,2-*trans* glycoside. With reactive alcohols, it is possible to get a mixture of glycosides, as they can undergo a reaction with the intermediate oxocarbenium ion.

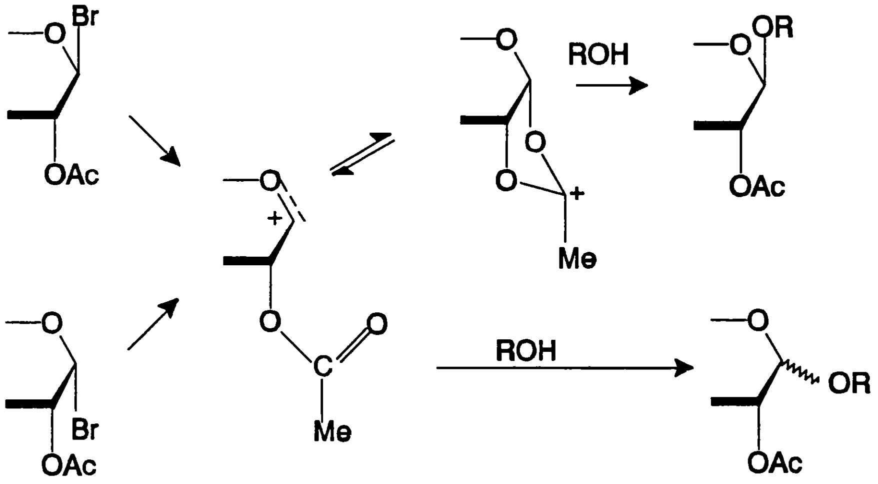


Figure 2.5

This type of assisted glycosylation has been widely exploited to form β-glucosides or galactosides, and α-mannosides, which are commonly found in naturally occurring oligosaccharides.

2.1.2 Glycosylation methods

2.1.2a Fischer glycosidation

One of the simplest ways to form a glycoside was by the reaction of a free sugar with an alcohol in the presence of an acid catalyst (Fischer glycosidation). The sugar aldehyde could react with the alcohol to give a glycoside. However, as sugars have more than one hydroxyl group able to participate in cyclic hemiacetal formation, this method allowed formation of glycopyranosides (6-member ring) and glycofuranosides (5-member ring), in varying α:β mixtures.

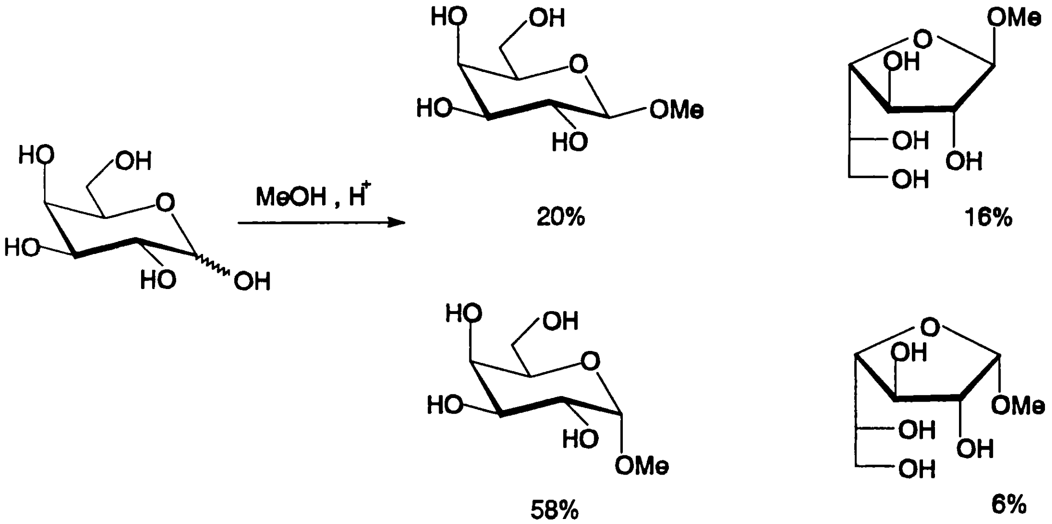


Figure 2.6

When galactose was treated with methanol under reflux in the presence of HCl, the major product was the α-pyranoside, but the β-pyranoside and β-furanoside were also formed in 20% and 16% yields respectively (Fig. 2.6)[103]. Because of the mixtures of products obtained, this method is not suitable for the synthesis of oligosaccharides, where a high degree of regio- and stereoselectivity is required.

2.1.2b The Koenigs-Knorr method

The Koenigs-Knorr glycosylation method was first reported in 1901[109]. The reaction utilised halo-sugars (chloro or bromo) as glycosyl donors, which exist predominantly in their more stable axial forms. The glycosyl halide was allowed to react with an alcohol by nucleophilic substitution in the presence of an insoluble silver salt, eg. Ag_2O or Ag_2CO_3, or in later versions of the procedure with mercury salts (Fig. 2.7). Improved yields were obtained when drying agents were added to reaction mixtures to remove the water formed during glycosylation[110]. Other silver salts have been investigated for use as the catalyst in this reaction - soluble salts such as silver triflate were found to give improved results in some cases[111].

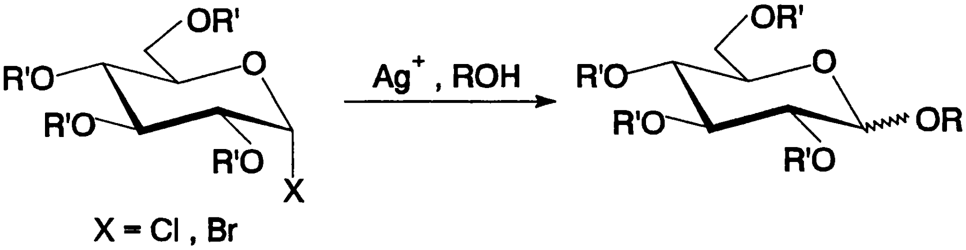


Figure 2.7

Stereoselectivity has been achieved in this type of reaction by exploiting several factors :

i) neighbouring group participation to form 1,2-*trans* glycosides

ii) *in situ* anomerisation of axial halides to the more reactive equatorial halides, to give axial glycosides

iii) use of heterogeneous catalysts for the formation of 1,2-*cis* glycosides

The reactivity of the glycosyl donor and acceptor could be increased by changes in their protection, and by changing the halide used (bromide > chloride). The use of silver triflate or silver perchlorate as promoter also gave increased reactivity. However, in many cases, especially when the glycosyl acceptor had a secondary hydroxyl group, yields were moderate or low. Another drawback was the instability of the glycosyl halide donors required, necessitating *in situ* generation, or the use of low temperatures.

2.1.2c Glycosyl fluorides as glycosyl donors

Glycosyl fluorides have been known since the 1920's, but have only recently became important as glycosyl donors, due to the discovery of fluorophilic agents for use as glycosylation promoters, and the development of more convenient methods for glycosyl fluoride synthesis. They have been prepared from thioglycosides by treatment with hydrogen fluoride, silver fluoride, dimethyl(methylthio)sulphonium tetrafluoroborate (DMTSB), or diethylaminosulphur trifluoride (DAST), or by the action of 2-fluoro-1-methylpyridinium tosylate on protected sugars with a free anomeric hydroxyl[103].

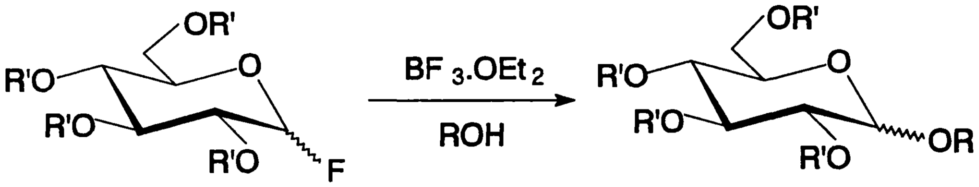


Figure 2.8

Fluoride is a poorer leaving group than chloride or bromide due to the high strength of the C-F bond. The greater stability of the fluoride group allowed base-catalysed modifications of protective groups on the donor which would not be possible with a glycosyl bromide or chloride[112]. Glycosyl fluorides can be activated by boron trifluoride etherate, trimethylaluminium, tin (II) chloride/perchlorate mixtures and other Lewis acids (Fig 2.8)[113,114].

2.1.2d Thioglycosides as glycosyl donors

Alkyl or aryl thioglycosides are able to react with sulphur specific reagents to form sulphonium cations, which are good leaving groups and easily displaced. The thioglycoside donors have been found to be exceptionally stable to protective group manipulations and often exist in crystalline forms.

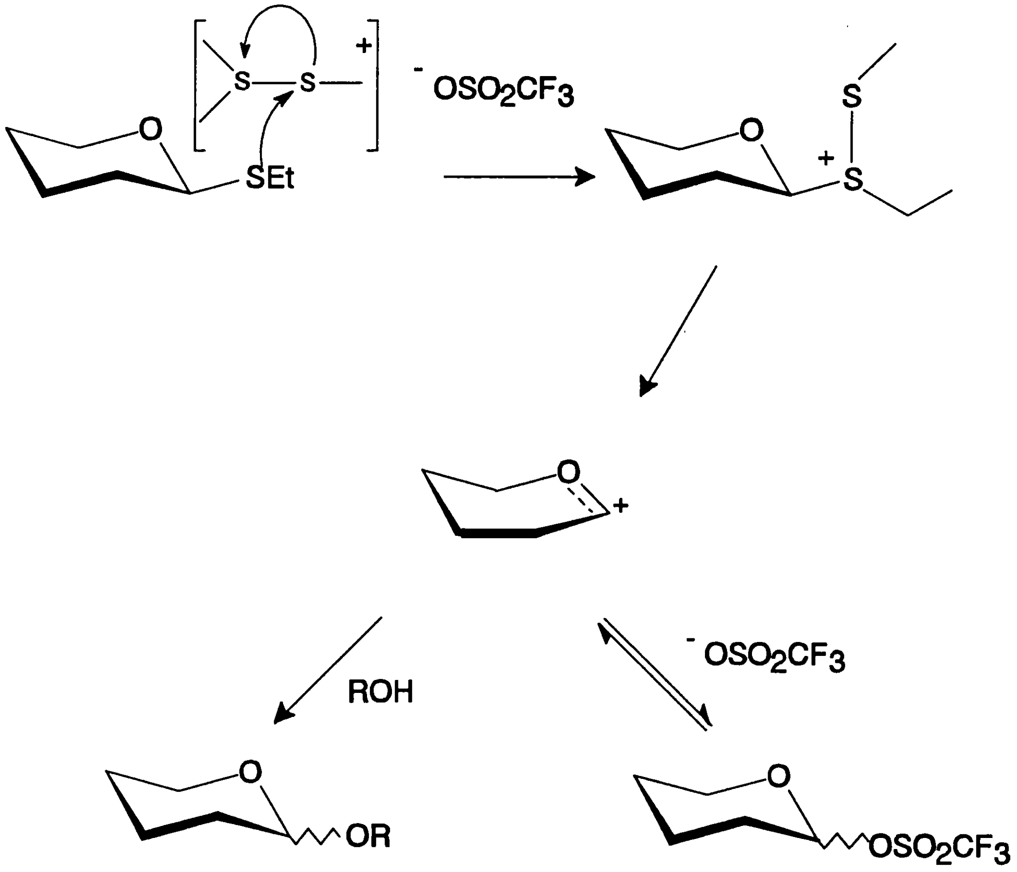


Figure 2.9

Originally thiophilic salts of mercury (II) and lead (II) were used as activators, but limited success in the glycosylation of sugar alcohols lead to the search for more potent thiophilic reagents[115]. Methyl triflate, dimethyl(methylthio)sulphonium triflate (DMTST) (Fig. 2.9), *N* -iodosuccinimide / triflic acid (NIS/TfOH), iodonium dicollidine perchlorate and iodonium dicollidine triflate have all been found effective[116-120]. Glycosylation by activation of thioglycosides with reagents such as these has become one of the most efficient methods for oligosaccharide synthesis.

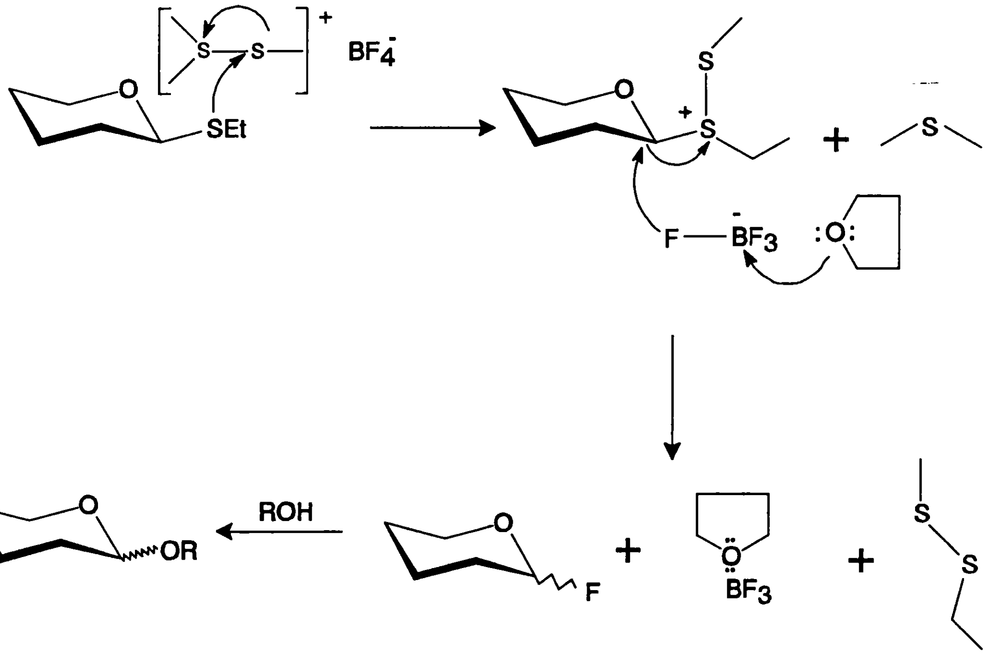


Figure 2.10

It was suggested that glycosylations with DMTSB, another thiophilic activator, may occur as a two-step process, with initial formation of a glycosyl fluoride, followed by activation of this intermediate by the boron trifluoride formed (Fig. 2.10)[121].

Activation of thioglycosides could also be achieved in two steps *via* conversion to the glycosyl halide *in situ* in the absence of promoter, eg treatment of a thioglycoside with bromine gave a glycosyl bromide, which could then be activated[122].

2.1.2e Glycosylation with glycosyl trichloroacetimidates

The glycosyl trichloroacetimidates, introduced by Schmidt have proved to be versatile glycosylating agents[123]. They are capable of undergoing nucleophilic displacement reactions under mild conditions. The trichloroacetimidates were formed by the reaction of a protected sugar alcohol with trichloroacetonitrile in the presence of base, to give either the α- or β- anomer depending upon the conditions used (Fig. 2.11)[125].

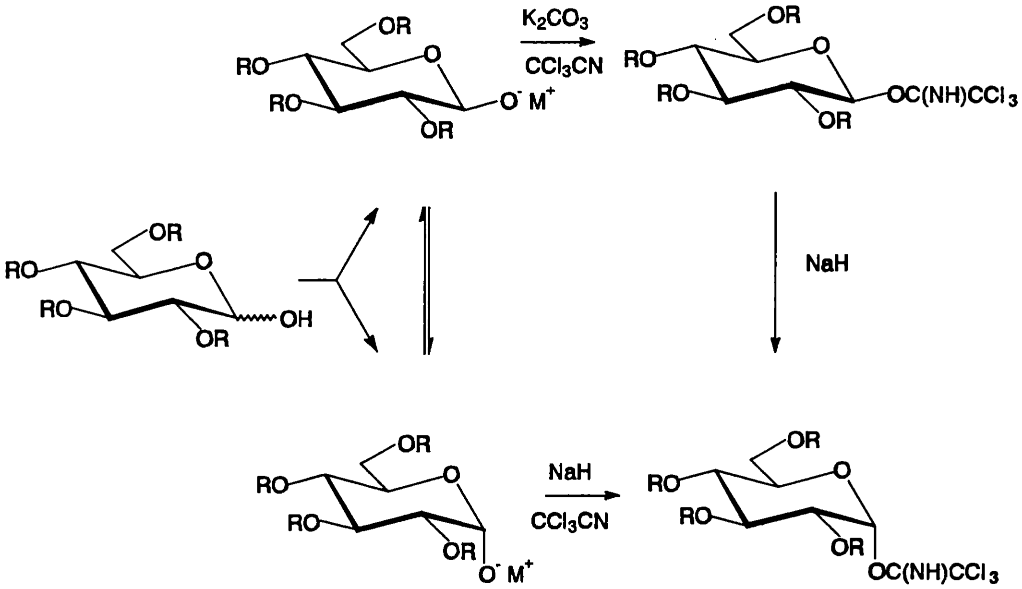


Figure 2.11

With potassium carbonate the β-imidate was the major product, but with sodium hydride the α-anomer was formed. This happened because the β-anion is able to react faster than the α-anion to give the β-imidate; this was stable to potassium carbonate but anomerised in the presence of sodium hydride to the more thermodynamically stable α-imidate. They have been used as glycosyl donors in the presence of boron trifluoride etherate, trimethylsilyl triflate and p-toluenesulphonic acid[126].

2.1.2f Glycosylation with glycosyl esters and orthoesters

1-*O*-acetylated and 1,2-orthoesters are able to act as glycosyl donors under the activation of Lewis acids eg trimethylsilyl triflate, tin (IV) chloride, zinc (II) chloride[103]. With the orthoesters, side products could be formed resulting from the release of alcohol from the orthoester, which could compete with the glycosyl acceptor to form a mixture of glycosides[110].

2.1.3 Stereoselectivity in *O*-glycosylation reactions

A high degree of stereoselectivity is required in the formation of *O*-glycosidic bonds in the construction of oligosaccharides. Essentially, there are four main types of glycosides found in natural products (excepting 2-deoxy compounds). These are the 1,2-*trans* configured α-mannosides (A) and β-gluco- or galactosides (B) and the 1,2-*cis* configured α-gluco- or galactosides (C) and β-mannosides (D) (Fig.2.12).

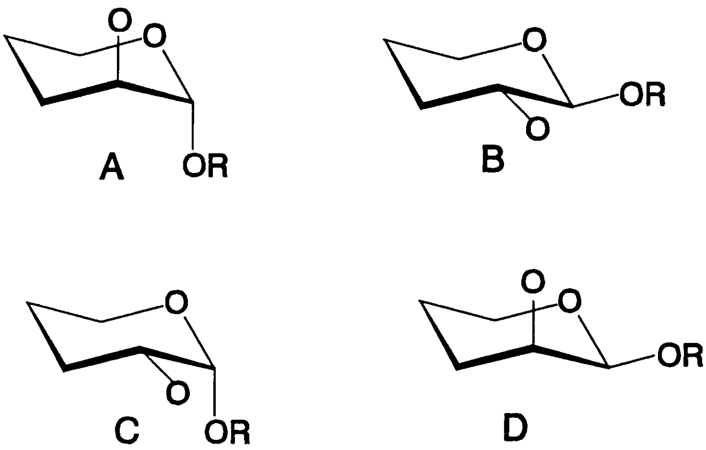


Figure 2.12

Different approaches are required for the formation of each type of glycoside, with the level of difficulty associated with each type increasing from A to D.

2.1.3a Formation of 1,2-*trans* glycosides

A common method of forming 1,2-*trans* glycosides was to use a sugar donor with a good leaving group at C-1 and an acyl (participating) neighbouring group at C-2 (Fig 2.5). Good yields of 1,2-*trans* glycosides could be obtained if the glycosyl acceptor was reactive, but poor yields were noted with some secondary sugar alcohols. Neighbouring group participation has failed to give stereospecificity in some cases where the acceptor was a weak nucleophile, such as a secondary carbohydrate hydroxyl[127].

2.1.3b 1,2-*trans* glycosylation with surface active insoluble catalysts

Surface active, insoluble catalysts such as silver silicate or silver salicylate were found to promote glycosylation with good 1,2-*trans* selectivity from glycosyl 1,2-*cis* halides. It was suggested that these reactions took place on the surface of the silver salt, and proceeded by S_N2 type mechanisms[108,110]. A solvent of low polarity was used to suppress side-reactions due to decomposition or anomerisation of the halide. Simple primary alcohols were the most successful acceptors, with secondary alcohols giving lower yields. A disadvantage of this type of reaction was the formation of water as a side product.

2.1.3c 1,2-*trans* or 1,2-*cis* glycosylation *via* nitrilium-nitrile intermediates

Schmidt *et al.* reported a 1,2-*trans* glycosylation procedure using trichloroacetimidates with non-participating protective groups (P) at position C-2 as sugar donors and nitrile solvents (Fig 2.13)[128]. Nitrilium-nitrile glycosylations offered the possibilities of selective 1,2-*trans* or 1,2-*cis* glycoside formation, depending on either kinetic or thermodynamic control over the intermediates formed[107].

They proposed that under S_N1 conditions an oxocarbenium ion formed from both α- and β-imidates, which could go on to react with the nitrile solvent to give axial and equatorial nitrilium-nitrile conjugates. If the reaction conditions were controlled kinetically (run at <-40°C), the faster formation of the axial nitrilium-nitrile conjugate determined the outcome of the reaction. The equatorial 1,2-*trans* glycoside was the major product in this case. If the reaction conditions were controlled thermodynamically, anomerisation of the nitrilium-nitrile conjugates should lead to the more thermodynamically stable equatorial conjugate. This should allow formation of the axial 1,2-*cis* glycoside; however in practice a mixture of glycosides could form in some cases, demonstrating the difficulty in controlling stereoselectivity in this way.

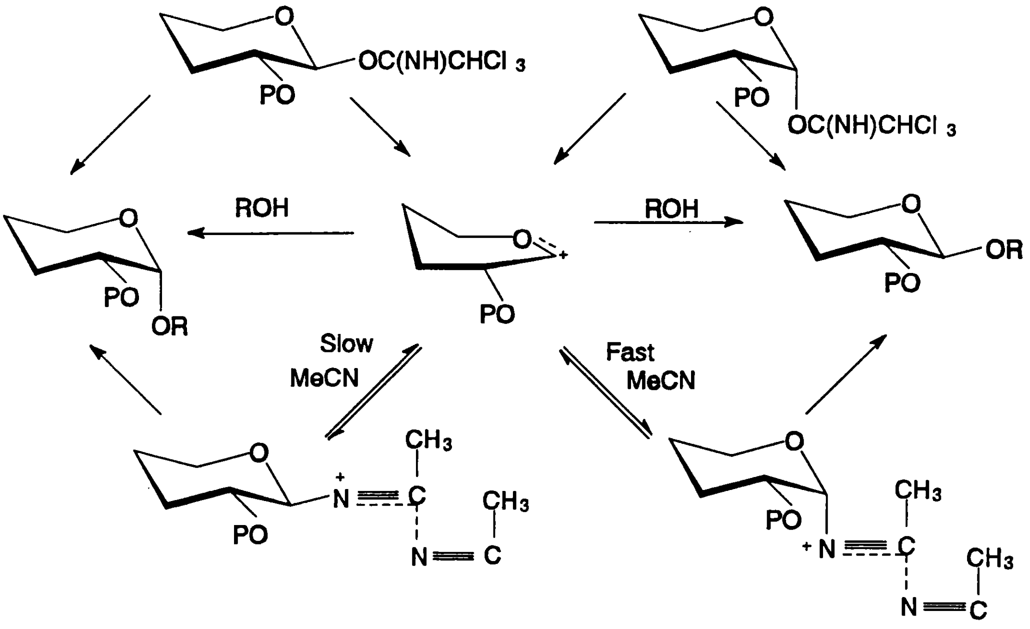


Figure 2.13

The principles of this type of glycosylation could be applied to other types of sugar donor which form an intermediate oxocarbenium ion eg. thioglycosides.

2.1.3d Formation of 1,2-*cis* glycosides

For the formation of 1,2-*cis* glycosides, the protecting group at C-2 of the glycosyl donor must be a non-participating substituent (i.e. non-acyl type). A benzyl group has often been used for this purpose, being non-participating and able to confer a higher reactivity on the sugar donor.

2.1.3e 1,2-*cis* glycosylation with halide ion catalysis

Although they are less stable than axial forms, equatorial 1,2-*trans* glycosyl halides can be synthesised and isolated, particularly the less reactive chlorides. These halides could undergo S_N2 type nucleophilic substitution when treated with an alcohol in a low polarity solvent (eg. dichloromethane, ether) in the presence of an active catalyst such as silver perchlorate to give 1,2-*cis* glycosides[129]. This procedure is only possible where the halide component is relatively stable.

Lemieux developed a halide ion catalysed glycosylation method which utilised the more stable axial halides as glycosyl donors, and exploited their tendency to undergo rapid, partial anomerisation in the presence of tetraalkylammonium halides in dichloromethane (Fig 2.14)[130].

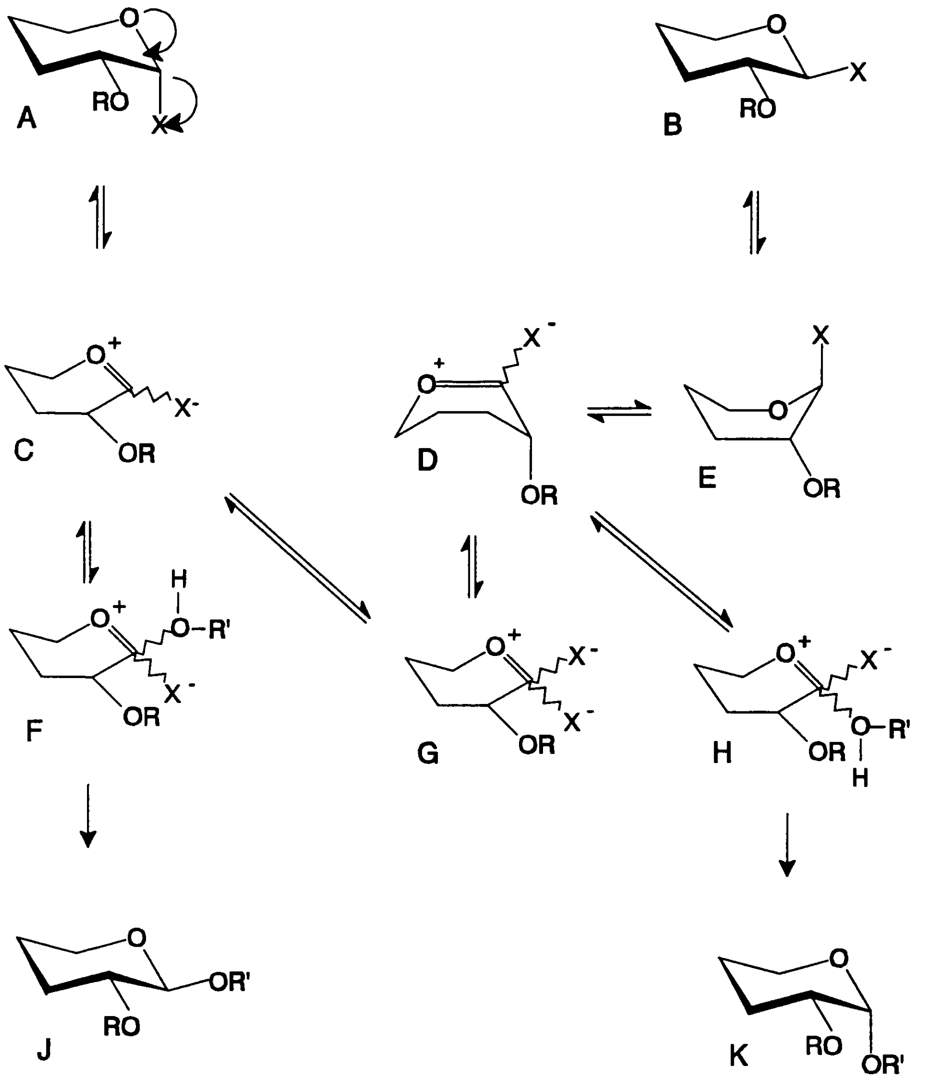


Figure 2.14

Lemieux proposed that the axial halide **A** existed in equilibrium with the more unstable equatorial halide **B**, and that the equilibration between them was catalysed by halide ions. In low polarity solvents ion pairs **C** and **D** could be produced from the axial and equatorial halides respectively. The two pairs were in equilibrium *via* the ion triplet **G**.

When an alcohol R'OH was added the two halides could react with inversion to give the glycosides **J** and **K**. The energy barrier for this reaction following the path **B→D→H→ K** was lower than for the corresponding path **A→C→F→ J**, allowing faster formation of the 1,2-*cis* glycoside than the 1,2-*trans* glycoside.

If this energy difference was large enough the reaction could be steered towards production of only the 1,2-*cis* glycoside. This could happen even if the initial concentration of the equatorial glycoside was low, as long as the establishment of the equilibrium between the two halides was fast enough.

This type of reaction required very reactive glycosyl donors and long reaction times. The use of more polar solvents resulted in reduced selectivity. It was found possible to substitute a more active catalyst eg. mercury (II) bromide/cyanide, silver perchlorate or silver triflate for the tetraalkylammonium halides[131]. Stronger catalysts could decrease the difference in the rates of formation of the glycosides, leading to poorer selectivity, though this could be countered by using lower reaction temperatures.

2.1.3f 1,2-*cis* glycosylation from thioglycosides

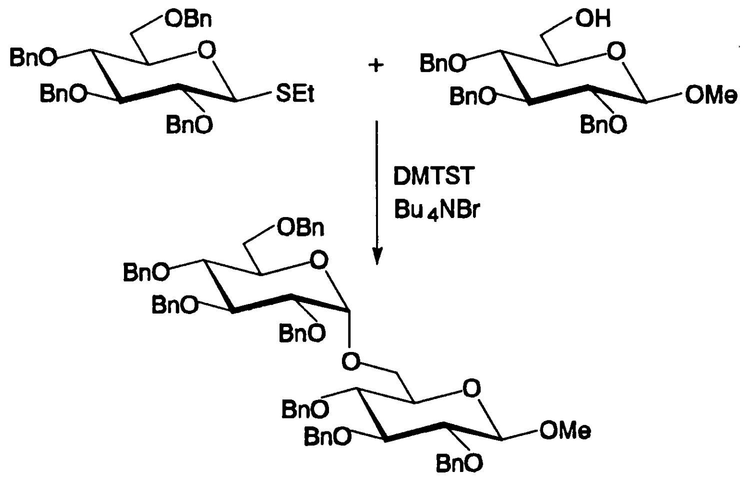


Figure 2.15

Andersson *et al.* used a modified form of the halide ion promoted glycosylation with a thioglycoside as the sugar donor to synthesise 1,2-*cis* glycosides (Fig 2.15)[132]. In a reaction using a thioglycoside with a non-participating group at C-2, with DMTST as promoter in the presence of a tetraalkylammonium bromide, the 1,2-*cis* product was the only product isolated. Examination of the reaction mixture by TLC showed that the corresponding glycosyl bromide was formed almost immediately from the thioglycoside. An alternative method was treatment of a thioglycoside with bromine or copper (II) bromide to generate the glycosyl bromide initially, which could then be used in a halide ion catalysed glycosylation (Fig. 2.16)[133].

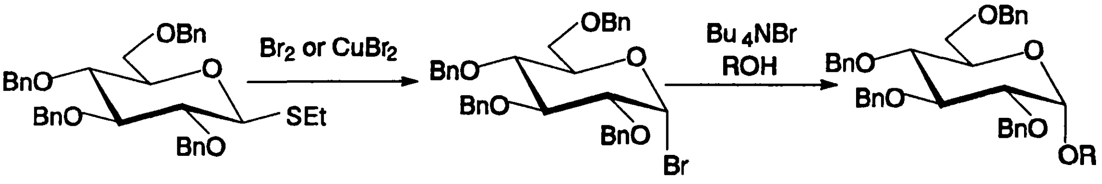


Figure 2.16

Iodonium ion promoted glycosylation with IDCP or IDCT as promoter could be used to form 1,2-*cis* glycosides from thioglycosides if the acceptor was a less reactive alcohol[119,120]. It was proposed that such acceptors would only react with the more reactive 1,2-*trans* iodosulphenium intermediate generated, giving the 1,2-*cis* glycoside. With reactive primary alcohols anomeric mixtures were obtained.

2.1.3g 1,2-*cis* glycosylation from trichloroacetimidates

Glycosylations with trichloroacetimidate donors with non-participating groups could be steered towards either 1,2-*trans* or 1,2-*cis* selectivity by choice of the donor anomer and of the promoter used (Fig. 2.17).

Boron trifluoride etherate promotion gave glycosylation with inversion; thus if the 1,2-*trans* imidate was used as donor a 1,2-*cis* glycoside was formed.

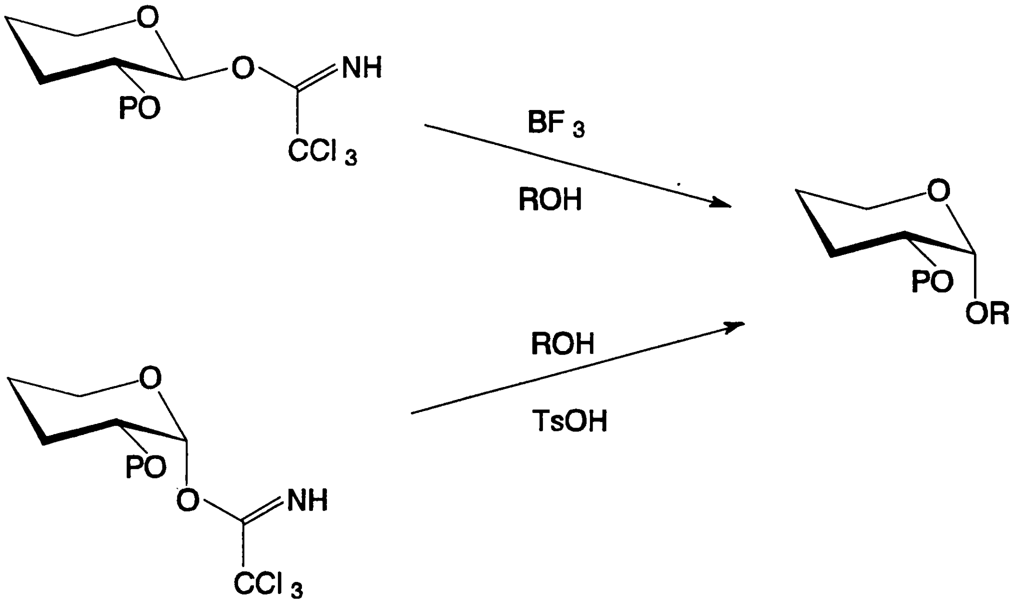


Figure 2.17

Glycosylation with trimethylsilyl triflate or *p*-toluenesulphonic acid promotion resulted in the more thermodynamically stable anomer and the axial 1,2-*cis* imidates gave axial 1,2-*cis* glycosides under these conditions[103].

2.2 GLYCOSYLATION OF SIALIC ACID

The synthesis of glycosides of sialic acid (Neu5Ac) has been of great interest because of the abundance of sialosides in naturally occurring carbohydrates. Many attempts have been made to synthesise both the α-sialosides and the unnatural β-glycosides. It has proved to be a challenging target however because of the inherent difficulties in glycosylating a structure such as sialic acid.

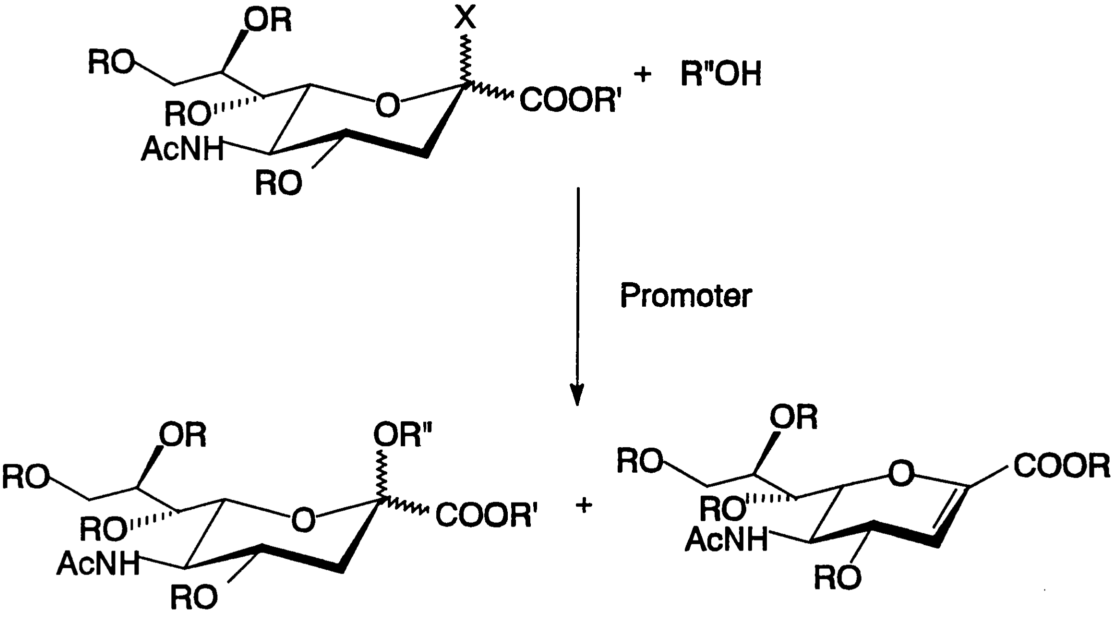


Figure 2.18

The carboxylic acid group at C-2 electronically disfavours oxonium ion formation, which is the intermediate associated with almost all known glycosylation reactions[139]. Glycoside formation is also inhibited by steric effects at the quaternary C-2. There is no substituent at C-3, so participating group strategies to direct the stereoselectivity of glycosylation reactions are not possible. The formation of a 2,3-dehydro derivative of the donor, formed by intramolecular elimination, was a common product of glycosylation reactions (Fig. 2.18).

2.2.1 The Koenigs-Knorr reaction in sialylation

The Koenigs-Knorr glycosylation method was the first approach used to form sialosides. Meindl *et al.* used a chloro-sialic acid donor with silver carbonate as promoter to form α-sialosides in 30-50% yield with various primary alcohols[134]. This type of reaction has proved generally to be successful with reactive alcohols, but if sterically hindered, unreactive alcohols are used the elimination side-product became predominant (Fig. 2.19).

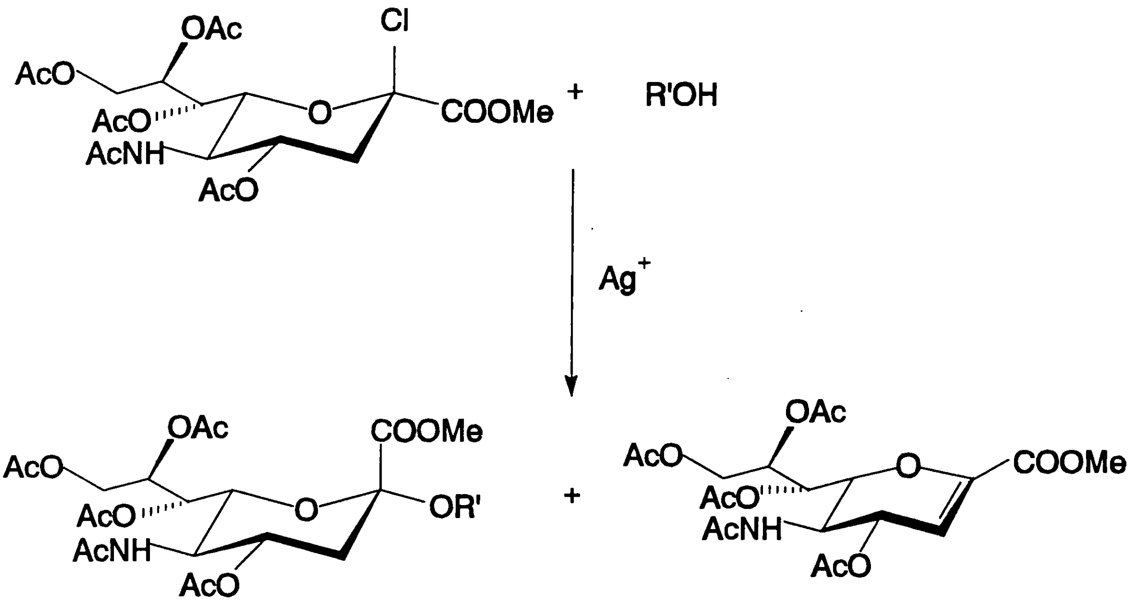


Figure 2.19.

Experiments were made to vary the nature of the silver promoter used in an attempt to improve yields. Eschenfelder and Brossmer had some success with silver polymaleate, obtaining α-sialosides in 55-65 % yield from primary alcohol acceptors[135]. Van der Vleugel *et al.* used silver salicylate to form the α-sialosides of primary and secondary alcohols in greatly improved yields (65-95 %), without formation of β-sialosides or elimination[136]. These salts of hydroxy carboxylic acids or dicarboxylic acids (especially those that can form a 6-member ring structure) were clearly better promoters than simple insoluble silver salts[137]. It was proposed that a concerted reaction can take place between a glycosyl halide, alcohol and the promoter on the surface of these silver salts, leading to stereoselective glycosylation with inversion of configuration.

2.2.2 The Williamson reaction in sialylation

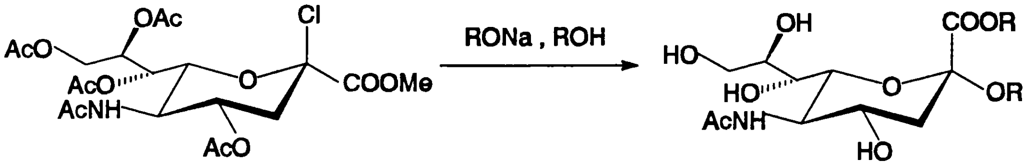


Figure 2.20

Lubineau and Le Gallic used Williamson- type reaction conditions to obtain methyl and ethyl α-sialosides from a β-sialyl chloride donor (Fig. 2.20)[138].

The sialylations proceeded with complete stereoselectivity and without formation of 2,3-dehydro products. However, transesterification and de-acetylation could not be avoided under these conditions.

2.2.3 Use of auxiliary groups at C-3 in sialylation

Several groups have attempted to prevent intramolecular elimination reactions and direct the stereoselectivity of sialylations by introducing a temporary auxiliary group at C-3.

Okamoto *et al.* prepared a diaxial bromo-derivative of sialic acid from a 2,3-dehydro sialic acid derivative by the addition of bromine (Fig. 2.21)[139]. When the di-bromide was reacted with primary and secondary sugar alcohols in the presence of silver triflate, only β-sialosides were obtained, due to steric blocking of the α-position by the C-3 bromo group. The auxiliary group was removed by triphenyltin hydride to give the β-sialosides in high overall yield.

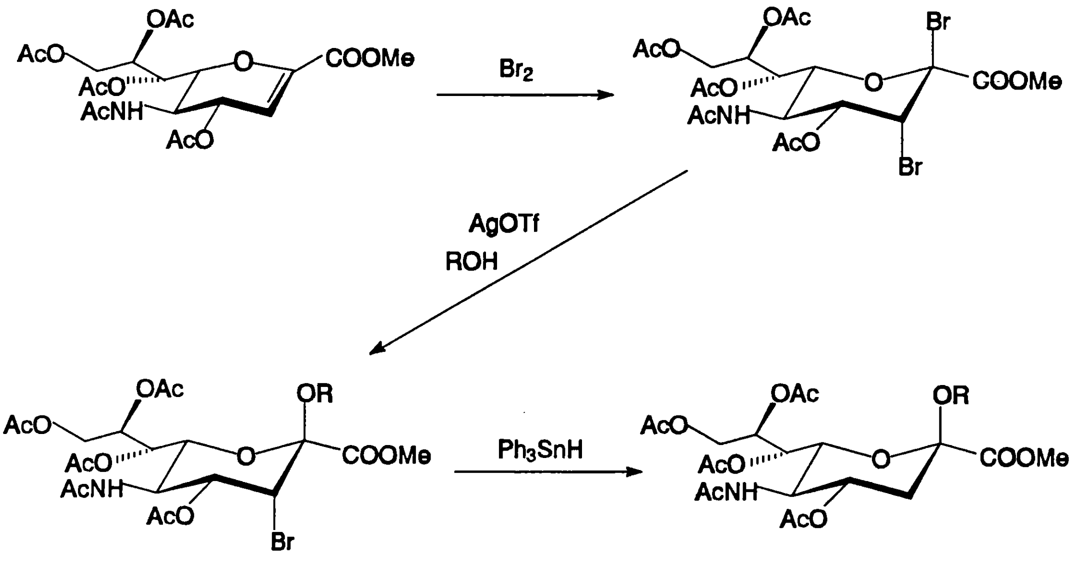


Figure 2.21

A different strategy was required to create α-sialosides with neighbouring group assistance. Itoh *et al.* investigated the use of equatorial phenylselenyl (PhSe) and phenylsulphenyl (PhS) groups at C-3[140]. Sialyl donors with a C-3 PhS group proved to be more successful than those with a PhSe group[141]. When used with a bromo-leaving group and $Hg(CN)_2$ / $HgBr_2$ as promoter, α-sialosides of secondary sugar alcohols could be obtained in reasonable yields (>60%) (Fig. 2.22). The major drawback of these syntheses was the multiple reaction steps required to prepare the auxiliary substituted donor.

