AMINO SUGARS AND THEIR GLYCOSIDES

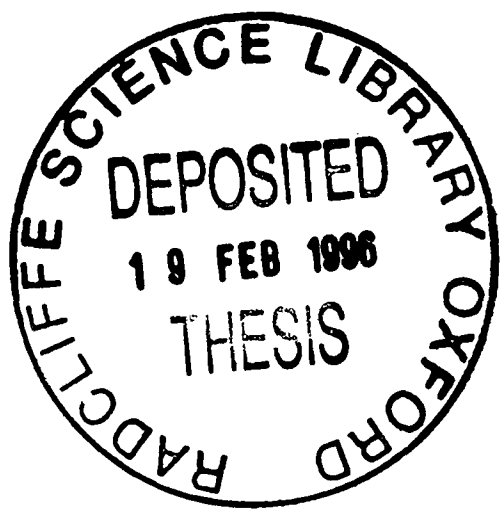
by

Neil A. Hindle

St. John's College, Oxford

1995

A thesis submitted in partial fulfilment of
the requirements of the degree of D. Phil.



To the Memory of

my father

Amino Sugars and their Glycosides

Neil A. Hindle, St. John's College, Oxford

D.Phil., 1995.

ABSTRACT

This thesis describes approaches to the transformation of simple carbohydrates into a polyhydroxylated pyrrolidine and the formation of its glucosides.

Chapter one describes the synthesis of the naturally occurring pyrrolidine 2,5-dideoxy-2,5-imino-**D**-mannitol. Synthesised from di-*O*-isopropylidene-**D**-glucose, the key steps are the introduction of nitrogen at C-5 with retention of configuration. Then cyclisation of the nitrogen onto the C-2 position with inversion to form the pyrrolidine ring. Reduction of the aldehyde furnished the polyhydroxylated heterocycle in 3.4% yield over 16 steps. The synthetic compound matched the naturally occurring compound in all respects.

Chapter two contains a review of commonly used glycosylation methods. It also describes the glycosylation of di-O-isopropylidene-α-**D**-glucose as a model system comparing the Koenig-Knorr method to the trichloroacetimidate method using several reaction conditions. Glycosylation of 2,5-dideoxy-2,5-imino-**D**-mannitol was carried out using the trichloroacetimidate method to synthese all four glucosides. Boron trifluoride etherate and trimethylsilyl trifluoromethanesulphonate were used as catalysts in dichloromethane, diethyl ether and acetonitrile under strictly anhydrous conditions. All four glucosides were prepared 1-*O*-(αβ-**D**-glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol and 3-*O*-(αβ-**D**-glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol. Biological screening carried out against a wide range of glycosidases and glycosyl transferases indicated that the glucosides showed little inhibition in comparison to 2,5-dideoxy-2,5-imino-**D**-mannitol.

Chapter three describes the isolation and identification of 1-*O*-(β-**D**-glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol from *Nephthytis poisonii* N.E.Br. a member of the Araceae family found in tropical Africa. Identification was made by comparison with the previously synthesised glucosides of 2,5-dideoxy-2,5-imino-**D**-mannitol. Investigations of *Hyacinthoides non-scriptus* (L.) chouard ex Rothm are also discussed.

Chapter four describes the synthesis of a diazidolactone that could be used to form a 1,5 disubstituted tetrazole. This would have a second nitrogen functionality in the molecule allowing the possibility of the inclusion of the tetrazole into a peptide sequence. The synthesis was carried out from **L**-gulono-1,4-lactone. An azido group was introduced selectively at C-2, this unexpectedly occurred with retention of configuration. A second azide was then introduced at C-5, this occurring with the more commonly observed inversion of configuration to afford the 2,5-diazido-2,5-dideoxy-**D**-manno-1,4-lactone.

Acknowledgements

I would firstly like to thank my supervisor Dr George Fleet for his unbounded energy and encouragement over the past seven years. My gratitude also goes to Drs Robert Nash and Alison Watson for all their help and support with the natural product isolations and identifications. Also for making Aberystwyth an enjoyable, even although wet place to visit. Also to Dr Mark Wormald for performing and interpreting endless NMR experiments.To Rhodi Griffiths for carrying out the enzyme studies.

My thanks also go to the all the technical staff of the Dyson Perrins laboratory without whose help this thesis would not have been possible. Particular thanks go to Elizabeth McGuiness, Val Lambourne, Tina Jackson, Levi and Ralph.

My appreciation also goes to Rachel, Helen, John, Katy and Andrew for proof-reading after which I accept responsibility for any remaining mistakes. Also to everybody in the Perkin Lab, not forgetting Little P., past and present for making the last three years an enjoyable experience. Particluarly Andy for helping to keep a perspective over the last two months. My thanks go to Jong 'just the one' Son, Thomas Krülle, Tilmann 'what van?' Brandstetter and Yves 'Oves' for their help with many problems for the past few years. Particular note must also go to past and present members of the HH gathering (including those how have fallen from old ways) not forgetting Joe 'madman' Wheatley, Mark 'farmer Giles' Andrews, Dave 'what have I done!' Hughes, Ken 'sleeply head' Hsia and Fez Fairbanks for many memorable nights and also the ones we can't remember. Thanks also go to all members of labs 52 and 53 for use of Perrin and Armarego and the ether still.

I treasure greatly the friendship of my colleagues at SJC and appreciate their help on all matters; Ian 'terry' Lilley, Grant 'beers' Taylor, Dave 'I've got an idea' Levinger, Tam 'Cherwell Swimmer' O'Connell, and 'Big' Stevie. Also I thank SJCBC for first allowing me near a boat and particluarly to Rachel for her advice, organising and fudge brownies. Others of SJCBC that must be mentioned are Sos for her complete trust, Ollie 'legs' Kingsbury, Glen 'one pot and what a race it was ' Leighton and Paul 'puddles' Pattenden.

My thanks would not be complete without the mention of all the members of OUWBC past and present including Ali, Emma for their leadership, OULRC '94 for an extra 12 inches at Henley when it mattered and especially the Osiris crews of 94 and 95; Emma, Jo, Mel, Vicky, Jane, Sophie, Dawn, Karen, Sarah, Emma, Kath, Jo, Jana and never forgetting Tessa.

Finally my warmest and deepest thanks must go to my family near and far for all their help and encouragement over the past years.

Contents

Abbreviations

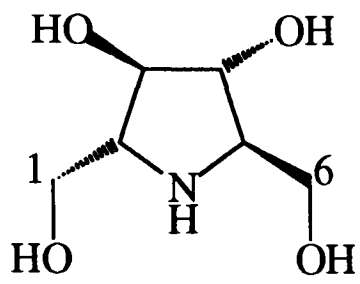
The following abbreviations are used in this thesis:

Ac	acetyl		
AIDS	acquired immunodeficiency syndrome	GCMS	gas chromatography-mass spectroscopy
AZT	azidothymindine	Glc	glucose
aq	aqueous	gp	glycoprotein
Bn	benzyl	HIV	human immuno-deficiency virus
Bz	benzoyl		
br	broad	IC_{50}	50% inhibition concentration
CAST	castanospermine		
DAG	diacetone-α-**D**-glucose	IMG	(1-imidazolylcarbonyl) glycosides
DCM	dichloromethane		
DMDP	2,5-dideoxy-2,5-imino-**D**-mannitol	IR	infra red
		K_i	equilibrium inhibition constant
DMF	*N*,*N*-dimethylformamide		
DMJ	deoxymannojirimycin	Lit	literature value
DMSO	dimethylsulphoxide	m	multiplet
DNA	deoxyribose nucleic acid	M.p.	melting point
DNJ	deoxynojirimycin	Man	mannose
d	doublet	mCPBA	*meta*-chloroperbenzoic acid
dd	double doublet		
ddd	double double doublet	Me	methyl
dt	double triplet	Ms	mesyl
ETDA	ethylenediamine-tetraacetic acid	NBS	*N*-bromosuccinimide
		nOe	nuclear Overhauser effect
Gal	galactose		