Kondo *et al.* developed a more convenient synthesis of a sialyl donor with an SPh C-3 auxiliary group by treating a 2,3 -dehydro derivative of sialic acid with phenylsulphenyl chloride[142]. With a chloro- leaving group and silver triflate as promoter, primary and secondary sugar alcohols were sialylated with α-selectivity in yields of 40-70%. The auxiliary PhS group was removed with tributylstannane after sialylation.

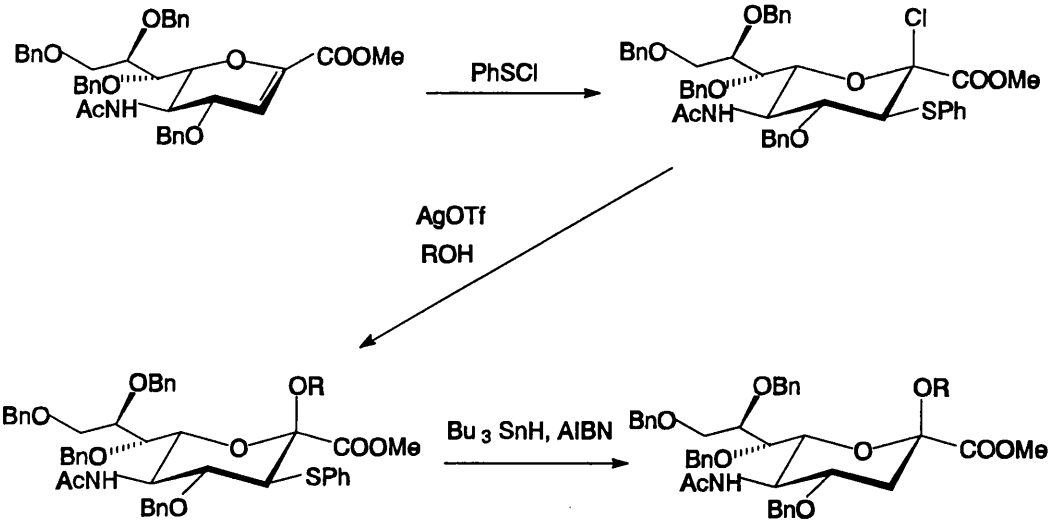


Figure 2.22

The application of auxiliary group assisted of approach has been limited by the need to introduce the auxiliary group to the donors, and to remove the auxiliary group after sialylation.

2.2.4 Sialylation with thio-sialosides as donors

The observed utility of thioglycosides as glycosyl donors in the case of other sugars led to attempts to use thio-sialosides as donors.

Hasegawa *et al.* activated a methyl-thio sialyl donor with DMTST to obtain sialosides of primary and secondary alcohols (Fig. 2.23)[143]. Under this type of activation the ratio of stereoisomers formed was found to be dependent on the donor anomer used, the solvent, and the nature of the acceptor. Dichloromethane as solvent tended to give β-anomers, while acetonitrile gave predominantly the α-anomer. Nitrilium-nitrile sialylation from thio-glycosides under kinetically controlled conditions favoured formation of the equatorial α-anomer. Alternative promoters (*N*-iodosuccinimide, methylsulphenyl bromide and silver triflate) have also been successfully used in the synthesis of α-sialosides[144,145].

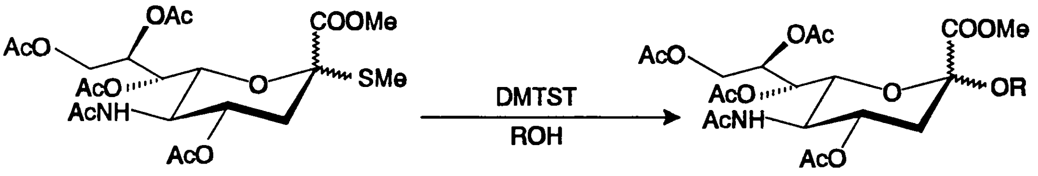


Figure 2.23

Sialylation of secondary sugar alcohols often resulted in formation of solely the α-anomer, presumably for steric reasons.

2.2.5 Sialylation from sialyl xanthate donors

Sialyl xanthate donors could be activated in a similar manner to thioglycosides, and have become of interest because of the good α-stereoselectivity they exhibited as sialyl donors.

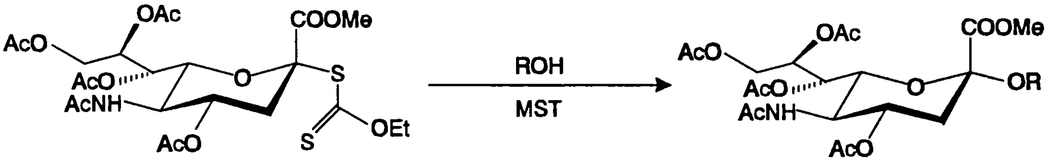


Figure 2.24

While DMTST has been widely used as a promoter for thio-sialosides, xanthates could be activated with milder catalysts such as methylsulphenyl triflate (MST) and phenylsulphenyl triflate (PST). In sialylations of primary and secondary carbohydrates, a sialyl xanthate donor with PST as promoter gave predominantly α-sialosides in 60-80% yield (Fig. 2.24)[146].

2.2.6 Sialylation from sialyl phosphites

Sialyl phosphites have recently been investigated as alternative donors to thioglycosides. Kondo *et al.* synthesised a di-benzyl phosphite sialyl donor, and utilised it in sialylations of primary and secondary sugar alcohols, with catalytic amounts of TMSOTf as promoter[147]. Yields of sialoside were between 70-85% with the α-anomers found as major components when acetonitrile was used as solvent.

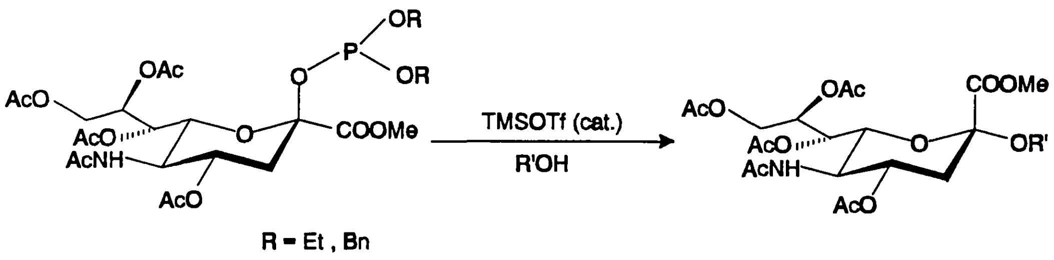


Figure 2.25

The requirement for only catalytic amounts of Lewis acid (eg TMSOTf) for activation offers an advantage over thiosialosides which need equivalent amounts of promoter, which can cause unwanted side reactions. Martin and Schmidt used a diethyl-phosphite donor in a sialylation of a 3',4'-unprotected lactose acceptor, and obtained a 55% yield of α-sialoside (Fig.2.25)[148]. The yield of α-sialoside from this method were superior to published yields obtained with other sialylation methods using the same acceptor.

2.3 SYNTHESES OF SIALYL LEWIS X TETRASACCHARIDE

The recognition of SLeX as a bio-active oligosaccharide involved in disease processes led to interest in synthesising this structure and its analogues for biological testing. As it is not easily available from biological sources, there was also a commercial interest in producing this compound. In the last seven years, a range of both chemical and enzymatic synthetic approaches have been developed.

2.3.1 Enzymatic methods

Chemo-enzymatic methods have been used to synthesise SLeX in preparative scale, and some analogues have also been produced enzymatically[149-151]. For this tetrasaccharide three enzymes (β-1,4-galactosyltransferase, α-2,3-sialyltransferase and α-1,3-fucosyltransferase) and three sugar nucleotides (UDP-galactose, CMP-*N*-acetylneuraminic acid and GDP-fucose) were needed, starting from a glucosamine core. A problem with this type of enzymatic reaction is "production inhibition", caused by the release of nucleotide phosphates. This was partly overcome by *in situ* regeneration of sugar nucleotides, requiring multi-enzyme systems. While these methods may ultimately prove to be superior to chemical syntheses, at this time they are limited by the availability and cost of the glycosyltransferases and donor sugar nucleotides necessary.

2.3.2 Chemical methods

Synthesis of the SLeX tetrasaccharide was first reported in 1991 by two groups, Nicolaou *et al.* and Hasegawa *et al* [152,153].

Nicolaou *et al.* began the synthesis with the construction of a lactosamine core which was selectively deprotected at C-3 of the glucosamine residue (Fig.2.26)[152]. Fucosylation with a fluoro-fucose donor in the presence of $AgClO_4$/ $SnCl_2$ gave the required trisaccharide, which was deprotected to give a Gal C-2,3,4 triol acceptor. A chloro-sialic acid derivative with a C-3 SPh auxiliary group was used as a sialyl donor to form the tetrasaccharide. During deprotection, intra-molecular lactone formation occurred between the carboxylic acid function of the Neu5Ac residue and C-2 of the Gal unit. Base hydrolysis and hydrogenolysis gave the free tetrasaccharide.

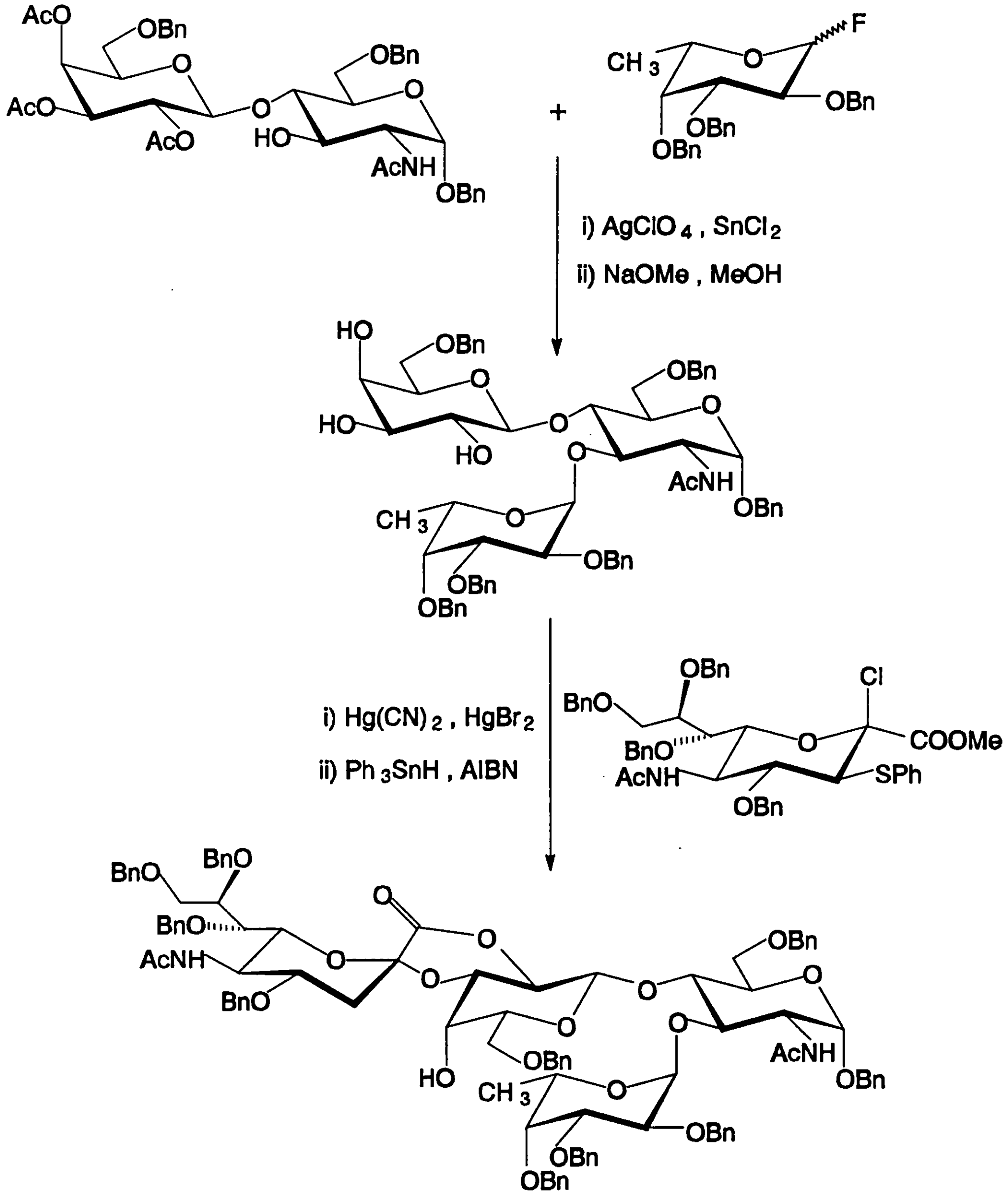


Figure 2.26

The group of Hasegawa has been at the forefront of the synthesis of SLeX gangliosides and their analogues[153-160]. Their approach to these syntheses has been marked by several characteristics :

i) The frequent use of thio-glycosides as glycosyl donors

ii) "Block" - type syntheses of oligosaccharides ie. constructing target molecules with pre-formed di/trisaccharides. The use of a sialic acid - galactose disaccharide donor over sialylation of a large oligosaccharide acceptor (Fig. 2.27) was especially favoured.

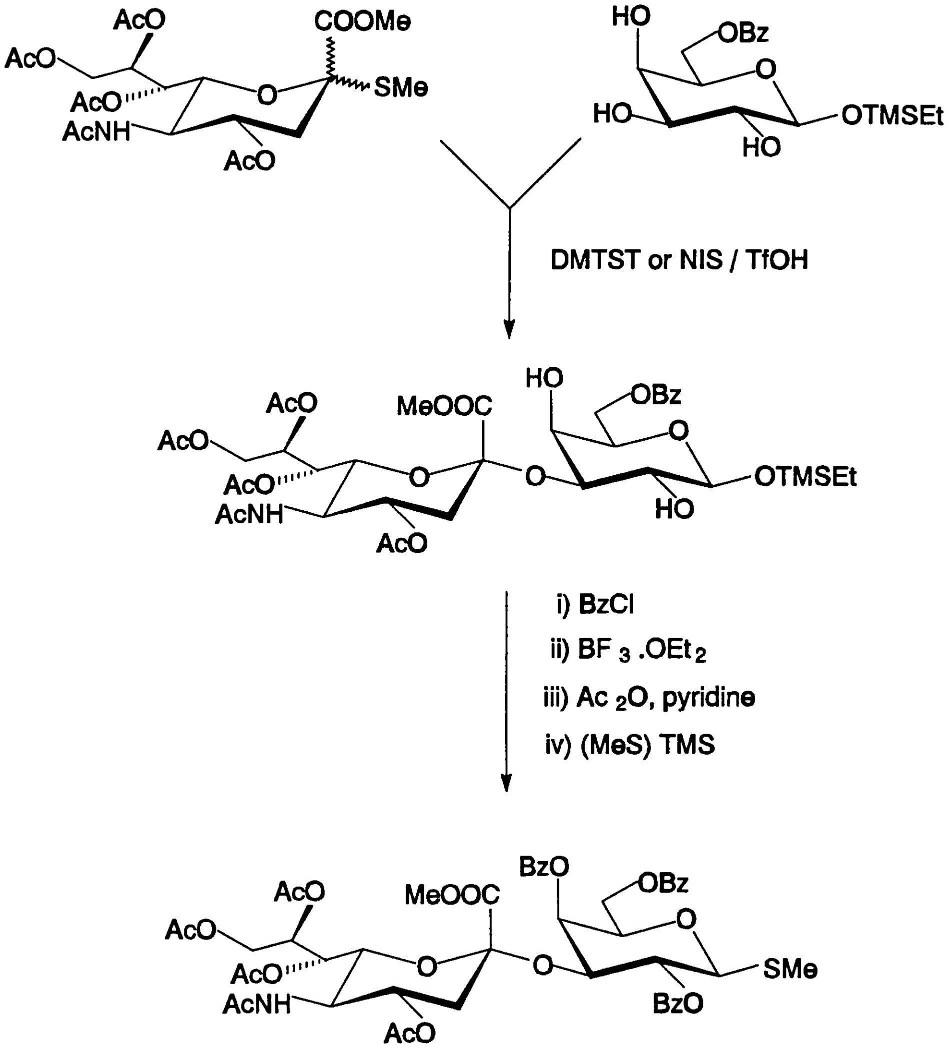


Figure 2.27

This type of donor was typically formed by sialylation of a C-2,3,4 unprotected galactose acceptor, which was found to be regioselective, independent of the sialylation method used.

iii) The use of the 2-(trimethylsilyl)ethyl group as temporary anomeric protection.

These techniques were used to form SLeX ganglioside (a hexasaccharide with a ceramide aglycone), positional isomers, and analogues with modified sugar units for testing as potential selectin ligands. A "practical" synthesis of a SLeX pentasaccharide with an ethyl galactoside at the reducing end of the epitope, aimed at providing material for biological testing, was recently described[161].

The initial strategy for the synthesis of this pentasaccharide was the formation of a fucosylated trisaccharide acceptor (Fig 2.28, R = protected ethyl galactose).

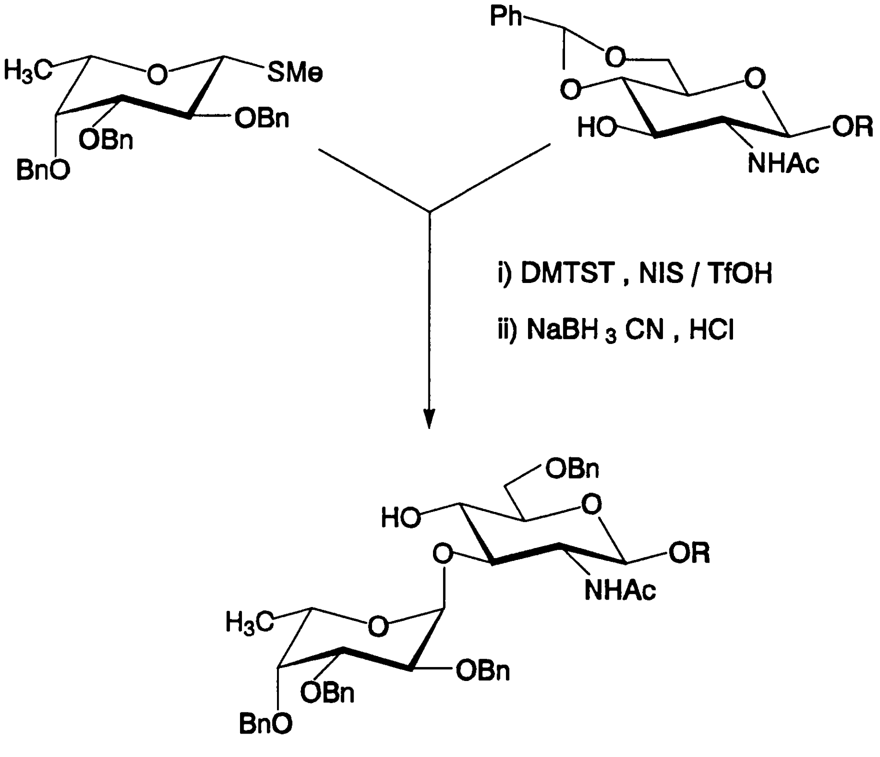


Figure 2.28

Glycosylation of the trisaccharide with a Neu5Ac-Gal thioglycoside donor in the presence of DMTST failed to produce the required pentasaccharide in this case (although successful glycosylation under these conditions with very similar acceptor/donor pairs had been previously reported). The suggested reason for this failure was that the C-4 position of the acceptor was sterically crowded.

This synthesis of the pentasaccharide could only be completed by using a temporary protective group at C-3 of the GlcNAc group, glycosylation with the disaccharide donor at C-4, and deprotection and fucosylation as the final steps (Fig. 2.29).

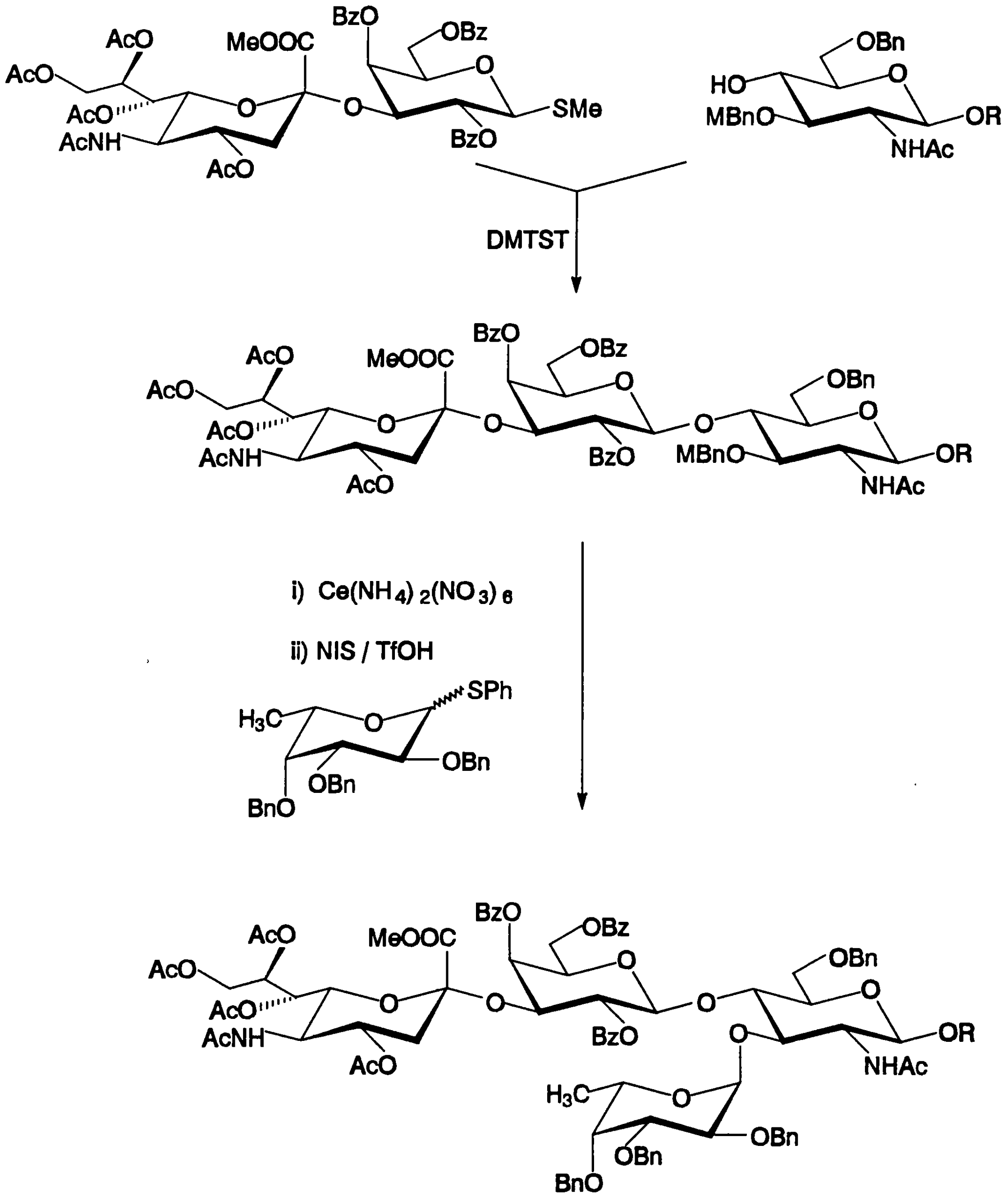


Figure 2.29

Danishefsky *et al.* reported the synthesis of SLeX containing a glycal in the place of the glucosamine residue (Fig. 2.30)[162]. The synthesis began by fucosylation of a 6-protected glucal. It was found that glycosylation with this acceptor and a fluoro-fucose donor was regioselective (C-3 : C-4 fucosylation in a ratio of 8:1), but α-stereoselectivity was only achieved when the fucose donor had a benzoyl protective group at C-4. A tri-benzylated donor gave a 5:3 α:β mixture. Galactosylation of this disaccharide with a trichloroacetimidate donor and boron trifluoride promotion gave the trisaccharide in 75% yield. Deprotection gave a triol acceptor, which was sialylated with a chloro-sialic acid donor in the presence of silver triflate.

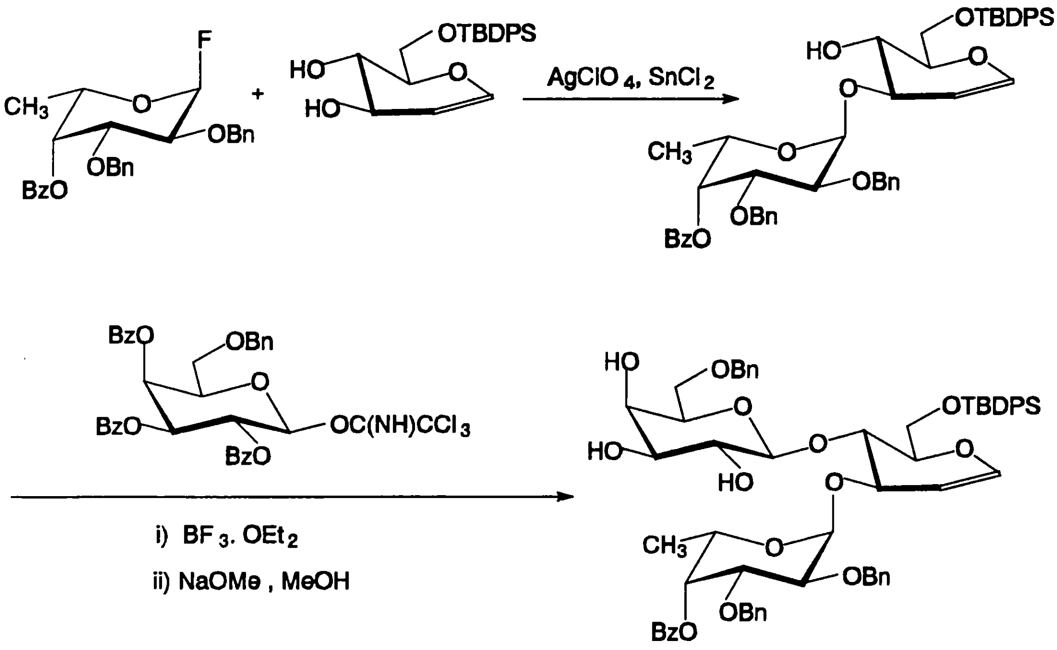


Figure 2.30

The glucal function still remained at the end of this synthesis; conversion to glucosamine required six reaction steps with the loss of nearly 90% of the starting tetrasaccharide.

Sprenghard *et al.* wished to synthesise a SLeX derivative which would be suitable for coupling to peptides, and they were able to synthesise an azido-SLeX derivative for this purpose in multi-gram scale[77,163].Fucosylation of a GlcNAc acceptor with a per-benzylated thiofucoside in the presence of tetrabutylammonium bromide/copper bromide gave the α-disaccharide stereoselectively in 92% yield.

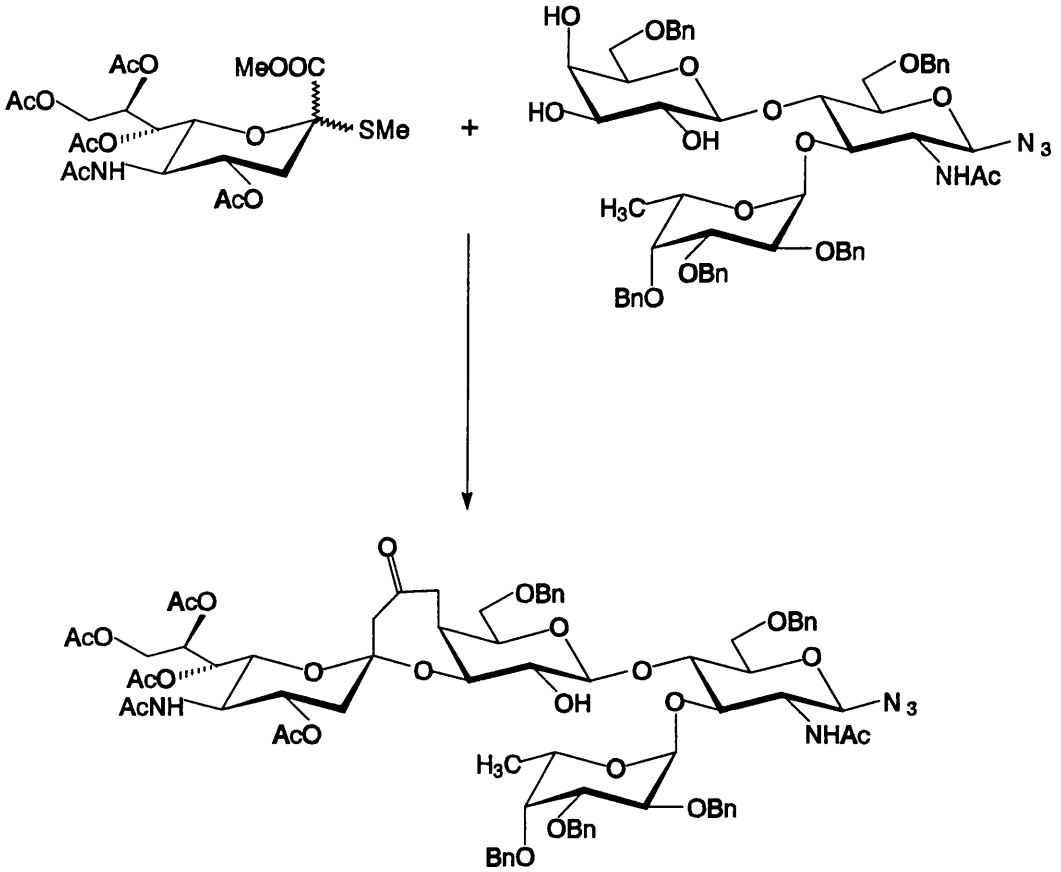


Figure 2.31

After reductive ring opening, reaction of the trisaccharide with a galactosyl trichloroactimidate donor was attempted. Difficulties were reported with the galactosylation reaction, and the trisaccharide was obtained in only 44% yield. A methylthio-galactoside donor was found to give poorer results. After deprotection, sialylation with a thio-sialoside donor gave the 1-4' intramolecular lactone as the major product (Fig. 2.31).

2.4 SYNTHESIS OF SIALIC ACID - NUCLEOSIDE CONJUGATES

The biologically active sialic acid - nucleoside conjugate KI-8110, and some analogues, had been synthesised by various methods.

2.4.1 Synthesis of conjugates under Koenigs Knorr conditions

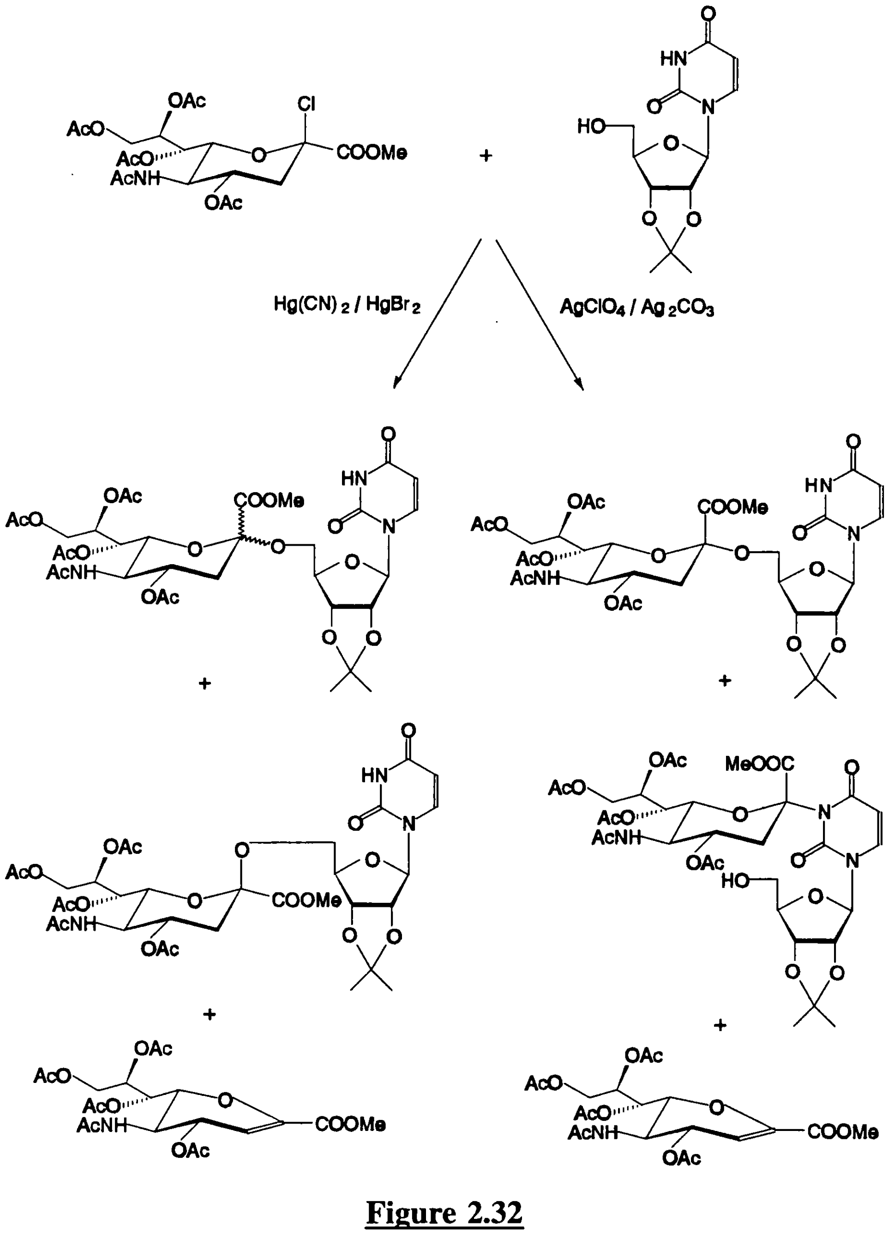


Figure 2.32

Kijima-Suda *et al.* had synthesised sialosides of 5-fluoro-2',3'-isopropylidene uridine and of 2',3'-di-*O*-acetylinosine by the use of Koenigs-Knorr type reactions, using an acetyl-protected chloro-sialic acid donor, and mercury (II) cyanide and mercury (II) bromide as promoters, but gave no details of the yields obtained[164].

Ogura *et al.* reported the synthesis under Koenigs-Knorr conditions of sialosides of uridine derivatives[165]. 2',3'-Isopropylidene uridine was reacted with a chloro-sialic acid donor in the presence of mercury (II) cyanide and mercury (II) bromide, or in the presence of silver perchlorate and silver carbonate (Fig. 2.32). When the mercury salts were used as promoters, the α-anomer was isolated in 30% yield, and the β-anomer in 10% yield. When the silver salts were used as promoters, no β-glycoside was isolated, but an α-*N*-linked sialoside was isolated in 10% yield instead. With both promoters the 2,3-dehydro derivative of the donor was also formed in 30% yield.

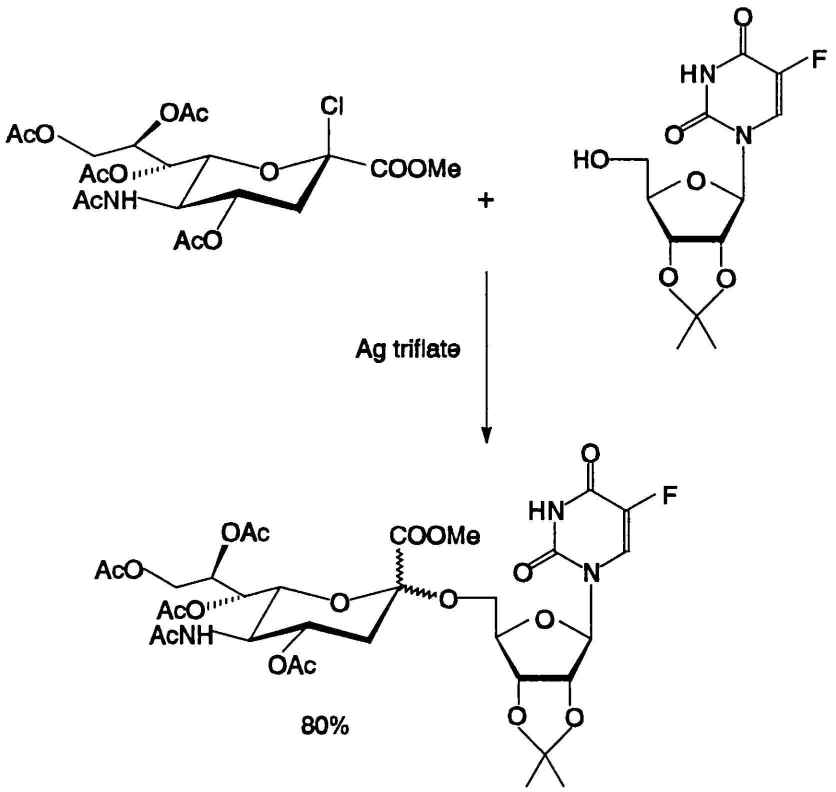


Figure 2.33

Sueda *et al.* reported that an 80% yield of sialoside (as a 3:1 α:β anomeric mixture) could be obtained when silver triflate was used as a promoter in a reaction between a chloro- sialic acid donor and 5-fluoro-2'3'-isopropylidene uridine (Fig. 2.33)[166].

Sato *et al.* compared the action of the promoters mercury (II) cyanide / mercury (II) bromide with silver triflate in the sialylation of 2',3'-di-*O*-acetylinosine[167]. The mercury salt promoted reaction at room temperature gave a 6% yield of sialylated nucleoside as a 1:1 α:β mixture (Fig. 2.34).

When silver triflate was used as promoter, the yield and anomeric ratio of sialosides isolated was dependent on the conditions employed. At room temperature,

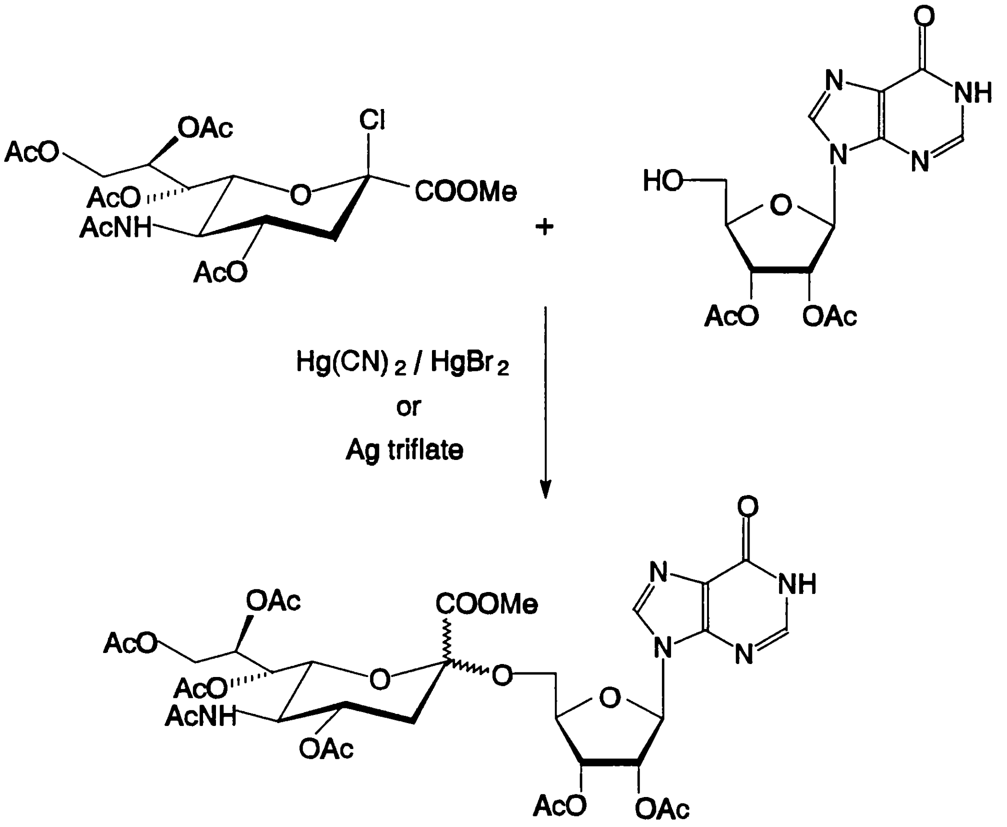


Figure 2.34

reaction in dichloromethane gave a 9% yield of sialylated nucleosides, reaction in

THF gave 13% and reaction in DMF gave 31%. A 1:2 α:β mixture was obtained with each solvent. Reaction in DMF at 0 °C gave 32% of a 1:2 α:β mixture, and at -20 °C gave 26% of a 3:10 α:β mixture.

2.4.2 Effect of intramolecular hydrogen bonding on sialylation of nucleosides

The yield of sialoside obtained in these reactions was surprisingly low considering that the acceptor had a primary hydroxyl group.

Whitfield *et al.* investigated the causes of this low reactivity[168]. They found that it was not possible to galactosylate several nucleosides (protected deoxycytidine, arabinocytidine and thymidine derivatives with a free 5'-OH) with

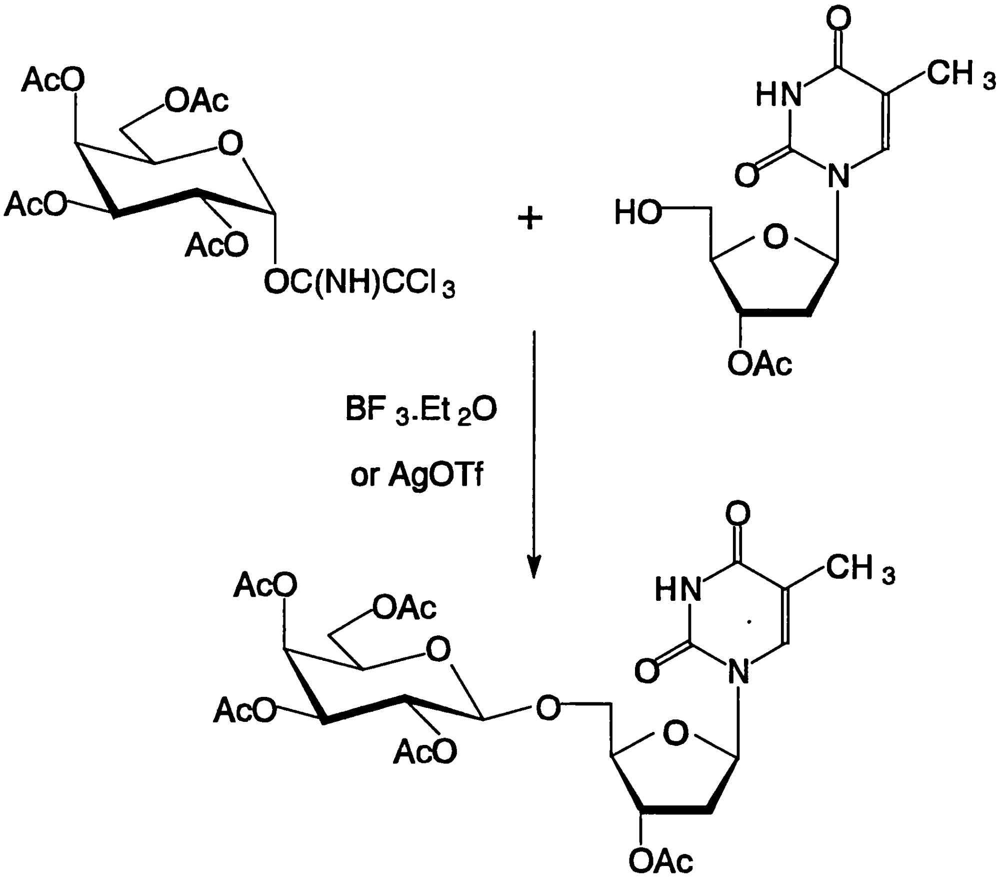


Figure 2.35

acetobromogalactose under Koenigs-Knorr conditions, but galactosylation could proceed if a per-acetylated galactosyl trichloroacetimidate was used as donor in Lewis acid (boron trifluoride or silver triflate) promoted reactions (fig. 2.35). Spectroscopic (NMR, IR) examination of the nucleosides suggested that it was possible for the nucleosides to exist in intramolecularly hydrogen bonded conformations. In the case of the deoxycytidine and thymidine derivatives, a conformation where a hydrogen bond between the hydrogen at the free hydroxyl group at position 5’ and the oxygen at position 2 on the base ring exists appeared to be predominant.

It was suggested that while participation in hydrogen bonding should make the 5’ oxygen a better nucleophile, the hydrogen abstraction that precedes glycoside formation may be more difficult if the hydrogen is stabilised by an intramolecular hydrogen bond.

2.5 SYNTHESIS OF THIO-OLIGOSACCHARIDES

The ability of natural *O*-linked oligosaccharides to act as inhibitors of selectin / leucocyte interactions has been demonstrated[71-75], but these compounds have relatively short *in vivo* half-lives as they are susceptible to the action of glycosidases. Thioglycosides have been shown to be more resistant to glycosidases and to acid hydrolysis than *O*-linked glycosides[169-172], so the synthesis of thio-linked analogues of selectin ligands was an interesting target.

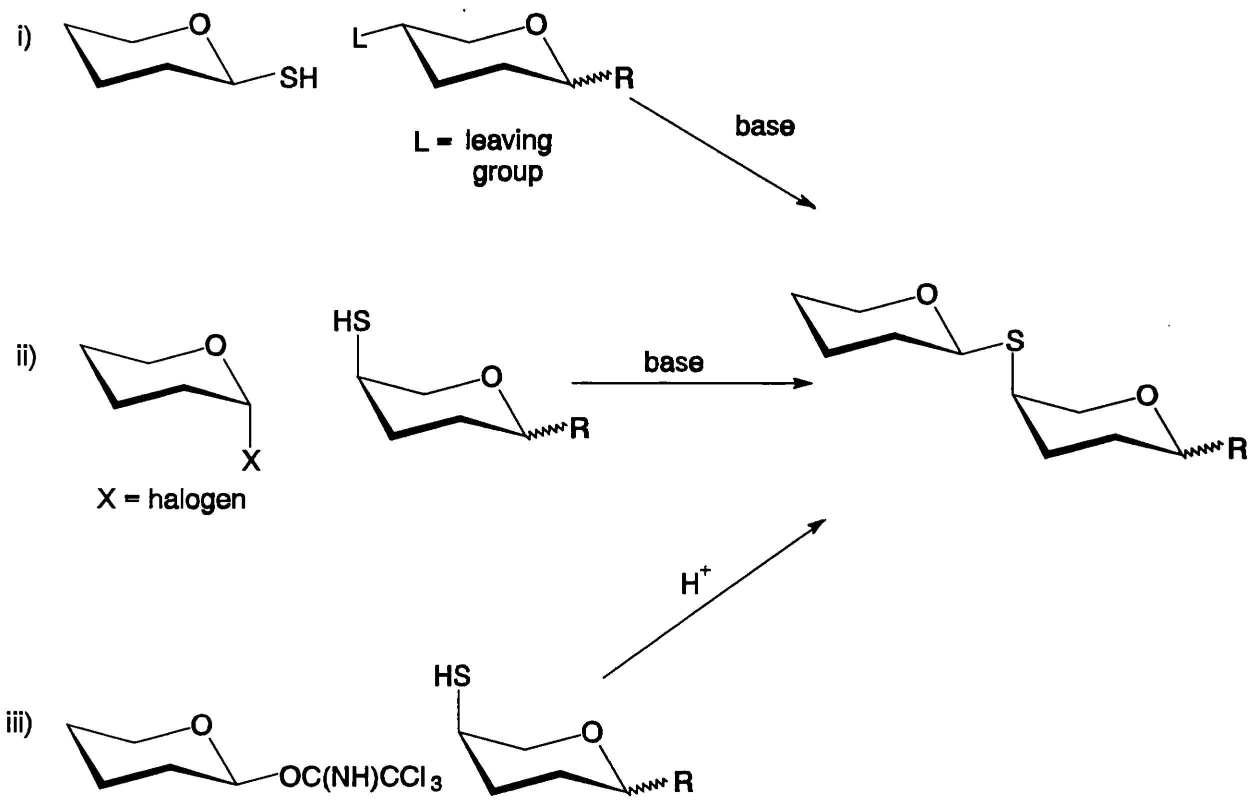


Figure 2.36

2.5.1 Formation of thioglycoside bonds

The methods previously employed for thioglycoside bond formation in thio-oligosaccharides were :-

i) anomeric *S*-alkylation
ii) base promoted *S*-glycosylation
iii) acid catalysed *S*-glycosylation.

Methods i) and ii) were most frequently reported[173], involving S_N2-type substitutions of either 1-thio donors on acceptors carrying a good leaving group (eg triflate, tosylate or halide) or of thiolate anions on glycosyl halides (Fig. 2.36). Method iii) has been reported with trichloroacetimidates as donors, with the stereoselectivity of the coupling reaction dependent on the protective groups of the donor[174].

2.5.2 Synthesis of 1-thioglycoses

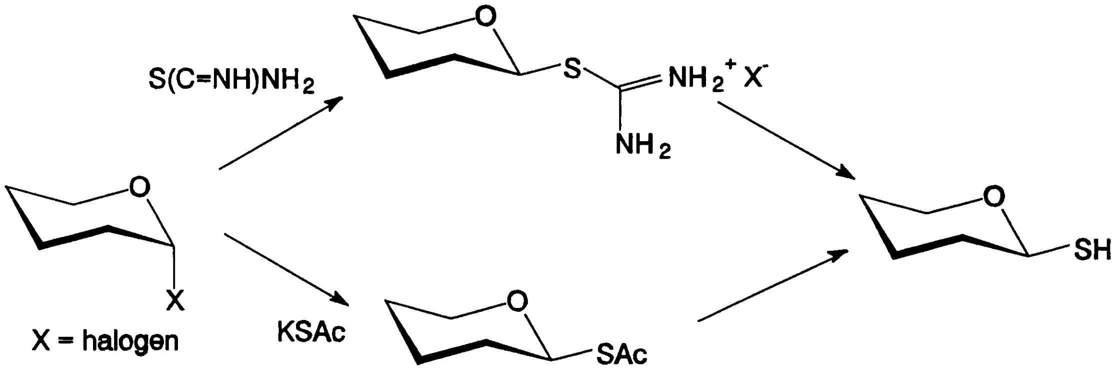


Figure 2.37

1,2-*trans*-Thioglycose donors have been synthesised from 1,2-*cis*-glycosyl halides either *via* thiourea derivatives or by reaction with potassium thioacetate and subsequent *S*-deacetylation (Fig. 2.37)[175].

1,2-*cis*-Thioglycoses could be synthesised by the reaction of 1,2-*trans*-glycosyl chlorides with potassium thioacetate, but this method was not straightforward as it required at first the isolation of the less stable anomer of the glycosyl halide. A 1-thio-α-L-fucopyranose was synthesised by the reaction of L-fucose with thioacetic acid in the presence of hydrogen chloride[175].

2.5.3 Anomeric *S*-alkylation

The anomeric *S*-alkylation approach to thiooligosaccharide synthesis required an S_N2-type substitution reaction of a 1-thioglycose (as glycosyl donor) with a sugar carrying a good leaving group (as glycosyl acceptor) in the presence of base. In this

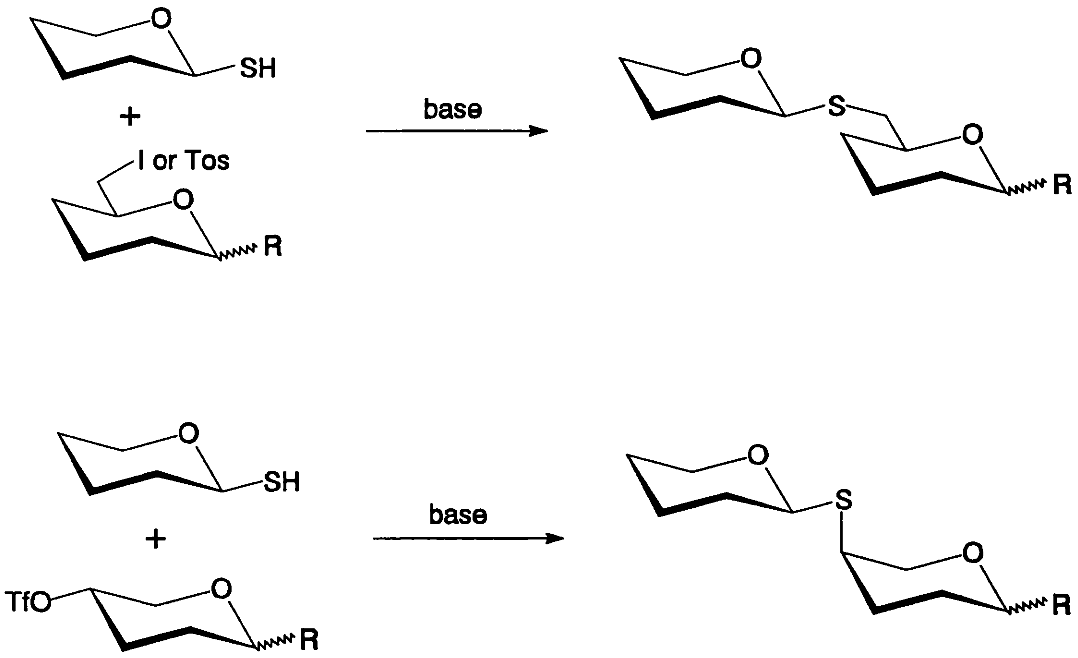


Figure 2.38

type of reaction the donor retains its anomeric configuration, while the acceptor undergoes an inversion of configuration at the position where substitution has occurred. These reactions were frequently performed in DMF, HMPA or THF in the presence of a crown ether, with preliminary formation of the sodium salt of the donor with sodium hydride. The type of leaving group on the acceptor could determine the success of formation of thio-linked disaccharides. It was possible to form 1,6-thio-linkages with iodo or tosyl leaving groups, but at secondary positions the more reactive triflate group was needed for bond formation (Fig. 2.38).

2.5.4 Syntheses of thio-linked analogues of Lewis X and sialyl Lewis X

Hashimoto *et al.* synthesised α-(1→3)-*S*-fucosyl-glucosamine disaccharides, structures that are included in the Lewis X epitope[175]. Anomeric *S*-alkylation was used to construct the disaccharides from a 1-thio-α-fucosyl donor and a 2,3-tosylepimino allopyranoside acceptor (Fig. 2.39). Sodium methoxide was used in the

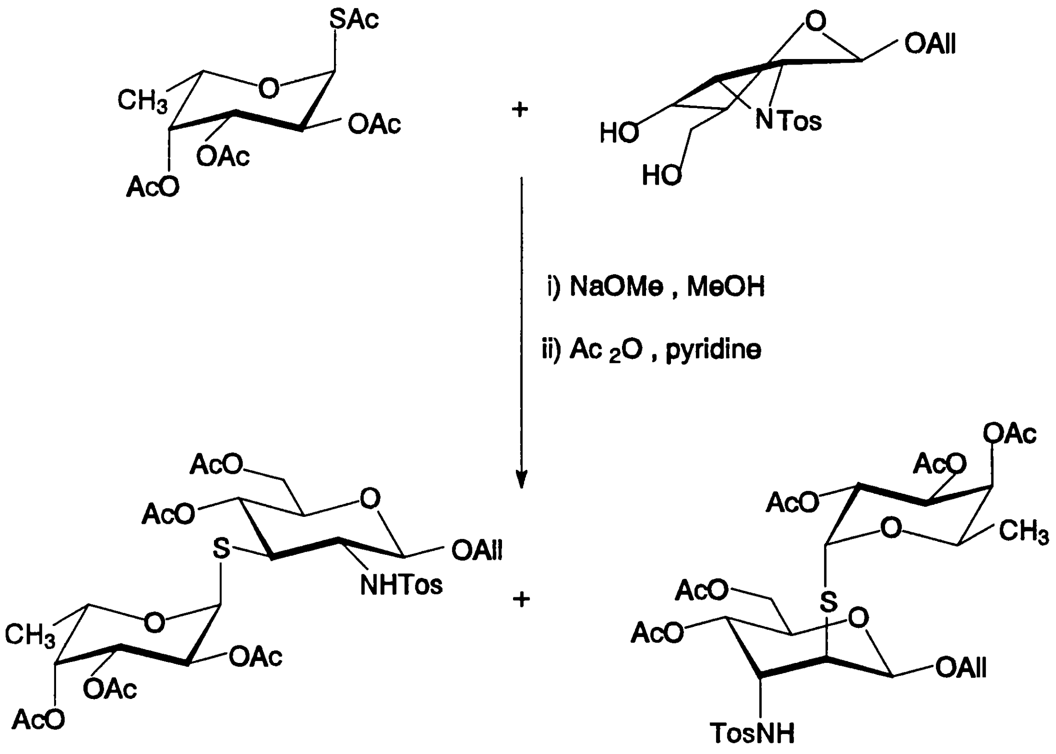


Figure 2.39

coupling reaction, resulting in the desired α-(1→3)-linked disaccharide and also a 1→2-linked product in a ratio of 2:1.

Aguilera and Fernández-Mayoralas attempted the synthesis of a Lewis X derivative where only the fucose residue was *S*-linked, as the α-fucosyl bond was known to be particularly acid sensitive[176]. An *N*-acetyl allosamine acceptor molecule with a leaving group at position C-3 was needed for formation of this structure. It was found to be not possible to introduce a triflate group at this position, while a tosylate group was not sufficiently reactive to allow a further substitution. A cyclic

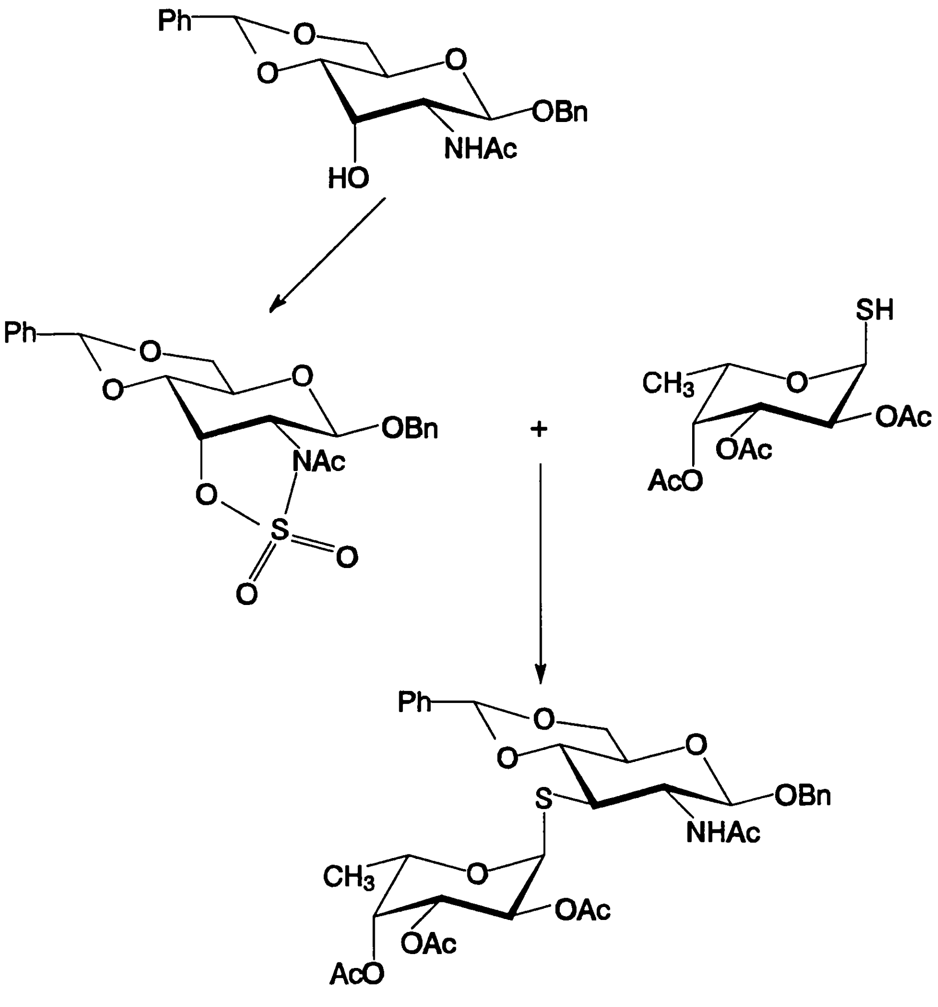


Figure 2.40

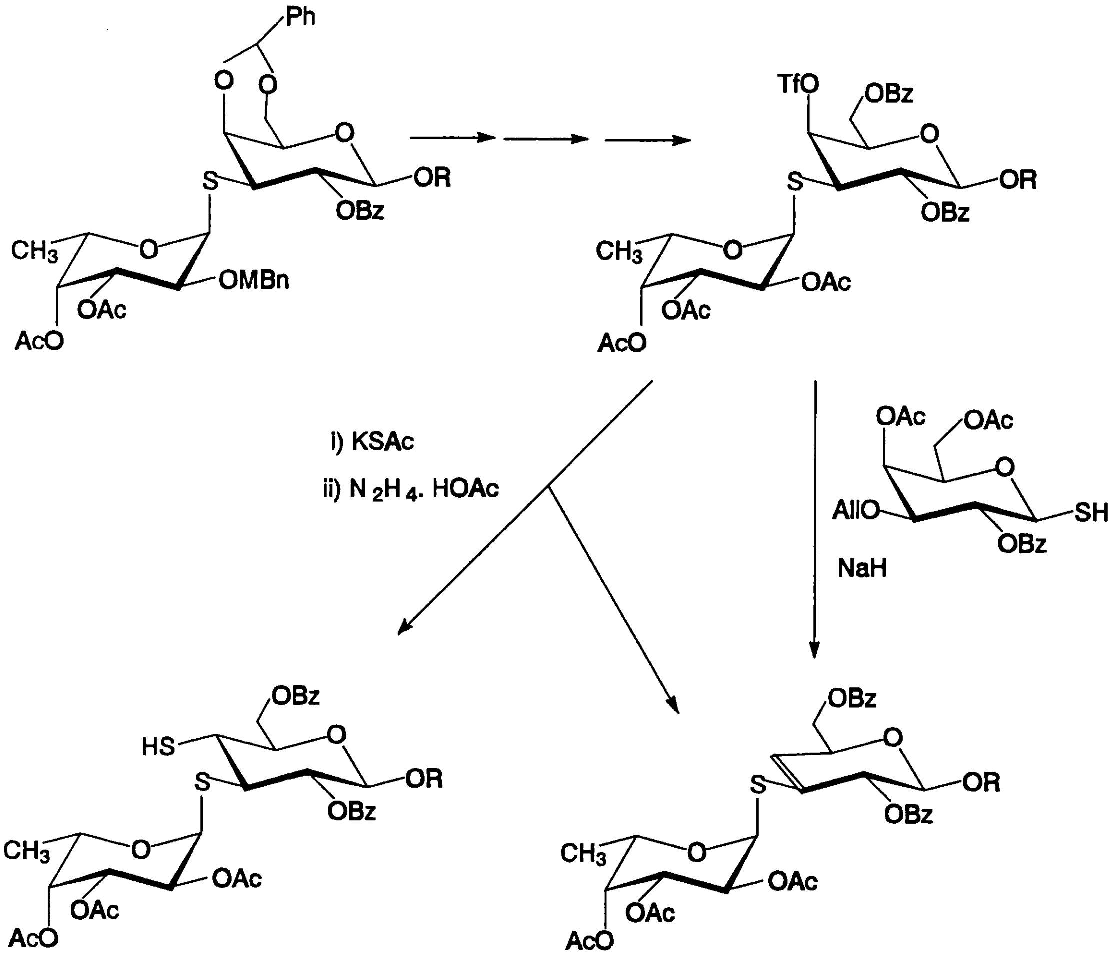


Figure 2.42

sulfamidate derivative of the allosamine residue was synthesised by reaction with sulphuryl diimidazole. This compound was reacted with a 1-thio-fucose to give the α-(1→3)-linked disaccharide in 50% yield (Fig. 2.40).

Eisele and Schmidt[174] synthesised thio-linked analogues of Lewis X and sialyl Lewis X using a combination of thio-glycosylation methods. A thio-linked fucose-galactose disaccharide was the basic unit required for these syntheses, and was formed by an acid catalysed *S*-glycosylation between a fucosyl trichloroacetimidate donor and a 3-thiogalactose acceptor (Fig. 2.41).

Two synthetic routes to the LeX trisaccharide structure were investigated, *S*-alkylation of a 1-thiogalactose and *S*-glycosylation of a galactosyl bromide. It was found that *S*-alkylation resulted only in an elimination reaction of the disaccharide, with no trisaccharide formation. The alternative route required first the introduction of a thiol-group to the disaccharide, a reaction that was successful but also accompanied by elimination (Fig. 2.42).

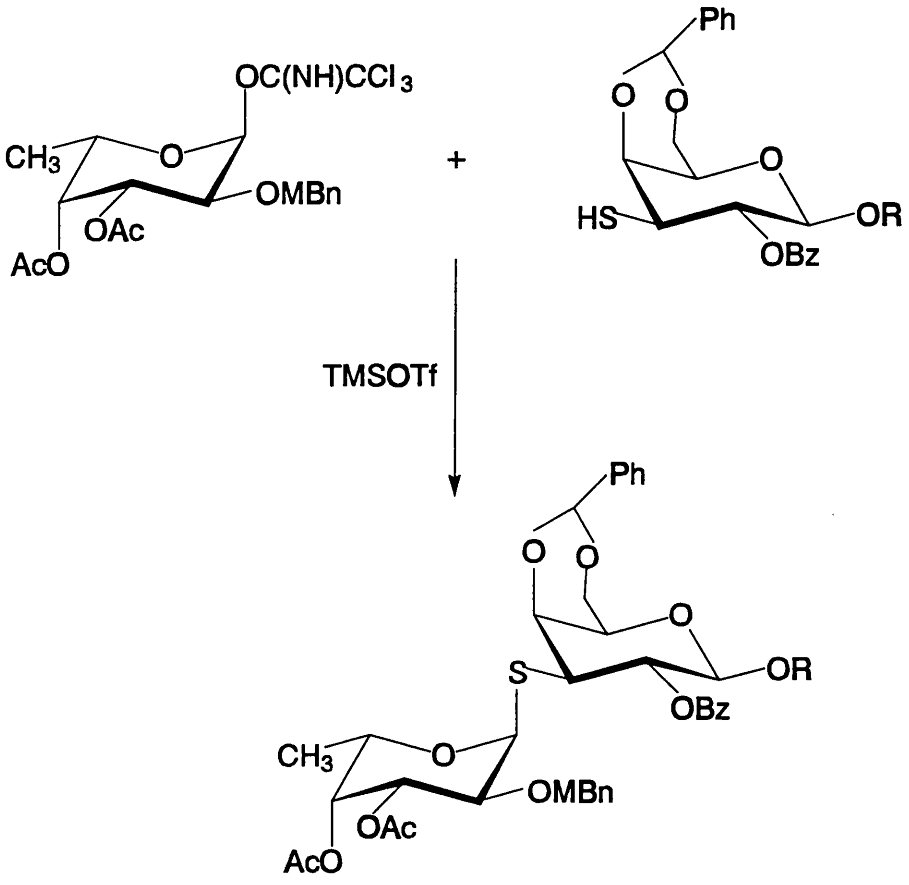


Figure 2.41

S-Glycosylation of the disaccharide with a galactosyl bromide in the presence of sodium hydride gave the desired trisaccharide in 70% yield. A similar reaction with a sialic acid-galactosyl bromide gave an *S*-linked SLeX analogue (Fig. 2.43).

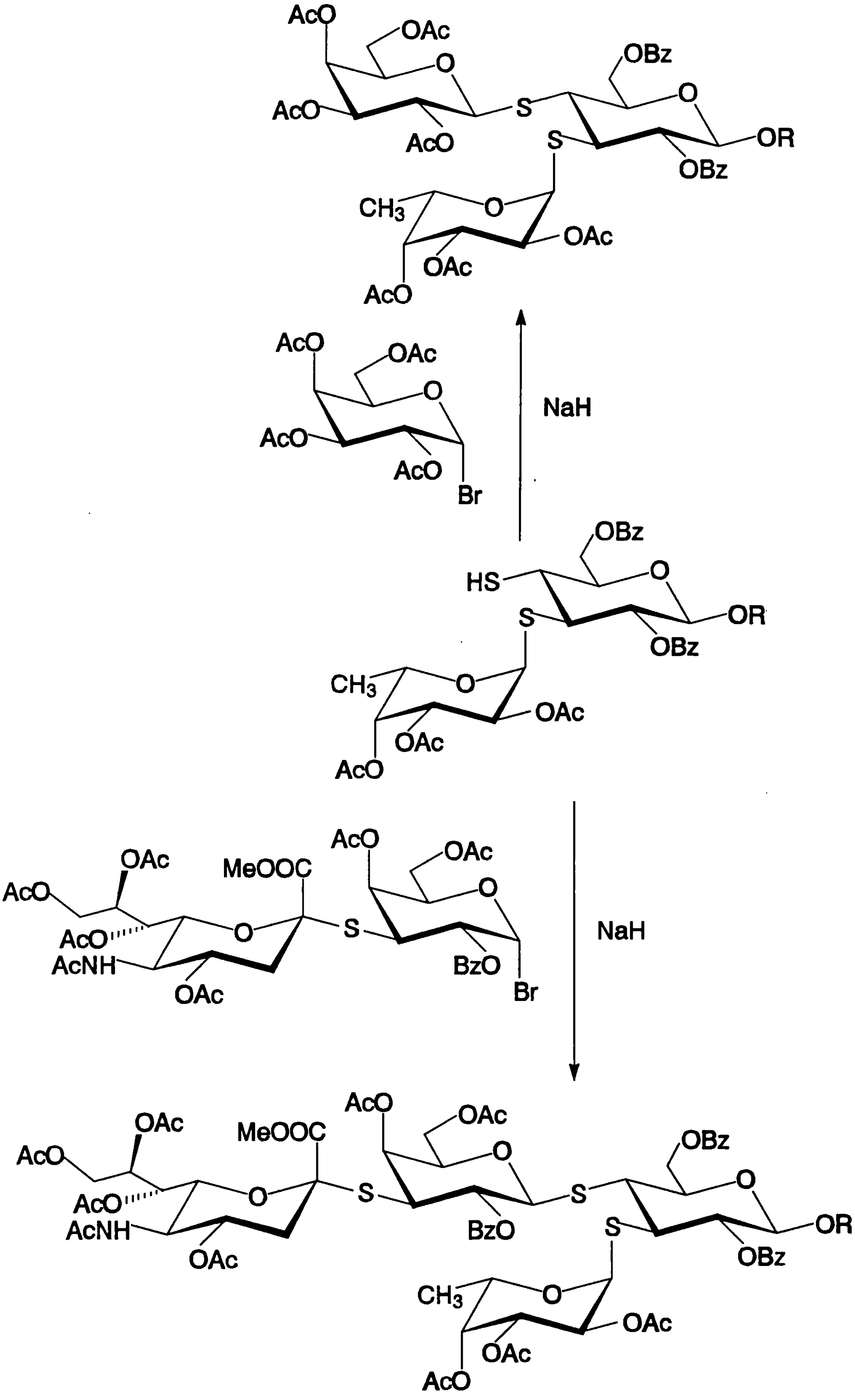


Figure 2.43

3 RESULTS AND DISCUSSION

3.1 LARGE SCALE SYNTHESIS OF SIALYL LEWIS X TETRASACCHARIDE

We attempted to develop an economical large scale synthesis of the SLeX tetrasaccharide, and also the related Lewis X trisaccharide to provide material for selectin binding studies. Large scale synthetic pathways should ideally :-

i) have a minimum number of steps
ii) lead to a reasonable overall yield
iii) use cheap, non-hazardous, easily obtained reagents
iv) avoid extreme reaction conditions eg very low temperatures.

Chromatographic purification between steps should be kept to a minimum, with purification by recrystallisation preferable. "One pot" reactions, where several steps are carried out consecutively without purification, are desirable.

3.1.3 Oligosaccharide synthesis strategies

Oligosaccharides can be synthesised using two different strategies. In a stepwise addition approach, sugar units are added sequentially to a lengthening chain. This requires careful consideration of protective group strategy, to allow selective unmasking of the next hydroxyl to be glycosylated in the chain, leaving the remainder of the protective groups intact. The alternative strategy is to form "blocks" of di- or trisaccharides which can be condensed together to form the desired product. This requires either differential glycosylation methods (as the leaving group at the reducing end must be unchanged during synthesis of the block, only being activated in the final formation of the construct), or temporary anomeric protection followed by formation of a suitable donor group. This method can suffer from the reduced reactivity of either acceptor or donor when they are oligosaccharides.

It was considered that a stepwise approach offered the better route to the construction of SLeX. A pathway that included the LeX trisaccharide as an intermediate would be advantageous, as this structure is also of interest for biological testing. Sialic acid was the most expensive unit to be used in the synthesis; losses of this material had to be avoided. The acceptor and donor sugars used would preferably be easily synthesised, stable structures which could be stored for extended periods.

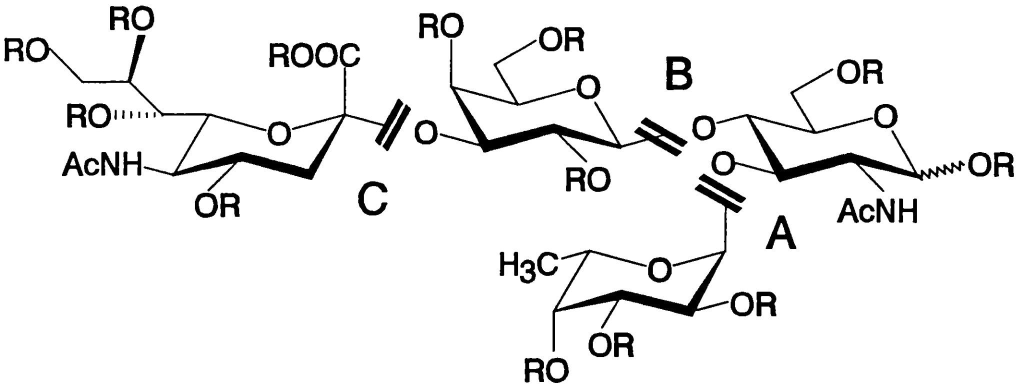
3.1.2 Formation of the sialyl Lewis X tetrasaccharide

The formation of the SLeX tetrasaccharide required the synthesis of three glycosidic bonds. Some consideration was needed of their optimal formation conditions.

i) Neu5Ac-α1,3-Gal - The most striking observation that could be made from the published data was the near universal use of a 2,3,4- unprotected galactose triol as the acceptor in formation of this bond[152,153,162,163]. Sialylation of such acceptors was found to be regio- and stereoselective, independent of the sialylation method used or the nature of the other parts of the acceptor. Use of C-3 auxiliary groups on the sialic acid donor was not considered advantageous, because of the loss of material occasioned by their introduction and removal. A chloro- or methylthio-sialoside donor were initially chosen.

ii) Gal-β1,4-GlcNAc - β-Galactosides are most easily formed by the use of a donor with a participating, acyl-type group at C-2. Comparable yields had previously been achieved with trichloroacetimidates, haloses or thioglycosides as donors[77]. Of these, thioglycosides are relatively cheap to prepare and have excellent stability to protective group manipulations. This donor needed removable protection at positions 2, 3 and 4 for subsequent sialylation. The glucosamine acceptor in this reaction required universal protection except at C-4. Conversion of the *N*-acetyl group to azide or *N*-phthaloyl had been used to avoid imidate formation, which can occur during glycosylation of this sugar, but required several additional synthetic steps.

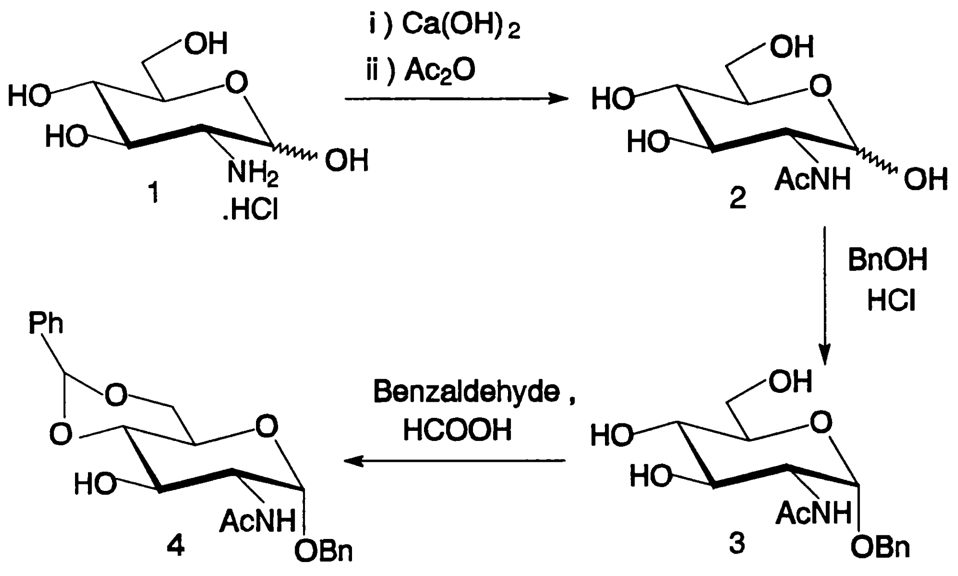
iii) Fuc-α1,3-GlcNAc - Stereoselective α-fucosylation was observed in many cases when an *N*-acetyl glucosamine protected with a benzylidene ring at C-4,6 was the acceptor[154,163]. High yields of fucosides could be obtained with halo-fucoses, trichloroacetimidates or thio-fucosides, which were generally protected with benzyl ethers. As with the galactosyl donors, thio-fucosides are cheaply prepared and stable.



The order of bond formation was decided as **A**, **B**, **C**.

3.1.3 Synthesis of glucosamine acceptor

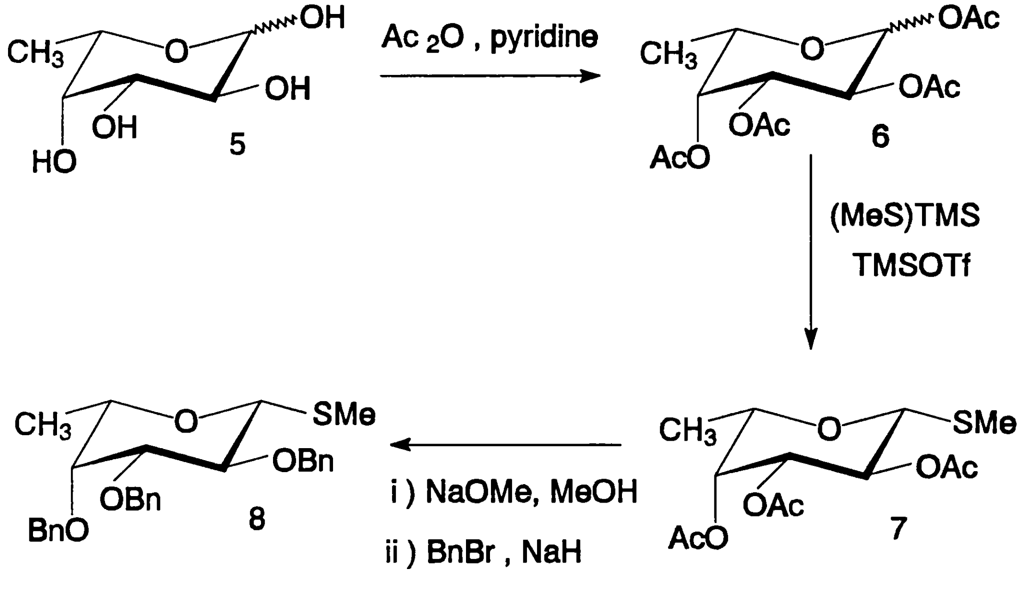
The glucosamine unit, an acceptor for two glycosylations, required anomeric protection. A benzyl group was chosen as it can be removed by hydrogenation at the



end of the oligosaccharide synthesis. Benzylidene protection of the C-4,6 positions was convenient, as it directs fucosylation and can be selectively opened to leave a free hydroxyl at C-4. Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside was the required starting unit.

This compound was synthesised in large scale in three steps from glucosamine hydrochloride **1**[177]. *N*-Acetylation was achieved by generation of the free base by treatment with calcium hydroxide, then addition of acetic anhydride to give **2** in 80% yield. Glycosylation with benzyl alcohol containing 2 % HCl gave predominantly the more stable α-anomer **3**. Benzylidene protection was carried out with benzaldehyde and formic acid resulting in the acceptor **4**.

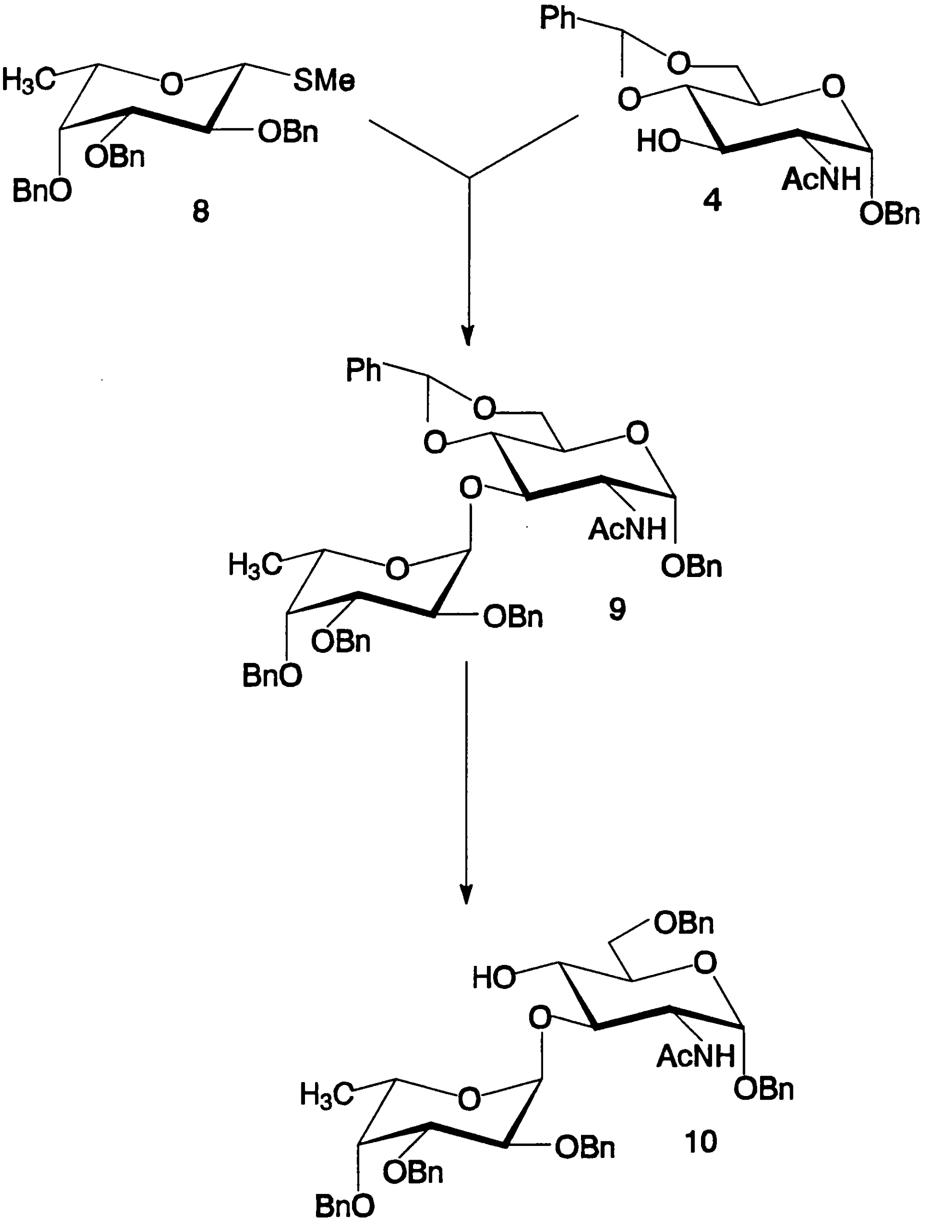
3.1.4 Synthesis of fucosyl donor



L-Fucose **5** was peracetylated with a pyridine / acetic anhydride mixture. The β-methylthio-fucoside **7** was formed in good yield from the peracetate **6** by reaction with (methylthio)trimethylsilane and trimethylsilyl triflate[155]. Acetate protective groups are incompatible with α-fucosylation; non-participating groups are needed. Deprotection with sodium methoxide in methanol was followed by benzylation with sodium hydride / benzyl bromide to give the required donor **8**.

3.1.5 α-Fucosylation experiments

Initially the thiophilic promoter DMTST[178] was selected for this fucosylation.



In a small scale reaction using 1 eq. from each sugar, 3 eq. promoter, 4Å-MS, and CH_2Cl_2 :THF 1:1 v/v as solvent (as the acceptor sugar was insufficiently soluble in CH_2Cl_2 alone), the α-disaccharide **9** was isolated in 40 % yield. Unreacted acceptor remained in the reaction mixture. This yield was improved by changing the solvent to THF alone, which perhaps allowed better dissolution of the acceptor. The concentration of acceptor / donor was increased from 0.02 mmol/mL in the initial experiment to 0.03 mmol/mL. These changes allowed more complete glycosylation of the acceptor, and the yield increased to 80 %.

While DMTST was a very successful promoter, it had some drawbacks as a reagent to be used in a large scale reaction. The methyl triflate required for synthesis of this salt is toxic, and the salt itself is unstable[178]. It can be stored as a solution in dichloromethane, but must be precipitated and used immediately before use as it is very hygroscopic. Blomberg *et al.* had introduced an alternative salt, DMTSB, for activation of thioglycosides[121]. This is a commercially available, crystalline solid, and is much less hygroscopic than DMTST. In a comparative experiment, 2 eq. of DMTSB were used in place of DMTST. This promoter was equally effective for this glycosylation, and α-selectivity was maintained.

In a large scale synthesis of this disaccharide, reaction of the two sugars in THF at room temperature was found to be complete after addition of only 1.5 eq DMTSB.

3.1.6 Reductive ring opening of benzylidene acetal

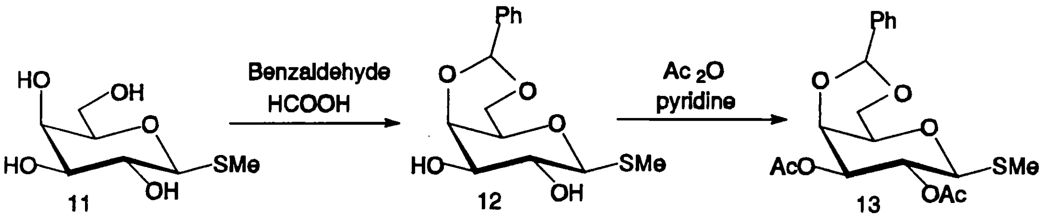
To obtain a suitable acceptor for galactosylation, the benzylidene acetal was subjected to reductive ring opening with sodium cyanoborohydride in the presence of HCl, following the method of Garegg *et al*[179]. This method allows opening of the acetal ring to leave the C-6 position protected as a benzyl ether, and C-4 as a free hydroxyl.

The reaction was successful when carried out at 0°C, with careful addition of acid to avoid complete acetal removal or cleavage of the sensitive fucosyl bond, and gave the acceptor **10** in 72 % yield. However, purification of this compound to free it from cyanoborohydride derivatives proved to be difficult. Chromatography through silica gel failed to remove these impurities.

Treatment of a solution of the reaction mixture with mixed ion exchange resin, followed by recrystallisation from methanol was a satisfactory solution to this problem.

In large scale experiments, it was desirable to avoid chromatographic separations. This was possible in the synthesis of **9** and **10**. Ring opening could be carried out on the crude mixture after glycosylation without deleterious effects, and with an isolated yield over two steps of 72%.