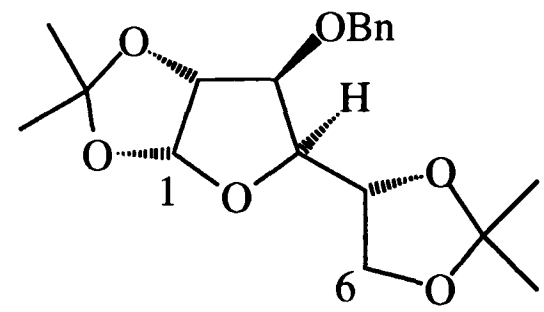
NMR	nuclear magnetic resonance
Ph	phenyl
py	pyridine
q	quartet
RNA	ribose nucleic acid
SIV	simian immuno- deficiency virus
s	singlet
TBMDS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
Triflate (Tf)	trifluormethanesulphonyl
Ts	*p*-toluenesulphonyl
tlc	thin layer chromatography
Z	benzyloxycarbonyl

Note Concerning Nomenclature

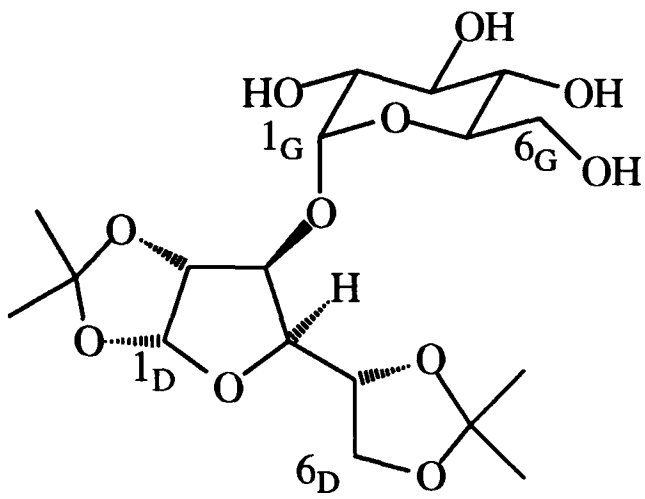
Spectroscopic data are assigned according to the numbering systems shown for the examples below, trivial names are used in conjunction with IUPAC nomenclature:-



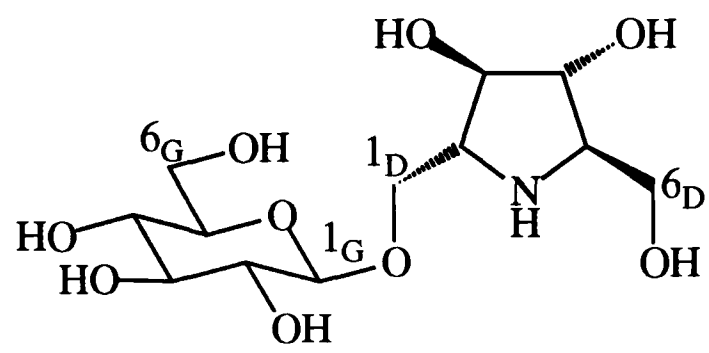
2,5-Dideoxy-2,5-imino-**D**-mannitol



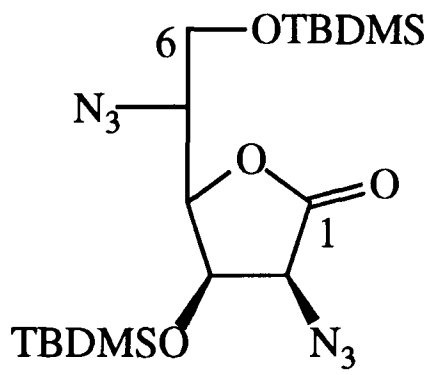
3-*O*-Benyl-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose



3-*O*-(α-**D**-Glucopyranosyl)1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose



1-*O*-(β-**D**-Glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol



2,5-Diazido-3,6-*O*-di(*tert*-butyldimethyl)silyl-2,5-dideoxy-**D**-manno-1,4-lactone

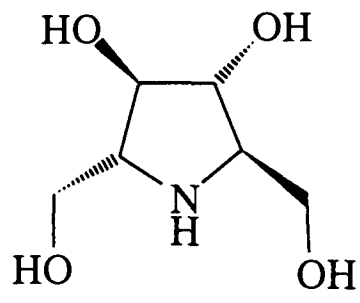
CHAPTER 1

CHAPTER 1

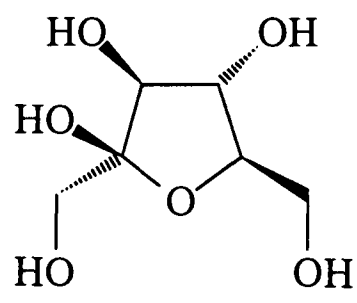
Synthesis of 2,5-dideoxy-2,5-imino-D-mannitol (DMDP)

1.1 Introduction

First extracted from the leaves of *Derris elliptica*,[1] 2,5-dideoxy-2,5-imino-**D**-mannitol (2*R*, 5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine) (**1.1**) is one of only a few simple hydroxylated pyrrolidines to have been found that are glycosidase inhibitors.[2,3] Previous studies have shown that it inhibits α-glucosidase (IC_{50} 3.3 μM) and β-glucosidase (IC_{50} 7.8 μM). A mimic of β-**D**-fructose (**1.2**), its occurrence in plants is believed to be as an anti-feedant, inhibiting glycosidases in insects' digestive fluids, thus preventing recognition of the plant by the insect.[4]



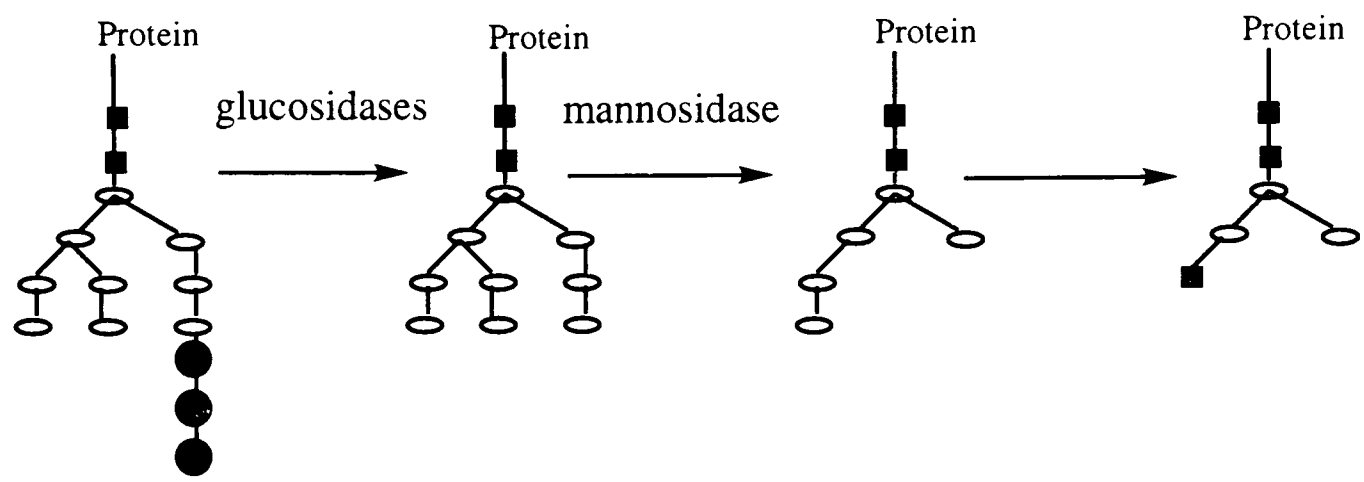
(1.1)



(1.2)

DMDP (**1.1**) has also been isolated from the seeds of *Lonchocarpus sericeus* and been shown to inhibit glycoprotein processing.[5] Glycoproteins are oligosaccharide structures that occur on the membrane surface of viruses, linked to the protein by glucosamine. The oligosaccharide is made up of various carbohydrates, but mainly consists of mannose and glucose units arranged in a complex array of branched chains. The biosynthesis of these *N*-linked oligosaccharides involves a series of reactions where higher

oligosaccharides are trimmed and further monosaccharides then added to the chain. For example, the chain $Glc_3Man_9GlcNAc_2$ is transferred to a membrane protein and then is rapidly trimmed by a series of glucosidases enzymes. After the removal of the glucose units, mannosidase I trims four of the α1,2-linked mannose units to leave $Man_5GlcNAc$, which after removal of a further two mannose units and addition of glucosamine gives GlcNAcManα1,3(Manα1,6) Man(βGlcNAc)$_2$-protein (Figure 1.1).



- *N*-acetyl glucosamine
- mannose
- glucose

Figure 1.1

DMDP (**1.1**) has been shown to inhibit glucosidase I the enzyme responsible for removal of the first glucose unit.[5] Specific inhibition of these glycoprotein processing enzymes would allow further investigation of the function and biosynthesis of *N*-linked glycoproteins.