3.1.7 β-Galactosylation experiments

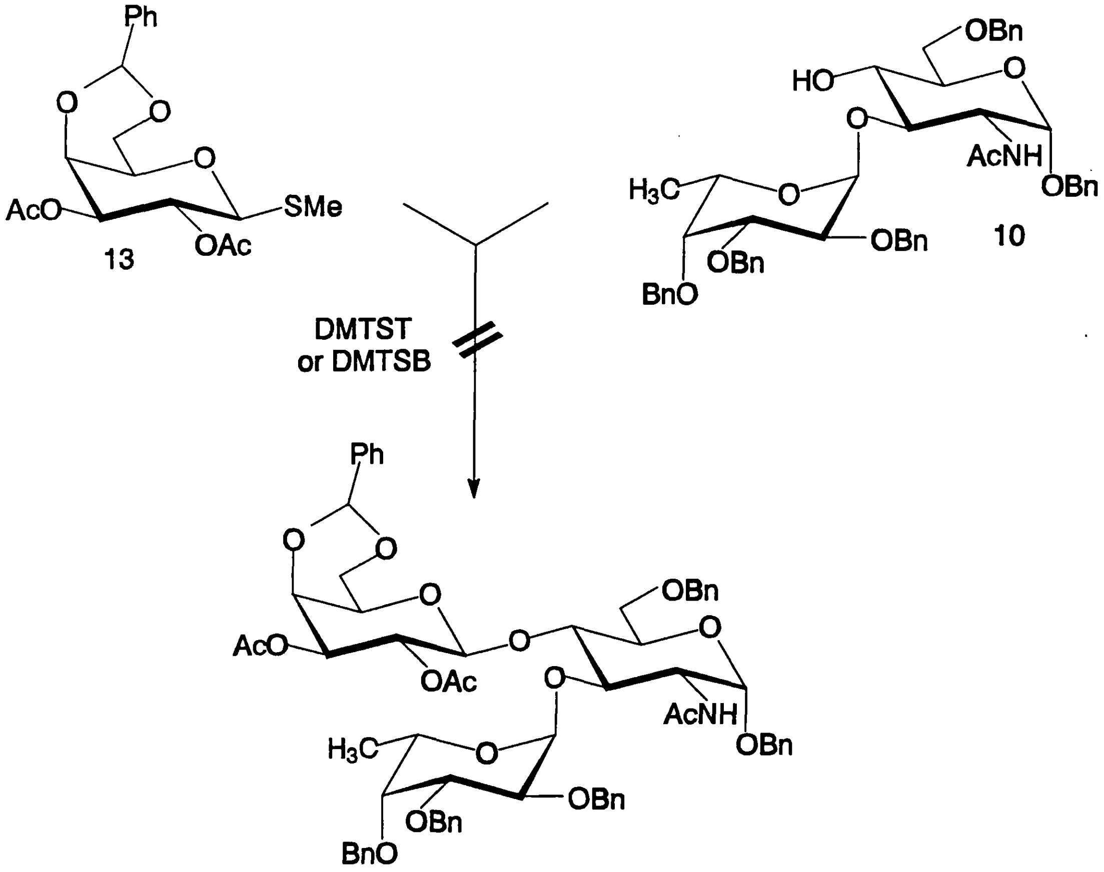


The initial donor chosen for these experiments was methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galctopyranoside **13**, prepared in two steps from methylthio-galactoside **11**, by benzylidene protection and acetylation. This donor had an acetyl group at C-2 to direct glycosylation, and could be formed into a triol for subsequent sialylation by deacetylation and reductive ring opening.

An initial DMTST promoted galactosylation between **10** and **13** in the presence of 4Å-MS in CH_2Cl_2 failed to give the expected trisaccharide **14**. The disaccharide remained in the mixture, while the galactose donor had undergone some degradation to a mixture of products.

This reaction was repeated many times, with alterations to dilution, temperature (0°C, room temperature), solvent (CH_2Cl_2, THF) and the promoter (DMTST, DMTSB). Monitoring of these reactions by TLC and FAB mass spectrometry revealed that the trisaccharide **14** was formed in low amounts. The disaccharide remained largely unreacted, while the galactose donor had formed multiple by-products, including a 1-OH derivative **16**.and its dimers **15**. When long reaction times were used, de-acetylation of the donor had also ocurred.

Galactose donor **13** was converted to its bromo-derivative with bromine, and a silver triflate promoted glycosylation was attempted. This reaction was also unsuccessful, with no trisaccharide detected by FAB-MS.



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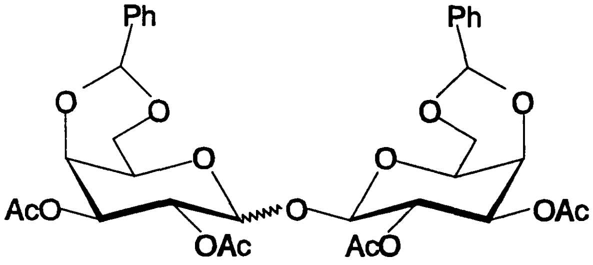

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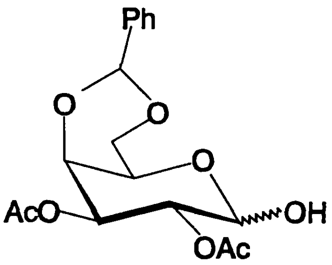
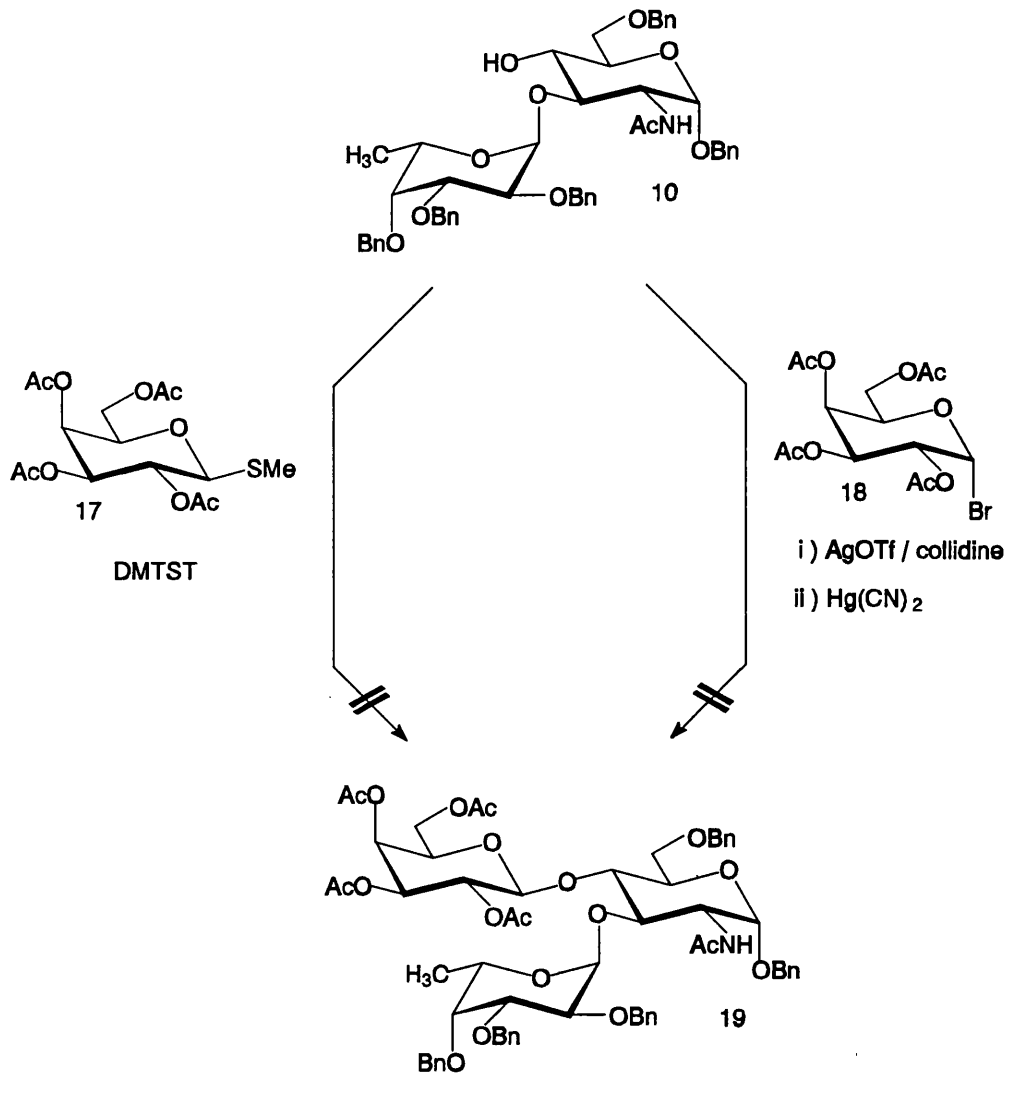


16

The failure of this type of galactosylation has been previously noted[161,163], and has been attributed to low reactivity of the acceptor and steric hindrance around the hydroxyl to be glycosylated. It was not possible to change the reactivity of this acceptor if the chosen pathway was to be pursued. It was possible to change the reactivity of the donor sugar however.

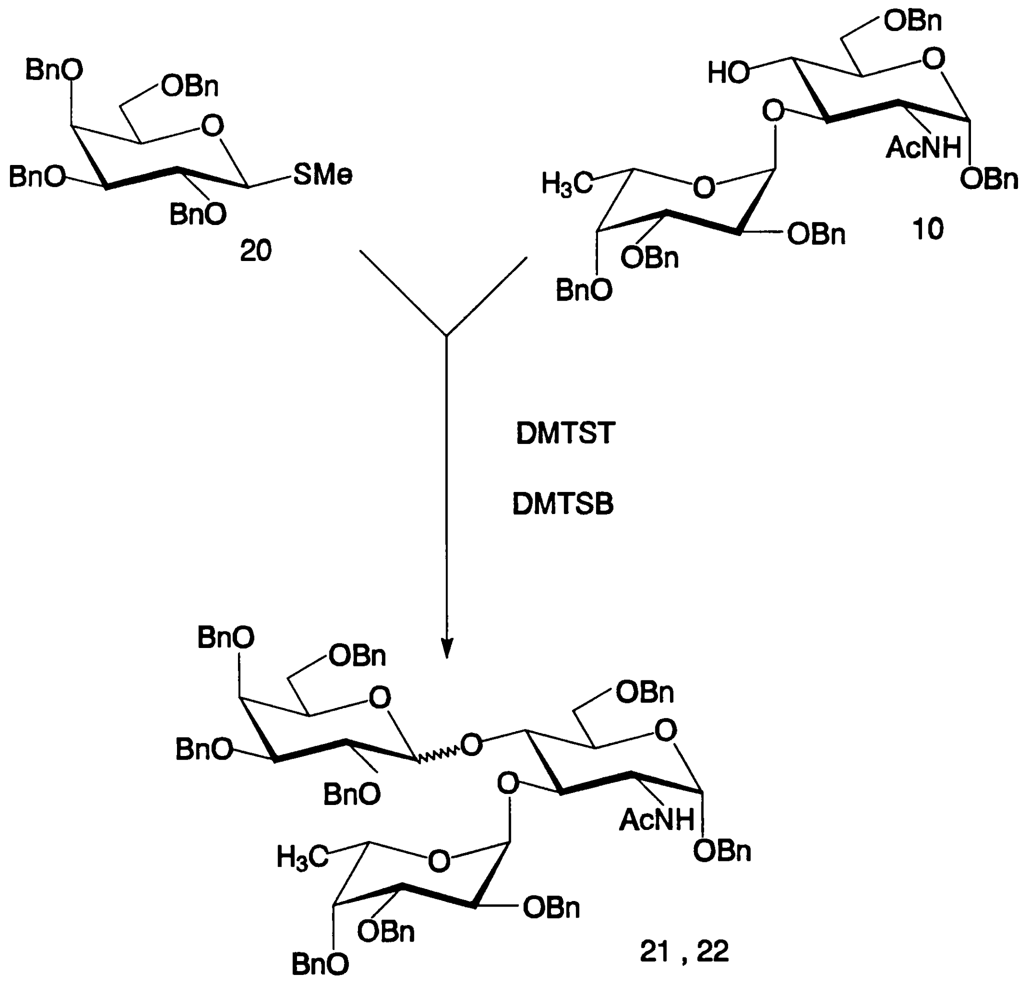
It has been demonstrated that the reactivity of sugar donors can be greatly influenced by the substituents they carry on their non-anomeric positions, and these differences have been exploited in "armed-disarmed" glycosylations. More reactive donors have alkoxy or deoxy groups at C-2. Less reactive donors have ester-type groups at C-2, or have 2,3- or 4,6- acetals. Such acetals may prevent formation of reactive intermediate glycocations.



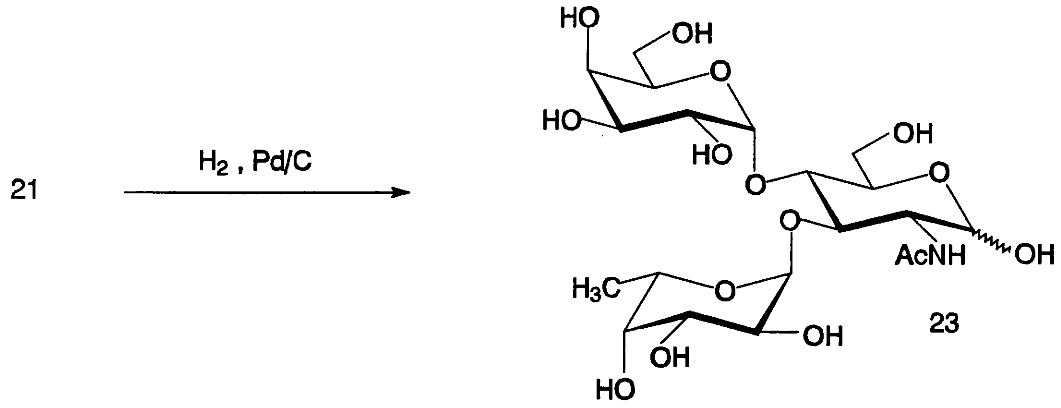
Our chosen donor possessed both of these "disarming" features, an acetate at C-2 and a 4,6-benzylidene acetal.

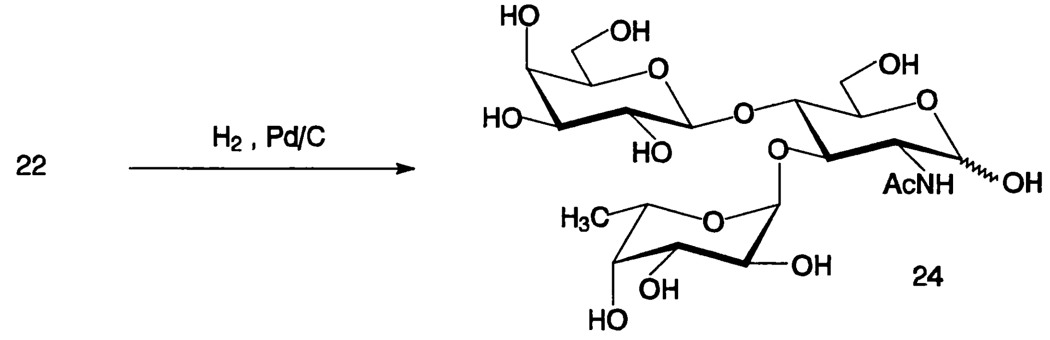
A reconsideration of the donor design was required.

Per-acetylated methylthio galactoside **17** and per-acetylated galactosyl bromide **18** were investigated as possible donors, as they lacked a disarming acetal but retained a directing group at C-2. A reaction between **17** and **10** in the presence of DMTST did not give trisaccharide **19**. Reactions between **18** and **10** in the presence of either AgOTf / collidine or $Hg(CN)_2$ were also unsuccessful. These results demonstrated that even without the benzylidene group, acetate protected donors were not sufficiently active to glycosylate this acceptor.



It is known that benzyl-type protective groups enhance the activity of sugar donors[103,104]. A fully benzylated methyl thiogalactoside (derived from **11** by treatment with benzyl bromide and sodium hydride) was used to test glycosylation of **10** with this type of donor. Reactions with DMTST and DMTSB as promoters were attempted. With DMTST at room temperature the trisaccharides **21** (α-galactoside) and **22** (β-galactoside) were formed in a ratio of approximately 2:3, with only traces of disaccharide remaining after 2 hours. The reaction with DMTSB was much slower, with disaccharide still present after 24 hours.





3.1.8 Synthesis of Lewis X trisaccharide and its α-galactosyl analogue

Trisaccharide **22** was not suitable for continuation of the synthesis of SLeX, as it could not be selectively deprotected to provide a suitable acceptor for sialylation. The LeX epitope can be easily obtained from this structure though; a larger scale reaction was carried out to prepare this trisaccharide.

An excess of donor (3.5 eq.) was used, with $CHCl_3$ as solvent, 4Å-MS, and 5 eq. DMTST, at a dilution of 0.06 mmol/mL relative to the donor (twice as concentrated than used for the initial test reaction). Under these conditions the trisaccharides were formed in 61 % yield.

Multiple chromatographic steps were required for separation of the two anomers, which were isolated in 35 % (β, **22**) and 26 % (α, **21**) yields respectively. The anomers were identified from their ^{1}H NMR spectra by the presence of a doublet at 4.84 ($J_{1,2}$ = 7.99Hz) for **22** and a narrow doublet at 5.12 ($J_{1,2}$ = 3.5 Hz) for **21**, and by the relative position of the H-6_{fuc} signals at 1.12 for **22** and 1.27 for **21**.

The two trisaccharides **21** and **22** were fully deprotected by catalytic hydrogenation to produce **23** and **24** (LeX) in 99% yield. Compound **23** is an analogue of the natural structure.

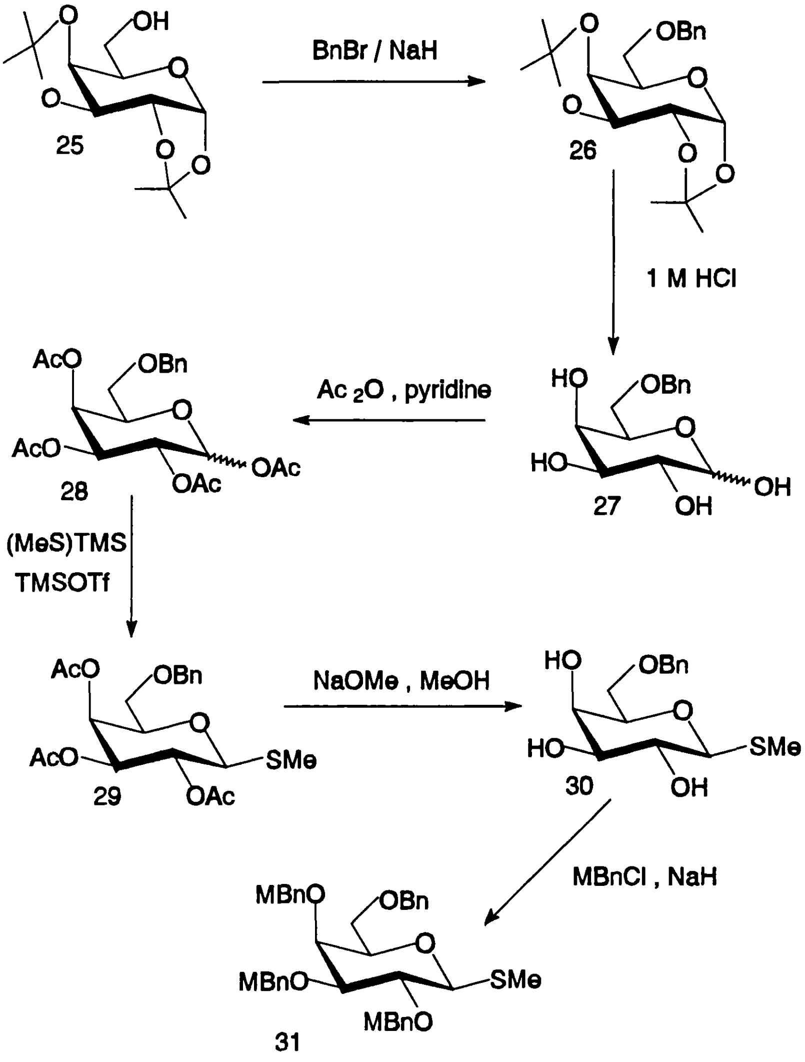
3.1.9 Synthesis of galactosyl donor with C-2,3,4 selectively removable protection

A galactosyl donor with semi-permanent protection at C-6, and selectively removable protection at C-2,3,4 was required. A benzyl group at C-6 would be the most convenient as it could be removed at the end of the synthesis with the rest of the semi-permanent protection. Benzyl-type protection at the other three positions appeared to offer the best hope of success. The 4-methoxybenzyl group was chosen for this purpose, as it can be removed by oxidative methods, leaving benzyl groups intact. A methylthio- leaving group was used at the anomeric position.

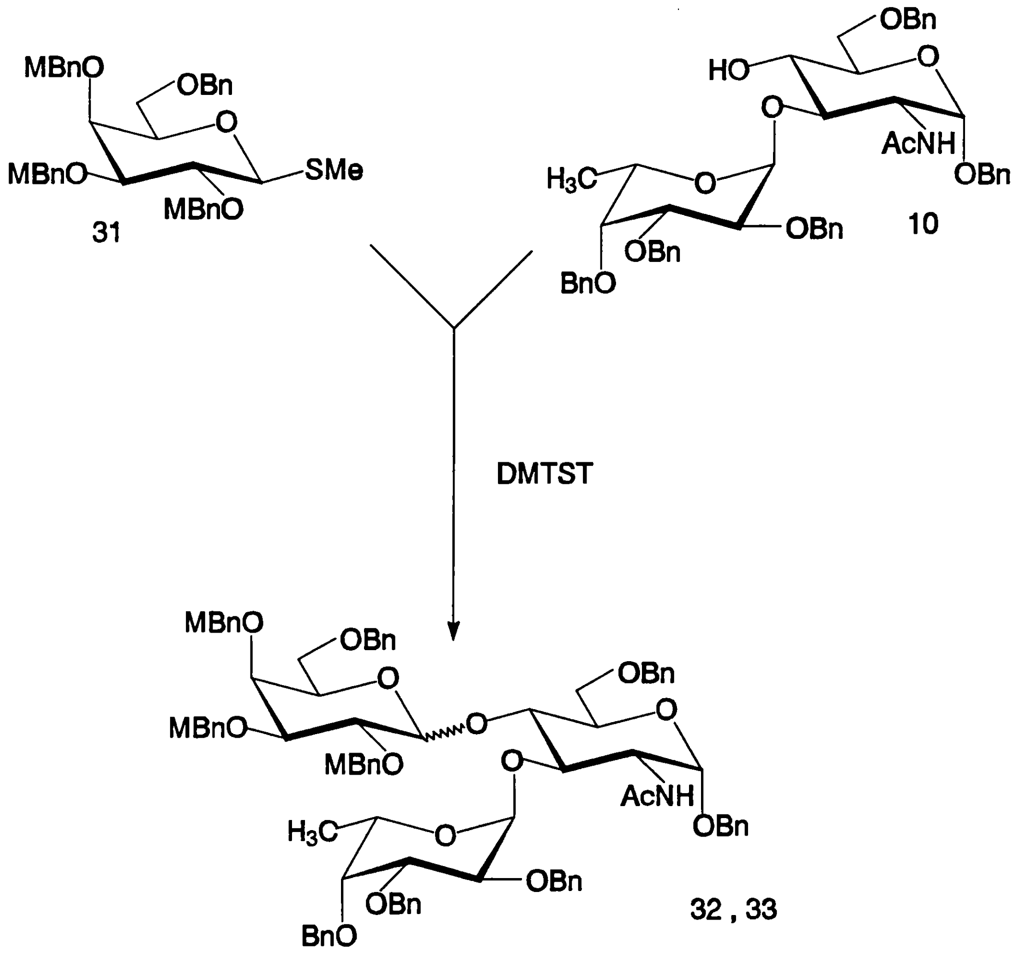
This donor was synthesised in large scale in six steps from commercially available 1,2:3,4-di-*O*-isopropylidene galactose **25**.

Treatment with benzyl bromide and sodium hydride gave the 6-benzyl derivative **26**, which was acetal deprotected with 1 M HCl solution to give **27**. Acetylation with acetic anhydride and pyridine, and subsequent treatment with (methylthio)trimethylsilane and trimethylsilyl triflate gave the methylthio-galactoside

29 as an anomeric mixture, the β-anomer of which could be precipitated from methanol. Zemplen deprotection and reaction with 4-methoxybenzyl chloride and sodium hydride gave the donor **31**.



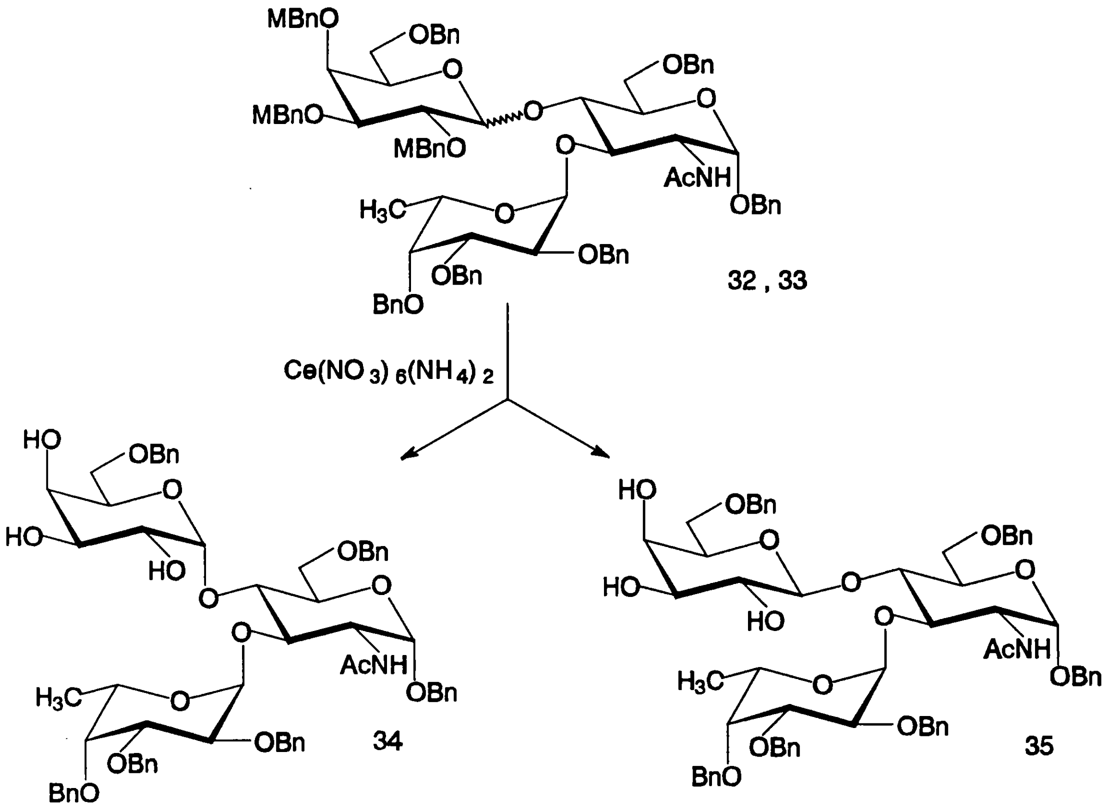
In a test reaction, galactosylation of **10** with with **31** in the presence of DMTST as promoter gave a mixture of the desired trisaccharides **32** (α-galactoside) and **33** (β-galactoside) in a combined isolated yield of 28 %.



It would have been advantageous if the galactosylation could be directed to favour the β-anomer, to avoid wasting material and a potentially difficult anomeric separation. As it was not possible to use a neighbouring group participation to achieve this, a nitrilium-nitrile type glycosylation under kinetic control was attempted[107]. The reaction was carried out with DMTST as promoter with acetonitrile as solvent, at -30°C. Unfortunately at this temperature no reaction occurred.

A large scale preparation of this trisaccharide was then carried out at room temperature. A twofold excess of donor was used, with 4.5 eq. DMTST and 4Å-MS in $CHCl_3$ at a dilution of 0.09 mmol/mL relative to the acceptor. The reaction mixture from this coupling was taken onto the next step without chromatographic separation.

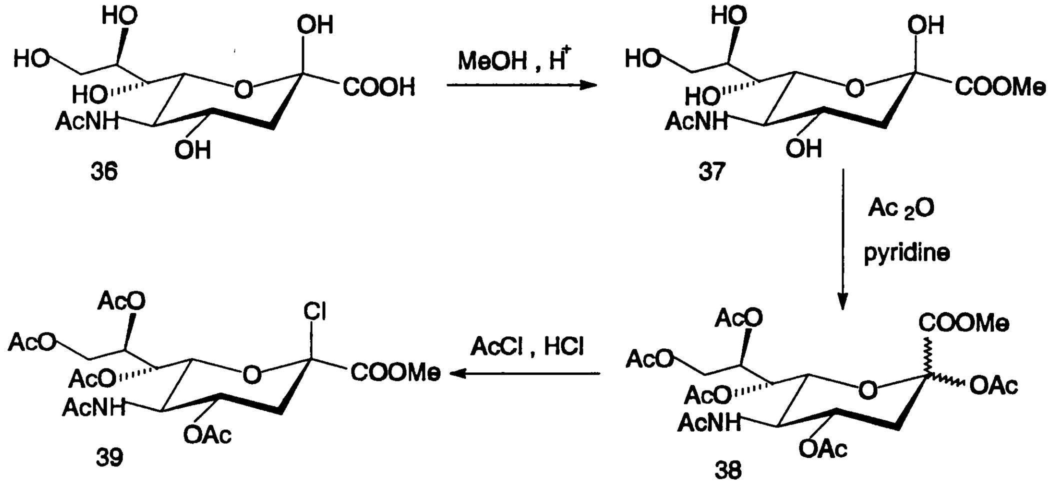
The literature claimed that it was possible to deprotect the 4-methoxybenzyl group by two methods, either by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[180], or with ceric ammonium nitrate (CAN)[181]. We investigated both of these methods for the deprotection of **32** and **33**. The reaction with DDQ proceeded very slowly, with deprotection still incomplete after 4 days at room temperature. Reaction with CAN in a 9:1 v/v mixture of acetonitrile:water was quicker with complete deprotection in under 1 hour. After deprotection it was possible to separate the two anomers by chromatography. to give **34** (α-galactoside, 23 % yield from **10**) and **35** (β-galactoside, 25 % yield from **10**).



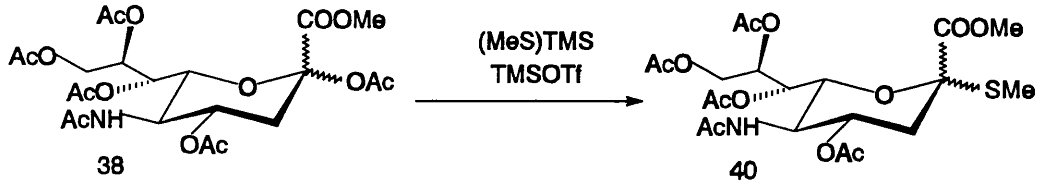
3.1.10 α-Sialylation experiments

Two sialic acid donors were synthesised to test the sialylation of **35**, a sialyl chloride and a methylthio-sialoside.

Sialic acid **36** was treated with methanol and Amberlite IR-120 (H^+) ion exchange resin to give the methyl ester **37**[182]. This was acetylated with acetic anhydride / pyridine to give the mixed anomeric acetates **38**[183]. The β-chloride **39** was synthesised by reaction of the acetates with acetyl chloride saturated with HCl[184].



The methylthio-sialoside **40** was synthesised using a modified version of Hasegawa's method by treatment of **38** with (methylthio)trimethylsilane and trimethylsilyl triflate[143].

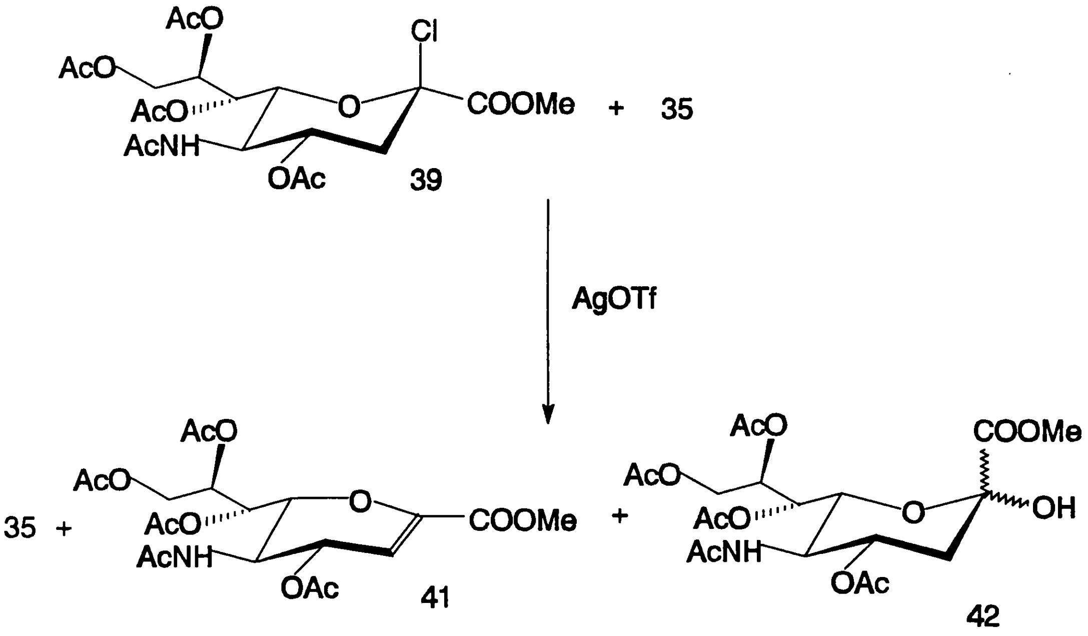


3.1.11 Sialylation with silver salts as promoters

The chloro- donor **39** was used with the soluble promoter silver triflate in attempted glycosylations with the triol acceptor **35**. This reaction was tested in tetrahydrofuran under the following conditions:-

i) at -15°C for 2 days
ii) at -15°C with di-*tert*-butylpyridine for 2 days
iii) at -15°C to room temperature
iv) at -15°C to room temperature with di-*tert*-butylpyridine

In trials i) and ii) tetrasaccharide could be detected by mass spectrometric analysis of reaction mixtures, but could not be distinguished by thin layer chromatography separation of the mixture. The trisaccharide remained unchanged in the mixture, with the sialyl donor forming the elimination product **41**, with traces of the 2-OH derivative **42** also present.



3.1.12 Sialylation with a methylthio-sialoside donor

The methylthio-sialoside **40** was tested in DMTST promoted glycosylations of **35**. Test reactions were made with variations in the solvent and temperature used.

Reaction of **35** with **40** at -15°C to room temperature in $CHCl_3$ gave a trace of tetrasaccharide **43** (by TLC), with unchanged **35**, and **42**.

Reaction in acetonitrile with addition of DMTST at -15°C gave an improved result, although **42** was still the major product. In an attempt to reduce this intramolecular elimination, the temperature was lowered to -30°C at the addition of DMTST, with the temperature allowed to rise to -15°C after 30 minutes. This variation gave an improvement in the yield of tetrasaccharide as observed by TLC.

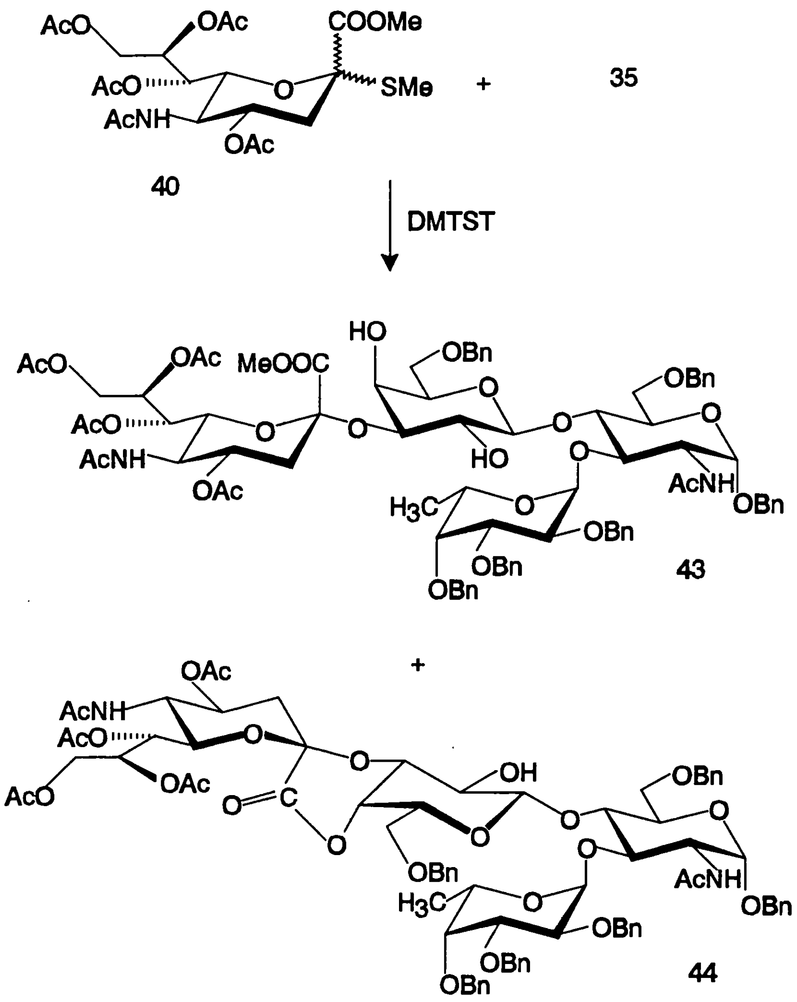
Some published reports of this type of sialylation advocated stirring the acceptor and donor together with 4Å-MS for some hours before cooling and addition of promoter. This technique was tried, with 5 hours stirring before addition of promote, and it appeared to offer a modest benefit.

Toluene and benzene were tried as solvents in reactions carried out at -70°C, but under these conditions **35** was insufficiently soluble to allow formation of **43**.

3.1.13 Large scale synthesis of tetrasaccharide

A large scale preparation of the tetrasaccharide was carried out using acetonitrile as solvent. The acceptor and donor were stirred together with 4Å-MS for 3 hours before promoter was added. DMTST was added at -30°C, with the reaction being continued at -15°C. A second portion of donor **40** and DMTST were added to the mixture after 24 hours, in an attempt to cause sialylation of all the trisaccharide acceptor.

This reaction afforded the expected tetrasaccharide **43** in 12% isolated yield. A second product isolated in 15% yield was found to have a mass 32 units less than **43**; it was determined to be the intramolecular 1→4' lactone **44**, formed by transesterification during glycosylation. The ^1H NMR spectra of **44** showed no signal at 3.5 (position of the methyl ester of **43**), with the H-2_{gal} signal at 4.23, and H-4_{gal} at 5.31.



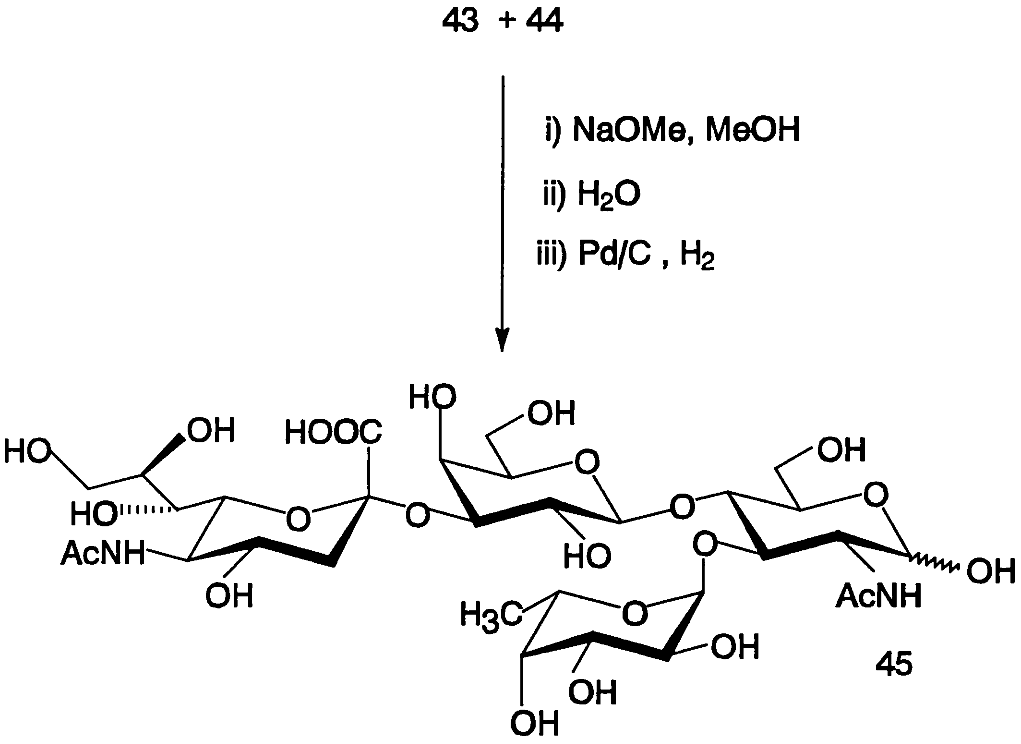
An inseparable mixture of **43** and **44** was also recovered, with the combined yield of tetrasaccharides amounting to 58%.

3.1.14 Deprotection of tetrasaccharides

There were two necessary steps for the deprotection of **43** and **44**, Zemplen deprotection and base hydrolysis to remove the acetates and ester / lactone function, and catalytic hydrogenation to remove the benzyl groups. Both orders of deprotection were tested.

Catalytic hydrogenation with acetic acid as solvent gave benzyl deprotection accompanied by some loss of the fucose and sialic acid residues. Deprotection in methanol proceeded without side reactions, but was rather slow. Following benzyl deprotection with Zemplen deprotection and base hydrolysis resulted in a complex mixture of products; these could have been formed by the action of the base on the free reducing sugar.

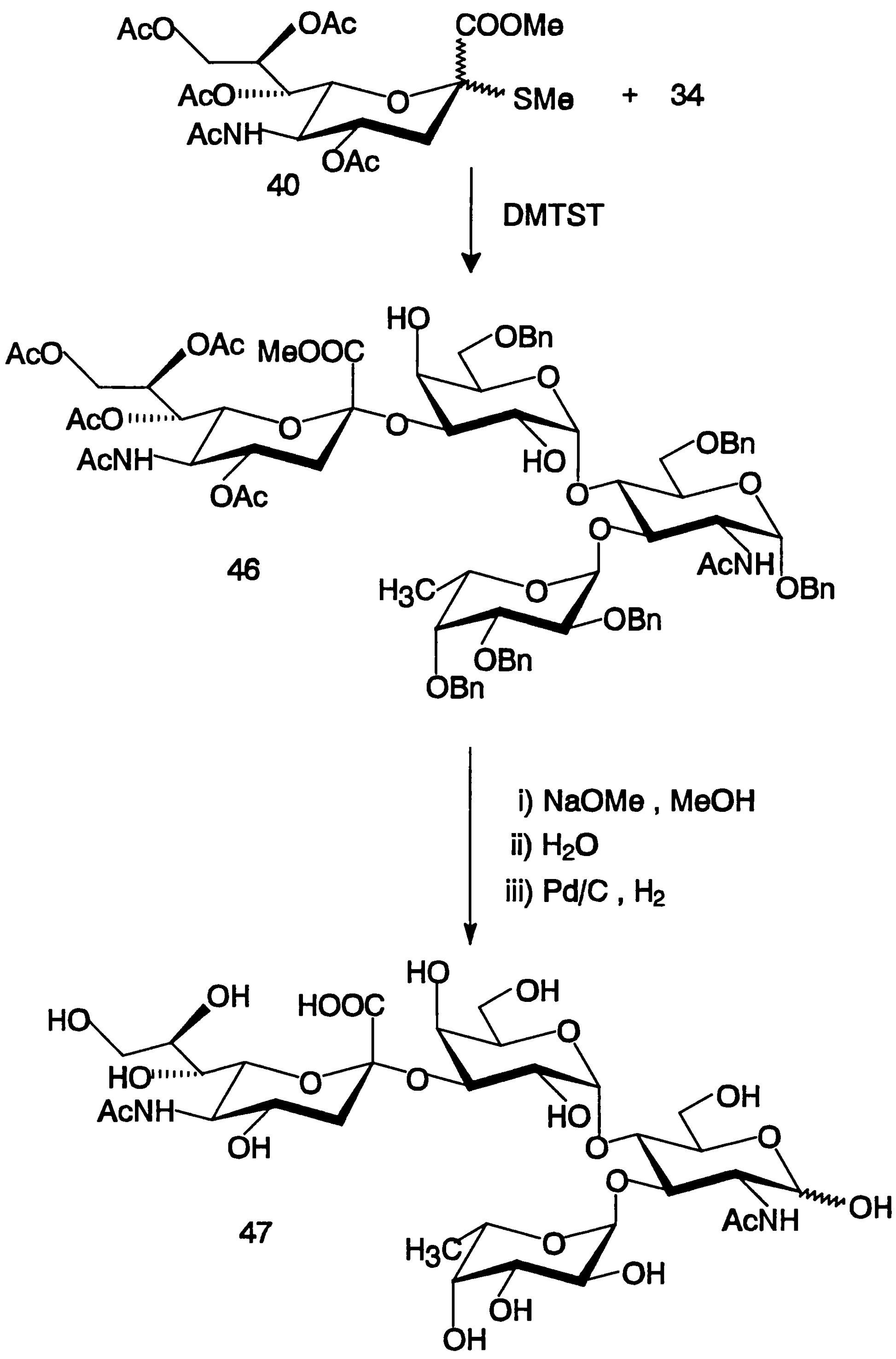
The alternate route, base deprotection followed by catalytic hydrogenation, was found to be superior, and gave the free tetrasaccharide **45** as an anomeric mixture in 78% yield.