1.1.a.i AIDS and HIV

In recent years the interest in glycoproteins has greatly increased due to the discovery of the complex structure on the surface of the retrovirus, HIV. The surface was found to contain various glycoproteins within its framework (Figure 1.2).

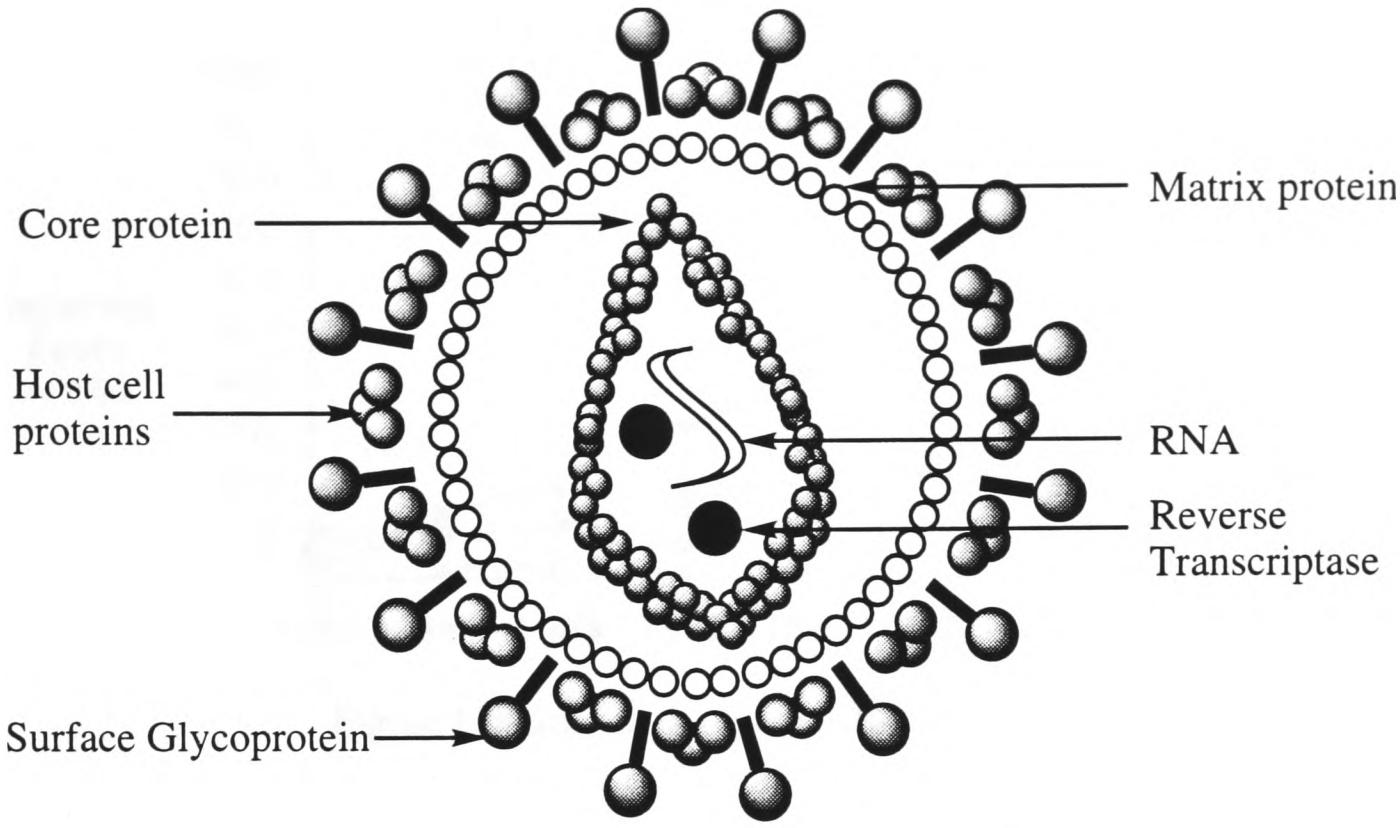


Figure 1.2 Systematic diagram of HIV virus

Although disputed by a few people,[6] HIV-1 and HIV-2 are now recognised as the viruses responsible for AIDS. In 1981, the occurrence was reported of five young previously healthy males having developed *Pneumocystis carinii* pneumonia, in three different Los Angles hospitals over a three month period.[7] This disease had previously only been observed in patients with severe suppression of their immune systems caused by drugs or disease. A month later 26 males, 20 from New York and six from California were reported to be suffering from Karposi's Sarcoma.[8] The New York university cancer registry had recorded only three cases of this sarcoma in men under 50 before 1979 in New York hospitals.

This large increase in cases of the complete collapse of immune systems, led in 1982 to a set of clinical guidelines being introduced, defining the symptoms associated with AIDS, thus starting the wide spread diagnosis and monitoring of the disease. By May 1985, ten thousand cases had been reported in the US. Since then the number of cases has slowly increased each year reaching one million in 1994 (Figure 1.3).[9]

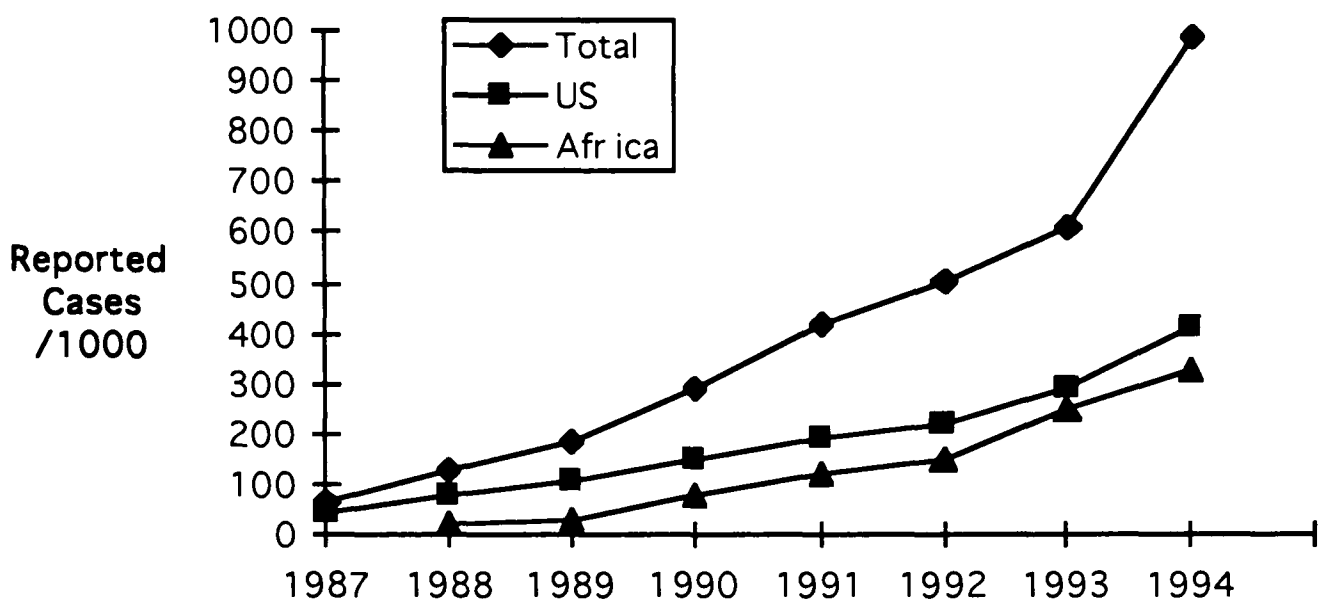


Figure 1.3 Growth in reported AIDS cases [10]

In 1983, a virus was discovered that showed close associations to the AIDS complex. Barré-Sinoussi[11] isolated a retrovirus from a patient with lymphadenopathy syndrome, a disease common in AIDS patients, then in the following year, Gallo[12] succeeded in propagating a similar virus in cell culture. After a long dispute, the Pasteur Institute in 1990[13] analysed the genetic structure of the two viruses. They showed the two to be so similar that it was very unlikely for them not to be the same. By international convention, the virus was named the human immunodeficiency virus (HIV).

1.1.a.ii The Origin of HIV

The origin of HIV has been the centre of great debate. The earliest evidence of a patient with HIV was the detection of HIV antibodies in frozen serum from Kinshashu, Zaire in 1959,[14] then also from Uganda in 1972.[15] These are the first recorded occurrences of HIV suggesting that the virus is new in humans. There have now been a number of hypotheses to the actual origin of the virus.

One hypothesis suggests that the human virus originated from simian immunodeficiency virus, SIV, found in monkeys. Each species of monkey is infected with one specific immunodeficiency virus which is unable to infect other species. Study of the

genetic material of these viruses has shown that the strain from the Sooty Mangabey monkey, SIVsm, bears a close resemblance to HIV-2.[16] The relationship is as close between SIVsm and HIV-2 as between different strains of SIV, with more than 70% of the RNA bases in common. Circumstantial evidence also points to SIVsm being the source of HIV-2. In Western Africa HIV-2 is more commonly found than HIV-1, particularly in Angola and Mozambique, the natural habitat of the Sooty Mangabey monkey. There still remains the question of the link between HIV-1 and SIV virus, although a virus from a captive chimpanzee in Gabon has showed a similar genetic structure to HIV-1.[17]

The jump across the species for SIV to become HIV would still be a significant barrier, although as a retrovirus the rate of mutation is very high. It is possible for a single host to be infected with many thousands of variants making it possible to have very rapid genetic evolution. The physical infection may have occurred in a number of ways, either from the hunting of monkeys for protein, where the contamination with infected blood may have occurred *via* skin abrasions or wounds. Another possibility was the use of monkey tissues for growth media in early trials of malaria[18] and polio vaccines.[19] Some of these trials in the early 1950's were carried out in Rwanda, Zaire and Burundi, now known to be the early epicentre of the AIDS epidemic in Africa.

1.1.a.iii The Spread of AIDS

The spread of the disease has been difficult to monitor due to the social stigma connected to it in western countries. In Africa diagnosing and monitoring is made difficult by the lack of communication between local doctors and the monitoring organisations, although, the biggest problem with monitoring the advancement of the disease is the long time period between infection and development of AIDS symptoms. This asymptomatic period in average is about ten years and maybe as long as twelve, thus the number of new AIDS cases at any time is the number of new HIV infections approximately ten years earlier. The rate of spread of HIV and AIDS in the western world is generally on the

increase although in particular groups the rate of infection has decreased. In male homosexuals the number of new HIV infections has dropped from 4.2 thousand in 1983 to 1.7 thousand in 1985,[20] this is mainly due to the change in behaviour brought about by an extensive educational program. In the heterosexual community there is still an upward trend in infection despite media coverage of the disease.

1.1.a.iv Pathology of HIV

As a retrovirus, HIV carries its genetic code as RNA, as opposed to DNA. Then, using the enzyme reverse transcriptase, the RNA is encoded into DNA and inserted into the DNA of the host cell. Retroviruses have no mechanism for the validation of the replication of RNA and this leads to a large number of mutations in the genetic sequence. This limitation of the virus allows it to mutate rapidly and is the main cause for the body's immune system becoming overwhelmed by the infection.

After initial infection there is a large response from the immune system with the patient becoming seriously ill. Then the disease enters the asymptomatic period, were the patient appears to be healthy. The virus was thought to be in a dormant state with the onset of AIDS being brought about by an independent trigger. It is now known that as opposed to being dormant there is a continuous fight between the virus and the immune system with the immune system finally losing, leading to the complete suppression of the body defences and minor infections becoming fatal.[21]

The initial contact between the virus and cell is through the glycoprotein, gp120. This mainly attacks the $CD4^+$ receptor protein that occurs on a number of cells. A large number of $CD4^+$ receptors occur on the T-helper lymphocytes, the effect of the virus being greater the more receptors on the cell surface, thus making the T-lymphocyte very vulnerable. Once attached to the $CD4^+$ receptor the gp120 binds non-covalently to a second glycoprotein, gp41 which is covalently bonded to the surface membrane of the virus. Gp41 also is responsible for the penetration of the cell membrane. At this point the RNA strands

cross into the host cell, along with reverse transcriptase. This encodes DNA from the RNA and inserts it into the host cell's genetic material. The cell then produces viral proteins and RNA for new viruses. Finally, the host cell bursts (lysis) and releases the replicated viruses into the body to infect other cells. The T-lymphocytes are the main cells that co-ordinate the immune system. When depleted by an infection the whole immune response is dramatically effected and as such is unable to fight off the virus which can then infect further T-cells. This finally causes complete collapse of the immune system.[22]

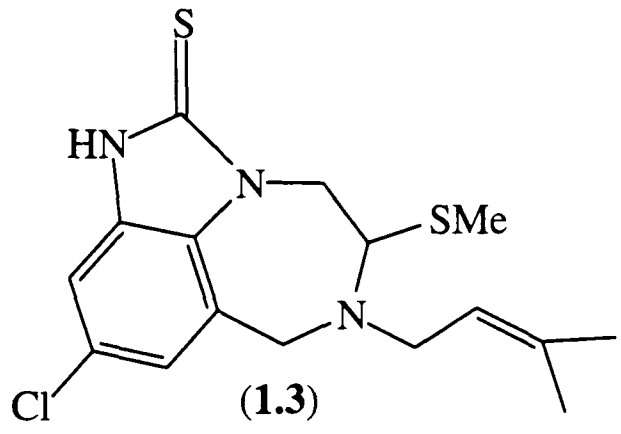
1.1.a.v Vaccines against HIV

Diseases caused by viruses are mainly treated with vaccines, smallpox, tuberculosis and polio for example. The difference between these viruses and HIV is the high number of mutations that can occur even within one patient. Another problem with vaccines is to produce an immune response that will combat the virus as the body's own initial immune response to the virus does not halt the infection. Although HIV replicates extremely fast, producing 10^8- 10^9 viruses a day,[23] it exists inside the host cell for the majority of its lifecycle. This effectively shields the virus from any agents introduced into the plasma to attack it. Another approach to a vaccine is the use of gp120 to stimulate the immune system. Unfortunately, as with the virus where gp120 is non-covalently bound to the membrane, free gp120 can attach itself to the $CD4^+$ receptors on the T-cells causing the immune system to kill the healthy T-cell.

1.1.a.vi Replication Inhibitors

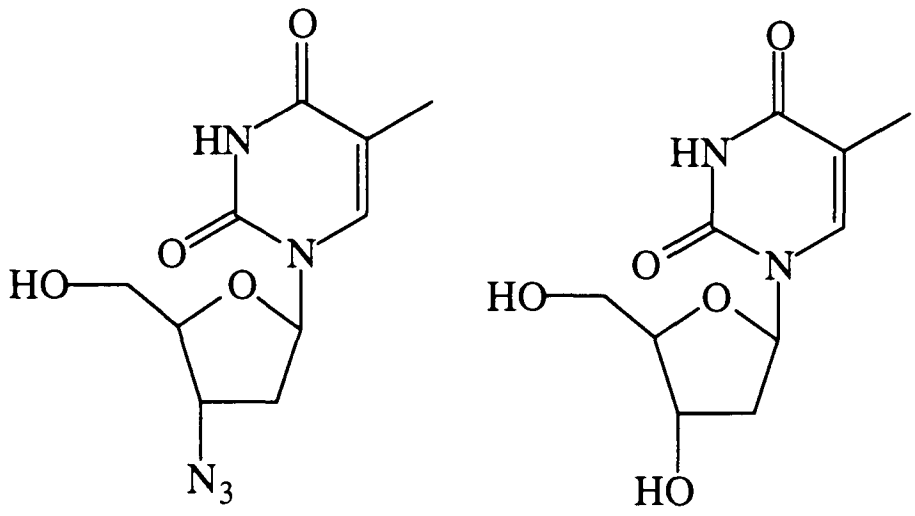
A major area of research has been the study of the virus lifecycle, in order to identify a point of attack for a drug, that will either slow down the disease or possibly even eradicate it. An important point at which the virus may be vulnerable, is the replication of

the RNA into DNA by reverse transcriptase. Mammalian cells do not contain reverse transcriptase and an agent that specifically inhibited it would probably be non-toxic to healthy cells. One such agent developed was the diazepinone compound (**1.3**).[22] Surprisingly though this has proved to be toxic to other human cells and cannot be used to attack HIV.



(**1.3**)

Another strategy to block the replication of RNA, has been the use of nucleoside analogues. One successful example has been the drug zidovudine (AZT, Retrovir®) (**1.4**). This consists of the natural base thymidine (**1.5**) with the C-3 hydroxyl replaced by an azido group (Figure 1.4).