3.1.15 Synthesis of an α-galactosyl analogue of SLeX

The trisaccharide **34** was also a suitable acceptor for sialylation, and would provide an analogous structure of SLeX. The sialylation of this trisaccharide was performed in the same way as for **35**, in a reaction with an excess of methylthio-sialoside **40** and DMTST. In this case the yield of the sialylated product **46** was considerably worse than in the case of the β-galactosyl acceptor. The tetrasaccharide was recovered in 12% yield, with unreacted trisaccharide and the sialic acid derivative **41** also present. No lactone products were isolated from this reaction mixture.

The protective groups were removed by Zemplen deprotection and base hydrolysis, followed by catalytic hydrogenation to give an anomeric mixture of tetrasaccharides **47**.



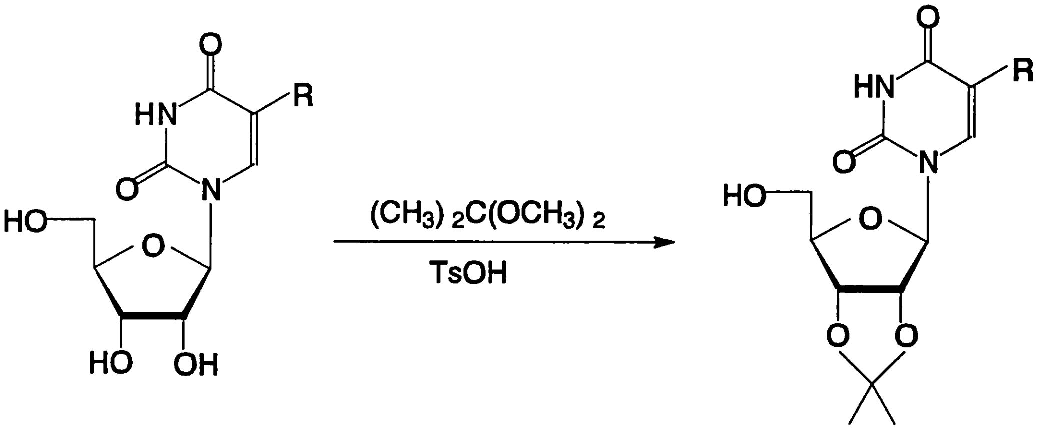
3.2 SYNTHESIS OF ANALOGUES OF KI-8110

A series of analogues of KI-8110 were synthesised to investigate structure/ activity relationships. Alterations were made by :

- changing the substituent at position 5 of the uracil ring
- using a 2-deoxyribose in place of ribose
- reduction of the carboxylic acid function of the sialic acid unit
- partial deprotection of the structure

3.2.1 Preparation of uridine derivatives

The isopropylidene protected uridine derivatives **51, 52, 53** and **54** required for use as sialyl acceptors were prepared in 62-77 % yield from commercially available uridine compounds **48, 49 50** and **51** by refluxing with dimethoxypropane in the presence of *p*-toluenesulphonic acid.



48 R = F

49 R = H

50 R = Br

51 R = Et

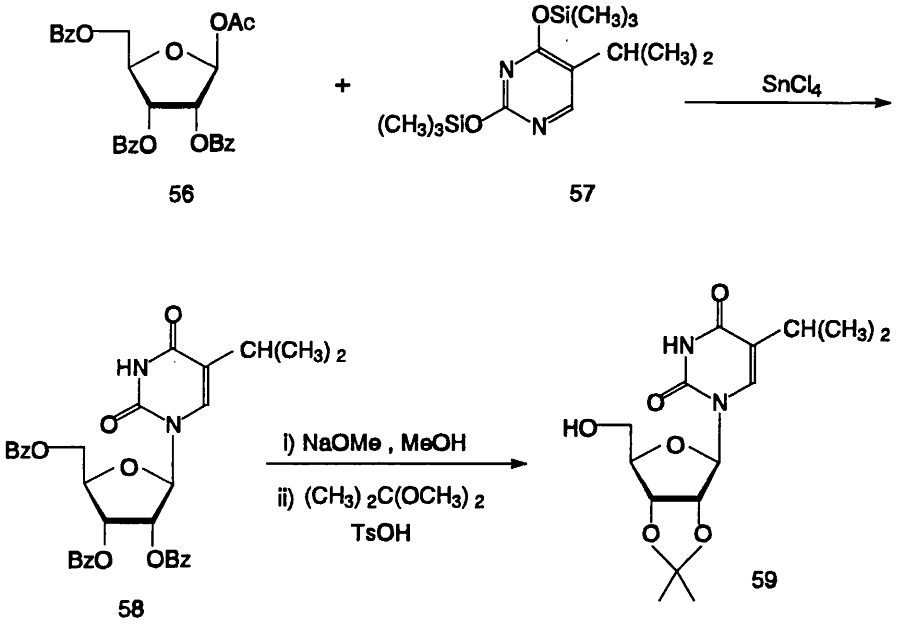
52 R = F

53 R = H

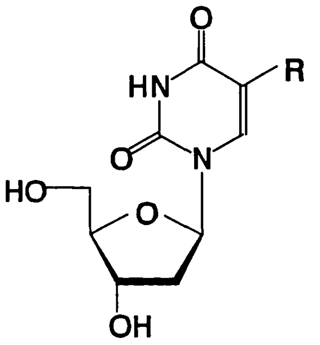
54 R = Br

55 R = Et

The uridine acceptor **59** was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose **56** and 5-isopropyl-2,4-di-*O*-trimethylsilyl uracil **57** in reaction with tin (IV) chloride. The product **58** of this reaction was Zemplen deprotected, and reacted with dimethoxypropane in the presence of *p*-toluenesulphonic acid to give the isopropylidene protected acceptor.



3.2.2 2-Deoxyuridine acceptors

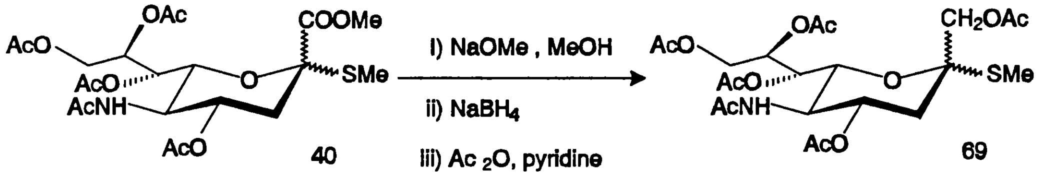


60	R = F	64	R = CH_2CH_3
61	R = H	65	R = $CH(CH_3)_2$
62	R = Br	66	R = $(CH_2)_3CH_3$
63	R = I	67	R = $(CH_2)_4CH_3$
		68	R = $(CH_2)_5CH_3$

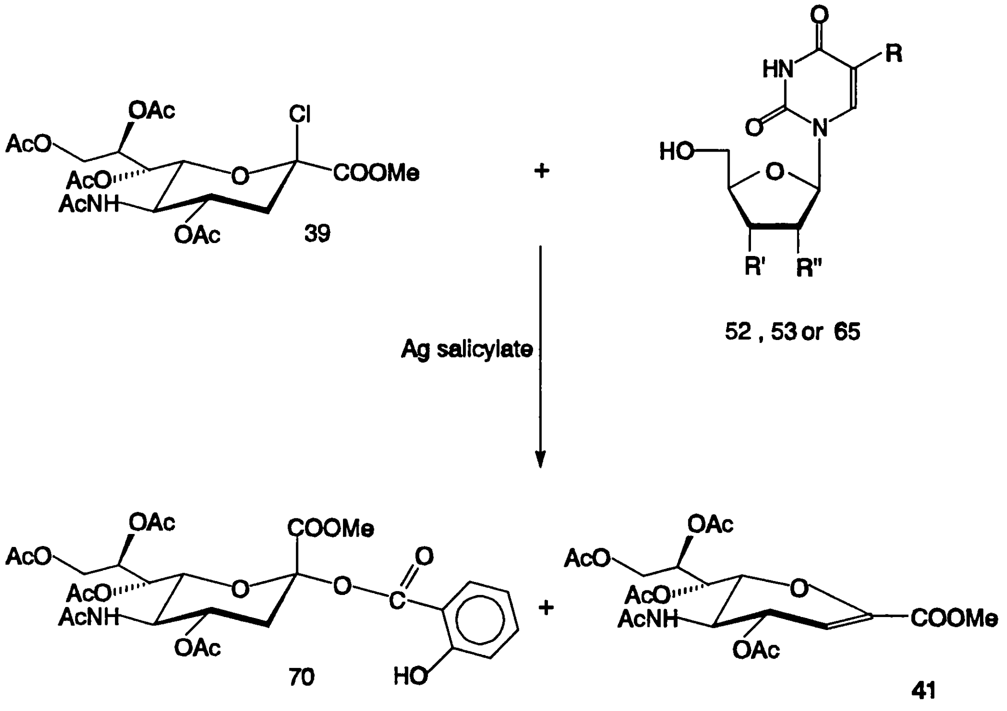
The 2-deoxyuridine acceptors **60** - **68** were a kind gift of Dr. Gruber of the Central Research Institute for Chemistry, Budapest.

3.2.3 Sialic acid donors

The chloro-sialic acid donor **39**[183] and the methylthio-sialoside donor **40**[143] were used in the sialylation experiments. A sialic acid derivative which had no carboxylic acid function was also prepared. Compound **40** was reduced with $NaBH_4$ and acetylated to give the 1-*O*-acetyl derivative **69**.



3.2.4 Sialylation of uridine derivatives with insoluble silver salts as promoters

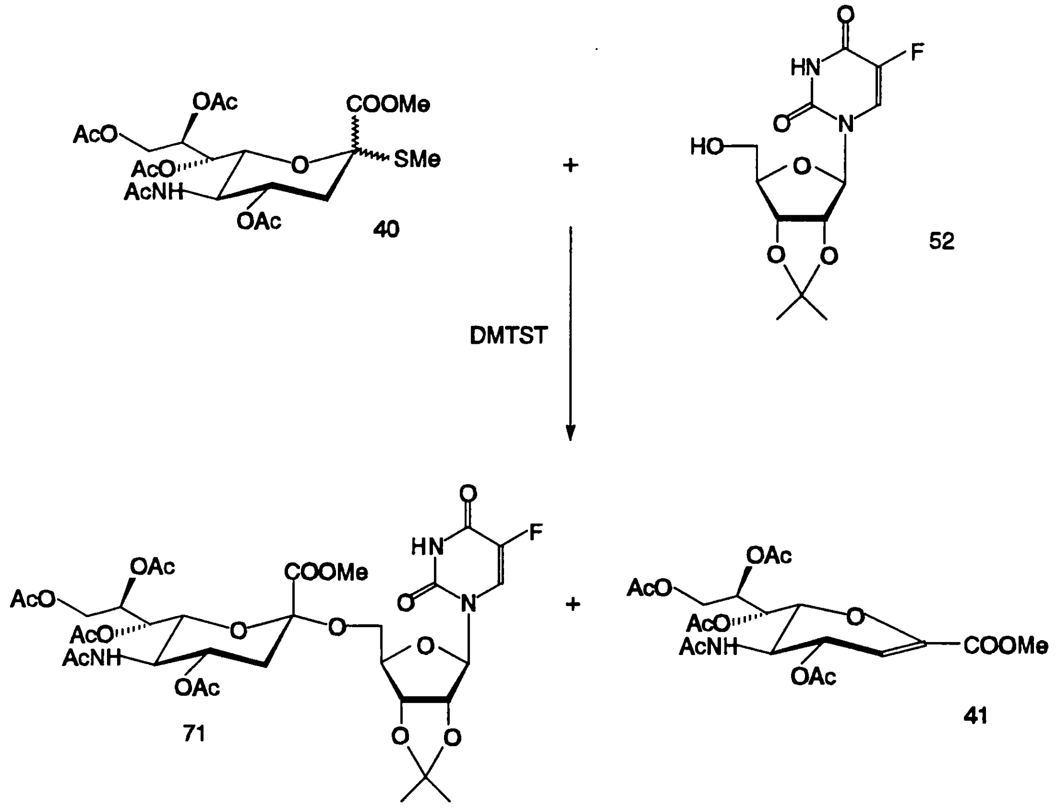


The chloro-sialoside donor **39** was used as a sialyl donor in silver salicylate promoted sialylation of the uridine acceptors **52**, **53** and **65**[136].

In each case, the major component isolated from the reaction mixtures was the salicyloyl sialoside **70**, with the sialic acid derivative **41** (formed by intramolecular elimination) also present. The desired sialic acid - nucleoside conjugates were not produced from these reactions.

3.2.5 Sialylation of uridine derivatives with thiosialoside donors

The methylthio-sialoside **40** was used as a donor in sialylations of the nucleoside acceptors with thiophilic promoters.



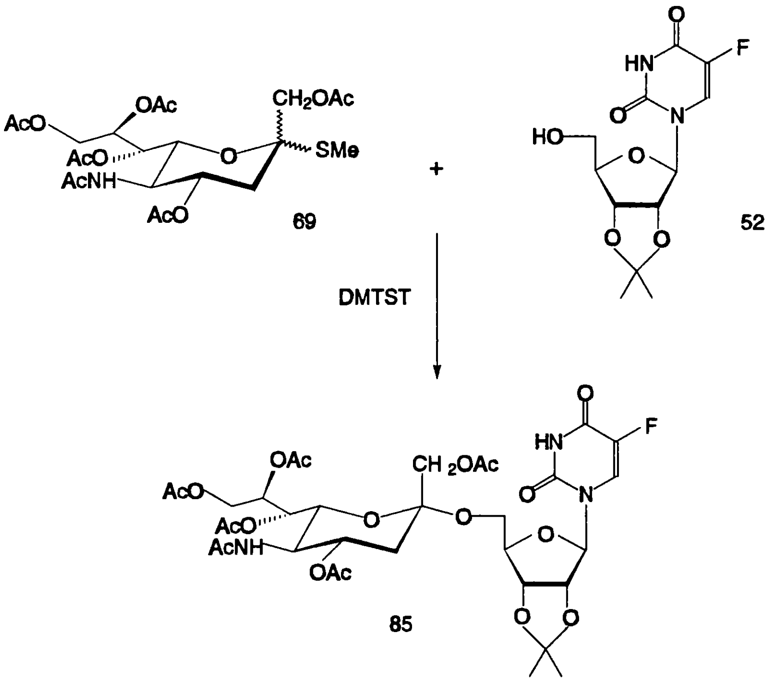
The 5-fluoro-uridine acceptor **52** was sialylated with **40** using DMTST as promoter, in acetonitrile at -15 °C for 2 days. The α-sialoside conjugate **71** was isolated in 10% yield, with the elimination product **41** found as the major product in 52% yield. The reaction was repeated using THF as solvent and room temperature, but in this case **41** was the sole product isolated with none of the desired conjugates.

In a similar reaction the 5-isopropyl-2'-deoxyuridine acceptor **65** was reacted with the sialyl donor **40** in a DMTST promoted reaction at -15 °C, using a 4:1 v/v mixture of acetonitrile: DMF as solvent. The sialic acid - nucleoside conjugate **81** was isolated from the reaction mixture in 10% yield as a 1:2 α:β anomeric mixture of sialosides.

The sialyl donor **40** was treated with bromine to obtain an intermediate bromide derivative, and then reacted with the 5-bromouridine acceptor **54** in the presence of silver salicylate. No sialic acid - nucleoside conjugates were found in the reaction mixture, with **41** present as the major product.

Copper (II) bromide was used as a promoter in a reaction between **40** and uridine acceptor **53** in acetonitrile at -15 °C for 2 days. Compound **41** was again the major product, with a trace of the nucleoside conjugate detected by FAB MS.

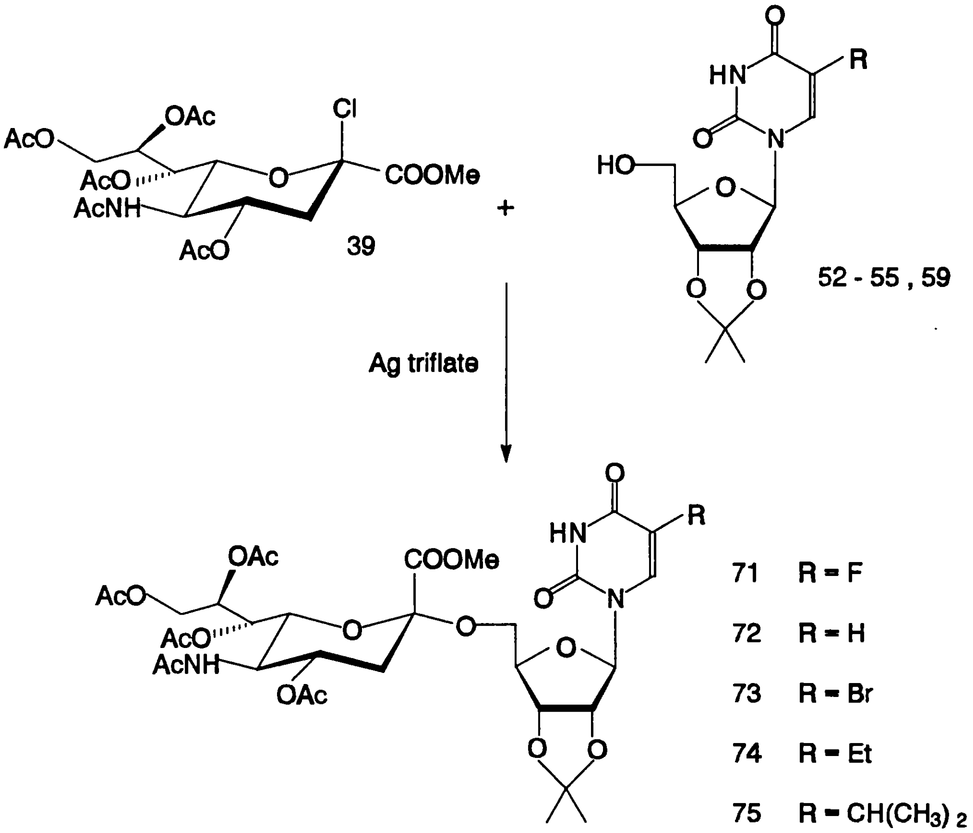
3.2.6 Glycosylation of a nucleoside acceptor with a reduced sialic acid derivative



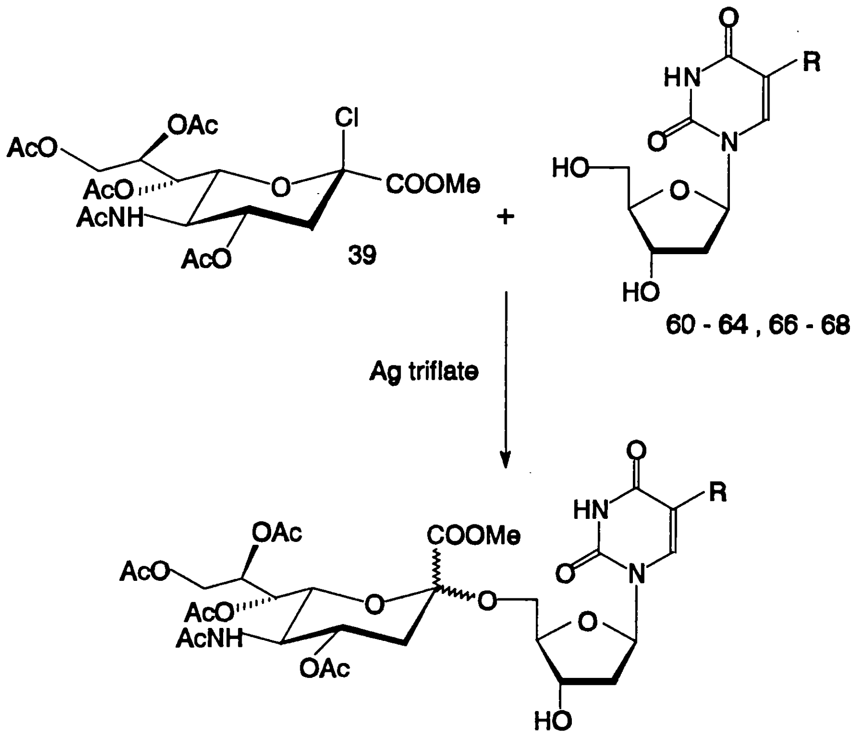
The methylthio- sialic acid derivative **69**, where the carboxylic acid function had been reduced and acetylated, was reacted with the 5-fluoro-uridine acceptor **52** in the presence of DMTST as promoter and $CHCl_3$ as solvent at room temperature for 20 hours. The α-linked nucleoside conjugate **85** was isolated from the reaction mixture in 32 % yield.

3.2.7 Sialylation of nucleoside acceptors with silver triflate as promoter

The homogenous promoter silver triflate was used in the sialylation of the nucleoside acceptors **52-55**, **59**, **60-64** and **66-68** with the chloro-sialic acid derivative **39**. Reactions were carried out at -15 °C in the presence of 4Å-MS. The elimination product **41** and the 2-OH derivative of the donor **42** were also isolated from reaction mixtures.



The isopropylidene protected sialic acid - nucleoside conjugates **71** - **75** were isolated in between 11 - 20% yield as the α-linked sialosides.

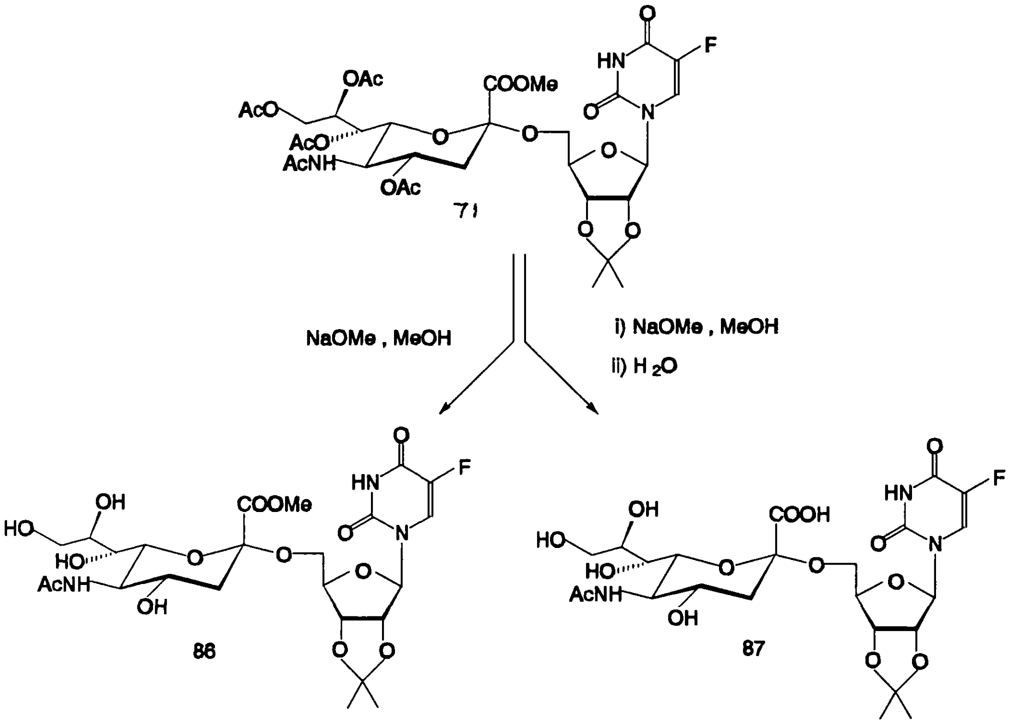


76	R = F	80	R = Et
77	R = H	82	R = $(CH_2)_3CH_3$
78	R = Br	83	R = $(CH_2)_4CH_3$
79	R = I	84	R = $(CH_2)_5CH_3$

The deoxy-ribose containing sialic acid - nucleoside conjugates **76** - **79** were formed in 11 - 17% yield and could only be isolated as α:β mixed sialosides. The 5-alkyl-2'-deoxyuridine conjugates **82-84** were isolated in 21-30 % yield as the α-linked sialosides.

3.2.8 Partial deprotection of sialic acid - nucleoside conjugates

Partial deprotection of the conjugate **52** was carried out. Zemplen deprotection gave the deacetylated derivative **86**. Zemplen deprotection followed by aqueous base hydrolysis gave the deacetylated free acid derivative **87**.



The nucleoside - sialic acid conjugates are currently under biological investigation.

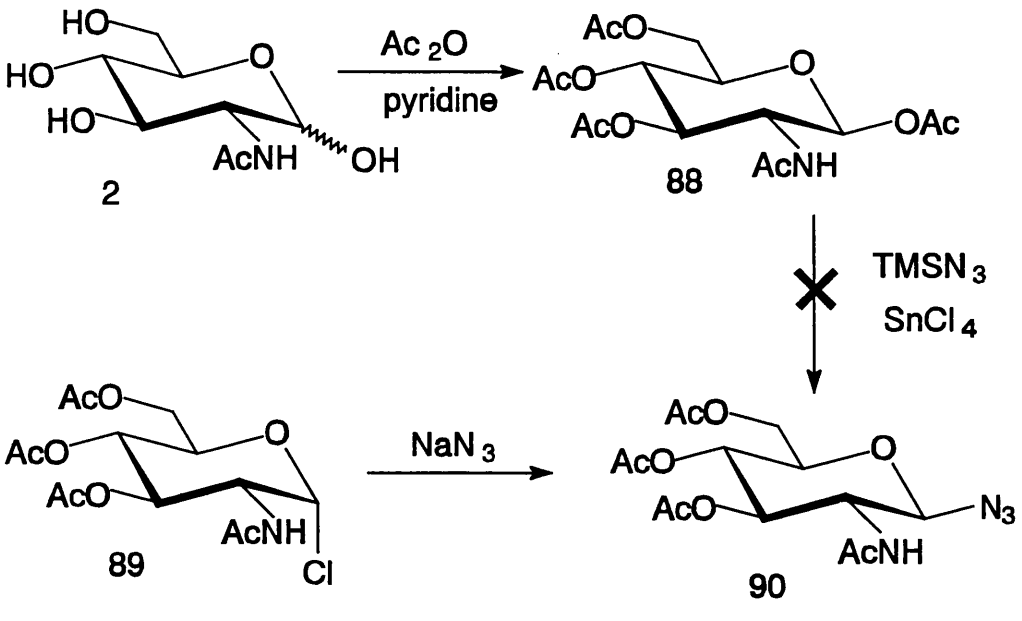
3.3 SYNTHESIS OF THIO-LINKED OLIGOSACCHARIDES

The ability of SLeX and related structures to inhibit inflammatory processes *via* their interaction with selectins has led to a search for more active or more biologically stable analogues. Thio-linked oligosaccharide analogues are of interest because of their increased stability over *O*-glycosides to enzymatic attack.

The synthesis of thio-linked analogues of the trisaccharides Lewis X and Lewis A was initially attempted. It was intended to form the two thio-linkages of the trisaccharides by displacement of sulphonate esters under basic conditions. The syntheses required firstly the formation of a suitably protected sugar unit to take the position of the glucosamine residue. It was decided to introduce an azide group at the anomeric position of this sugar, as this group may be reduced after formation of the conjugates to give a glycosylamine. Peptides or lipids could then be linked to the conjugate *via* an amide bond.

3.3.1 Synthesis of allosamine derivative

Displacement of sulphonate esters to form thio-linkages results in inversion of configuration at the linkage position, so the central glucosamine unit had to be

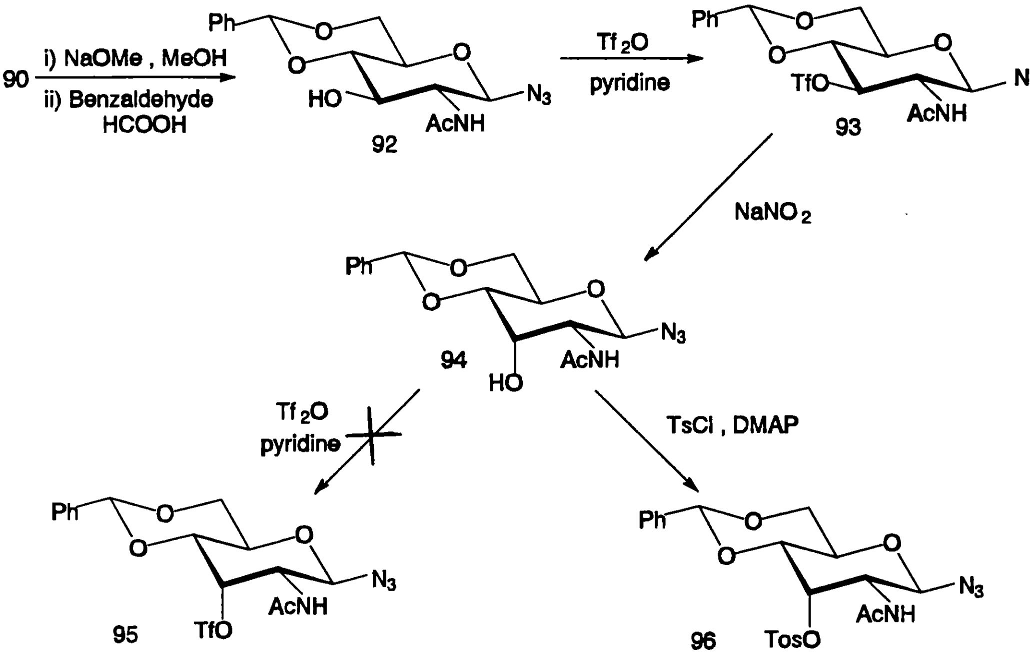


converted into an intermediate with opposite configurations at position C-3 or C-4. It was decided to attempt a linkage at position C-3 first, requiring synthesis of an allosamine derivative. Inversion of configuration at C-4 could be attempted after formation of a disaccharide.

Compound **2** was peracetylated by reaction with acetic anhydride and pyridine to give **88**. Introduction of an azide function was initially attempted by reaction of **88** with trimethylsilyl azide in the presence of tin (IV) chloride[185]. The reaction proceeded very slowly with little azide formed after 24 hours. An alternative method of azide introduction was necessary.

The acetylated chloride **89** was synthesised from **88** by reaction with acetyl chloride saturated with HCl. The chloride reacted smoothly with sodium azide in a phase-transfer-catalysed reaction to give the required azide **90** in 65% yield[186].

A derivative with semi-permanent protection at C-6, temporary protection at C-4 and a free hydroxyl at position C-3 was required. A 4,6-benzylidene acetal would be suitable, as later reductive ring opening would leave only a benzyl group at C-6. Zemplen deacetylation of **90** and protection as the 4,6-benzylidene acetal with benzaldehyde and formic acid gave the 3-unprotected derivative **92**.



Reaction of **92** with triflic anhydride gave the 3-triflate **93**. The configuration at position C-3 was inverted by reaction of the triflate with sodium nitrite in DMF to give the allosamine derivative **94** in 61% yield[187].

3.3.2 Synthesis of 3-sulphonate esters of allosamine

A 3-sulphonate ester at position C-3 of the allosamine was required as a 'leaving group' in the coupling reaction. Initial attempts were made to synthesise the 3-triflate, as this was expected to be the best leaving group. Ester formation was attempted using triflic anhydride, with CH_2Cl_2: pyridine 15:1 v/v as solvent, at -15 °C, -15 to 0°C and -15°C to room temperature. In each case, unreacted starting material and a mixture of unidentified products were seen on TLC examination of reaction mixtures. Examination of the mixtures by FAB MS showed a major component having a mass of 448; this was 18 mass units less than the desired product **95**. This component could not be isolated from the mixtures following chromatography.

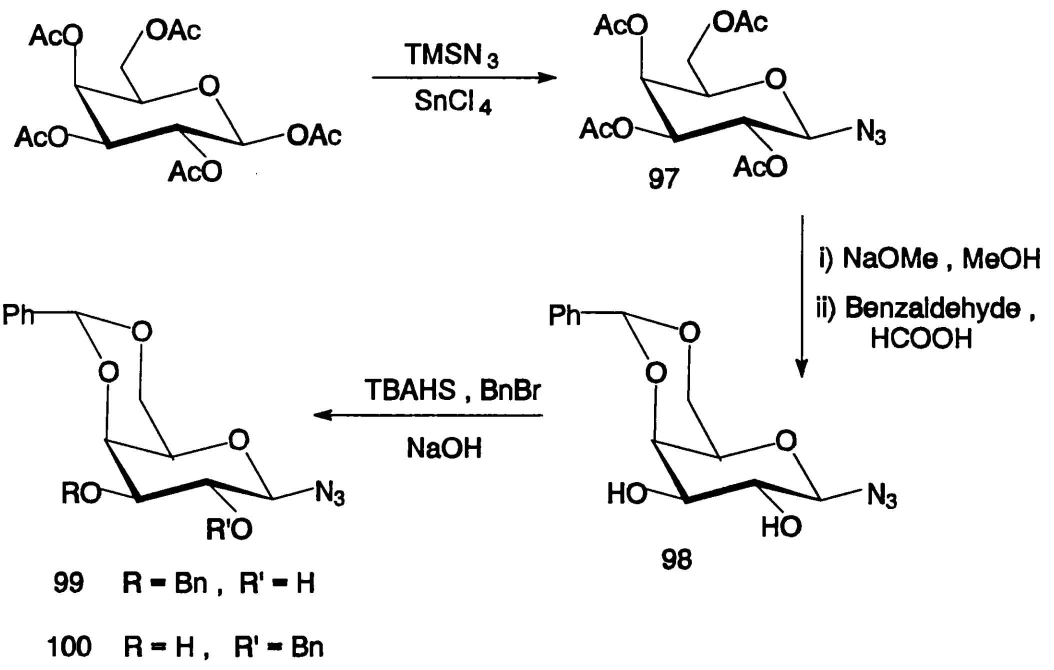
Substitution at C-3 with the less reactive tosylate was attempted next, by treatment of **94** with tosyl chloride and dimethylaminopyridine in DMF. Examination of the reaction mixture by TLC after 18 hours showed unreacted starting material, and one major component. Examination of this mixture by FAB MS showed a component with a mass of 488, the mass of the tosylate **96**. However this component decomposed when chromatographic separation was attempted.

The failure of these reactions to introduce a sulphonate ester at position C-3 could be due to interference from the neighbouring 2-acetamido group. As the acetamido group of the glucosamine residue may be replaced without affecting the affinity of SLeX for selectins[188], it was decided to attempt the synthesis of analogues where the central glucosamine was replaced by a glucose residue.

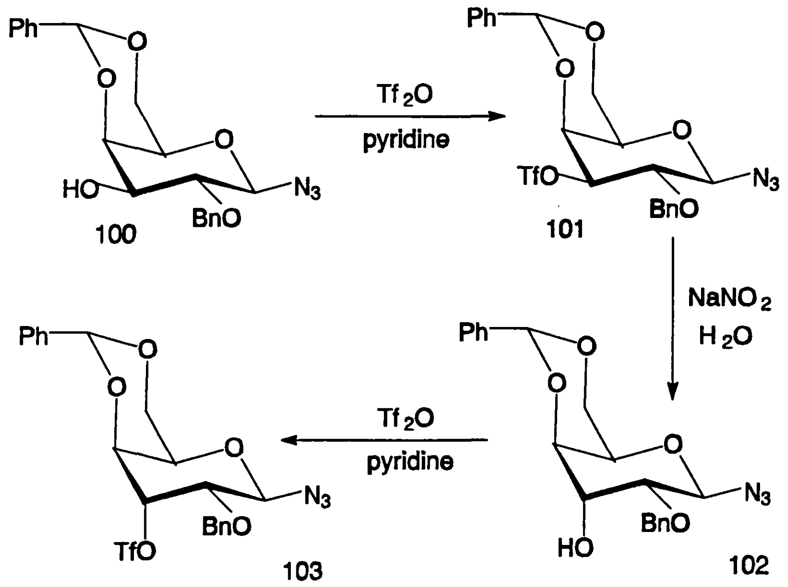
3.3.3 Synthesis of gulose derivative

Synthesis of a gulose residue was required, as inversion of configuration at positions C-3 and C-4 would occur after coupling reactions to give the *gluco-* form. The gulose derivative was synthesised in six steps from galactose peracetate.

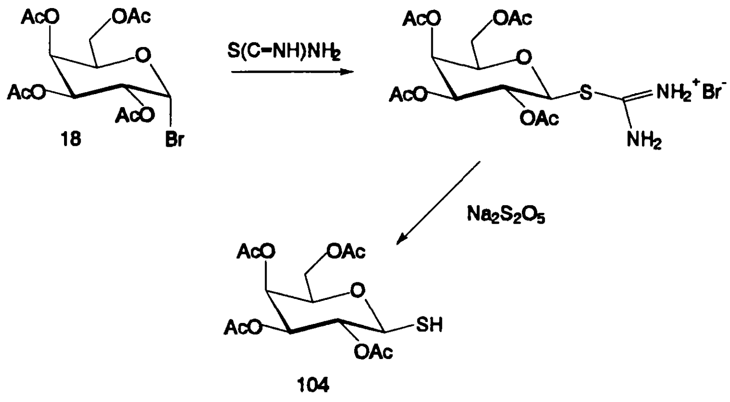
1,2,3,4,6-Penta-*O*-acetyl-β-D-galactopyranose was treated with trimethylsilyl azide and tin (IV) chloride to give the azide **97**. Zemplen deprotection followed by treatment with benzaldehyde and formic acid gave the 4,6-benzylidene acetal **98**. Mono-benzylation of **98** was carried out under phase-transfer catalysed conditions to give the 3-benzyl derivative **99** in 15 % yield and the 2-benzyl **100** derivative in 22 % yield[189].

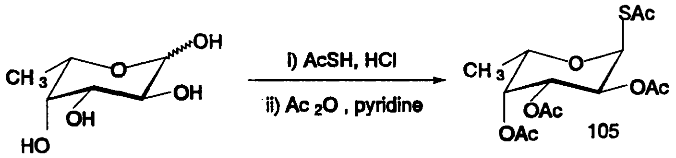


Compound **100** was converted to the 3-triflate **101** by treatment with triflic anhydride in CH_2Cl_2: pyridine 15:1 v/v. The configuration at position C-3 was inverted by reaction of **101** with sodium nitrite in DMF to give the gulose derivative **102**. The displacement of triflate in this case only proceeded when the reaction mixture was heated at 60 °C and a trace of water was added. The gulose compound was treated with triflic anhydride and pyridine to give the 3-triflate **103**.



3.3.4 Synthesis of 1-thio sugars



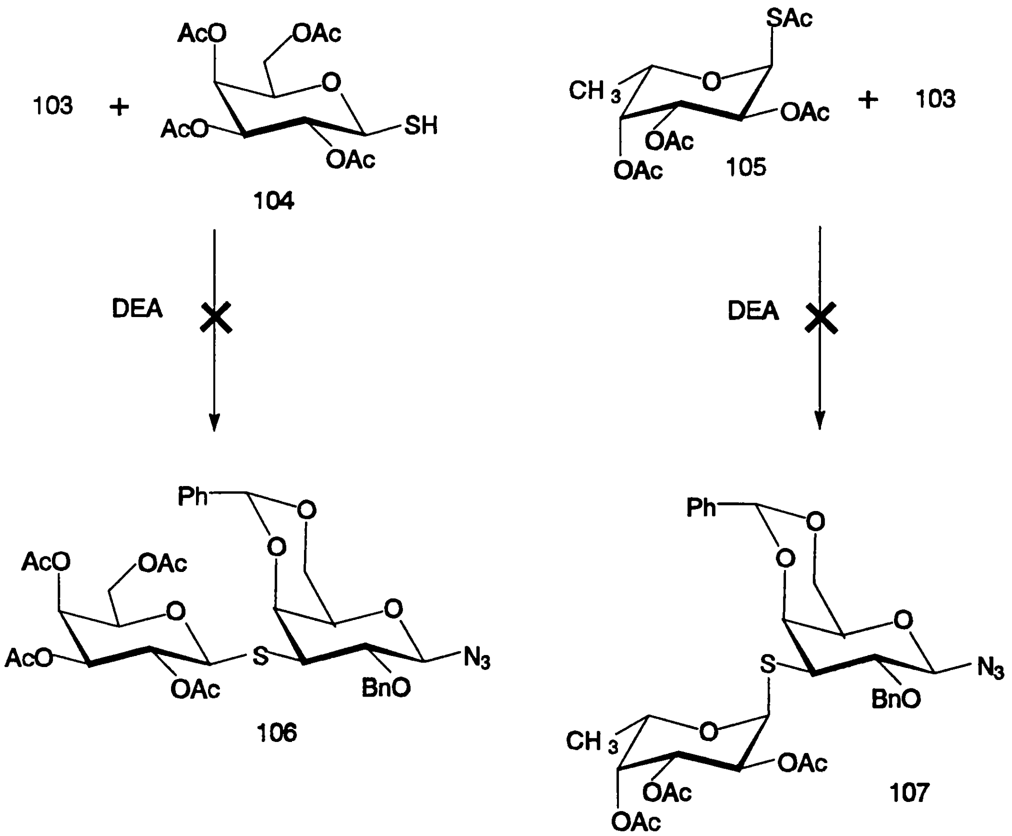


Derivatives of fucose and galactose with a thio function at the anomeric position were required for conjugation to the synthesised gulose derivative. The anomeric configuration of these sugars would be maintained during the coupling reaction, as anomerisation of glycosylthio anions is very slow. The appropriate anomers (β for the galactose, α for the fucose) of the thio-sugars were synthesised.

The 1-thio-galactose derivative **104** was synthesised from peracetylated galactosyl bromide **18** in 85 % yield by reaction with thiourea, followed by treatment with sodium metabisulphite[190].

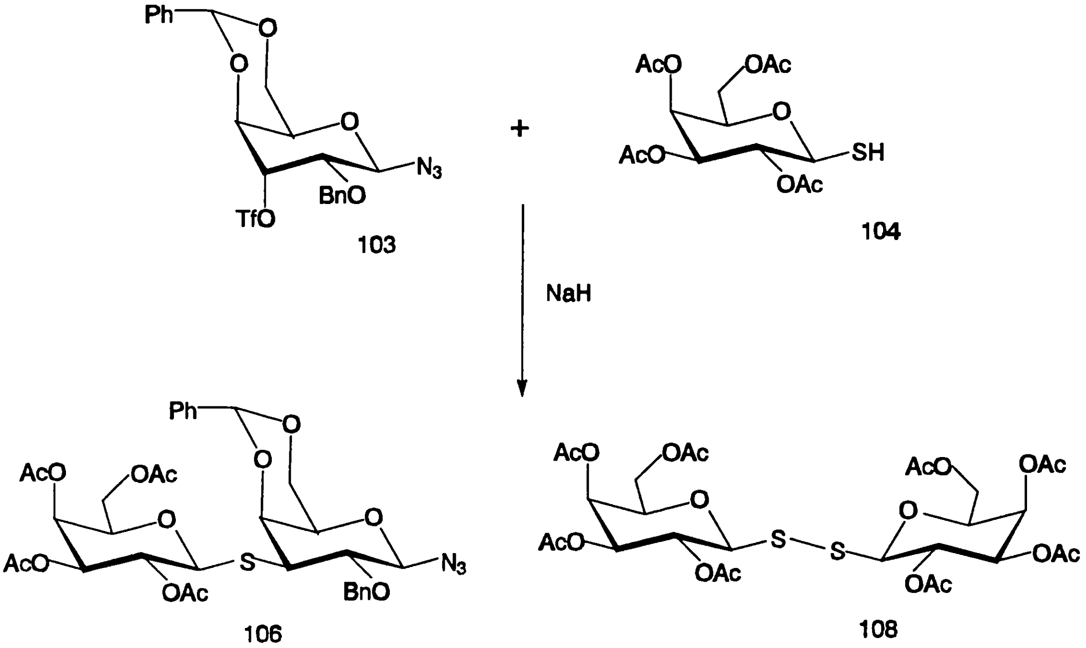
The 1-thioacetate fucose derivative **105** was synthesised in 17% yield from fucose by treatment with thioacetic acid saturated with HCl, and subsequent acetylation[175].