AZT (**1.4**) Thymidine (**1.5**)

Figure 1.4

During replication, the AZT (**1.4**) is incorporated into the growing DNA chain through the remaining C-5 hydroxyl then further transcription cannot proceed as extra bases cannot be added as C-3 is blocked by the azido group.

A double blind trial[24] was carried out with 282 patients where AIDS had developed as *Pneumocystis carii* pneumonia. During the 24 week period of the trial, 19 patients (13.8%) on the placebo and one on AZT (0.6%) died. The patients taking the active drug were also less susceptible to other opportunistic infections. In another trial, AZT was given to patients who had been infected with HIV but had not developed any AIDS related infections. In the Concorde trial,[25] 1749 HIV infected individuals were randomised. Half the group were given AZT and half the group were given a placebo, until the time that they showed symptoms of the disease. The number of clinical endpoints (AIDS or death) in the Concorde trial was 347, with no statistically significant difference between the two groups. The trial showed no benefit in the use of AZT in symptom free HIV infected adults. These trials show that it is of benefit to patients to receive AZT after the development of AIDS related infections have been diagnosed or their $CD4^+$ cell count drops below a threshold.

During the numerous trials of AZT several widespread adverse side-effects have been observed.[26] While receiving the drug many patients complained of suffering from nausea, insomnia and prolonged headaches. The more adverse effects of AZT treatment were anaemia (a drop in the number of red blood cells), leukopenia and neutropenia (associated with a drop in the number of white bloods cell), this occurred in 34% of recipients after six weeks of treatment. During the trial, 45% of the trial group had grade three bone marrow suppression, with greater suppression found in patients with advanced AIDS. It was noted that there were no cases of renal, hepatic or gastrointestinal adverse effects in the trial.

The current policy on AZT treatment is to postpone treatment until on onset of AIDS, or a low $CD4^+$ cell count is detected, and then treated with low dosage of AZT. During treatment, close monitoring of the haemoglobin level is carried out, with blood

transfusions being administered if bone marrow suppression becomes acute. This has the effect of prolonging the patient's life through the initial stages of AIDS.

1.1.a.vii Glycoprotein Processing Inhibitors

A further area where the virus may be targeted is the production and trimming of the glycoproteins. Gp120 consists of more than 20 potential *N*-glycosylation sites.[27] Each of these sites has the potential to have a complex carbohydrate structure comprising of mannose, glucose or galactose units attached to it (Figure 1.5).[28]

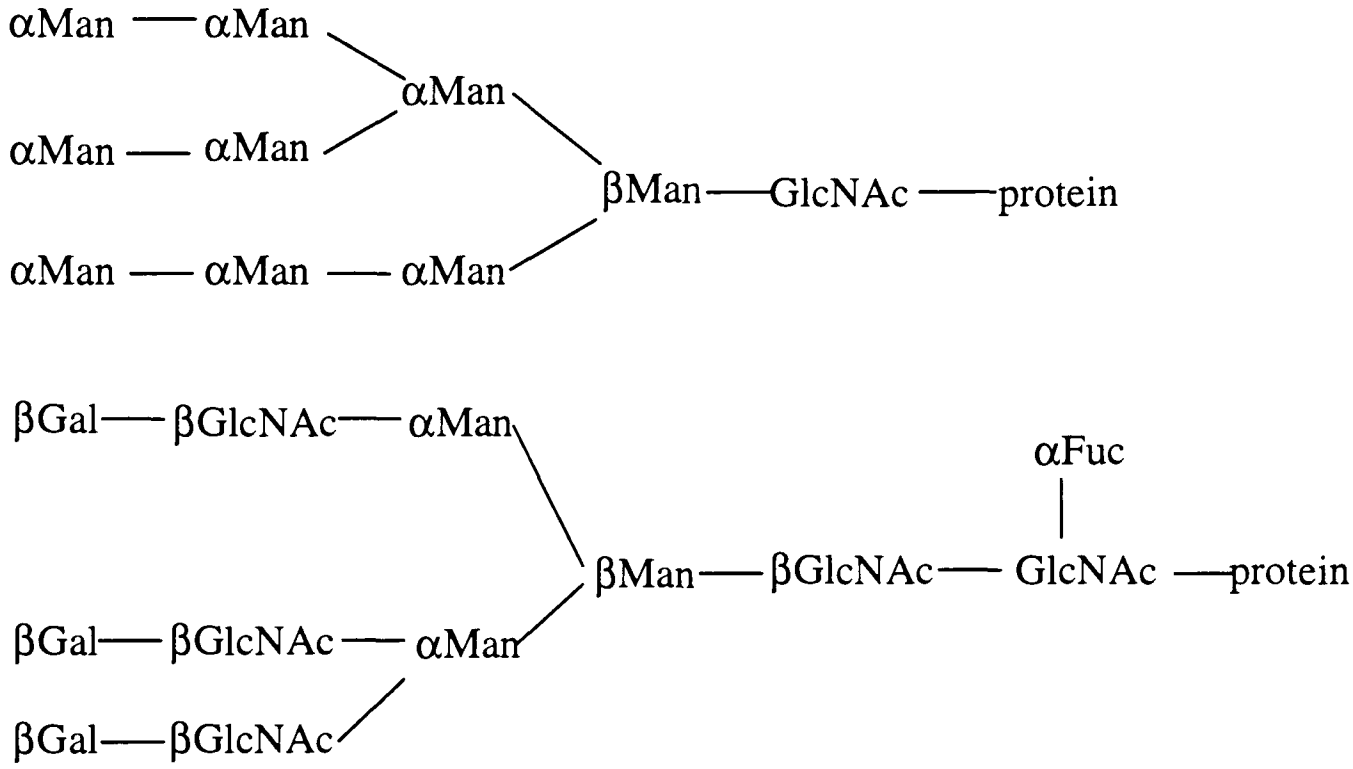
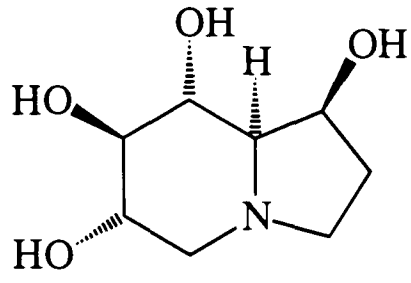
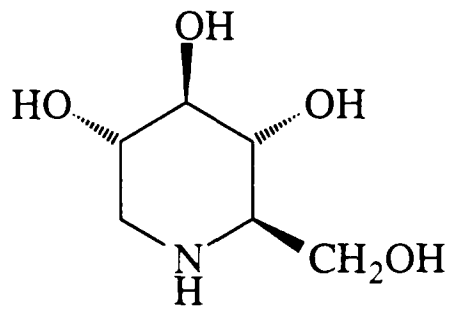


Figure 1.5. Two common oligosaccharides from gp120.

If the carbohydrate structures are removed from gp120 then the binding of gp120 to the $CD4^+$ receptors is greatly reduced.[29] Three naturally occurring plant alkaloids have been shown to inhibit the enzymes that are responsible for the elaboration of the final structures leading to the oligosaccharides of gp120.[30] Castanospermine (CAST) (**1.6**), from Australian chestnuts, *Castanospermum australe*, [31] deoxynoijrimycin (DNJ) (**1.7**) from *streptomyce spp.*[32] (Figure 1.6) and DMDP (**1.1**) have a striking affect on the formation of grouped cells (syncytia) by the action of gp120 binding to target cells. This effect has been seen on HIV-1 (table 1.1)[30] and also HIV-2 cells.