3.3.5 Synthesis of thio-linked conjugates



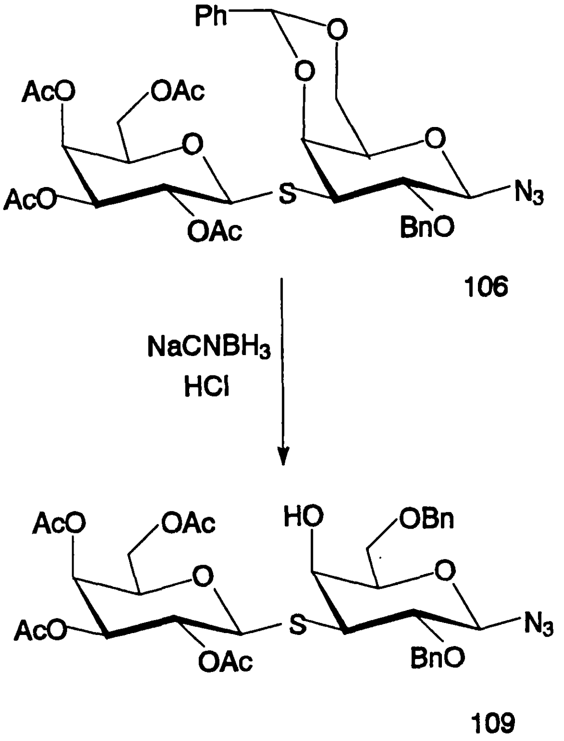
Initially conjugation reactions between **104** and **105** with **103** were attempted with diethylamine in DMF. The thio-linked disaccharides **106** and **107** were not formed in these reactions, with unreacted triflate **103** remaining in the mixtures.

The disulphide linked dimer form of **104** could be isolated from a reaction mixture so 1,4-dithioerythreitol was added to reaction mixtures to prevent disulphide formation. The required products were not formed under these conditions however.



A reaction between **103** and **104** was attempted with sodium hydride as base in DMF. The thio-linked conjugate **106** was formed, with the disulphide **108** as a major side product. Partially de-acetylated compounds were also present in the mixture.

The conjugate **106** was subjected to reductive ring opening with sodium cyanoborohydride with THF saturated with HCl to give **109**.



Conclusions

The Lewis X and sialyl Lewis X epitopes were synthesised on gram scale. A major difficulty encountered in the syntheses of these oligosaccharides was the formation of a β-galactosyl linkage to a disaccharide acceptor. It could only be achieved using a galactosyl donor with benzyl-type protection, leading to a loss of stereoselectivity. Sialylation of a trisaccharide diol acceptor gave the expected tetrasaccharide and also a lactone formed by intramolecular transesterification.

A series of analogues of the disaccharide nucleoside KI-8110 were synthesised by silver ion promoted sialylation of protected nucleosides. The biological properties of these conjugates are under investigation.

An attempt was made to synthesise thio-linked analogues of the Lewis X and Lewis A trisaccharides. Difficulties were encountered in the synthesis of 3-sulphonate esters of *N*-acetylallosamine, a key intermediate in these syntheses.

4 EXPERIMENTAL

EXPERIMENTAL

General methods.

Purification was achieved by chromatography through Sorbsil C60-H40/60, using mobile phases as stated. Reaction progress was monitored by thin layer chromatography on Kieselgel 60 F_{254} using mobile phases as stated. Visualisation was by UV light, iodine, or charring with sulphuric acid. The ion exchange resin used was Amberlite IR-120(H^+). The solvents used in reaction mixtures were water free. Reactions were carried out at room temperature unless otherwise stated.

^{1}H NMR spectra were obtained with a Bruker AM 500 instrument operating at a field of 500 MHz. Chemical shifts are reported in ppm downfield from internal TMS.

Mass spectra were run with a VG Analytical ZAB-SE instrument using fast atom bombardment (FAB) techniques - 20kV Cs^+ ion bombardment, with 2 μL of appropriate matrix, either 3-nitrobenzyl alcohol or thioglycerol with NaI (MeOH) solution added when necessary to produce natriated species when no protonated molecular ions were observed, or on a Fisons matrix assisted laser desorption time of flight spectrometer (MALDI TOF) with a N_2 laser operating at 337nm and 5 μL of 2,5-dihydroxybenzoic acid as matrix.

Reagents

Dimethyl(methylthio)sulphonium trifluoromethanesulphonate (DMTST)

Methyl disulphide (0.24 g, 2.54 mmol), and methyl trifluoromethanesulphonate (0.41 g, 2.54 mmol) were stirred in dichloromethane (5 mL) for 2 days. Ether (40 mL) was added, and the precipitated crystals were removed by filtration (580 mg, 92%): ^{1}H NMR ($CDCl_3$) δ 3.16 (s, 6H, $2CH_3S$), 2.81 (s, 3H, CH_3S).

Silver salicylate

Salicylic acid (6.8g, 15mmol) was dissolved in 96% ethanol (23 mL). The pH of the solution was adjusted to 8 with 25% aqueous ammonia. Silver nitrate (8.1g, 4.8mmol) was dissolved in ethanol: water 1:1 v/v (24 mL) and slowly added to the salicylic acid solution at 60 °C. The mixture was cooled to room temperature after 30 minutes. The precipitate was collected, washed with ethanol, washed with ether and recrystallised from acetonitrile.

Synthesis of sialyl Lewis x and Lewis x oligosaccharides

Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (4)

Glucosamine hydrochloride **1** (100 g, 463.8mmol) was dissolved in cold water (560 mL) and cooled to 5°C. Calcium hydroxide (20g) was gradually added in portions. A mixture of acetic anhydride: methanol 7:10 v/v (170 mL) was added dropwise over 3 hours at 5-10°C. Sulphuric acid 10% (37 mL) was added. The resulting suspension was filtered through Celite. The filtrate was evaporated and the residue was recrystallised from methanol (200 mL) to give **2** (82 g, 80%).

Compound **2** (80 g, 361.6 mmol) was dissolved in benzyl alcohol (1345 mL) containing 2% w/w HCl and the mixture was stirred at 85°C for 2 hours, then at 70°C under vacuum for 1 hour. The solution was cooled to 0°C and a solution of K_2CO_3 (200 g in 300 mL water) was added. The mixture was stirred overnight, the phases were separated, and the organic phase was dried over $MgSO_4$. The mixture was concentrated, and the residue was treated with ether (1 L). The resulting solid was filtered off, dried, and recrystallised from ethanol (500 mL) to give **3** (27 g, 24%).

Compound **3** (10 g, 32.15 mmol) was dissolved in benzaldehyde (45 mL) and formic acid (15 mL) and stirred at room temperature for 90 minutes. Ether (700 mL) was added, and the resulting precipitate was filtered and dried, Recrystallisation from methanol (100 mL) gave **4** (9.1 g, 71%): R_f 0.4 ($CHCl_3$: EtOH 10:0.5 v/v); ^{1}H NMR ($CDCl_3$) δ 7.30-7.38 (m, 10H, 10ArH), 5.80 (d, 1H, NH), 5.58 (s, 1H, CHAr), 4.94 (d, 1H, H-1, $J_{1,2}$= 3.8 Hz), 4.50, 4.75 (2d, 2H, CH_2Ar), 4.24 (m, 2H, H-5, H-6), 3.96 (t, 1H, H-6'), 3.88 (m, 1H, H-2), 3.77 (t, 1H, H-4), 3.60 (t, 1H, H-3), 1.99 (s, 3H, NAc); FAB MS $C_{22}H_{25}NO_6$ (399.45) *m/z* (%) 422 $[M+Na]^+$ (57), 400 $[M+H]^+$ (17), 286 (100).

α-L-Fucopyranosyl tetraacetate (6α) and β-L-Fucopyranosyl tetraacetate (6β)

L-Fucose **5** (1.0 g, 6.04 mmol) was dissolved in dry pyridine (10 mL) and acetic anhydride (7.0 g, 68.6 mmol) was added. The reaction mixture was kept at 0°C for 12 hours. The reaction mixture was concentrated and toluene (3x10 mL) was distilled off. The residue was purified by chromatography using chloroform/ether 10:2 (v/v) as the mobile phase, to give **6α** (750 mg, 37 %): R_f 0.55 (chloroform:ether 5:1 v/v); ^{1}H NMR ($CDCl_3$) δ 6.35 (d, 1H, H-1, $J_{1,2}$ = 2.9 Hz), 5.33 (m, 3H, H-2, H-3, H-4), 2.28 (m, 1H, H-5), 2.18, 2.15, 2.00, 1.98 (4s, 12H, 3 OAc), 1.16 (d, 3H, H-6). FAB MS $C_{14}H_{20}O_9$ (332.3) m/z (%) 355 $[M+Na]^+$ (86), 273 (74), 153 (100), 111 (96); and **167β** (210 mg, 10 %): R_f 0.45 (chloroform:ether 5:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.69 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 5.32 - 5.27 (m, 2H, H-2, H-4), 5.07 (dd, 1H, H-3), 3.95 (m, 1H, H-5), 2.17, 2.14, 2.00, 1.99 (4s, 12H, 3 OAc), 1.20 (d, 3H, H-6); FAB MS $C_{14}H_{20}O_9$ (332.3) m/z (%) 355 $[M+Na]^+$ (100), 273 (68), 153 (66), 111 (62) and a mixture of **6α** and **6β** (1.0 g, 49 %).

Methyl 2,3,4-Tri-O-acetyl-1-thio-ß-L-fucopyranoside (7)

A mixture of **6β** and **6α** (2.1 g, 6.32 mmol) was dissolved in dichloromethane (12 mL). Trimethylsilyl trifluoromethanesulphonate (1.64 g, 6.95 mmol) and (methylthio)trimethylsilane (1.13 g, 9.48 mmol) were added, and the mixture was stirred at room temperature for 2 days. The reaction mixture was diluted with dichloromethane (120 mL) and washed with sat. $NaHCO_3$ (20 mL), and water (20 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform as the mobile phase, to give **7** (1.82 g, 90 %): R_f 0.35 (hexane:ethyl acetate 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.26 (dd, 1H, H-4), 5.22 (t, 1H, H-2), 5.03 (dd, 1H, H-3), 4.34 (d, 1H, H-1), 3.83 (m, 1H, H-5), 1.96, 2.05, 2.15, 2.17 (4s, 12H, 3 OAc, MeS), 1.20 (d, 3H, H-6); FAB MS $C_{13}H_{20}O_7S$ (320.36) m/z (%) 343 $[M+Na]^+$ (100), 273 (8), 153 (10), 111 (10).

Methyl 2,3,4-Tri-O-benzyl-1-thio-ß-L-fucopyranoside (8)

Compound **7** (800 mg, 2.38 mmol) was dissolved in methanol (5 mL). Sodium methoxide (20 mg, 0.37 mmol) was added and the mixture was stirred at room temperature for 10 minutes. The solution was neutralised with ion exchange resin and filtered. The filtrate was evaporated and the residue was dissolved in dry DMF (4.5 mL). The solution was cooled to 0°C, sodium hydride (60%) (445 mg, 18.54 mmol) was added and the mixture was stirred for 30 minutes. Benzyl bromide (1.18 g, 11.1 mmol) was added and the solution was stirred at room temperature for 12 hours. Methanol (1.2 mL) was added and the mixture was evaporated. The residue was taken up in chloroform (15 mL), filtered and extracted with water (3.3 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using hexane/ether/methanol 12:7:1 (v/v/v) as the mobile phase, to give **8** (770 mg, 69.9 %): R_f 0.3 (hexane:ethyl acetate 5:1 v/v); ^1H NMR ($CDCl_3$) δ 7.43 - 7.29 (m, 15H, 15 Ar-H), 5.03 - 4.07 (m, 6H, 3 CH_2-Ar), 4.32 (d, 1H, H-1), 3.87 (t, 1H, H-2), 3.64 (t, 1H, H-4), 3.59 (dd, 1H, H-3), 3.52 (m, 1H, H-5), 2.23 (s, 3H, MeS), 1.24 (d, 3H, H-6): FAB MS $C_{28}H_{32}O_4S$ (464.60) m/z (%) 487 $[M+Na]^+$ (100), 181 (32), 91 (99).

Benzyl 3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (9)

Compound **4** (1.0 g, 2.51 mmol), and **8** (1.16 g, 2.51 mmol) and 4Å-MS (1.5 g) in CH_2Cl_2 (50 mL) and tetrahydrofuran (50 mL) were stirred together at room temperature. DMTST (1.94 g, 7.52 mmol) was added and the mixture was stirred for 14 hours. Triethylamine (0.4 mL) was added, and the mixture was filtered and evaporated. The residue was taken up in CH_2Cl_2 (140 mL) and washed with water. The organic layer was dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform/ethyl acetate 10:2 (v/v) to give **9** (958 mg, 46.9%): R_f 0.42 ($CHCl_3$:EtOAc 10:2 v/v); ^1H NMR ($CDCl_3$) δ 7.40-7.23 (m, 20H, 20 ArH), 6.48 (d, 1H, NH), 5.59 (s, 1H, CHAr), 4.96 (d, 1H, CH_a-Ar), 4.47 (d, 1H, CH_b-Ar), 3.91 (dd, 1H, H-3'), 1.42 (s, 3H, NAc), 1.07 (d, 3H, H-6'); FAB MS $C_{49}H_{53}NO_{10}$ (815.96) m/z (%) 838 $[M+Na]^+$ (62).

Benzyl 3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-acetamido-6-O-benzyl-2-deoxy-α-D-glucopyranoside (10)

Compound **9** (390 mg, 0.47 mmol) was dissolved in tetrahydrofuran (10 mL). 4Å-Molecular sieves (500 mg) and a few grains of methyl orange indicator were added. Sodium cyanoborohydride (440 mg, 7.05 mmol) was added with stirring. Ether saturated with HCl was added very slowly until a permanent pink colour was obtained. The reaction mixture was stirred at room temperature for 3 days. The mixture was filtered and evaporated. The residue was taken up in chloroform (10 mL) and washed with saturated $NaHCO_3$ (2x1.8ml). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform/ethyl acetate 10:2 (v/v) to give **10** (291mg, 75.7%): R_f 0.36 ($CHCl_3$:EtOAc 10:2 v/v); ^{1}H NMR ($CDCl_3$) δ 7.41-7.16 (m, 25H, ArH), 5.94 (d, 1H, NH), 1.42 (s, 3H, NAc), 1.17 (d, 3H, H-6'); FAB MS $C_{49}H_{55}NO_{10}$ (817.98) m/z (%) 840 $[M+Na]^+$(100), 747 (10), 360 (40), 338.

Large scale procedure

Benzyl 3-*O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (10).

A mixture of **4** (6.94 g, 17.39 mmol), **8** (8.07 g, 17.39 mmol) and molecular sieves 4Å (13 g) in tetrahydrofuran (650 mL) was stirred at room temperature. DMTSB (5.0 g, 25.5 mmol) was added and the mixture was stirred for 2 h. The reaction was monitored by TLC using $CHCl_3$:EtOAc 5:1 v/v as the mobile phase. Triethylamine (7 mL) was added, the mixture was filtered and the filtrate was concentrated. The residue was taken up in chloroform (300 mL) and washed with water (50 mL), saturated $NaHCO_3$ solution (50 mL) and water (50 mL). The organic layer was dried with $MgSO_4$, filtered and concentrated. Ethanol/benzene 1:1 v/v (40 mL) was evaporated from the residue, giving 15.6 g of a crude mixture containing benzyl3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**9**). The residue was taken up in THF (130 mL) then methyl orange indicator (50 mg) and sodium cyanoborohydride (10 g, 159.13 mmol) were added at 0 °C. THF saturated with HCl was added very slowly until a permanent pink colour was obtained. More THF/HCl (2x10 drops) was added, and the reaction mixture was stirred between 25-40 °C for 4 h. The reaction was monitored by TLC using chloroform:EtOAc 5:1 v/v as the mobile phase. The mixture was neutralised with triethylamine and concentrated. The residue was taken up in chloroform (350 mL) and washed with water (70 mL), saturated $NaHCO_3$ solution

(70 mL). The organic layer was treated with Amberlite MB-1 ion exchange resin (60 g), dried over $MgSO_4$, and concentrated. The product **10** was crystallised from methanol (30 mL) (10.2 g 72 %).

Methyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside (12)

Methyl 1-thio-ß-D-galactopyranoside **11** (202 mg, 0.96 mmol), benzaldehyde (0.4 ml) and formic acid (0.4 ml) were stirred together at room temperature for 40 minutes. Ether (10 mL) was added. The precipitate formed was collected and purified by chromatography using dichloromethane/methanol 10:0.5 (v/v) to give **12** (157 mg, 54.6 %): R_f 0.30 (dichloromethane:methanol 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 7.48 (m, 3H, 2 Ar-H), 7.37 (m, 3H, 3 Ar-H), 5.54 (s, 1H, CH-Ar), 4.37 (dd, 1H, H-6'), 4.28 (d, 1H, H-1, $J_{1,2}$=9.4 Hz), 4.26 (m, 1H, H-4), 4.05 (dd, 1H, H-6), 3.93 (t, 1H, H-2), 3.66 (m, 1H, H-3), 3.55 (s, 1H, H-5), 2.26 (s, 3H, SMe); FAB MS $C_{14}H_{18}O_5S$ (298.36) m/z (%) 299 $[M+H]^+$ (27), 251 (53), 193 (32), 107 (100).

Methyl 2,3-di-O-acetyl-4,6-O-benzylidene-1-thio-ß-D-galactopyranoside (13)

12 (130 mg,0.43 mmol) was dissolved in pyridine (4 mL) and cooled to 5°C. Acetic anhydride (0.25 mL) was added, and the mixture was stirred at 5°C for 1 hour, then at room temperature for 16 hours. The solution was evaporated. Toluene (4 mL) was added and evaporated. The residue was purified by chromatography using hexane/ ethylacetate 1:1 (v/v) to give **13** (136 mg, 81.8%): Rf 0.5 (hexane:ethylacetate 1:1): ^1H NMR ($CDCl_3$) δ 7.49 (m, 2H, Ar-H), 7.37 (m,3H,Ar-H), 5.50 (s, 1H, CH-Ar), 5.48-5.54 (m, 1H, H-2), 5.00 (dd, 1H, H-3), 4.42 (m, 1H, H-4), 4.40 (d, 1H, H-1), 4.37 (dd, 1H, H-6'), 4.02 (dd, 1H, H-6), 3.60 (s, 1H, H-5), 2.24 (s, 3H, SMe), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc); FAB MS $C_{18}H_{22}O_7S$ (382.44) m/z (%) 405 $[M+Na]^+$ (100), 335 (77), 277 (45), 254 (23), 229 (20), 176 (78), 149 (72), 136 (74), 105 (77).

Benzyl *O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (14)

Run A : Compound **13** (14 mg, 0.036 mmol) and disaccharide **10** (30 mg, 0.03 mmol) with 4Å-MS (50 mg) were dissolved in CH_2Cl_2 (5 mL). DMTST (23 mg, 0.09 mmol) was added to the mixture, and it was stirred at room temperature for 24 hours.

Run B : Compound **13** (127 mg, 0.15 mmol) and disaccharide **10** (50 mg, 0.13 mmol) with 4Å-MS (75 mg) were dissolved in CH_2Cl_2 (5 mL). DMTST (80 mg, 0.31 mmol) was added to the mixture, and it was stirred at room temperature for 16 hours. Triethylamine (0.3 mL) was added, and the mixture was filtered. The filtrate was evaporated and the residue taken up in $CHCl_3$ (5 mL). The solution was washed with water (2x 1 mL), dried over $MgSO_4$, Filtered and concentrated. The residue was purified by chromatography using CH_2Cl_2:acetone 10:0.5 v/v as the mobile phase to give unreacted **10** and compound **15** (9mg, 17%): R_f 0.48 (CH_2Cl_2:acetone 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 7.34-7.52 (m, 10H, 10ArH), 6.48 (d, 1H, H-1b, $J_{1,2}$= 4.0Hz), 5.51, 5.45 (2s, 2H, 2CHAr), 5.28 (m, 2H, H-2a, H-3b), 4.87 (dd, 1H, H-3a), 4.59 (d, 1H, H-1a, $J_{1,2}$= 8.1Hz), 4.46 (d, 1H, H-4a), 4.25-4.31 (m, 4H, H-2b, H-4b, H-6a, H-6b), 3.99, 3.96 (2dd, 2H, H-6'a, H-6b'), 3.81 (s, 1H, H-5b), 3.47 (s, 1H, H-5a), 2.11, 2.10, 2.04, 2.01 (4s, 12H, 4OAc); FAB MS $C_{34}H_{38}O_{15}$ (686.66) *m/z* (%) 709 $[M+Na]^+$ (100), 567 (14), 335 (37).

Run C : Compound **13** (25 mg, 0.06 mmol) and disaccharide **10** (65 mg, 0.07 mmol) with 4Å-MS (60 mg) were dissolved in CH_2Cl_2 (5 mL) and cooled to 0°C. DMTST (45 mg, 0.17 mmol) was added to the mixture at 0°C, and it was stirred at room temperature for 16 hours.

Run D : Compound **13** (30 mg, 0.08 mmol) and disaccharide **10** (40 mg, 0.05 mmol) with 4Å-MS (50 mg) were dissolved in THF (5 mL). DMTSB (20 mg, 0.10 mmol) was added to the mixture, and it was stirred at room temperature for 2 hours. The mixture was filtered and the filtrate diluted with CH_2Cl_2 (10 mL). The solution was washed with saturated $NaHCO_3$ solution (1.5 mL) and water (1.5 mL), then dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography using $CHCl_3$: MeOH 10:1 v/v to give **16** (15mg, 53%): R_f 0.65 ($CHCl_3$: MeOH 10:1 v/v); ^1H NMR ($CDCl_3$) δ 7.48, 7.35 (2m, 5H, 5ArH), 6.38 (d, 1H, H-1, $J_{1,2}$= 3.7Hz), 5.50 (s, 1H, CHAr), 5.17 (dd, 1H, H-3), 4.45 (d, 1H, H-4), 4.41 (dd, 1H, H-2), 4.25 (dd, 1H, H-6), 4.02 (dd, 1H, H-6'), 3.84 (s, 1H, H-5), 2.14, 2.10 (2s, 6H, 2OAc); FAB MS $C_{17}H_{20}O_8$ (352.35) *m/z* (%) 375 $[M+Na]^+$ (100), 293 (22).

Run E : Compound **13** (9 mg, 0.02 mmol) and disaccharide **10** (10 mg, 0.01 mmol) with 4Å-MS (10 mg) were dissolved in THF (2 mL). DMTSB (3 mg, 0.03 mmol) was added to the mixture, and it was stirred at room temperature for 16 hours.

Run F : Compound **13** (12 mg, 0.03 mmol) and disaccharide **10** (10 mg, 0.012 mmol) with 4Å-MS (20 mg) were dissolved in $CHCl_3$ (3 mL). DMTST (8 mg, 0.03 mmol) was added to the mixture, and it was stirred at room temperature for 4 hours.

Run G : Compound **13** (9 mg, 0.023 mmol) was dissolved in THF (5 mL) and bromine (4.4 mg, 0.027 mmol) was added. The mixture was stirred for 30 minutes and disaccharide **10** (10 mg, 0.012 mmol) with 4Å-MS (20 mg) was added. The mixture was cooled to -15°C, silver triflate (9 mg, 0.034 mmol) in THF (1 mL) was added dropwise, and the mixture was stirred at this temperature for 2 hours.

Methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-ß-D-galactopyranoside (17)

1,2,3,4,6-penta-*O*-acetyl-α,ß-D-galactopyranose (1.0g, 2.56mmol), (methylthio)trimethylsilane (452mg, 3.76mmol) and trimethylsilyl trifluoromethanesulfonate (694mg, 2.94mmol) in dichloromethane (10 mL) was stirred overnight at room temperature. The reaction mixture was diluted with 50ml dichloromethane (50 mL) and washed with 1M Na_2CO_3 solution, dried over $MgSO_4$ and concentrated. The residue was recrystallised from ethyl acetate/hexane 1:1 (v/v) to give **17** (740mg, 77%): R_f 0.37 (hexane:EtOAc 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.42 (dd, 1H, H-4), 5.26 (t, 1H, H-2), 5.04 (dd, 1H, H-3), 4.38 (d, 1H, H-1), 4.13 (m, 2H, H-5, H-6'), 2.20, 2.16, 2.06, 2.03, 1.97 (5s, 15H, 4OAc, SCH_3); FAB MS $C_{15}H_{22}O_9S$ (378.40) m/z (%)= 401 $[M+Na]^+$ (100), 331 (56)

2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (18)

1,2,3,4,6-Penta-*O*-acetyl-α,ß-D-galactopyranose (100mg, 0.25mmol) was dissolved in acetic acid (4 mL) and was treated with HBr in acetic acid (30% , 4 mL) at 0°C, and then stirred for 30 minutes at room temperature. The reaction mixture was evaporated and toluene (3x3 mL) was distilled off from the residue. The residue was purified by chromatography using chloroform/ether 5:2 (v/v) to give **18** (82mg, 80%): R_f 0.55 (hexane:EtOAc 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 6.71 (d, 1H, H-1), 5.52 (d, 1H, H-4), 5.42 (dd, 1H, H-2), 5.03 (dd, 1H, H-3), 4.50 (t, 1H, H-6'), 4.16 (m, 2H, H-6, H-5); FAB MS $C_{14}H_{19}BrO_9$ (411.19) m/z (%) 423 $[M+Na]^+$ (100), 411 $[M+H]^+$ (8).

Benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (19)

Run A : Compound **17** (10 mg, 0.03 mmol) and disaccharide **10** (20 mg, 0.02 mmol) with 4Å-MS (10 mg) were dissolved in CH_2Cl_2 (3 mL). DMTST (23 mg, 0.09 mmol) was added to the mixture, and it was stirred at room temperature for 4 hours.

Run B : Compound **18** (13 mg, 0.03 mmol) and disaccharide **10** (20 mg, 0.02 mmol) were dissolved in CH_2Cl_2 (2 mL) and 4Å-MS (10 mg) was added. The mixture was cooled to -25°C and silver triflate (7.5 mg, 0.03mmol) and collidine (3.5 mg, 0.03 mmol) in CH_2Cl_2 (1 mL) was added dropwise. The mixture was stirred at -25°C for 1 hour and then at -15°C for 2 hours.

Run C : Compound **18** (13 mg, 0.03 mmol) and disaccharide **10** (20 mg, 0.02 mmol) were dissolved in nitromethane:toluene 1:1 v/v (2 mL) and 4Å-MS (20 mg) and calcium sulphate (10 mg) were added. The mixture was cooled to -15°C, mercury (II) cyanide (13 mg, 0.05 mmol) was added, and the mixture was stirred at this temperature for 12 hours.

2,3,4,6-tetra-O-benzyl-1-thio-ß-D-galactopyranoside (20)

A mixture of **17** (300mg, 0.79mmol) and sodium methoxide (47mg, 3.17mmol) in methanol (5 mL) was stirred at room temperature for 2 hours and neutralised with ion exchange resin. The solution was evaporated and the residue was taken up in dimethylformamide (10 mL). Sodium hydride 60% (190mg, 4.76mmol) was added at 0°C. The mixture was stirred at 0°C for 30 minutes and then at room temperature for 3 hours. Benzyl bromide (814mg, 4.76mmol) was added dropwise at 0°C, and the mixture was stirred at room temperature for 3 hours, then evaporated. The residue was purified by chromatography with hexane/ether/methanol 12:7:0.3 (v/v/v) to give **20** (384mg, 85%): R_f 0.42 (hexane:EtOAc 1:1 v/v); ^1H NMR ($CDCl_3$) δ 7.42-7.20 (m, 20H, 20ArH), 4.98 (d, 1H, H-4), 4.95-4.42 (6d, s, 16H, $4CH_2Ar$), 4.33 (d, 1H, H-1), 3.59 (t, 1H, H-2), 3.58 (m, 4H, H-3, H-5, H-6', H-6); FAB MS $C_{35}H_{38}O_5S$

(570.21) m/z (%) 593 $[M+Na]^+$ (50), 571 $[M+H]^+$ (5).

Benzyl *O*-(2,3,4,6-tetra-*O*-benzyl-α,β-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tetra-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (21, 22)

A mixture of **20** (2.44 g, 4.28 mmol) and **10** (1.0 g, 1.22 mmol) was stirred at room temperature with 2 g molecular sieves 4Å in chloroform (20 mL). DMTST (950 mg, 3.68 mmol) was added and the mixture was stirred for 1 h. A further 600 mg (2.32 mmol) DMTST was added and the mixture was stirred for 3 h. Chloroform (150 mL) was added and the solution was washed with 2 x 40 mL saturated $NaHCO_3$ solution. The organic layer was separated and dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using hexane:chloroform:ether:acetonitrile 3:10:3:1 v/v/v/v as the mobile phase to give **22** (β-galactoside) (583mg, 36%): R_f 0.44 (hexane:chloroform:ether:acetonitrile 3:10:3:1 v/v/v/v); ^{1}H NMR ($CDCl_3$) δ 7.43-7.24 (m, 45H, ArH), 5.77 (d, 1H, NH), 5.29 (d, 1H, H-1_{fuc}), 1.83 (s, 3H, NHAc), 1.12 (d, 3H, H-6_{fuc}); FAB MS $C_{83}H_{89}NO_{15}$ (1340.54) *m/z* (%) 1363 $[M+Na]^+$ (100), 1273 (12); and **21** (α-galactoside) (423mg, 26%): R_f 0.48 (hexane:chloroform:ether:acetonitrile 3:10:3:1 v/v/v/v); ^{1}H NMR ($CDCl_3$) δ 7.01-7.46 (m, 45H, ArH), 5.92 (d, 1H, NH), 5.36 (d, 1H, H-1_{fuc}), 5.12 (d, 1H, H-1_{gal}, $J_{1,2}$=3.5Hz), 1.36 (s, 3H, NHAc), 1.27 (d, 3H, H-6_{fuc}); FAB MS $C_{83}H_{89}NO_{15}$ (1340.54) *m/z* (%) 1363 $[M+Na]^+$ (100), 1273 (12), 471 (10).

***O*-(ß-D-Galactopyranosyl)-(1→4)-*O*-(α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-α,ß-D-glucopyranose (24).**

10% Pd/C (1000 mg) was added to **22** (583 mg, 0.43 mmol) in 10 mL acetic acid/methanol 3:1 v/v. The mixture was stirred under H_2 for 2 days at 40 °C, then filtered. The filtrate was lyophilised to give **24** (230 mg, 100%): R_f 0.7 (MeOH:H_2O 4:1 v/v); ^{1}H NMR (D_2O) δ 4.94 (t, H-$1_{Fuc\ \alpha,\beta}$), 4.68 (d, H-1_{GlcNAc}), 4.34 (d, H-1_{Gal}, $J_{1,2}$=7.7 Hz), 1.87, 1.85 (2s, NAc_α, NAc_β), 1.02 (d, H-6_{Fuc}); MALDI-TOF MS $C_{20}H_{35}NO_{15}$ (529.48) *m/z* (%) 568 $[M+K]^+$ (31), 552 $[M+Na]^+$ (100), 375 (29), 332 (32), 273 (34).

O-(α-D-Galactopyranosyl)-(1→4)-*O*-(α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-α,ß-D-glucopyranose (23).

10% Pd/C (800 mg) was added to **21** (423 mg, 0.315 mmol) in 10 mL acetic acid/methanol 3:1 v/v. The mixture was stirred under H_2 for 2 days at 40 °C, then filtered. The filtrate was lyophilised to give **23** (165 mg, 99%): R_f 0.66 (MeOH:H_2O 4:1 v/v); ^{1}H NMR (D_2O) δ 8.06 (d, NH), 4.72 (d, H-$1_{GlcNAcß}$, $J_{1,2}$=7.2Hz), 1.89, 1.86 (2s, NAc_α, $NAc_ß$), 1.05 (d, H-6_{Fuc}); MALDI-TOF MS $C_{20}H_{35}NO_{15}$ (529.48) *m/z* (%) 568 $[M+K]^+$ (31), 552 $[M+Na]^+$ (100), 375 (29), 332 (32), 273 (34).

6-*O*-Benzyl-1,2,3,4-di-*O*-isopropylidene-D-galactopyranose (26)

1,2,3,4-di-O-isopropylidene-D-galactopyranose **25** (25.0g, 96.15mmol) in 50ml DMF was added dropwise at 0°C to a suspension of sodium hydride 60% (5.67g, 140.35mmol) in 50ml DMF. The mixture was stirred at room temperature for 2.5 hours, then benzyl bromide (24.0g, 140.35mmol) was added dropwise at 0°C. The mixture was stirred at room temperature overnight. The mixture was evaporated, and xylene (2x15ml) was distilled from the residue. The residue was taken up in 500ml ether and washed with 2x100ml water. The organic layer was evaporated, the residue was taken up in 300ml methanol and extracted with 2x150ml hexane. The methanol phase was evaporated to give **26** as a crude residue (34.2g). A portion was purified by column chromatography for analytical purposes using chloroform/ethyl acetate 1:1 (v/v) as the mobile phase: R_f 0.4 ($CHCl_3$:EtOAc 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 5H, ArH), 5.56 (d, 1H, H-1), 4.52-4.66 (m, 3H, CH_2Ar, H-4), 4.31 (m, 2H, H-2, H-3), 4.03 (t, 1H, H-5), 3.71, 3.63 (2m, 2H, H-6, H-6'), 1.61, 1.54, 1.47, 1.35 (4s, 12H, $4CH_3$); FAB MS $C_{19}H_{26}O_6$ (350.40) *m/z* (%) 373 $[M+Na]^+$ (55), 351 $[M+H]^+$ (35), 335 (35), 181 (37), 91 (100).

6-*O*-Benzyl-α,ß-D-galactopyranose (27)

Compound **26** (34.2g) was dissolved in 60 mL dioxane:1N HCl 1:1 (v/v) and heated at 100°C for 3 hours. The dioxane was evaporated, and the solution was neutralised with 1M NaOH. The solution was evaporated and 3x5 mL benzene was distilled off to give 27.6g of **27** as a crude residue. An analytical sample was purified by column chromatography using chloroform/methanol 4:1 (v/v) as the mobile phase to give **27** as an anomeric mixture: R_f 0.55 ($CHCl_3$:MeOH 1:1 v/v); ^1H NMR (D_2O) δ 7.27 (m, 5H, ArH), 5.09 (d, 1H, H-1α), 4.48 (s, 2H, CH_2Ar_a), 3.32-4.51 (8m, CH_2Ar_b, sugar H); FAB MS $C_{13}H_{18}O_6$ (270.27) m/z (%) 293 $[M+Na]^+$ (100), 91 (70)

1,2,3,4 tetra-*O*-acetyl-6-*O*-benzyl-α,ß-D-galactopyranose (28)

Compound **27** (27.6g) was dissolved in 170 mL pyridine, and cooled to 0°C. Acetic anhydride (193 mL) was added slowly, and the mixture was stirred at 0°C for 30 minutes, then at room temperature for 16 hours. The mixture was evaporated, and 2x40 mL toluene was distilled off. The residue was taken up in 300 mL $CHCl_3$, and washed with 60 mL water. The organic layer was dried over $MgSO_4$, filtered and evaporated, to give 30.7g crude residue. An analytical sample was prepared by column chromatography using hexane/ethyl acetate 8:7 v/v as the mobile phase to give **28α**: and **28β**: R_f 0.45 (α), 0.43 (ß) (CHC13:EtOAc 10:1 v/v); ^1H NMR ($CDCl_3$) α δ 7.31 (m, 5H, ArH), 6.36 (d, 1H, H-1, $J_{1,2}$=2.9 Hz), 5.56 (d, 1H, H-4), 5.32 (m, 2H, H-2, H-3), 4.53, 4.41 (2d, 2H, CH_2Ar), 4.28 (t, 1H, H-5), 3.54, 3.47 (2m, 2H, H-6, H-6'), 2.12, 2.04, 2.00, 1.98 (4s, 12H, 4OAc); ß δ= 7.31 (m, 5H, ArH), 5.71 (d, 1H, H-1, $J_{1,2}$=8.1Hz), 5.52 (d, 1H, H-4), 5.32 (dd, 1H, H-2), 5.07 (dd, 1H, H-3), 4.56, 4.40 (2d, 2H, CH_2Ar), 3.98 (t, 1H, H-5), 3.55, 3.47 (2m, 2H, H-6, H-6'), 2.10, 2.06, 2.03, 2.00 (4s, 12H, 4OAc); FAB MS $C_{21}H_{26}O_{10}$ (438.42) *m/z* (%) 461 $[M+Na]^+$ (100), 379 (22), 91 (100).

Methyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-1-thio-ß-D-galactopyranoside (29)

Crude **28** (30.7g) was dissolved in 20 mL CH_2Cl_2. Methylthio-trimethylsilane (13.2g, 105mmol) and trimethylsilyl triflate (18.2g, 77mmol) were added. The mixture was diluted with 20 mL CH_2Cl_2 and stirred at room temperature for 16 hours. The mixture was cooled to 0°C and 100 mL 1M potassium carbonate solution was added to the stirred solution. The mixture was diluted with CH_2Cl_2 (250 mL) and the organic layer was separated and washed with 100 mL water. The organic layer was separated, dried over $MgSO_4$, filtered and evaporated. Methanol was added to the residue, and the resulting white precipitate of **29** (12.82g, 42%) was filtered off. The filtrate was evaporated, and the residue was purified by column chromatography using hexane/ethyl acetate 8:7 v/v as the mobile phase to give a further 4.34g (15%) of **29**: R_f 0.57 (hexane:EtOAc 8:7 v/v); ^1H NMR ($CDCl_3$) δ 7.31 (m, 5H, ArH), 5.51 (d, 1H, H-4), 5.22 (t, 1H, H-2), 5.05 (dd, 1H, H-3), 4.54, 4.42 (2d, 2H, CH_2Ar), 4.37 (d, 1H, H-1, $J_{1,2}$=9.7 Hz), 3.57, 3.46 (2m, 2H, H-6, H-6'), 2.17 (s, 3H, SMe), 2.05, 2.04, 1.97 (3s, 9H, 3OAc); FAB MS $C_{20}H_{26}SO_8$ (426.47) *m/z* (%) 449 $[M+Na]^+$ (100), 379 (22).

Methyl 6-*O*-benzyl-1-thio-ß-D-galactopyranoside (30)

Compound **29** (12.80g, 30.05mmol) and sodium methoxide (300mg, 5.63mmol) were taken up in 150 mL methanol and stirred at 40°C for 2 hours. The mixture was neutralised with ion exchange resin, filtered and evaporated to give **30** (8.75g, 97%) as a white solid: R_f 0.5 ($CHCl_3$:MeOH 5:1 v/v); ^1H NMR (DMSO-d6) δ= 7.31 (m, 5H, ArH), 4.47 (s, 2H, CH_2Ar), 4.12 (d, 1H, H-1, $J_{1,2}$=8.8Hz), 2.04 (s, 3H, SMe); FAB MS $C_{14}H_{20}SO_5$ (300.36) *m/z* (%) 345 $[M+2Na]^+$ (14), 323 $[M+Na]^+$ (100), 91 (82).

Methyl 6-*O*-benzyl-2,3,4-tri-*O*-(4-methoxybenzyl)-1-thio-ß-D-galactopyranoside (31)

Compound **30** (8.75g, 29.16mmol) in 30 mL DMF was added slowly at 0°C to sodium hydride 60% (9.61g, 240mmol) suspended in 50 mL DMF. The mixture was stirred for 45 minutes at room temperature, then cooled to 0°C. 4-Methoxybenzyl chloride (37.5g, 240mmol) was added slowly. The mixture was stirred at room temperature for 16 hours. The mixture was cooled to 0°C and methanol was added to the solution until gas evolution ceased. The mixture was evaporated, and 4x10 mL xylene and 20 mL benzene were distilled off. The residue was taken up in 300 mL $CHCl_3$ and washed with 75 mL $NaHCO_3$ solution and 100 mL water. The organic layer was evaporated. The residue was taken up in 100 mL acetonitrile and extracted with 3x200 mL hexane. The acetonitrile phase was separated and evaporated. The residue was taken up in methanol and the resulting precipitate was filtered off. The solid was recrystallised from di-isopropyl ether to give **31** as a white solid (15.61g, 78.7%): R_f 0.45 (hexane/$CHCl_3$/EtOAc 7:7:2 v/v/v); ^1H NMR ($CDCl_3$) δ 7.27, 6.84 (2m, 17H, ArH), 4.88 (d, 1H, CHAr), 4.76 (q, 2H, CHAr), 4.66 (s, 2H, CHAr), 4.56

(d, 1H, CHAr), 4.43 (q, 2H, CHAr), 4.31 (d, 1H, H-1, $J_{1,2}$=9.6Hz), 3.92 (d, 1H, H-4), 3.84, 3.81, 3.80 (3s, 9H, CH_3OAr), 3.56 (m, 3H, H-5, H-6, H-6'), 2.20 (s, 3H, SMe); FAB MS $C_{38}H_{44}O_8S$ (660.79) *m/z* (%) 684 $[M+Na]^+$ (100), 479 (22), 441 (37), 326 (37).

Benzyl *O*-(6-*O*-Benzyl-2,3,4-tri-*O*-[4-methoxybenzyl]-α,ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (32,33).

Compound **10** (7.67 g, 9.38 mmol) and **31** (15.49 g, 23.47 mmol) with 15 g molecular sieves 4Å in 100 mL chloroform were stirred together at room temperature for 30 minutes. DMTST (11.7 g, 45.35 mmol) was added and the mixture was stirred for 3 h. Triethylamine was added until the pH of the mixture was basic. The mixture was diluted with 200 mL chloroform and filtered. The filtrate was washed with 100 mL water, 100 mL saturated $NaHCO_3$ solution, and another 100 mL water. The organic layer was separated and evaporated to give 26.0g of crude residue which was deprotected without further purification. An analytical sample was prepared by column chromatography using 1,2-dichloroethane:hexane:ether:acetonitrile 10:3:5:1 v/v/v/v as the mobile phase to give **32** and **33** as a mixture: R_f 0.55 (1,2-dichloroethane:hexane:ether:acetonitrile 10:3:5:1 v/v/v/v); ^1H NMR ($CDCl_3$) δ 7.67-7.16 (m, ArH), 5.87 (d, NHα), 5.66 (d, NHß), 5.38 (d, H-1_{fuc}α), 5.22 (d, H-1_{fuc}ß), 5.14 (d, H-1_{gal}α), 3.81, 3.80, 3.78 (3s, CH_3OAr), 1.78 (s, NHAcß), 1.37 (s, NHAcα), 1.27 (d, H-6_{fuc}α), 1.07 (d, H-6_{fuc}ß); FAB MS $C_{86}H_{95}NO_{18}$ (1430.62) *m/z* (%) 1452 $[M+Na]^+$ (100), 1361 (11), 515 (23), 360 (17).

Benzyl*O*-(6-*O*-Benzyl-α,ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (34, 35). Compounds **32** and **33** (26.0g) was taken up in 400 mL acetonitrile:water 9:1 v/v and cooled to 0 °C. Ammonium cerium (IV) nitrate (100 g, 182.4 mmol) was slowly added and the mixture was stirred at room temperature for 1 h. 2000 mL dichloromethane was added, and the mixture was washed with 800 mL saturated $NaHCO_3$ solution and filtered. The organic layer was separated and concentrated. 500 mL hexane was added to the residue and heated with stirring until boiling, then the supernatant liquid was decanted. This was repeated with a further 500 mL hexane, and 500 mL petroleum ether. The residue was taken up in 400 mL ether:ethyl acetate 2:1 v/v and washed with 100 mL water. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using dichloromethane:acetonitrile 2:1 v/v as the mobile phase to give **34** (α-anomer, 2.29 g, 23% from **10**): R_f, 0.45 (CH_2Cl_2:MeCN 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.39-7.17 (m, 30H, ArH), 5.18 (d, 1H, H-1_{gal}, $J_{1,2}$= 3.3Hz), 5.15 (d, 1H, H-1_{fuc}, $J_{1,2}$= 3.7), 5.03 (d, 1H, H-$1_{glcNAc,}$ $J_{1,2}$= 3.1 Hz), 4.26 (dd, 1H, H-3_{gal}), 1.40 (s, 3H, NHAc), 1.19 (d, 3H, H-6_{fuc}); FAB MS $C_{62}H_{71}NO_{15}$ (1069.17) *m/z* (%) 1092 $[M+Na]^+$ (100), 1000 (10), 441 (47), 326 (33), 199 (49), 173 (47); and **35** (ß-anomer, 2.50g, 25% from **10**): R_f, 0.5 (CH_2Cl_2:MeCN 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.28-7.06 (m, 30H, ArH), 5.27 (d, 1H, H-1_{fuc}), 5.07 (d, 1H, H-1_{glcNAc}), 1.36 (s, 3H, NHAc), 1.04 (d, 3H, H-6_{fuc}); FAB MS $C_{62}H_{71}NO_{15}$ (1069.17) *m/z* (%) 1092 $[M+Na]^+$ (100), 1000 (12), 441 (47), 326 (39).

Methyl 5-Acetamido-3,5-dideoxy-D-*glycero*-ß-D-*galacto*-2-nonulopyranosonate (37)

5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid **(36)** (100 mg, 0.32 mmol) was suspended in methanol (5 mL) and Amberlite IR 120 (H^+) (67 mg) added. The reaction mixture was stirred at room temperature for 3 days. The ion exchange resin was filtered off and washed with methanol. The filtrate was evaporated and the residue was purified by chromatography using chloroform/methanol/water 5:6:2 (v/v/v) as the mobile phase, to give **37β** (96 mg, 92 %): R_f 0.6 (chloroform:methanol:water 5:6:2 v/v); ^{1}H NMR ($CDCl_3$) δ 4.00 - 3.94 (m, 2H, H-4, H-6), 3.83 (t, 1H, H-5), 3.76 (s, 3H, CH_30), 3.74 (dd, 1H, H-9'), 3.63 (dd, 1H, H-8), 3.53 (dd, 1H, H-9), 3.46 (d, 1H, H-7), 2.22 (dd, 1H, H-3_{eq}), 1.82 (dd, 1H, H-3_{ax}); FAB MS $C_{12}H_{21}NO_9$ (323.29) *m/z* (%) 346 $[M+Na]^+$ (100), 324 $[M+H]^+$ (3), 307 (7), 286 (15).

Methyl 5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-ß-D-*galacto*-2-nonulopyranosonate (38β) and Methyl 5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (38α).

Compound **37** (96 mg, 0.297 mmol) was dissolved in pyridine (12 mL) and acetic anhydride (14 mL) was added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated and toluene (3x5 mL) was distilled off. The residue was purified by chromatography using ethyl acetate/methanol 10:0.5 (v/v) as the mobile phase, to give the α-anomer **38α** (33 mg, 21 %): R_f 0.35 (ethyl acetate:methanol 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 5.36 (dd, 2H, NH, H-7, $J_{7,8}$=7.0 Hz), 5.19 (dd, 1H, H-8), 5.04 - 4.99 (m, 1H, H-4), 4.68 (dd, 1H, H-6), 4.36 (dd, 1H, H-9'), 4.16 (m, 1H, H-5), 4.06 (dd, 1H, H-9), 3.76 (s, 3H, CH_3O), 2.56 (dd, 1H, H-3_{eq}), 2.07 (dd, 1H, H-3_{ax}), 2.12, 2.09, 2.02, 1.89 (4s, 18H, 5 OAc, NHAc); FAB MS $C_{22}H_{31}NO_{14}$ (533.49) m/z (%) 556 $[M+Na]^+$ (37), 534 $[M+H]^+$ (5), 474 (18), 414 (100), 372 (7), 312 (5), 252 (11), 196 (12), 154 (17), 137 (22), 106 (9); and the β-anomer **38β** (88 mg, 56 %): R_f 0.30 (ethyl acetate:methanol 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ= 5.37 (dd, 1H, H-7, $J_{7,8}$=5.3 Hz), 5.31 - 5.22 (m, 2H, H-4, NH), 5.06 (dd, 1H, H-8), 4.49 (dd, 1H, H-9'), 4.15-4.07 (m, 3H, H-5, H-6, H-9), 3.79 (s, 3H, CH_3O), 2.55 (dd, 1H, H-3_{eq}), 2.14 (dd, 1H, H-3_{ax}), 2.16, 2.08, 2.04, 1.89 (4s, 15H, 5 OAc, NHAc); FAB MS $C_{22}H_{31}NO_{14}$ (533.49) *m/z* (%) 556 $[M+Na]^+$ (38), 534 $[M+H]^+$ (1), 474 (26), 414 (100), 372 (4), 312 (4), 252 (10), 210 (7), 196 (12), 154 (22), 137 (24), 106 (11).

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-chloro-D-glycero-ß-D-galacto-2-nonulopyranosonate (39)

A mixture of **38α** and **38ß** (120 mg, 0.22 mmol) was dissolved in acetyl chloride (15 mL), which had been saturated with HCl, at 0°C. The reaction mixture was then stirred at room temperature for 24 hours. The reaction mixture was concentrated with toluene (3x2 mL). The residue was purified by chromatography using ethyl acetate as the mobile phase, to give **39** (100 mg, 87 %): R_f 0.42 (ethyl acetate); ^1H NMR ($CDCl_3$) δ 5.51 (d, 1H, NH), 5.47 (dd, 1H, H-7), 5.38 (m, 1H, H-4), 5.16 (m, 1H, H-8), 4.43 (dd, 1H, H-9'), 4.36 (dd, 1H, H-6), 4.21 (m, 1H, H-5), 4.08 (m, 1H, H-9), 3.87 (s, 3H, CH_3O), 2.76 (dd, 1H, H-3_{eq}), 2.27 (dd, 1H, H-3_{ax}), 1.90, 2.05, 2.09, 2.12 (4s, 15H, 4 OAc, NHAc); FAB MS $C_{20}H_{28}ClNO_{12}$ (509.88) *m/z* (%) 532 $[M+Na]^+$ (47), 496 (100), 436 (52), 376 (7), 326 (17), 286 (22).

Methyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-

ß-D-*galacto*-2-nonulopyranoside)onate (40β) and Methyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranoside)onate (40α)

A mixture of **38α** and **38β** (50 mg, 0.09 mmol) was dissolved in dichloromethane (1 mL). Trimethylsilyl trifluormethanesulphonate (26 mg, 0.11 mmol) and (methylthio)trimethylsilane (23 mg, 0.18 mmol) were added. The reaction mixture was kept at room temperature for 2 days, then diluted with dichloromethane (20 mL). The solution was washed with water (2 mL), saturated $NaHCO_3$ (2 mL) and water (2 mL) again. The organic layer was dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using ethyl acetate/methanol 10:0.5 (v/v) as the mobile phase, to give the β-anomer **40β** (30 mg, 64 %): R$_f$ 0.55 (ethyl acetate:methanol 10:0.5 v/v); ^{1}H NMR (CDCl$_3$) δ 5.43 (m, 1H, H-7, J$_{7,8}$=2.6 Hz), 5.34 (d, 1H, NH), 5.26 (m, 1H, H-4), 5.16 (m, 1H, H-8), 4.78 (dd, 1H, H-9'), 4.29 (dd, 1H, H-6), 4.18 - 4.04 (m, 2H, H-9, H-5), 3.80 (s, 3H, CH$_3$O), 2.52 (dd, 1H, H-3$_{eq}$), 2.16 (t, 1H, H-3$_{ax}$), 2.13, 2.11, 2.06, 2.04, 1.90 (5s, 18H, 4 OAc, AcNH, SCH$_3$); FAB MS $C_{22}H_{31}NO_{14}$ (533.47) *m/z* (%) 544 [M+Na]$^+$ (100), 414 (60), 329 (27); and the α-anomer **40α** (14 mg, 29%): R$_f$ 0.50 (ethyl acetate:methanol 10:0.5 v/v); ^{1}H NMR (CDCl$_3$) δ 5.36 (dd, 1H, H-7), 5.28 (d, 1H, NH), 5.26 (m, 1H, H-4), 5.07 (m, 1H, H-8), 4.49 (dd, 1H, H-9'), 4.32 (dd, 1H, H-6), 4.22 (m, 2H, H-9, H-5), 3.79 (s, 3H, OCH$_3$), 2.54 (dd, 1H, H-3$_{eq}$), 2.09 (t, 1H, H-3$_{ax}$), 2.16, 2.08, 2.03, 1.89 (4s, 18H, 4 OAc, NHAc, SCH$_3$); FAB MS $C_{22}H_{31}NO_{14}$ (533.47) *m/z* (%) 544 [M+Na]$^+$ (100), 414 (52), 329 (18).

Methyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α,ß-D-*galacto*-2-nonulopyranoside)onate (40) (Large scale procedure)

A solution of **37** (2.87 g, 9.28 mmol) in pyridine (33.9 mL) was cooled to -15°C and acetic anhydride (38.4 mL) was added dropwise. The mixture was stirred for 2 hours at 0°C and at room temperature for 40 hours. The reaction mixture was concentrated and toluene (3x15 mL) was evaporated from the residue. The residue (5.04 g) was taken up in 1,2-dichloroethane (75 ml) and molecular sieves (MS-4A) (1.25 g), (methylthio)trimethylsilane (4.18 g, 34.85 mmol) and trimethylsilyl trifuoromethanesulphonate (2.23 g, 9.45 mmol) were added. The reaction mixture was stirred at 50°C for 6 hours. The reaction mixture was cooled and 1 M Na_2CO_3 solution (60 mL) was added while stirring. The layers were separated, and the aqueous layer was filtered. The organic layer was washed with water (60 mL).The combined aqueous portions were extracted with 1,2-dichloroethane (50 mL). The combined organic portions were dried over $MgSO_4$, filtered and evaporated, to give **40** as a white foam (1.47 g, 91%).

Benzyl *O*-(6-*O*-Benzyl-3-*O*-[Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (43)

Run A : Compounds **39** (12 mg, 0.02 mmol) and **35** (5 mg, 0.004 mmol) were dissolved in THF (1 mL), 4Å-MS (20 mg) were added and the mixture was cooled to -15 °C. Silver triflate (12 mg, 0.04 mmol) in THF (1 mL) was added dropwise, and the mixture was stirred at -15 °C for 2 days.

Run B : Compounds **39** (3.5 mg, 0.007 mmol) and **35** (5 mg, 0.004 mmol) and di-*tert*-butylpyridine (2.5 mg, 0.014 mmol) were dissolved in THF (1 mL), 4Å-MS (20 mg) were added and the mixture was cooled to -15 °C. Silver triflate (3.5 mg, 0.014 mmol) in THF (1 mL) was added dropwise, and the mixture was stirred at -15 °C for 2 days.

Run C : Compounds **39** (12 mg, 0.02 mmol) and **35** (5 mg, 0.004 mmol) were dissolved in THF (1 mL), 4Å-MS (20 mg) were added and the mixture was cooled to -15 °C. Silver triflate (30 mg, 0.11 mmol) in THF (1.5 mL) was added dropwise, and the mixture was stirred at -15 °C for 2 hours, then at room temperature for 14 hours.

Run D : Compounds **39** (7 mg, 0.014 mmol) and **35** (5 mg, 0.004 mmol) and di-*tert*-butylpyridine (5 mg, 0.028 mmol) were dissolved in THF (1 mL), 4Å-MS (20 mg) were added and the mixture was cooled to -15 °C. Silver triflate (7 mg, 0.028 mmol) in THF (1 mL) was added dropwise, and the mixture was stirred at -15 °C for 2 hours, then at room temperature for 14 hours.

Compounds **41** and **42** could be isolated from these mixtures. Compound **41**: R_f 0.40 (chloroform:methanol 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 6.00 (d,1H, H-3), 5.60 (m, 1H, H-6), 5.50 (dd, 1H, H-7), 5.35 (m, 1H, H-8), 4.60 (dd, 1H, H-4), 4.39 (m, 2H, H-5, H-9'), 4.18 (dd, 1H, H-9), 3.79 (s, 3H, OCH_3),2.11, 2.07, 2.05, 2.04, 1.92 (5s, 15H, 4 OAc, NHAc); FAB MS $C_{20}H_{27}NO_{12}$ (473.28) *m/z* (%) 496 $[M+Na]^+$ (100), 474 $[M+H]^+$ (7), 436 (56), 414 (63). Compound **42β** R_f 0.33 (chloroform:methanol 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 5.41 (d, 1H, NH), 5.32 (dd, 1H, H-7), 5.24 (m, 2H, H-4, H-8), 4.42 (dd, 1H, H-9'), 4.21 (dd, 1H, H-6), 4.15 (m, 1H, H-5), 4.03 (m, 1H, H-9), 3.86 (s, 3H, OCH_3), 2.29 (t, 1H, H-3_{ax}), 2.13, 2.09, 2.02, 2.01, 1.89 (5s, 15H, 5OAc); FAB MS $C_{20}H_{29}NO_{13}$ (491.45) *m/z* (%) 514 $[M+Na]^+$ (100), 492 $[M+H]^+$ (8), 454 (67), 414 (36).

Run E : Compounds **40** (12 mg, 0.02 mmol) and **35** (5 mg, 0.004 mmol) were dissolved in $CHCl_3$ (1 mL) and 4Å-MS (25 mg) was added. The mixture was cooled to -15 °C and DMTST (24 mg, 0.09 mmol) was added. The mixture was stirred at room temperature for 18 hours.

Run F : Compounds **40** (7 mg, 0.014 mmol) and **35** (5 mg, 0.004 mmol) were dissolved in CH_3CN (1 mL) and 4Å-MS (15 mg) was added. The mixture was cooled to -15 °C and DMTST (7 mg, 0.028 mmol) was added. The mixture was stirred at -15 °C for 18 hours.

Run G : Compounds **40** (2.4 mg, 0.004 mmol) and **35** (2.5 mg, 0.002 mmol) were dissolved in CH_3CN (0.5 mL) and 4Å-MS (20 mg) was added. The mixture was cooled to -30 °C and DMTST (7 mg, 0.028 mmol) was added. The mixture was stirred at -30 °C for 30 minutes and then at -15 °C for 18 hours.

Run H : Compounds **40** (2.4 mg, 0.004 mmol) and **35** (2.5 mg, 0.002 mmol) were dissolved in CH_3CN (0.5 mL), 4Å-MS (20 mg) was added and the mixture was stirred at room temperature for 5 hours. The mixture was cooled to -30 °C and DMTST (7 mg, 0.028 mmol) was added. The mixture was stirred at -30 °C for 30 minutes and then at -15 °C for 18 hours.

Benzyl *O*-(6-*O*-Benzyl-3-*O*-[Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (43) and Benzyl *O*-(3-*O*-{4"-[5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylolide]}-6-*O*-

benzyl-ß-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (44). (Large scale procedure)

Compounds **35** (2.44 g, 2.28 mmol) and **40** (2.38 g, 4.56 mmol) were dissolved in acetonitrile (125 mL) and stirred with molecular sieves (4Å) (6.1 g) at 20 °C for 5 h. The reaction mixture was cooled to -30 °C, and DMTST (3.53 g, 13.69 mmol) and molecular sieves (4Å) (2.5 g) were added. The mixture was stirred at -30 °C for 10 minutes and then at -15°C for 24 h. A second portion of **40** (2.38 g, 4.56 mmol) was added. The mixture was cooled to -30 °C and DMTST (3.53 g, 13.69 mmol) with molecular sieves (4Å) (2.5 g) were added. The mixture was stirred at -30 °C for 10 minutes, then at -15 °C for 24 h. Triethylamine (4 mL) was added, and the temperature of the mixture was allowed to rise to 20 °C. The mixture was filtered and concentrated. The residue was taken up in dichloromethane (300 mL) and washed with saturated $NaHCO_3$ solution (50 mL) and water (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by multiple column chromatography using ether:ethyl acetate:methanol 10:4:0.25 v/v/v, 1,2-dichloroethane:tetrahydrofuran 2:1 v/v, toluene:acetonitrile 1:1 v/v as mobile phases, to give **43** (410 mg, 12 %): ^{1}H NMR ($CDCl_3$) δ 7.39-7.17 (m, 30H, ArH), 5.47 (m, 1H, H-8_{sia}), 5.42 (d, 1H, H-1_{fuc} $J_{1,2}$=3.2Hz), 5.34 (dd, 1H, H-7_{sia}), 5.15 (m, 2H, NH_{sia}, H-1_{glcNAc}), 4.93 (m, 2H, H-4_{sia}, CH_2Ar_a), 3.54 (s, 3H, OMe), 2.72 (dd, 1H, H-$3_{sia,eq}$), 2.10, 2.09, 2.06, 2.02 (4s, 12H, 4OAc), 1.92 (s, 6H, 2NAc), 1.14 (d, 3H, H-6_{fuc}); MALDI TOF MS $C_{82}H_{98}N_2O_{27}$ (1542.62) *m/z* (%) 1581 $[M+K]^+$ (52), 1565 $[M+Na]^+$ (100), 1475 (20); and **44** (513 mg, 15 %): ^{1}H NMR ($CDCl_3$) δ 7.39-7.06 (m, 30H, 30ArH), 5.38 (m, 3H, H-8_{sia}, H-4_{sia}, NH_{sia}), 5.32 (d, 1H, H-1_{fuc}, $J_{1,2}$=3.4Hz), 5.31 (d, 1H, H-4_{gal}), 5.24 (dd, 1H, H-7_{sia}), 5.14 (d, 1H, H-1_{glcNAc}, $J_{1,2}$=3.5Hz), 4.94 (d, 1H, CH_2Ar_a), 4.79 (d, 1H, H-1_{gal}, $J_{1,2}$=9.3Hz), 4.34 (dd, 1H, H-3_{gal}), 4.23 (t, 1H, H-2_{gal}), 2.49 (t, 1H, H-$3_{sia,\ ax}$), 2.28 (dd, 1H, H-$3_{sia,\ eq}$), 2.16, 2.03, 1.90, 1.89, 1.88, 1.70 (6s, 18H, 4OAc, 2NAc), 1.10 (d, 3H, H-6_{fuc}); MALDI TOF MS $C_{82}H_{96}N_2O_{26}$ (1510.34) *m/z* (%) 1549 $[M+K]^+$ (47), 1533 $[M+Na]^+$ (100), 1443 (50), 1353 (27); and a mixture of **43** and **44** (1.13 g, 32 %).

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic

acid)-(2→3)-*O*-ß-D-galactopyranosyl)-(1→4)-[*O*-(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α,ß-D-glucopyranose (45)

A mixture of **43** and **44** (1.23 g, 0.797 mmol) was dissolved in methanol (32 mL) and sodium methoxide (255 mg, 4.72 mmol) was added. The reaction mixture was stirred at 35 °C for 24 h then water (6.2 mL) was added. The mixture was stirred at room temperature for 6 h and neutralised with Amberlite IR-120 (H^+) ion exchange resin. The resin was filtered off and the filtrate was concentrated to give the deacetylated acid. The residue was dissolved in methanol (130 mL) and hydrogenated over Pd/C (10%) (2.2 g) for 5 days at 35 °C. The mixture was filtered and concentrated. The residue was purified by column chromatography using acetonitrile:water 3:1 v/v as the mobile phase. After lyophilisation, **45** was obtained as a white solid (511 mg, 78%): R_f 0.41 (acetonitrile:water 3:1 v/v); ^{1}H NMR δ (D_2O) 4.95 (m, 1H, H-1 $_{fuc}$), 4.68 (q, 1H, H-1 $_{GlcNAc}$), 4.39, 4.37 (2d, 1H, H-1$_{Gal}$), 2.62 (dd, 1H, H-3$_{sia,eq}$), 1.88 (s, 6H, 2 NAc), 1.65 (t, 1H, H-3$_{sia,ax}$), 1.03, 1.01 (2d, 3H, H-6$_{fuc}$); MALDI TOF MS $C_{31}H_{52}N_2O_{23}$ (820.75) *m/z* (%) 897 $[M+2K]^+$ (10), 882 $[M+Na+K]^+$ (25), 866 $[M+2Na]^+$ (47), 859 $[M+K]^+$ (24), 843 $[M+Na]^+$ (100), 575 (55).

Benzyl *O*-(6-*O*-Benzyl-3-*O*-[Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-α-D-galactopyranosyl)-(1→4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (46).

Compounds **34** (307 mg, 0.288 mmol) and **40** (300 mg, 0.575 mmol) were dissolved in acetonitrile (16 mL) and stirred with molecular sieves (4Å) (1 g) at 20 °C for 3 h. The reaction mixture was cooled to -30 °C, and DMTST (445 mg, 1.72 mmol) and molecular sieves (4Å) (400 mg) were added. The mixture was stirred at -30 °C for 10 minutes and then at -15 °C for 24 h. A second portion of **40** (300 mg, 0.575 mmol) was added. The mixture was cooled to -30 °C and DMTST (445 mg, 1.72 mmol) with molecular sieves (4Å) (400 mg) were added. The mixture was stirred at -30 °C for 10 minutes, then at -15 °C for 24 h. Triethylamine (1 mL) was added, and the temperature of the mixture was allowed to rise to 20 °C. The mixture was filtered and concentrated. The residue was taken up in dichloromethane (30 mL) and washed with saturated $NaHCO_3$ solution (5 mL) and water (5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using ether:ethyl acetate:methanol 10:4:0.4 v/v/v as the mobile phase, to give **46** (80 mg, 18 %): R_f 0.55 (1,2 dichloroethane: tetrahydrofuran 2:1 v/v); ^{1}H NMR δ ($CDCl_3$) 7.14 -7.39 (m, 30H, ArH), 5.23 (d, 2H, H-1_{fuc}, H-1_{gal}), 5.18 (d, 1H, NH_{sia}), 5.01 (d, 1H, H-1_{glcNAc}), 4.93 (m, 1H, H-8_{sia}), 2.67 (dd, 1H, H-$3_{eq\ sia}$), 1.96, 1.98, 2.02, 2.11 (4s, 12H, 4 OAc), 1.56, 1.85 (2s, 6H, 2NAc), 1.14 (d, 3H, H-6_{fuc}); MALDI TOF MS $C_{82}H_{98}N_2O_{27}$ (1542.62) *m/z* (%) 1581 $[M+K]^+$ (23), 1565 $[M+Na]^+$ (100), 1476 (24), 1224 (22), 1150 (16).

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-α-D-galactopyranosyl)-(1→4)-[*O*-(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α,ß-D-glucopyranose (47)

Compound **46** (50 mg, 0.032 mmol) was dissolved in methanol (1.25 mL) and sodium methoxide (10 mg, 0.185 mmol) was added. The reaction mixture was stirred at room temperature for 24 h then water (0.25 mL) was added. The mixture was stirred at room temperature for 2 h and neutralised with Amberlite IR-120 (H^+) ion exchange resin. The resin was filtered off and the filtrate was concentrated to give the deacetylated acid. The residue was dissolved in methanol (10 mL) and hydrogenated over Pd/C (10%) (100 mg) for 7 days at room temperature. The mixture was filtered and concentrated to give **47** (25mg, 96%): R_f 0.37 (acetonitrile: water 3:1 v/v); ^{1}H NMR δ ($CDCl_3$) 2.58 (dd, 1H, H-$3_{eq\ sia}$), 1.86, 1.91 (2s, 6H, 2NAc), 1.66 (t, 1H, H-$3_{ax\ sia}$), 1.03 (m, 3H, H-6_{fuc}); MALDI TOF MS $C_{31}H_{52}N_2O_{23}$ (820.75) *m/z* (%) 882 $[M+K+Na]^+$ (57), 859 $[M+K]^+$ (94), 843 $[M+Na]^+$ (100), 585 (61), 360 (60).

Synthesis of nucleoside conjugates

5-fluoro-2',3'-O-isopropylidene-uridine (52)

A mixture of 5-fluoro-uridine (100 mg, 0.38 mmol) and p-toluenesulphonic acid (5 mg, 0.02 mmol) in 2,2-dimethoxypropane (15 mL) was stirred under reflux for 3 hours. The mixture was evaporated, and the residue was taken up in ethyl acetate (20 mL), washed with water (3 mL), and then with saturated $NaHCO_3$ solution (3 mL). The organic phase was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1.5 (v/v) as the mobile phase to give **52** (71mg, 62%); R_f 0.38 (chloroform:methanol 10:1.5 v/v); ^{1}H NMR (DMSO-d_6) δ 8.16 (d, 1H, H-1', $J_{1',2'}$=7.0 Hz), 5.81 (s, 1H, H-6), 4.86 (dd, 1H, H-3'), 4.74 (dd, 1H, H-2'), 4.09 (m, 1H, H-4'), 3.57 (m, 2H, H-5'), 2.07 (s, 1H, OH), 1.46 and 1.27 (2s, 6H, 2 CH_3); FAB MS $C_{12}H_{15}FN_2O_6$ (302.22) *m/z* (%) 325 $[M+Na]^+$ (65), 303 $[M+H]^+$ (8).

2',3'-O-isopropylidene uridine (53)

Uridine (250 mg, 1.02 mmol) and p-toluenesulphonic acid (13 mg, 0.05mmol) in 2,2- dimethoxypropane (13mL) were stirred together under reflux for 3 hours. The mixture was evaporated and the residue taken up in chloroform (25 mL), which was washed with 3ml saturated $NaHCO_3$, dried with $MgSO_4$, and filtered. The mixture was evaporated, and the residue purified by chromatography using chloroform/methanol 10:1 (v/v) to give **53** (190mg, 65%) R_f 0.30 (chloroform:methanol 10:1); ^{1}H NMR (DMSO-d6) δ 7.77 (d, 1H, H-6), 5.82 (s, 1H, H-1'), 5.60 (d, 1H, H-5), 4.87 (m, 1H, H-3'), 4.73 (m, 1H, H-2'), 4.06 (m, 1H, H-4'), 3.50 - 3.59 (m, 2H, H-5'), 2.07 (s, 1H, OH), 1.46 (s, 3H, CH_3), 1.27 (s, 3H, CH_3); FAB MS $C_{12}H_{16}N_2O_6$ (284.21) *m/z* (%) 307 $[M+Na]^+$(100), 285 $[M+H]^+$ (22), 173 (16), 113 (37).

5-bromo-2'3'-O-isopropylidene uridine (54)

5-bromo uridine (250 mg, 0.77 mmol) and p-toluenesulphonic acid (10 mg, 0.04 mmol) in 2,2-dimethoxypropane (10 mL) were stirred together under reflux for 3 hours. The mixture was evaporated and the residue taken up in ethyl acetate (25 mL). This was washed with water (2mL), and then with saturated $NaHCO_3$ solution (3mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was suspended in ether (10 mL), and the resulting precipitate **54** (162 mg, 54%) was collected. R_f 0.25 (chloroform:methanol 10:0.5 v/v); ^{1}H NMR (DMSO-d6) δ 5.81 (d, 1H, H-1), 4.91 (m, 1H, H-3'), 4.74 (m, 1H, H-2'), 4.10 (m, 1H, H-4'), 3.58 (m, 2H, H-5'), 1.97 (s, 1H, OH), 1.46 (s, 3H, CH_3), 1.27 (s, 3H, CH_3); FAB MS

$C_{12}H_{15}BrN_2O_6$ (362.93) *m/z* (%) 386 $[M+Na]^+$ (63), 364 (7), 287 (25), 213 (32), 176 (100).

5-ethyl-2',3'-O-isopropylidene-uridine (55)

A mixture of 5-ethyl-uridine (450 mg, 1.65 mmol), 2,2-dimethoxypropane (25 mL) and p-toluenesulphonic acid (100 mg, 0.4mmol) was stirred under reflux for 18 hours. The mixture was evaporated, the residue was taken up in ethyl acetate (35 mL), and washed with water (2x1.5 mL) and saturated $NaHCO_3$ solution (2x2 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform/methanol 10:0.7 (v/v) as the mobile phase to give **55** (400 mg, 77 %); R_f 0.60 ($CHCl_3$:MeOH 10:2 v/v); ^1H NMR (DMSO-d6) δ 8.28 (s, 1H, NH), 7.59 (s, 1H, H-6), 5.83 (s, 1H, H-1'), 4.87 (m, 1H, H-3'), 4.72 (m, 1H, H-2'), 4.06 (d, 1H, H-4'), 3.52 - 3.61 (m, 2H, H-5'), 2.18 (m, 2H, CH_2), 1.47 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.03 (t, 3H, CH_3); FAB MS $C_{14}H_{20}N_2O_6$ (312.47) *m/z* (%) 357 $[M+2Na]^+$ (12), 335 $[M+Na]^+$ (100), 313 $[M+H]^+$ (15), 141 (60), 133 (60).

5-isopropyl-2',3',5'-O-benzoyl-uridine (58)

1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribose **56** (3.93 g, 7.79 mmol) was dissolved in 1,2-dichloroethane. 5-isopropyl-2,4-di-O-trimethylsilyl-uracil **57** (2.43 g, 8.15 mmol) and tin (IV) chloride (1.15 mL) were added. The reaction was stirred at room temperature for 16 hours. Sodium carbonate (2.6 g) was added and then water (2.9 mL) was added dropwise. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was recrystallised from ethanol (17 mL) to give **58** (2.8g, 60%); R_f 0.4 ($CHCl_3$:ether 7:3 v/v); ^1H NMR ($CDCl_3$) δ 8.35 (s, 1H, NH), 8.12 (dd, 2H, 2ArH), 7.98 (2dd, 4H, 4ArH), 7.60-7.33 (m, 9H, 9ArH), 7.06 (s, 1H, H-6), 6.41 (d, 1H, H-1', $J_{1,2}$=6.4), 5.92 (dd, 1H, H-3'), 5.76 (t, 1H, H-2'), 4.84 (dd, 1H, H-5_a'), 4.69 (m, 1H, H-4'), 4.65 (dd, 1H, H-5_b'), 2.68 (m, 1H, CH), 0.95, 0.88 (2d, 6H, $2CH_3$); FAB MS $C_{33}H_{30}N_2O_9$ (598.59) *m/z* (%) 621 $[M+Na]^+$ (100), 599 $[M+H]^+$ (5), 445 (60).

5-isopropyl-2',3'-O-isopropylidene-uridine (59)

Compound **58** (2.0 g, 3.34 mmol), sodium methoxide (562 mg, 10.03 mmol) and methanol (40 mL) were stirred together at 40°C for 16 hours. The mixture was neutralised with ion exchange resin and evaporated. Ether (50 mL) was added to the residue. The resulting solid **58a** (870mg, 91%) was filtered off. [R_f 0.5 ($CHCl_3$:MeOH:H_2O 10:3:0.5 v/v/v); ^1H NMR (D_2O) δ 7.60 (s, 1H, H-6), 5.82 (d, 1H, H-1'), 4.23 (dd, 1H, H-3'), 4.15 (t, 1H, H-2'), 4.02 (m, 1H, H-4'), 3.82 (dd, 1H, H-5_a'), 3.73 (dd, 1H, H-5_b'), 2.72 (m, 1H, CH), 1.02 (2d, 6H, 2 CH_3); FAB MS $C_{12}H_{18}N_2O_6$ (286.28) *m/z* (%) 309 $[M+Na]^+$ (32), 287 $[M+H]^+$ (3), 195 (27)].

Compound **58a** (800 mg, 2.79 mmol) and p-toluenesulphonic acid (40 mg, 0.16 mmol) in 2,2 -dimethoxypropane (30 mL) were refluxed for 3 hours, then evaporated. The residue was taken up in $CHCl_3$ (30 mL), and washed with water and saturated $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:0.5 (v/v) to give **59** (640mg, 70%); R_f 0.37 ($CHCl_3$:MeOH 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 7.02 (s, 1H, H-6), 5.49 (d, 1H, H-1'), 5.11 (dd, 1H, H-3'), 4.99 (dd, 1H, H-2'), 4.26 (m, 1H, H-4'), 3.89 (d, 1H, H-5_a'), 3.80 (m, 1H, H-5_b'), 2.93 (bs, 1H, OH), 2.89 (m, 1H, CH), 1.57, 1.35 (2s, 6H, 2CH_3), 1.14 (d, 6H, 2CH_3); FAB MS $C_{15}H_{22}N_2O_6$ (326.35) *m/z* (%) 371 $[M+2Na]^+$ (15), 349 $[M+Na]^+$ (100), 327 $[M+H]^+$ (27), 176 (42), 155 (100)

Methyl 5-Acetamido-1,4,7,8,9-penta-O-acetyl-3,5-dideoxy-2-thio-ß-D-*glycero*-D-*galacto*-2-nonulopyranoside (69)

Compound **40** (300 mg, 0.57 mmol) was dissolved in methanol (10 mL) and the solution cooled to 0°C. Sodium methoxide (56 mg, 1.03 mmol) in methanol (10 mL) was added dropwise and the reaction was stirred at 0°C for 12 hours. The mixture was neutralised with ion exchange resin, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:3 (v/v) to give methyl **40a** (Methyl 5-Acetamido-3,5-dideoxy-2-thio-D-*glycero*-α,ß-D-*galacto*-2-nonulopyranoside)onate (71 mg, 35%); R_f 0.35 ($CHCl_3$:MeOH 10:3 v/v); ^1H NMR (D_2O) δ 3.87 (s, 3H, OCH_3), 3.90-3.80 (m, 3H, H-7, H-8, NH), 3.72 (m, 1H, H-4), 3.64 (dd, 1H, H-9'), 2.82 (dd, 1H, H-3eq), 2.02 (s, 3H, NAc), 1.85 (t, 1H, H-3ax); FAB MS $C_{13}H_{23}NO_8S$ (353.38) *m/z* (%) 376 $[M+Na]^+$ (100), 354 $[M+H]^+$ (10), 329

(22).

Compound **40a** (66 mg, 0.18 mmol) was dissolved in methanol (5 mL) and cooled to 0 °C. Sodium borohydride (66 mg, 1.7 mmol) in methanol (2 mL) was added dropwise at 0°C. The mixture was stirred for 3 hours, then neutralised with ion exchange resin and evaporated. The residue was dissolved in pyridine (2.4 mL) at 0°C and acetic anhydride (2.8 mL) was added dropwise. The reaction mixture was stirred at 0°C for 3 hours, and at room temperature for 16 hours. The mixture was evaporated and toluene (2 x 5 mL) was added and evaporated from the residue. The residue was taken up in $CHCl_3$ (20 mL), washed with saturated $NaHCO_3$ solution, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:0.5 (v/v) to give **69** (61mg, 63%); R_f 0.4 ($CHCl_3$:MeOH 10:0.5 v/v); ^{1}H NMR ($CDCl_3$) δ 5.37 (m, 1H, H-7), 5.28 (m, 2H, H-4, NH), 5.14 (m, 1H, H-8), 4.50 (dd, 1H, H-9'), 3.80 (s, 2H, OCH_2), 2.53 (dd, 1H, H-3eq), 2.11, 2.10, 2.02, 2.01, 2.00 (5s, 18H, 5OAc, SMe), 1.88 (s, 3H, NAc); FAB MS $C_{22}H_{33}NO_{12}S$ (535.55) *m/z* (%) 558 $[M+Na]^+$ (100), 544 (40), 428 (65), 414 (22)

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-2',3'-O-isopropylidene-β-D-ribofuranosyl]-5-fluoro-uracil (71)

Method A: A mixture of compounds **52** (78 mg, 0.25 mmol), **39** (135 mg, 0.25 mmol), and silver salicylate (95 mg, 0.38 mmol) in $CHCl_3$:DMF 16:1 v/v (9 mL) was stirred at room temperature for 20 hours. The reaction mixture was filtered, and the filtrate was evaporated. The residue was taken up in $CHCl_3$ (20 mL), and washed with saturated $NaHCO_3$ solution (3 mL), then dried over $MgSO_4$. The solution was evaporated, and the residue was purified by chromatography using $CHCl_3$:methanol 10:0.7 (v/v) as the mobile phase to give **70** (153 mg, 74 %); R_f 0.43 ($CHCl_3$:methanol 10:0.7 v/v); ^{1}H NMR (DMSO-d_6) δ 10.17 (s, 1H, OH), 7.84 (m, 1H, Ar-H), 7.57 (m, 1H, Ar-H), 7.04 (m, 2H, 2 Ar-H), 5.26 (dd, 1H, H-7), 5.15 (m, 1H, H-4), 4.95 (m, 1H, H-8), 4.23 (m, 2H, H-6, H-9'), 3.98 (m, 2H, H-5, H-9), 3.71 (s, 3H, OCH_3), 2.60 (dd, 1H, H-3_{eq}), 1.98 (t, 1H, H-3_{ax}), 2.02, 1.93, 1.70 and 1.64 (4 s, 15H, 3 OAc, NHAc); FAB MS $C_{27}H_{33}N_1O_{14}$ (611.24) *m/z* (%) 634 $[M+Na]^+$ (100), 414 (58).

Method B: A mixture of compounds **52** (50 mg, 0.16 mmol), **40** (175 mg, 0.33 mmol) and molecular sieves MS-4Å (100 mg) in acetonitrile (5 mL) was stirred at room temperature for 5 hours. The reaction mixture was cooled to -40°C and DMTST (258 mg, 1.0 mmol) was added, then the mixture was stirred at -15°C for 2 days. Triethylamine (0.2 mL) was added, and the mixture was filtered. The filtrate was evaporated, the residue was taken up in dichloromethane (20 mL) and washed with water, saturated $NaHCO_3$ solution and dried over $MgSO_4$. After evaporation, the residue was purified by chromatography with chloroform:methanol 10:0.5 (v/v) to give **41** (78 mg, 52%); R_f 0.41 (chloroform:methanol 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 6.00 (d,1H, H-3), 5.60 (m, 1H, H-6), 5.50 (dd, 1H, H-7), 5.35 (m, 1H, H-8), 4.60 (dd, 1H, H-4), 4.39 (m, 2H, H-5, H-9’), 4.18 (dd, 1H, H-9), 3.79 (s, 3H, OCH_3),2.11, 2.07, 2.05, 2.04, 1.92 (5s, 15H, 4 OAc, NHAc); FAB MS $C_{20}H_{27}NO_{12}$ (473.28) *m/z* (%) 496 $[M+Na]^+$ (100), 474 $[M+H]^+$ (7), 436 (56), 414 (63) and **71** (12 mg, 10%) R_f 0.28 (chloroform:methanol 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 7.83 (d, 1H, H-6), 5.94 (d, 1H, H-1’), 5.38 (m, 1H, H-8”), 5.32 (dd, 1H, H-7”), 5.25 (d, 1H, NH), 4.88 (m, 1H, H-4”), 4.84 (dd, 1H, H-3’), 4.69 (dd, 1H, H-2’), 4.44 (m, 1H, H-5’), 4.29 (dd, 1H, H-9”$_a$), 4.20 (m, 1H, H-4’), 4.16 - 4.01 (m, 3H, H-5”, H-6”, H-9”$_b$), 2.57 (dd, 1H, H-3”$_{eq}$), 2.14, 2.12, 2.04, 2.01 (4s, 12H, 4 OAc), 1.96 (t, 1H, H-3”$_{ax}$), 1.88 (s, 3H, NAc), 1.37, 1.20 (2s, 6H, 2 CH_3); FAB MS $C_{32}H_{42}FN_3O_{82}$ (775.19) *m/z* (%) 820 $[M+2Na]^+$ (15), 798 $[M+Na]^+$ (47), 716 (5), 474 (11), 414 (63), 286 (24).

1-[5’-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2’,3’-O-isopropylidene-ß-D-ribofuranosyl] uracil (72)

Compounds **39** (52 mg, 0.10 mmol) and **53** (90 mg, 0.31 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (37 mg, 0.14 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours. The mixture was filtered and evaporated. The residue was taken up in ethyl acetate (5 mL) and washed with saturated $NaHCO_3$ solution. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) as the mobile phase to give **72** (15 mg, 20 %); R$_f$ 0.28 ($CHCl_3$:MeOH 10:0.7 v/v); ^{1}H NMR ($CDCl_3$) δ 8.63 (bs, 1H, NH), 7.57 (d, 1H, H-6), 5.91 (d, 1H, H-1'), 5.71 (d, 1H, H-5), 5.37 (m, 1H, H-8"), 5.31 (m, 1H, H-7"), 5.26 (d, 1H, NH"), 4.87 (m, 1H, H-4"), 4.83 (dd, 1H, H-3'), 4.72 (dd, 1H, H-2'), 4.38 (d, 1H, H-4'), 4.29 (dd, 1H, H-9"), 4.02 - 4.16 (m, 4H, H-5', H-5", H-6", H-9"), 3.84 (s, 3H, OCH_3), 3.54 (dd, 1H, H-5'), 2.54 (dd, 1H, H-3"$_{eq}$), 2.16, 2.15, 2.06, 2.04 (4s, 12H, 4 OAc), 1.94 (t, 1H, H-3"$_{ax}$), 1.90 (s, 3H, NAc), 1.61, 1.37 (2s, 6H, 2 CH_3); FAB MS $C_{32}H_{43}N_3O_{18}$ (757.42) *m/z (%)* 802 [M+2Na]$^+$ (10), 780 [M+Na]$^+$ (100), 758 [M+H]$^+$ (5).

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2',3'-O-isopropylidene-ß-D-ribofuranosyl]-5-bromo-uracil (73)

Compounds **54** (120 mg, 0.23 mmol) and **39** (106 mg, 0.29 mmol) were dissolved in tetrahydrofuran (5 mL) and 4Å molecular sieves (120 mg) were added. The mixture was cooled to -15°C and silver triflate (100 mg, 0.39 mmol) in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 5 hours. The mixture was filtered and evaporated. The residue was taken up in ethyl acetate (5 mL), and washed with saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:acetone 2:1 (v/v) as the mobile phase to give **73** (26 mg, 13 %); R$_f$ 0.33 ($CHCl_3$:acetone 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H, H-6), 5.94 (d, 1H, H-1'), 5.37 (m, 1H, H-8"), 5.32 (m, 2H, H-7", NH"), 4.90 (m, 1H, H-4"), 4.85 (m, 1H, H-3'), 4.69 (m, 1H, H-2'), 4.42 (d, 1H, H-4'), 4.27 (dd, 1H, H-9"), 4.23 (dd, 1H, H-5'), 4.03 - 4.13 (m, 3H, H-5", H-6", H-9"), 3.80 (s, 3H, OCH_3), 3.58 (dd, 1H, H-5'), 2.71 (dd, 1H, H-3"$_{eq}$), 2.15, 2.12, 2.03, 2.01 (4s, 12H, 4 OAc), 1.94 (t, 1H, H-3"$_{ax}$), 1.88 (s, 3H, NAc), 1.58, 1.37 (2s, 6H, 2 CH_3); FAB MS $C_{32}H_{42}BrN_3O_{18}$ (835.45) *m/z (%)* 880 [M+2Na]$^+$ (5), 860 [M+Na]$^+$ (25), 836 [M+H]$^+$ (2), 808 (10), 641 (10), 414 (52).

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2',3'-O-isopropylidene-ß-D-ribofuranosyl]-5-ethyl-uracil (74)

Compounds **39** (100 mg, 0.19 mmol) and **55** (74 mg, 0.23 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (75 mg, 0.29 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours. The mixture was filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:acetone 2:1 (v/v) as the mobile phase to give **74** (30 mg, 19 %); R_f 0.33 ($CHCl_3$:acetone 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 8.47 (bs, 1H, NH), 7.21 (d, 1H, H-6), 5.83 (d, 1H, H-1'), 5.38 (m, 1H, H-8"), 5.33 (dd, 1H, H-7"), 5.24 (d, 1H, NH"), 4.88 (m, 1H, H-4"), 4.84 (m, 1H, H-3'), 4.79 (m, 1H, H-2'), 4.29 (dd, 1H, H-9"), 3.80 (s, 3H, OCH_3), 3.564 (dd, 1H, H-5'b), 2.57 (dd, 1H, H-3"$_{eq}$), 2.14, 2.11, 2.04, 2.01 (4s, 12H, 4 OAc), 1.95 (t, 1H, H-3"$_{ax}$), 1.88 (s, 3H, NAc), 1.58, 1.36 (2s, 6H, 2 CH_3), 1.11 (t, 3H, CH_3); FAB MS $C_{42}H_{47}N_3O_{18}$ (785.37) *m/z* (%) 830 $[M+2Na]^+$ (7), 808 $[M+Na]^+$ (100), 786 $[M+H]^+$ (4).