Castanospermine (**1.6**)



DNJ (**1.7**)

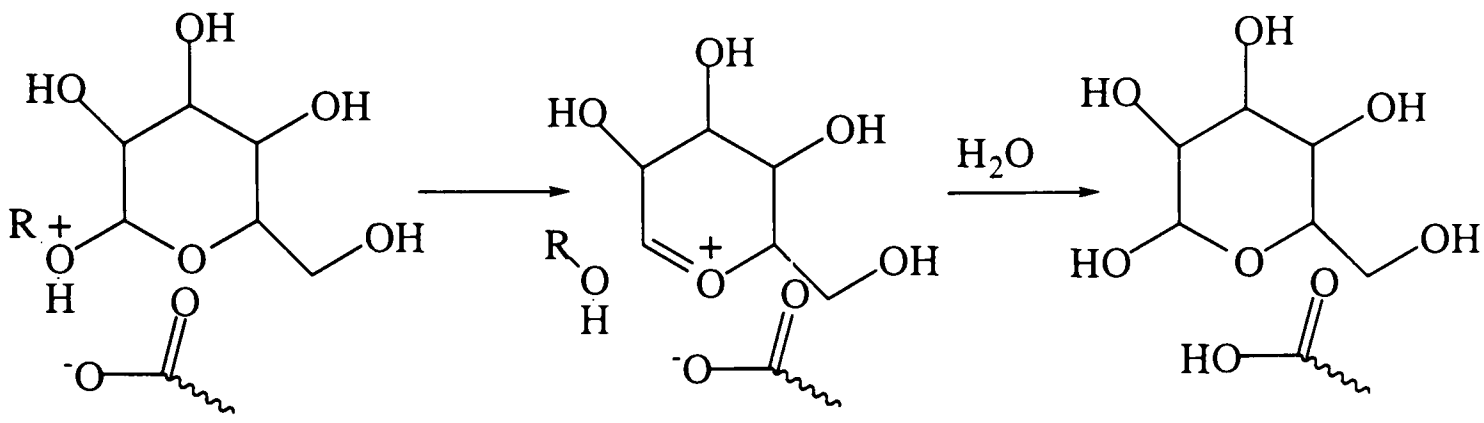
Figure 1.6

Alkaloid	Alkaloid dose mmol/ l	Syncytia formation
Control	0	abundant
CAST (**1.6**)	2.5	very rare
CAST (**1.6**)	0.62	rare
DNJ (**1.7**)	1.0	rare
DMDP (**1.1**)	2.0	rare
AZT (**1.4**)	0.05	very rare

Table 1.1

Although a large dose of DMDP (**1.1**) is required to produce the same effect as AZT (**1.4**). The study showed it to be non-toxic. Wild mice freely ate a diet of laboratory prepared meal containing 5% DMDP (**1.1**) for 7-8 days and showed no adverse affects apart from reversible weight loss.

DNJ (**1.7**) is a competitive inhibitor and it has been proposed that the nitrogen atom is protonated at the active site of the enzyme by a carboxylic acid residue and that it is the protonated form (**1.8**) that acts as the inhibitor (Figure 1.7), it having an analogous structure to the oxonium ion intermediate (**1.9**) that occurs during the hydrolysis of the oligosaccharide.[33]



R = ogliosaccharide

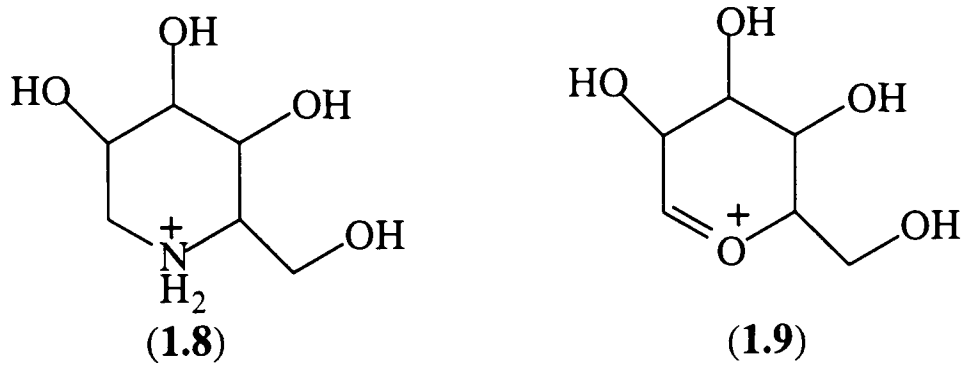
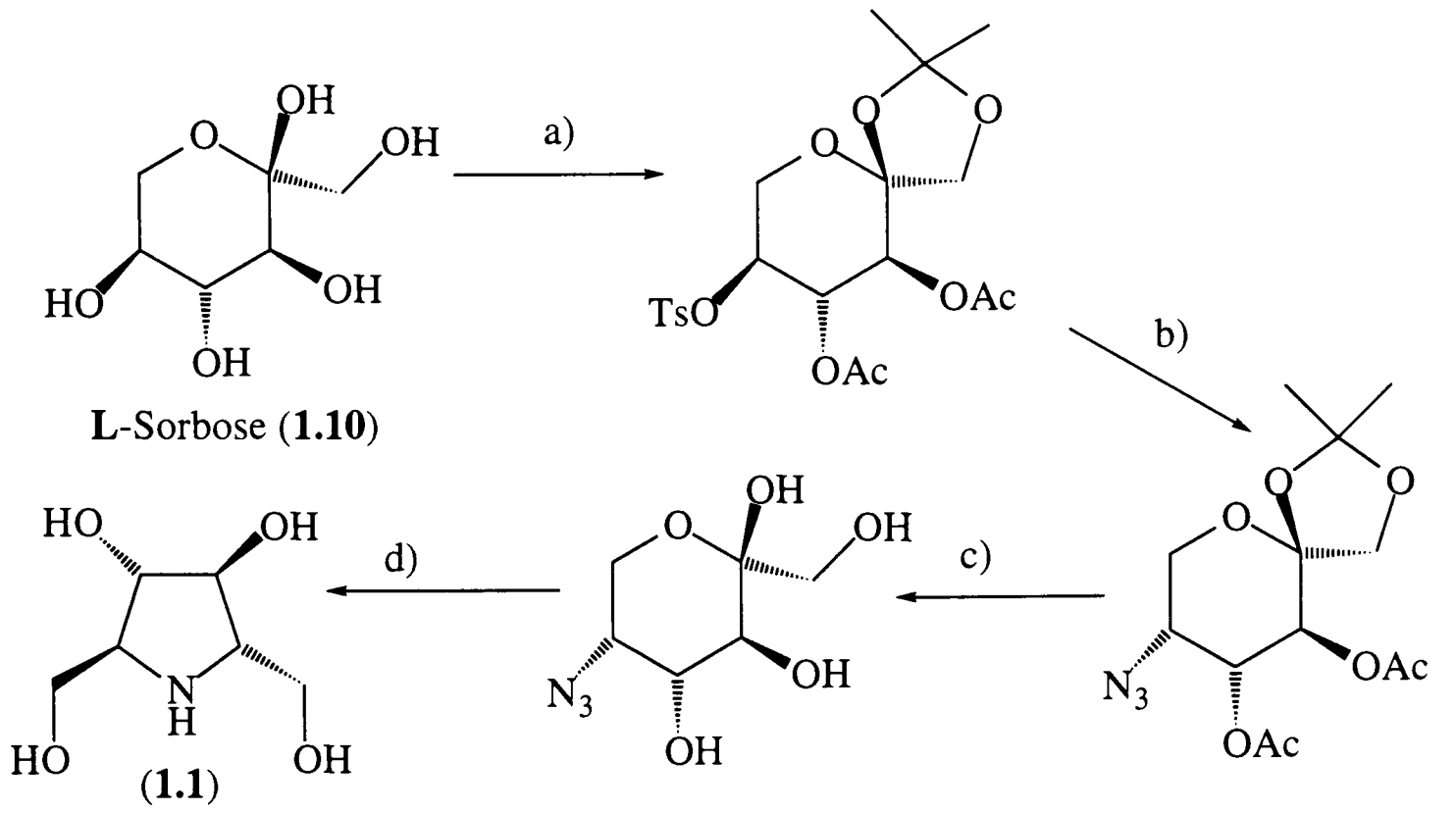


Figure 1.7

1.1.b Previous Syntheses of DMDP (1.1)

Synthesis of DMDP (**1.1**) was first carried out by Card.[2] Starting from **L**-Sorbose (**1.10**) the C-1 and C-2 are firstly protected as an acetonide. A tosyl ester was introduced at the least hindered site, C-5. Then displaced with an azide *via* a S_N2 process with inversion. After removal of the various protecting groups, the azide was then reduced to the amine which under the reaction conditions cyclises to DMDP (**1.1**) (Figure 1.8).



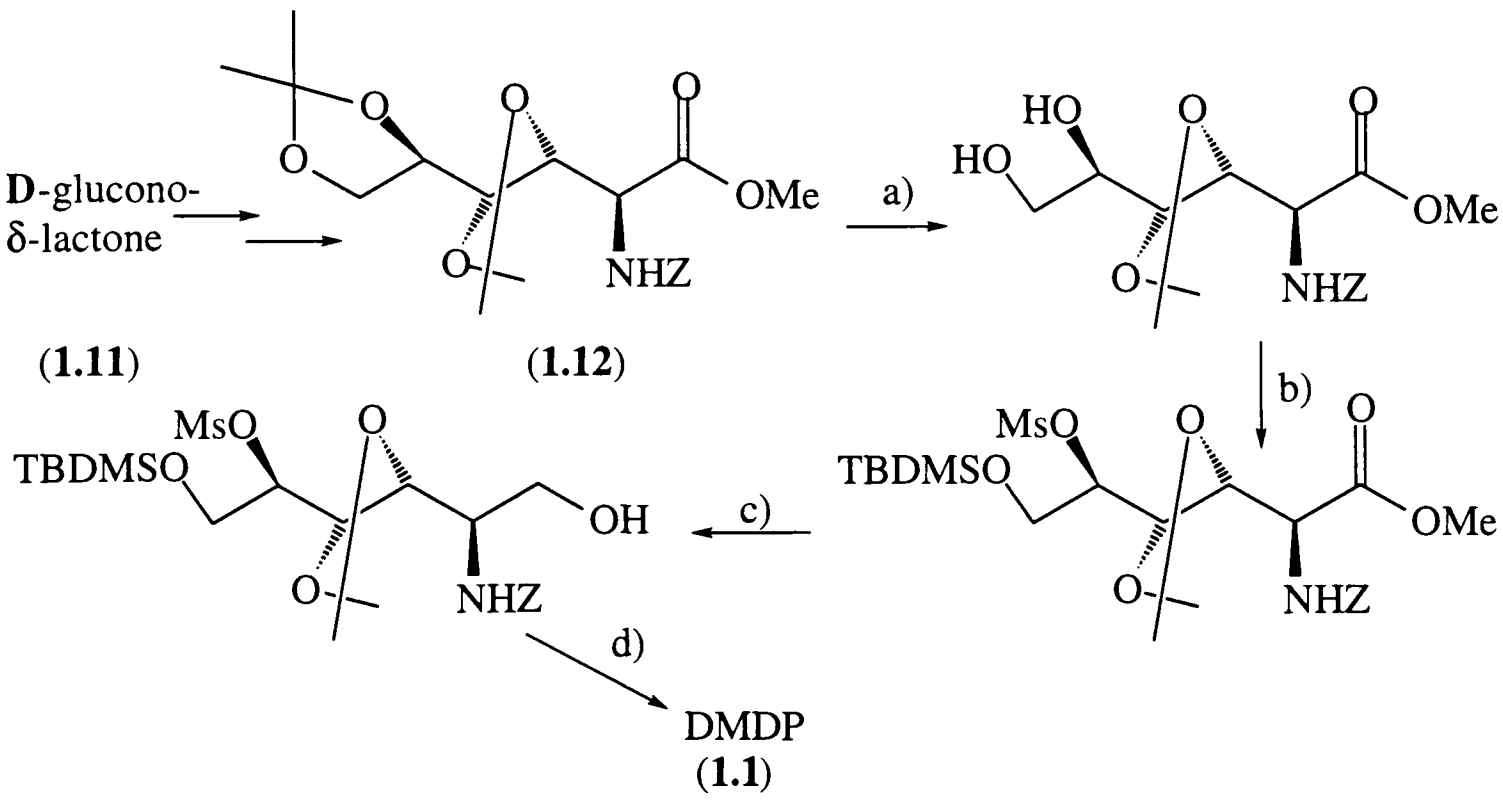
L-Sorbose (**1.10**)

a) i. $Me_2C(OMe)_2$, H^+ ii.TsCl, py iii. Ac_2O, py

b) LiN_3, DMF c) i. NaOMe, MeOH ii. H^+

d) H_2, Pd/C, EtOH

Figure 1.8



a) Dowex (H^+), methanol

b) i. TBDMSCl, imidazole, DMF ii. MsCl, THF

c) $LiAlH_4$, THF

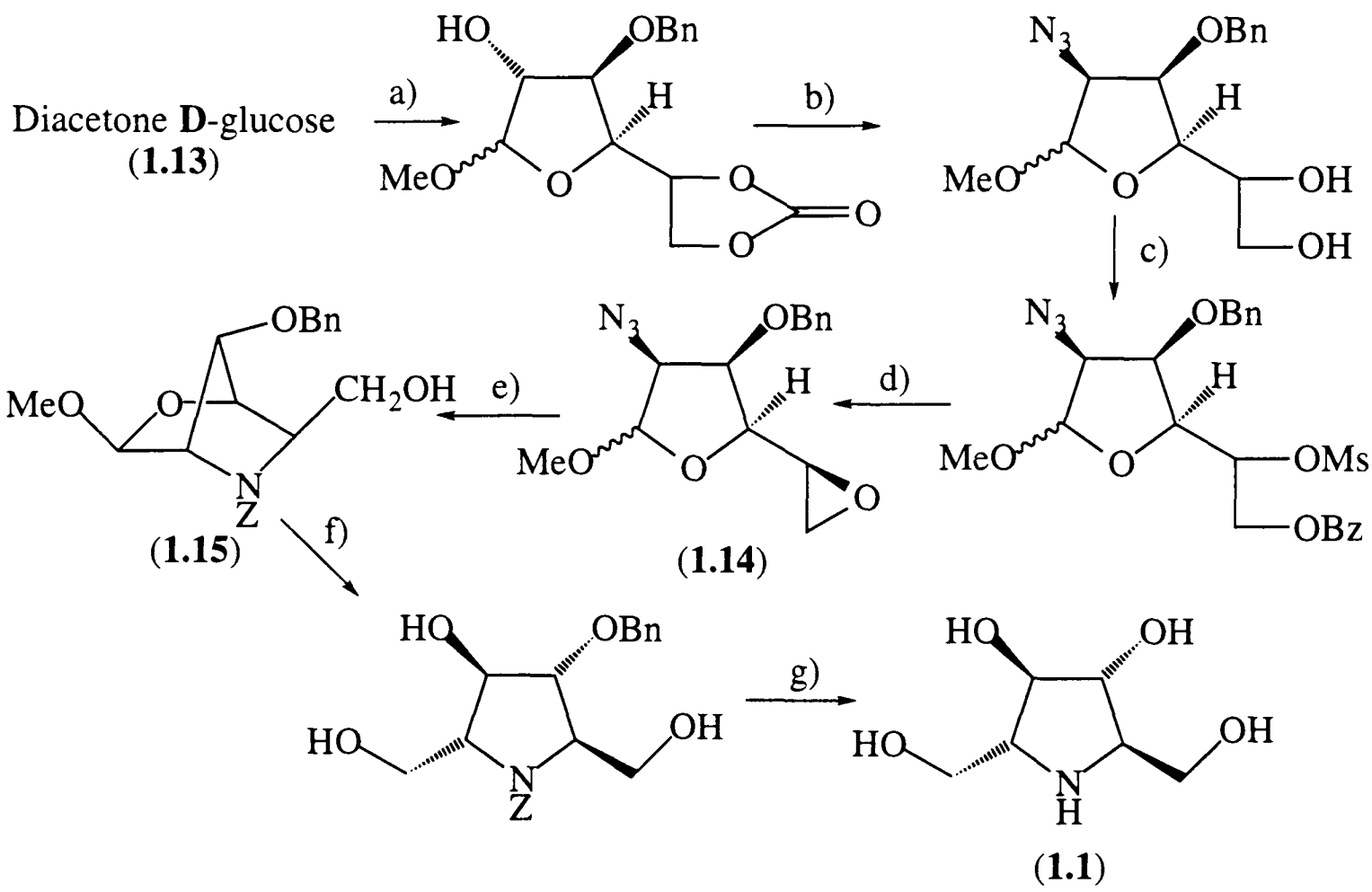
d) Dowex (H^+), methanol; sodium acetate, Pd/C, methanol, H_2

Figure 1.9

Another synthesis of DMDP (**1.1**) was carried out by Park (Figure 1.9).[34] Starting from **D**-glucono-δ-lactone (**1.11**), the mannonate (**1.12**) was prepared as described by Vasella.[35]

The terminal acetonide is removed followed by protection of the primary hydroxyl as a TBDMS ether. A mesyl ester is formed at C-5, followed by reduction of the terminal ester. The pyrrolidine ring is then formed by removal of the Z protecting group which cyclises *via* displacement of the mesyl ester with inversion of configuration. Final acid hydrolysis gives DMDP (**1.1**) in 60% overall yield from the mannonate (**1.12**).