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-2',3'-O-isopropylidene-ß-D-ribofuranosyl]-5-isopropyl-uracil (75)

Compounds **39** (91 mg, 0.18 mmol), **59** (75 mg, 0.23 mmol) and 4Å-MS (100 mg) were added to tetrahydrofuran (5 mL) and cooled to -15°C. Silver triflate (75 mg, 0.29 mmol) in tetrahydrofuran (2 mL) was added dropwise to the stirred mixture. The mixture was stirred at -15°C for 16 hours, then filterd and evaporated. The residue was taken up in ethyl acetate (20 mL), and washed with saturated $NaHCO_3$ solution. The organic layer was dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:acetone 2:1 (v/v) to give **75** (16mg, 11%); R_f 0.24 ($CHCl_3$:acetone 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 1H, NH), 7.06 (s, 1H, H-6), 5.71 (d, 1H, H-1'), 5.38 (m, 1H, H-8"), 5.30 (dd, 1H, H-7"), 5.17 (d, 1H, NH"), 4.85 (m, 3H, H-4", H-3', H-2'), 3.79 (s, 3H, OCH_3), 3.54 (dd, 1H, H-5$_b$'), 2.86 (m, 1H, CH), 2.57 (dd, 1H, H-3"eq), 2.12, 2.09, 2.03, 2.00 (4s, 12H, 4OAc), 1.92 (t, 1H, H-3"ax), 1.86 (s, 3H, NAc), 1.59, 1.35 (2s, 6H, 2CH_3), 1.14 (2d, 6H, 2CH$_3$); FAB MS $C_{35}H_{49}N_3O_{18}$ (799.76) *m/z* (%) 844 $[M+2Na]^+$ (23), 822 $[M+Na]^+$ (100)

1-[5'-O-(Methyl5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α,β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]-5-fluoro-uracil (76)

Compound **39** (91 mg, 0.18 mmol) and 5-fluoro-2'-deoxy-uridine **60** (66 mg, 0.27 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (64 mg, 0.25 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours, then filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution (3 mL). The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) as the mobile phase to give **76** as an anomeric mixture (13 mg, 11 %, α:β=2:1); R_f 0.34 ($CHCl_3$:MeOH 10:1 v/v); ^1H NMR ($CDCl_3$) δ 8.05 (d, H-6$_\alpha$), 7.74 (d, H-6$_\beta$), 6.28 (t, H-1'$_\alpha$), 6.20 (t, H-1'$_\beta$), 4.95 (dd, H-9''$_\beta$), 4.93 (m, H-4''$_\alpha$), 4.48 (dd, H-9''$_\alpha$), 3.82 (s, 3H, $OCH_{3,\alpha,\beta}$), 3.64 (dd, 1H, H-5'b), 2.62 (dd, H-3''$_{eq,\alpha}$), 2.45 (m, H-3''$_{eq,\beta}$, H-2'), 2.14, 2.01 (2s, 4 OAc$_\alpha$), 2.15, 2.03 (2s, 4 OAc$_\beta$), 1.94 (t, H-3''$_{ax,\alpha}$), 1.88 (s, NAc$_\alpha$), 1.87 (s, NAc$_\beta$); FAB MS $C_{29}H_{38}FN_3O_{17}$ (719.46) *m/z* (%) 764 $[M+2Na]^+$ (25), 742 $[M+Na]^+$ (100), 720 $[M+H]^+$ (4), 414 (55).

1-[5'-O-(Methyl5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α,β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]-uracil (77)

Compound **39** (91 mg, 0.18 mmol) and 2'-deoxy-uridine **61** (61 mg, 0.27 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (64 mg, 0.25 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours, then filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution (3 mL). The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) as the mobile phase to give **77** as an anomeric mixture (13 mg, 11 %, α:β=2:1); R_f 0.32

(CHCl$_3$:MeOH 10:1 v/v); ^{1}H NMR (CDCl$_3$) δ 9.26 (s, NH$_\beta$), 8.98 (s, NH$_\alpha$), 7.80 (s, H-6$_\alpha$), 7.58 (s, H-6$_\beta$), 6.32 (t, H-1'$_\alpha$), 6.22 (t, H-1'$_\beta$), 5.88 (d, H-5$_\beta$), 5.75 (d, H-5$_\alpha$), 5.48 (m, H-8''$_\alpha$), 5.32 (d, NH''$_\alpha$), 5.22 (m, H-4''$_\alpha$), 4.38 (dd, H-9''$_\alpha$), 3.81 (s, 3H, OCH$_{3,\alpha,\beta}$), 3.64 (dd, 1H, H-5'b), 2.58 (dd, H-3''$_{eq,\alpha}$), 2.14, 2.02 (2s, 4 OAc$_{\alpha,\beta}$), 1.95 (t, H-3''$_{ax,\alpha}$), 1.88 (s, NAc$_{\alpha,\beta}$); FAB MS $C_{29}H_{39}N_3O_{17}$ (701.39) *m/z* (%) 746 [M+2Na]$^+$ (23), 724 [M+Na]$^+$ (89), 702 [M+H]$^+$ (4), 414 (25).

1-[5'-O-(Methyl5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α,β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]-5-bromo-uracil (78)

Compound **39** (91 mg, 0.18 mmol) and 5-bromo-2'-deoxy-uridine **62** (82 mg, 0.27 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (64 mg, 0.25 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours, then filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated NaHCO$_3$ solution. The organic layer was separated, dried with MgSO$_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) as the mobile phase to give **78** as an anomeric mixture (24 mg, 17 %, α:β=3:1); R$_f$ 0.35 (CHCl$_3$:MeOH 10:1 v/v); ^{1}H NMR (CDCl$_3$) δ 9.26 (s, NH$_\beta$), 8.94 (s, NH$_\alpha$), 8.23 (s, H-6$_\alpha$), 7.83 (s, H-6$_\beta$), 6.30 (t, H-1'$_\alpha$), 6.14 (t, H-1'$_\beta$), 5.45 (m, 2H, H-8''$_{\alpha,\beta}$, H-7''$_{\alpha,\beta}$), 5.32 (d, 1H, NH''$_{\alpha,\beta}$), 4.41 (dd, H-9''$_\alpha$), 3.84 (s, OCH$_{3,\alpha}$), 3.83 (s, OCH$_{3\beta}$), 3.68 (dd, 1H, H-5'b), 2.72 (dd, H-3''$_{eq,\alpha}$), 2.50 (dd, H-3''$_{eq,\beta}$, H-2'), 2.14, 2.03, 2.00 (3s, 4 OAc$_\alpha$), 2.15, 2.03, 2.01 (3s, 4 OAc$_\beta$), 1.95 (t, H-3''$_{ax,\alpha}$), 1.89 (s, NAc$_\alpha$), 1.88 (s, NAc$_\beta$); FAB MS $C_{29}H_{38}BrN_3O_{17}$ (780.46) *m/z* (%) 826 [M+2Na]$^+$ (15), 804 [M+Na]$^+$ (45), 782 [M+H]$^+$ (4), 414 (62).

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]-5-iodo-uracil (79)

Compound **39** (91 mg, 0.18 mmol) and 5-iodo-2'-deoxy-uridine **63** (96 mg, 0.27 mmol) were dissolved in tetrahydrofuran (5 mL) and 4Å-molecular sieves (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (64 mg, 0.25 mmol) dissolved in tetrahydrofuran (2 mL) was added slowly dropwise. The reaction was stirred at -15°C for 6 hours, then filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) to give **79** as an anomeric mixture (17mg, 12%, α:ß 5:1); R_f 0.32 ($CHCl_3$:MeOH 10:1 v/v); ^{1}H NMR ($CDCl_3$) δ 9.03 (bs, NH_{β}), 8.73 (bs, NH_{α}), 8.23 (s, H-6_{α}), 7.86 (s, H-6_{β}), 6.30 (t, H-1_{α}), 6.07 (t, H-1_{β}), 5.42 (m, H-8"$_{\alpha,\beta}$, H-7"$_{\alpha,\beta}$), 5.27 (d, NH"$_{\alpha,\beta}$), 4.94 (m, H-4"$_{\alpha,\beta}$), 4.42 (dd, H-9"$_{\alpha}$), 3.85 (s,$OCH_{3\alpha}$), 3.83 (s, $OCH_{3\beta}$), 3.70 (dd, H-5'$_{b}$), 2.82 (dd, H-3"$_{\alpha,eq}$), 2.54 (dd, H-3"$_{\beta,eq}$), 2.45 (m, H-2'$_{a}$), 2.15, 2.03, 2.00 (3s, 4OAc$_{\alpha}$), 1.89 (s, NAc$_{\alpha}$); FAB MS $C_{29}H_{38}IN_3O_{17}$ (827.51) *m/z* (%) 872 $[M+2Na]^+$ (40), 850 $[M+Na]^+$ (100), 828 $[M+H]^+$ (2), 612 (37).

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]-5-ethyl-uracil (80)

Compound **39** (91 mg, 0.18 mmol) and 5-ethyl-2'-deoxy-uridine **64** (67 mg, 0.27 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (64 mg, 0.25 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours, then filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution (3 mL). The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform/methanol 10:1 (v/v) as the mobile phase to give **80** (13 mg, 10 %); R_f 0.32 ($CHCl_3$:MeOH 10:1 v/v); ^{1}H NMR ($CDCl_3$) δ 8.64 (bs, 1H, NH), 7.48 (s, 1H, H-6), 6.36 (t, 1H, H-1'), 5.44 (m, 2H, H-8'', H-7''), 5.30 (d, 1H, NH), 4.88 (m, 1H, H-4"), 4.42 (dd, 1H, H-9"a), 3.81 (s, 3H, OCH_3), 3.69 (dd, 1H, H-5'b), 2.59 (dd, 1H, H-3"$_{eq}$), 2.34 (q, 2H, CH_2), 2.15, 2.06, 2.03 (3s, 12H, 4 OAc), 1.93 (t, 1H, H-3"$_{ax}$), 1.88 (s, 3H, NAc), 1.11 (t, 3H, CH_3); FAB MS $C_{31}H_{43}N_3O_{17}$ (729.29) *m/z* (%) 773 $[M+2Na]^+$ (7), 752 $[M+Na]^+$ (100), 730 $[M+H]^+$ (4), 414 (60).

1-[5'-O-(Methyl5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α,β-D-*glycero*-D-

galacto-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]5-isopropyl-uracil (81)

A mixture of **40** (93 mg, 0.18 mmol), 5-isopropyl-2'-deoxy-uridine **65** (73 mg, 0.27 mmol) and molecular sieves 4Å (100 mg) in acetonitrile:dimethylformamide 4:1 v/v (25 mL) was stirred at -15°C and DMTST (140 mg, 0.54 mmol) was added. The mixture was stirred at - 15°C for two days. The reaction mixture was neutralised with triethylamine (0.1 mL) and filtered. The filtrate was concentrated and xylene (3x5 mL) was evaporated from the residue. The residue was taken up in ethyl acetate (20 mL), washed with water (3 mL), dried over $MgSO_4$ and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) as the mobile phase to give **81** as an anomeric mixture (12 mg , 10 %, α:β=1:2); R_f 0.35 ($CHCl_3$:MeOH 10:1); ^{1}H NMR ($CDCl_3$) δ 8.85 (s, NH_β), 8.61 (s, NH_α), 7.38 (s, H-6_α), 7.06 (s, H-6_β), 6.35 (t, H-1'$_\alpha$), 6.03 (t, H-1'$_\beta$), 5.06 (m, H-4''$_\alpha$), 4.98 (dd, H-9''$_\beta$), 4.89 (m, H-4''$_\beta$), 4.42 (dd, H-9''$_\alpha$), 3.81 (s, $OCH_{3,\alpha}$), 3.80 (s, $OCH_{3,\beta}$), 2.60 (dd, H-3''$_{eq,\alpha}$), 2.51 (dd, H-$3_{eq,\beta}$), 2.15, 2.05, 2.03, 2.01, 2.00 (5s, 4 $OAc_{\alpha,\beta}$), 1.88 (s, NAc_α), 1.87 (s, NAc_β), 1.15 (2s, 6H, 2 CH_3); FAB MS $C_{32}H_{45}N_3O_{17}$ (743.56) *m/z* (%)= 787 $[M+2Na]^+$ (10), 766 $[M+Na]^+$ (100), 744 $[M+H]^+$ (7), 414 (46).

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]-5-butyl-uracil (82)

Compound **39** (91 mg, 0.18 mmol) and 5-butyl-2'-deoxy-uridine **66** (76 mg, 0.27 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (64 mg, 0.25 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours, then filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution (3 mL). The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) as the mobile phase to give **35** (38 mg, 28 %); R_f 0.37 ($CHCl_3$:MeOH 10:1 v/v); ^{1}H NMR ($CDCl_3$) δ 8.57 (bs, 1H, NH), 7.46 (s, 1H, H-6), 6.38 (t, 1H, H-1'), 5.43 (m, 2H, H-8'', H-7''), 5.29 (d, 1H, NH), 4.90 (m, 1H, H-4''), 4.42 (dd, 1H, H-9''a), 3.81 (s, 3H, OCH_3), 3.69 (dd, 1H, H-5'b), 3.45 (bs, 1H, OH), 2.62 (dd, 1H, H-3''$_{eq}$), 2.40

(m, 1H, H-2'), 2.31 (q, 2H, CH_2), 2.16, 2.15, 2.03, 2.00 (4s, 12H, 4 OAc), 1.96 (t, 1H, H-3''$_{ax}$), 1.88 (s, 3H, NAc), 1.50, 1.34 (2m, 4H, 2 CH_2), 0.89 (t, 3H, CH_3); FAB MS $C_{33}H_{47}N_3O_{17}$ (757.31) *m/z* (%) 802 $[M+2Na]^+$ (9), 780 $[M+Na]^+$ (100), 758 $[M+H]^+$ (4), 414 (52).

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]-5-pentyl-uracil (83)

Compound **39** (91 mg, 0.18 mmol) and 5-pentyl-2'-deoxy-uridine **67** (80 mg, 0.27 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (64 mg, 0.25 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours, then filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution (3 mL). The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) as the mobile phase to give **83** (42 mg, 30 %): R_f 0.38 ($CHCl_3$:MeOH 10:1 v/v); ^1H NMR ($CDCl_3$) δ 8.51 (bs, 1H, NH), 7.45 (s, 1H, H-6), 6.37 (t, 1H, H-1'), 5.46 (m, 1H, H-8''), 5.40 (d, 1H, H-7''), 5.32 (d, 1H, NH), 4.92 (m, 1H, H-4''), 4.43 (dd, 1H, H-9''a), 3.81 (s, 3H, OCH_3), 3.69 (dd, 1H, H-5'b), 3.43 (bs, 1H, OH), 2.62 (dd, 1H, H-3''$_{eq}$), 2.40 (m, 1H, H-2'), 2.27 (t, 2H, CH_2), 2.15, 2.03, 2.00 (3s, 12H, 4 OAc), 1.95 (t, 1H, H-3''$_{ax}$), 1.88 (s, 3H, NAc), 1.50, 1.30 (2m, 4H, 3 CH_2), 0.87 (t, 3H, CH_3); FAB MS $C_{34}H_{49}N_3O_{17}$ (771.32) *m/z* (%) 816 $[M+2Na]^+$ (8), 794 $[M+Na]^+$ (100), 772 $[M+H]^+$ (4), 590 (49), 549 (51), 414 (42).

1-[5'-O-(Methyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-2'-deoxy-ß-D-ribofuranosyl]-5-hexyl-uracil (84)

Compounds **39** (91 mg, 0.18 mmol) and 5-hexyl-2'-deoxy-uridine **68** (84 mg, 0.27 mmol) were dissolved in tetrahydrofuran (5 mL), and molecular sieves 4Å (100 mg) were added. The mixture was cooled to -15°C and stirred. Silver triflate (64 mg, 0.25 mmol) dissolved in tetrahydrofuran (2 mL) was added dropwise. The reaction mixture was stirred at -15°C for 6 hours, then filtered and evaporated. The residue was taken up in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution.

The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 10:1 (v/v) as the mobile phase to give **84** (30 mg, 21 %); R_f 0.38 ($CHCl_3$:MeOH 10:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.47 (s, 1H, H-6), 6.36 (t, 1H, H-1'), 5.47 (m, 1H, H-8'', H-7''), 5.30 (d, 1H, NH), 5.27 (dd, 1H, H-7''), 4.90 (m, 1H, H-4"), 4.60 (m, 1H, H-4'), 4.42 (dd, 1H, H-9"a), 3.82 (s, 3H, OCH_3), 3.72 (dd, 1H, H-5'b), 2.62 (dd, 1H, H-3"$_{eq}$), 2.29 (t, 2H, CH_2), 2.15, 2.03, 2.01 (3s, 12H, 4 OAc), 1.94 (t, 1H, H-3"$_{ax}$), 1.88 (s, 3H, NAc), 1.56 - 1.20 (m, 8H, 4 CH_2), 1.11 (t, 3H, CH_3); FAB MS $C_{35}H_{51}N_3O_{17}$ (785.36) *m/z* (%) 830 $[M+2Na]^+$ (10), 808 $[M+Na]^+$ (94), 786 $[M+H]^+$ (4), 414 (100).

1-[5'-O-(5-Acetamido-1,4,7,8,9-penta-O-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)-2',3'-O-isopropylidene-β-D-ribofuranosyl]-5-fluoro-uracil (85)

Compound **69** (56 mg, 0.10 mmol) and **52** (40 mg, 0.13 mmol) were dissolved in $CHCl_3$ (5 mL) and 4Å-MS (100 mg) were added. DMTST (77 mg, 0.30 mmol) was added, and the reaction mixture was stirred at room temperature for 20 hours. The mixture was filtered, and $CHCl_3$ (15 mL) was added to the filtrate. The filtrate was washed with saturated $NaHCO_3$ solution, dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography using chloroform:acetone 2:1 (v/v) to give **85** (25mg, 32%); R_f 0.22 ($CHCl_3$:acetone 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 9.26 (d, 1H, NH), 7.42 (d, 1H, H-6), 5.62 (d, 1H, NH'), 5.45 (d, 1H, H-1'), 5.25 (m, 3H, H-8", H-7", H-4"), 5.14 (dd, 1H, H-3'), 5.02 (dd, 1H, H-2'), 4.66 (dd, 1H, H-9"), 2.33 (dd, 1H, H-3"eq), 2.12, 2.08, 2.04, 2.01, 1.99 (5s, 15H, 5OAc), 1.88 (s, 3H, NAc), 1.74 (t, 1H, H-3"ax), 1.75, 1.36 (2s, 6H, $2CH_3$); FAB MS $C_{33}H_{44}FN_3O_{18}$ (789.70) *m/z* (%) 844 $[M+2Na]^+$ (15), 812 $[M+Na]^+$ (100), 528 (37), 428 (82).

1-[5'-O-(Methyl 5-acetamido--3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-2',3'-O-isopropylidene-β-D-ribofuranosyl]-5-fluoro-uracil (86)

Compound **71** (27 mg, 0.03 mmol) was dissolved in methanol (3 mL) and sodium methoxide (8 mg, 0.15 mmol) was added. The mixture was stirred at room temperature for 2 days. The mixture was neutralised with ion exchange resin, filtered and concentrated. The residue was purified by column chromatography using chloroform: methanol 2:1 v/v as the mobile phase to give **86** (11 mg, 52%); R_f 0.65 ($CHCl_3$:MeOH 2:1 v/v); ^1H NMR (CD_3OD) δ 7.96 (d, 1H, H-6), 5.88 (d, 1H, H-1'), 4.96 (m, 1H, H-8''), 3.83 (s, 3H, OCH_3), 2.67 (dd, 1H, H-3''eq), 2.03 (s, 3H, NAc), 1.80 (t, 1H, H-3''ax), 1.53, 1.35 (2s, 6H, $2CH_3$); FAB MS $C_{24}H_{34}FN_3O_{14}$ (607.53) *m/z* (%) 652 $[M+2Na]^+$ (45) 630 $[M+Na]^+$ (100).

1-[5'-O-(5-acetamido--3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-2',3'-O-isopropylidene-β-D-ribofuranosyl]-5-fluoro-uracil (87)

Compound **71** (27 mg, 0.03 mmol) was dissolved in methanol (3 mL) and sodium methoxide (8 mg, 0.15 mmol) was added. The mixture was stirred at room temperature for 2 days. 2 drops of water were added and the mixture was stirred at 25-30°C for 24 hours. The mixture was neutralised with ion exchange resin, filtered and evaporated. The residue was purified by chromatography using chloroform:methanol 2:1 (v/v) to give **87** (11mg, 52%); R_f 0.65 ($CHCl_3$:MeOH 2:1 v/v); ^1H NMR (CD_3OD) δ 7.96 (d, 1H, H-6), 5.88 (d, 1H, H-1'), 4.96 (m, 1H, H-8"), 3.83 (s, 3H, OCH_3), 2.67 (dd, 1H, H-3"eq), 2.03 (s, 3H, NAc), 1.80 (t, 1H, H-3"ax), 1.53, 1.35 (2s, 6H, $2CH_3$); FAB MS $C_{24}H_{34}FN_3O_{14}$ (607.53) *m/z* (%) 652 $[M+2Na]^+$ (45), 630 $[M+Na]^+$ (100).

Synthesis of thio-oligosaccharides

2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (88)

Compound **2** (15.0 g, 67.87 mmol), was dissolved in pyridine (100 mL) and cooled to 0°C. Acetic anhydride (158 mL) was added dropwise and the mixture was stirred at room temperature for 24 hours. The mixture was concentrated, and toluene was added to the residue and evaporated to give **88** (22.78 g, 86%): R_f 0.41 (EtOAc); ^1H NMR δ ($CDCl_3$) 6.13 (d, 1H, NH), 5.74 (d, 1H, H-1, $J_{1,2}$= 9 Hz), 5.14-5.22 (m, 2H, H-3, H-4), 4.45 (m, 1H, H-2), 4.20 (m, 1H, H-6), 4.02 (m, 1H, H-6'), 3.97 (m, 1H, H-5) 2.15, 2.04, 2.10, 2.00, 1.90 (5s, 15H, 4OAc, NAc); FAB MS $C_{16}H_{23}NO_{10}$ (389.36) *m/z* (%) 412 $[M+Na]^+$ (42), 390 $[M+H]^+$ (5), 352 (5), 330 (100).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (89)

Compound **88** (15.7g, 40.35 mmol) was dissolved in acetyl chloride saturated with HCl (100 mL) at -5 °C and stirred at room temperature for 18 hours. The mixture was concentrated and toluene (3x10 mL) was added to the residue and evaporated. The residue was recrystallised from acetonitrile (75 mL) to give **89** (6.9g, 47%): R_f 0.57 (EtOAc); ^{1}H NMR δ ($CDCl_3$) 6.18 (d, 1H, H-1, $J_{1,2}$= 3.6 Hz), 5.81 (d, 1H, NH), 5.31 (t, 1H, H-3), 5.20 (t, 1H, H-4), 4.53 (m, 1H, H-2), 4.28 (m, 2H, H-6, H-6'), 4.13 (m, 1H, H-5), 2.06, 2.05, 2.02, 1.98 (4s, 12H, 3OAc, NAc); FAB MS $C_{14}H_{20}ClNO_8$ (365.77) *m/z* (%) 390 (7), 388 $[M+Na]^+$ (23), 368 (37), 366 $[M+H]^+$ (100), 331 (30), 324 (40).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (90)

Compound **89** (6.0g, 16.43 mmol), tetrabutylammonium hydrogensulphate (5.58g, 16.43 mmol), and sodium azide (4.26g, 65.53 mmol) were taken up in CH_2Cl_2 (60 mL). Saturated $NaHCO_3$ solution (60 mL) was added and the mixture was stirred vigourously for 1 hour. The mixture was diluted with EtOAc (600 mL), the organic phase was separated and washed with saturated $NaHCO_3$ solution (2x75 mL), water (2x75 mL) and brine (2x75 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated. Di-isopropyl ether (40 mL) was added to the residue to precipitate **90** (3.99g, 65%): R_f 0.45 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.95 (d, 1H, NH), 5.26 (t, 1H, H-3), 5.09 (t, 1H, H-4), 4.77 (d, 1H, H-1, $J_{1,2}$= 9.1 Hz), 4.25 (m, 1H, H-6), 4.14 (m, 1H, H-6'), 3.92 (q, 1H, H-5), 3.79 (m, 1H, H-2), 2.08, 2.02, 2.01, 1.96 (4s, 12H, 3OAc, NAc); FAB MS $C_{14}H_{20}N_4O_8$ (372.34) *m/z* (%) 395 $[M+Na]^+$ (59), 373 $[M+H]^+$ (100), 330 (96).

2-Acetamido-2-deoxy-β-D-glucopyranosyl azide (91)

Compound **90** (3.79g, 10.18 mmol) was dissolved in methanol (30 mL) and sodium methoxide (200mg, 3.70 mmol) was added. The mixture was stirred at 40 °C for 2 hours. The mixture was neutralised with ion exchange resin, filtered and evaporated to give **91** (2.32g, 93%): R_f 0.27 (CH_2Cl_2:MeOH 10:0.5 v/v); ^{1}H NMR (CD_3OD) δ 4.59 (d, 1H, H-1, $J_{1,2}$= 9.2 Hz), 3.65-3.77 (m, 3H, H-2, H-5, H-6), 3.47 (t, 1H, H-3), 2.01 (s, 3H, NAc); FAB MS $C_8H_{14}N_4O_5$ (246.23) *m/z* (%) 269 $[M+Na]^+$ (68), 247 $[M+H]^+$ (100), 204 (53),

.2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl azide (92)

Compound **91** (2.44 g, 9.92 mmol) was suspended in benzaldehyde (10 mL) and formic acid (6 mL) was added. The mixture was stirred for 2 hours, then di-isopropyl ether (100 mL) was added. The resulting precipitate was filtered off and recrystallised from methanol to give **92** (2.37 g, 71 %): R_f 0.70 (CH_2Cl_2:MeOH 10:1.5 v/v); ^1H NMR (CD_3OD) δ 7.49, 7.34 (2m, 5H, ArH), 5.61 (s, 1H, CHAr), 4.63 (d, 1H, H-1), 4.32 (m, 1H, H-2), 3.81 (m, 3H, H-3, H-4, H-6), 3.56 (m, 2H, H-5, H-6'), 2.00 (s, 3H, NAc); FAB MS $C_{15}H_{18}N_4O_5$ (334.34) *m/z* (%) 357 (26) $[M+Na\}^+$, 335 (100) $[M+H]^+$, 307 (49), 292 (26).

2-Acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-trifluoromethanesulphonyl-β-D-glucopyranosyl azide (93)

Compound **92** (2.52 g, 7.54 mmol) was dissolved in pyridine (16 mL) and CH_2Cl_2 (240 mL) was added. The mixture was cooled to -15°C and triflic anhydride (3 mL, 14.04 mmol) was added dropwise. The mixture was stirred at -15°C for 18 hours and then at 0°C for 5 hours. Cold CH_2Cl_2 (750 mL) was added and the mixture was washed with ice cold 10% $KHSO_4$. A white precipitate formed in the organic phase and this was filtered off to give **93** (802 mg). The filtrate was washed with ice cold saturated $NaHCO_3$ and water, dried with $MgSO_4$, filtered and evaporated to give a further portion of **93** (1980 mg, total yield 79 %); R_f0.65 ($CHCl_3$:EtOAc 10:3 v/v); ^1H NMR ($CDCl_3$) δ 7.37, 7.45 (2m, 5H, ArH), 5.87 (d, NH), 5.58 (s, 1H, CHAr), 5.51 (t, 1H, H-3), 4.48 (m, 1H, H-2), 3.83 (m, 2H, H-4, H-6), 3.71 (m, 1H, H-6'), 3.52 (m, 1H, H-5), 2.07 (s, 3H, NAc); FAB MS $C_{16}H_{17}F_3N_4O_7S$ (466.41) *m/z* (%) 489 (17) $[M+Na]^+$, 467 (100) $[M+H]^+$, 449 (46), 424 (38)

2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranosyl azide (94)

Compound **93** (1490 mg, 3.19 mmol) was dissolved in 25 mL DMF and sodium nitrite (700 mg, 10.14 mmol) was added. The mixture was stirred at room temperature for 18 hours. The mixture was concentrated and the residue was taken up in CH_2Cl_2: MeOH 10:2 v/v (30 mL) and the salts were filtered off. The filtrate was concentrated and the residue was taken up in $CHCl_3$: ether 1:1 v/v. The resulting solid **94** was filtered off (660 mg, 62%); R_f 0.48 (CH_2Cl_2: MeOH 10:0.5 v/v); ^1H

NMR (CD_3OD) δ 7.33, 7.50 (2m, 5H, ArH), 5.65 (s, 1H, CHAr), 4.09 (m, 1H, H-2), 4.00 (dd, 1H, H-6), 3.72 (dd, 1H, H-6'), 3.31 (t, 1H, H-3), 2.00 (s, 3H, NAc); FAB MS $C_{15}H_{18}N_4O_5$ (334.34) *m/z* (%) 357 (44) [M+Na}$^+$, 335 (83) [M+H]$^+$, 305 (21), 292 (7).

2-Acetamido-4,6-*O*-benzylidene-2-deoxy-3-trifluoromethanesulphonyl-β-D-allopyranosyl azide (95)

Compound **94** (645 mg, 1.93 mmol) was dissolved in CH_2Cl_2:pyridine 15:1 v/v (20 mL) and cooled to -15 °C. Triflic anhydride (0.65 mL) was added dropwise. The mixture was stirred at:-

i) -15 °C overnight.
ii) -15 °C for 30 minutes, then at 0 °C for 4 hours.
iii)-15 °C for 30 minutes, then at room temperature for 2 hours.

2-Acetamido-4,6-*O*-benzylidene-2-deoxy-3-(4-toluenesulphonyl)-β-D-allopyranosyl azide (96)

Compound **94** (253 mg, 0.757 mmol) was dissolved in DMF (3 mL). Dimethylaminopyridine (138mg, 1.13 mmol) was added and the mixture was cooled to 0 °C. 4-Toluenesulphonyl chloride (215mg, 1.13 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was concentrated and the residue was taken up in ether. The precipitate formed was filtered off, and the filtrate was concentrated. The residue was purified by chromatography to give **96** (24mg, 8%): R_f 0.78 (CH_2Cl_2:MeOH 10:0.4 v/v); FAB MS $C_{22}H_{24}N_4O_7S$ (488.49) *m/z* (%) 511 [M+Na]$^+$ (19), 489 [M+H]$^+$ (11).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide (97)

1,2,3,4,6-Penta-*O*-acetyl-α,β-D-galactopyranose (15.0 g, 38.46 mmol) was dissolved in dry CH_2Cl_2 (210 mL), then trimethylsilyl azide (6.2 mL) and $SnCl_4$ (2.35 mL) were added. The mixture was stirred at room temperature for 24 hours. The reaction mixture was diluted with CH_2Cl_2 (500 mL), and washed with 1M KF solution, saturated $NaHCO_3$ solution and brine, then dried over $MgSO_4$, filtered and evaporated to give **97** (13.96 g, 97 %): R_f 0.74 (hexane/EtOAc 8:7 v/v); ^{1}H NMR

($CDCl_3$) δ 5.41 (d, 1H, H-4), 5.17 (t, 1H, H-2), 5.04 (dd, 1H, H-3), 4.60 (d,1H, H-1, $J_{1,2}$=10 Hz), 4.19 (m, 2H, H-6), 4.00 (m, 1H, H-5), 2.15-1.98 (4s, 12H, 4 OAc); FAB MS $C_{14}H_{19}N_3O_9$ (373.32) *m/z* (%) 396 $[M+Na]^+$ (100), 374 $[M+H]^+$ (35), 331 (23).

4,6-*O*-Benzylidene-β-D-galactopyranosyl azide (98)

Compound **97** (13.96g, 37.32 mmol) was suspended in methanol (100 mL) and sodium methoxide (200mg, 3.7mmol) was added. The mixture was stirred at room temperature for 2 h, then neutralised with Amberlite IR-120 H^+ ion exchange resin, filtered and evaporated. Benzaldehyde (15 mL) and formic acid (15mL) were added to the residue, and the mixture was stirred for 50 minutes at room temperature. Ether: di-isopropyl ether 1:3 v/v (150mL) was added and the mixture was left at -15°C for 18 hours. The resulting solid was filtered off, washed with di-isopropyl ether and dried to give **98** (9.70g, 88%): R_f 0.4 (CH_2Cl_2: MeOH 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 7.49, 7.37 (2m, 5H, ArH), 5.54 (s, 1H, CHAr), 4.57 (d, 1H, H-1, $J_{1,2}$ = 7.9 Hz), 4.36 (dd, 1H, H-6), 4.23 (d, 1H, H-4), 4.07 (dd, 1H, H-6'), 3.67 (m, 2H, H-2, H-3), 3.58 (d, 1H, H-5); FAB MS $C_{13}H_{15}N_3O_5$ (293.28) *m/z* (%) 316 (45) $[M+Na]^+$, 294 (23) $[M+H]^+$, 251 (70).

3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl azide (99) and **2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl azide (100)**

Compound **98** (7.18g, 24.5mmol) was dissolved in CH_2Cl_2 (430 mL)and tetrabutylammonium hydrogen sulphate (1.663g, 4.9mmol), benzyl bromide (7.18g, 42mmol) and 5% NaOH solution (36 mL) were added. The mixture was stirred under reflux for 18 hours then allowed to cool. The layers were separated, and the organic layer was washed with water, dried with $MgSO_4$, filtered and evaporated. The residue was taken up in hexane and kept at -15°C for 1h. The supernatant liquid was decanted. The residue was recrystallised from Hex:EtOAc 1:1 to give **99** (1.44g, 15%): R_f 0.43 (Hex:EtOAc 1:1 v/v); ^1H NMR ($CDCl_3$) δ 7.51, 7.36 (2m, 10H, ArH), 5.45 (s, 1H, CHAr), 4.76, 4.67 (2d, 2H, CH_2Ar), 4.57 (d, 1H, H-1, $J_{1,2}$= 8.5 Hz), 4.32 (dd, 1H, H-6), 4.18 (d, 1H, H-4), 4.02 (dd, 1H, H-6'), 3.96 (t, 1H, H-2), 3.48 (m, 2H, H-3, H-5); FAB MS $C_{20}H_{21}N_3O_5$ (383.41) *m/z* (%) 406 $[M+Na]^+$ (38), 384

$[M+H]^+$ (14), 341 (91). The mother liquor was evaporated and the residue purified by column chromatography with Hex: EtOAc 1:1 and 2:3 v/v as successive mobile phases to give **100** (2.06g, 22%): R_f 0.39 (Hex:EtOAc 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.51, 7.38 (2m, 10 H, ArH), 5.55 (s, 1H, CHAr), 4.89, 4.80 (2d, 2H, CH_2Ar), 4.63 (d, 1H, H-1, $J_{1,2}$= 8.5 Hz), 4.36 (dd, 1H, H-6), 4.22 (d, 1H, H-4), 4.08 (dd, 1H, H-6'), 3.76 (dd, 1H, H-3), 3.56 (m, 2H, H-2, H-5); FAB MS $C_{20}H_{21}N_3O_5$ (383.41) *m/z* (%) 406 $[M+Na]^+$ (22), 384 $[M+H]^+$ (7), 341 (100).

2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-trifluoromethanesulphonyl-β-D-galactopyranosyl azide (101)

Compound **100** (1.40g, 3.65mmol) was dissolved in pyridine:CH_2Cl_2 1:15 v/v (128 mL) and the solution was cooled to -15°C. Trifluoromethanesulphonic anhydride (2mL, 11.84mmol) was added dropwise. The mixture was stirred at -15°C overnight, then diluted with 150 mL cold CH_2Cl_2 and washed successively with ice cold 10% $KHSO_4$ solution, saturated $NaHCO_3$ solution and water. The organic layer was dried with $MgSO_4$, filtered and evaporated to give **101** as a yellow semi-solid (1.79g, 95%) R_f 0.78 (Hex:EtOAc 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.52, 7.38 (2m, 10H, ArH), 5.59 (s, 1H, CHAr), 4.87 (dd, 1H, H-3), 4.82, 4.73 (2d, 2H, CH_2Ar), 4.75 (d, 1H, H-1, $J_{1,2}$=8.2), 4.48 (d, 1H, H-4), 4.41 (dd, 1H, H-6), 4.09 (dd,1H, H-6'), 3.88 (dd, 1H, H-2), 3.57 (d, 1H, H-5); FAB MS $C_{21}H_{20}F_3N_3O_7S$ (515.48) *m/z* (%) 538 (19) $[M+Na]^+$, 516 (35) $[M+H]^+$, 514 (51), 473 (100).

2-*O*-Benzyl-4,6-*O*-benzylidene-β-D-gulopyranosyl azide (102)

Compound **101** (1.79g, 3.47 mmol) was dissolved in DMF (36 mL). Sodium nitrite (2.34g, 33.9 mmol) and water (0.05 mL) were added and the mixture was stirred at 60°C for 18 h. The mixture was concentrated and xylene was added and evaporated from the residue. The residue was taken up in $CHCl_3$ and filtered. The filtrate was concentrated and the residue was purified by chromatography to give **102** (842mg, 63%). R_f 0.52 (Hex:EtOAc 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.51, 7.42 (2m, 10H, ArH), 5.54 (s, 1H, CHAr), 5.04 (d,1H, H-1, $J_{1,2}$= 8.7 Hz), 4.81, 4.63 (2d, 2H, CH_2Ar), 4.36 (dd, 1H, H-6), 4.18 (t, 1H, H-3), 4.13 (dd, 1H, H-4), 4.06 (dd, 1H, H-6'), 3.86 (d, 1H, H-5), 3.64 (dd, 1H, H-2); FAB MS $C_{20}H_{21}N_3O_5$ (383.41) *m/z* (%)

406 $[M+Na]^+$ (36), 384 $[M+H]^+$ (11), 341 (14), 329 (63).

2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-trifluoromethanesulphonyl-β-D-gulopyranosyl azide (103)

Compound **102** (825mg, 2.15 mmol) was dissolved in CH_2Cl_2 : pyridine 15:1 v/v and cooled to -15°C. Triflic anhydride was added dropwise and the mixture was stirred at -15°C for 18 hours. The mixture was diluted with cold CH_2Cl_2, and washed with ice cold 10% $KHSO_4$, saturated $NaHCO_3$ and water. The mixture was dried over $MgSO_4$, filtered and evaporated. The residue was purified by chromatography with hexane: EtOAc 8:7 v/v to give tiriflate (447mg, 40%). R_f 0.70 (Hex:EtOAc 8:7 v/v); ^{1}H NMR ($CDCl_3$) δ 7.31-7.46 (m, 10H, ArH), 5.56 (s, 1H, CHAr), 5.20 (t, 1H, H-3), 5.05 (d, 1H, H-1, $J_{1,2}$= 8.8), 4.73 (bs, 2H, CH_2Ar), 4.38 (dd, 1H, H-6), 4.25 (d, 1H, H-4), 4.10 (dd, 1H, H-6'), 3.84 (s, 1H, H-5), 3.74 (dd, 1H, H-2); FAB MS $C_{21}H_{20}F_3N_3O_7S$ (515.48) *m/z* (%) 538 (21) $[M+Na]^+$, 514 (37) $[M-H]^+$, 488 (17), 473 (100).

2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranose (104)

Compound **18** (1.0g, 2.43 mmol) was dissolved in acetone (3 mL) and thiourea (185mg, 2.43mmol) was added. The mixture was refluxed for 15 minutes. The mixture was cooled and the salt was filtered off. The salt was added to sodium metabisulphite (384mg, 2.02 mmol) in water (1.5 mL) with 1,2-dichloroethane (3 mL) and the mixture was refluxed for 10 minutes. The phases were separated, and the organic phase was washed with water (1 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated to give **104** (753mg, 85%): R_f 0.45 (CH_2Cl_2:EtOAc 10:1 v/v); ^{1}H NMR ($CDCl_3$) δ 5.41 (d, 1H, H-4), 5.16 (t, 1H, H-2), 5.00 (dd, 1H, H-3), 4.52 (t, 1H, H-1), 4.10 (m, 2H, H-6, H-6'), 3.93 (t, 1H, H-5), 2.35 (d, 1H, SH), 2.14, 2.07, 2.03, 1.96 (4s, 12H, 4OAc); FAB MS $C_{14}H_{20}O_9S$ (364.37) *m/z* (%) 387 $[M+Na]^+$ (14), 365 $[M+H]^+$ (27), 331 (100).

2,3,4-Tri-*O*-acetyl-1-thio-α-L-fucopyranose (105)

Thioacetic acid (10 mL) was saturated with HCl at -10 °C. L-Fucose (984mg, 6 mmol) was added and the mixture was stirred at 0 °C for 10 minutes and then at room temperature for 1 hour. The mixture was concentrated. Pyridine (10 mL) was added to the residue and the solution was cooled to -10 °C. Acetic anhydride (7 mL) was added dropwise, and the mixture was stirred at room temperature for 18 hours. The mixture was concentrated and the residue was purified by chromatography with ether:hexane 10:7 v/v as the mobile phase to give **105** (365mg,, 17%): R_f 0.53 (CCl_4:ether 10:4 v/v); ^1H NMR ($CDCl_3$) δ 6.21 (d, 1H,H-1, $J_{1,2}$= 5.3 Hz), 5.45 (q, 1H, H-2), 5.26 (d, 1H, H-4), 5.04 (dd, 1H, H-3), 4.04 (q, 1H, H-5), 2.39 (s, 3H, SAc), 2.16, 1.99,1.98 (3s, 9H, 3OAc),1.13 (d, 3H, H-6); FAB MS $C_{14}H_{20}O_8S$ (348.38) *m/z* (%) 371 $[M+Na]^+$ (21), 349 $[M+H]^+$ (9), 273 (100).

3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl azide (106)

Compound **104** (848mg, 2.33mmol) was dissolved in DMF (7 mL) and cooled to 0°C. Sodium hydride 60% (56 mg, 2.33mmol) was added and the mixture was stirred for 10 minutes. Compound **103** (600 mg, 1.16mmol) in DMF (3 mL) was added dropwise at 0°C. The reaction mixture was stirred and allowed to warm to room temperature. After 3 hours, the mixture was evaporated and xylene was added and evaporated. The residue was taken up in CH_2Cl_2 (150 mL) and washed with saturated $NaHCO_3$ and brine. The mixture was dried with $MgSO_4$, filtered and evaporated. The residue was purified by chromatography to give **106** (351mg, 41%): R_f 0.42 (Hex:EtOAc 1:1 v/v); ^1H NMR ($CDCl_3$) δ 7.33-7.51 (m, 10H, ArH), 5.51 (s, 1H, CHAr), 5.35 (d, 1H, H-4a), 5.17 (t, 1H, H-2a), 5.06 (d, 1H, H-1a, $J_{1,2}$= 10.2 Hz), 4.91, 4.71 (2d, 2H, CH_2Ar), 4.86 (dd, 1H, H-3a), 4.65 (d, 1H, H-1b, $J_{1,2}$= 8.3 Hz), 4.36 (dd, 1H, H-6b), 4.24 (d, 1H, H-4b) 4.01-4.14 (m, 3H, H-6a, H-6'a, H-6b), 3.74 (dd, 1H, H-2b), 3.65 (t, 1H, H-5a), 3.58 (s, 1H, H-5b), 3.15 (dd, 1H, H-3b); FAB MS $C_{34}H_{39}N_3O_{13}S$ (729.76) *m/z* (%) 862 (23) $[M+Cs]^+$, 419 (14), 392 (53), 331 (33), 286 (100) and the disulphide **108** (453mg, 53%) R_f 0.36 (Hex:EtOAc 1:1 v/v); ^1H NMR ($CDCl_3$) δ 5.44 (d, 2H, 2H-4), 5.34 (t, 2H, 2H-2), 5.09 (dd, 2H, H-3), 4.57 (d, 2H, 2H-1, $J_{1,2}$= 10 Hz), 4.21 (m, 2H, 2H-6), 4.12 (m, 2H, 2H-6'), 4.03 (t, 2H, 2H-5), 2.16, 2.09, 2.04, 1.98 (4s, 24H, 8OAc); FAB MS $C_{28}H_{38}O_{18}S_2$ (726.41) *m/z* (%) 859 $[M+Cs]^+$ (41), 749 $[M+Na]^+$ (3), 419 (9), 392 (20), 331 (100) 286 (83).

3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-*O*-benzyl-6-*O*-benzyl-β-D-galactopyranosyl azide (109)

Compound **106** (20mg, 0.027mmol) was dissolved in THF (0.5 mL) and sodium cyanoborohydride (21mg, 0.33mmol) and a grain of methyl orange were added. The mixture was cooled to 0°C and THF saturated with HCl was added dropwise until gas evolution ceased and a permanent pink colour was obtained. The mixture was stirred at this temperature for 30 minutes and then neutralised with triethylamine. The mixture was concentrated and the residue was purified by chromatography with hexane:EtOAc 1:1 v/v as the mobile phase to give **109** (9.5mg, 47%): R_f 0.47 (Hex:EtOAc 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.31-7.41 (m, 10H, ArH), 5.37 (d, 1H, H-4a), 5.29 (t, 1H, H-2a), 4.86-4.92 (m, 3H, H-1a, $J_{1,2}$= 10.2 Hz, H-3a, CH_2Ar), 4.64 (m, 2H, H-1b, $J_{1,2}$= 8.3 Hz, CH_2Ar), 4.05 (m, 2H, H-6a, H-6'a), 3.73 (m, 2H, H-6b, H-6'b), 3.58 (m, 2H, H-5a, H-5b), 3.09 (dd, 1H, H-3b), 3.02 (d, 1H, H-4b), 2.14, 2.09, 2.05, 1.98 (4s, 12H, 4OAc); FAB MS $C_{34}H_{41}N_3O_{13}S$ (731.77) *m/z* (%) 754 $[M+Na]^+$ (24), 732 $[M+H]^+$ (11), 331 (78).

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