Another method of producing DMDP (**1.1**) from **D**-glucose was reported using diacetone glucose (**1.13**) as starting material (Figure 1.10).[36]

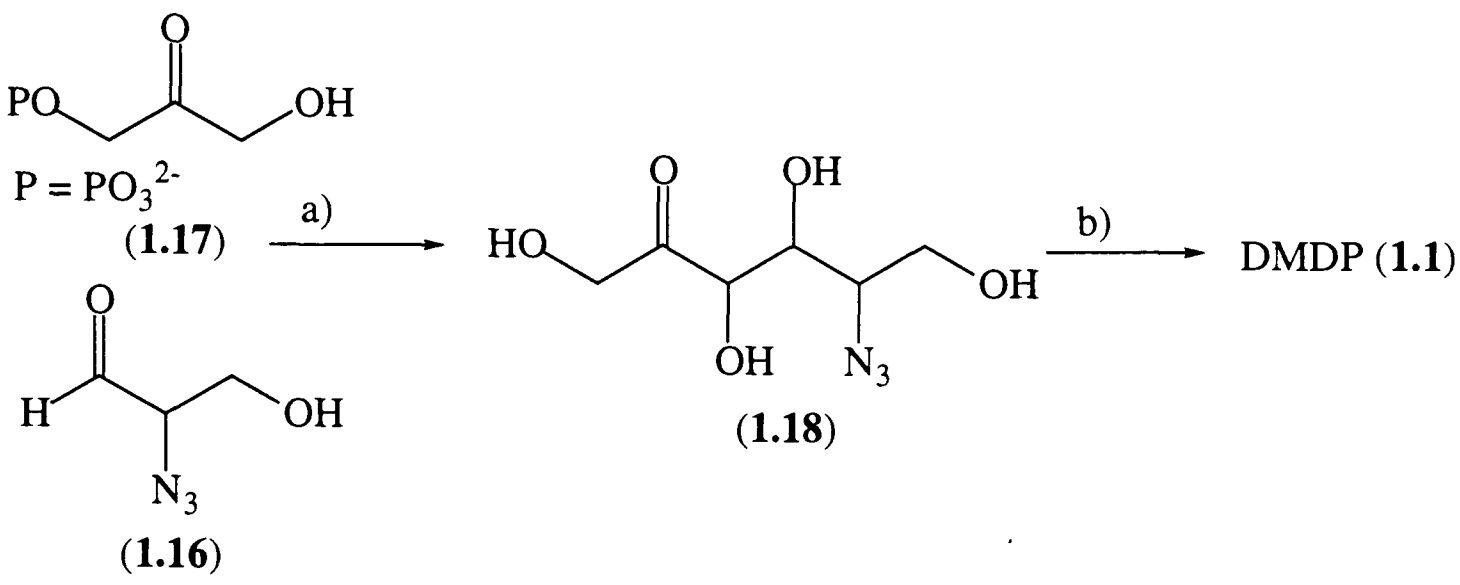


a) i. BnBr, NaH, THF ii. aq AcOH iii. $(MeO)_2CO$, NaOMe iv. MeOH, H^+
b) i. Tf_2O, py, DCM ii. NaN_3, DMF iii. NaOMe, MeOH
c) i. BzCl, py ii. MsCl, py d) NaOMe, DMF e) i. Pd/ H_2, EtOH ii. ZCl
f) i. aq TFA ii. $NaBH_4$ g) Pd/ H_2, AcOH

Figure 1.10

The key step of this synthesis is formation of the pyrrolidine ring by cyclisation of the amine formed by reduction of the azide (**1.14**). A major problem with the cyclisation is the epoxide being reduced before cyclisation of the amine can occur to form the bicycle (**1.15**).

A synthesis of DMDP (**1.1**) which does not use a sugar as the starting material uses aldolase enzymes to form the six carbon chain.[37] Starting from the racemic aldol (**1.16**) this is condensed with dihydroxyacetone phosphate (**1.17**) using fructose-1,6-diphosphate aldolase to give a diastereomeric mixture. The phosphates are removed by enzyme then reduction of the azide (**1.18**) occurs with hydrogen and palladium. Cyclisation of the amine occurs from the least hindered face for the ketone to give DMDP (**1.1**).



a) i. FDP aldolase ii. Phosphatase
b) H_2, Pd

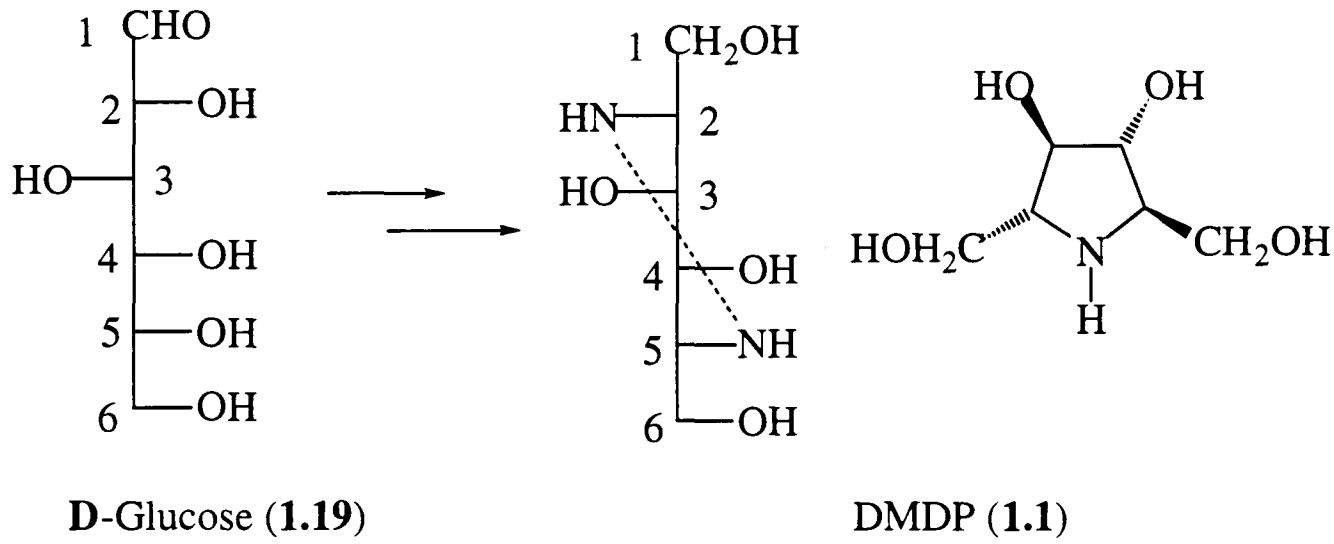
Figure 1.11

As the stereochemistry of the cyclisation is only controlled by the bulk of the top face, then this must lead to some production of epimer at C-5, giving rise to a mixture of products (Figure 1.11).

A stereoselective synthesis of DMDP (**1.1**) starting from a carbohydrate would require the minimal use of protection groups and steric control during the insertion of nitrogen at two carbon centres to give a single diastereomer of the pyrrolidine ring.

1.2 Results and Discussion

When first isolated as a natural product, the structure of DMDP (**1.1**) was determined by NMR.[1] It has six functionalised carbon atoms with four contiguous chiral centres. Common starting materials for previous syntheses have been six carbon sugars, which are commercially available and of high stereochemical purity. For this synthesis **D**-glucose (**1.19**), was chosen. As well as being cheap, the chemistry of **D**-glucose (**1.19**) has been very widely reported and as such, the first steps of any synthesis are likely to have been previously performed and optimised.



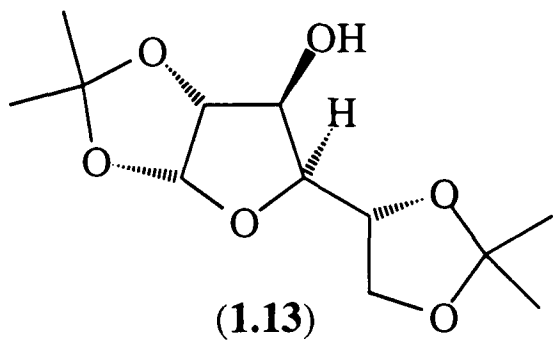
D-Glucose (**1.19**) DMDP (**1.1**)

Figure 1.12

Comparison of **D**-glucose (**1.19**) with DMDP (**1.1**) (Figure 1.12), shows that a synthesis would require the introduction of nitrogen between C-2 and C-5 in order to form the pyrrolidine ring. It would be necessary for this to be carried out with retention of configuration at C-5, possible by two inversions and a single inversion at C-2. Reduction of the aldehyde would then give the required product.

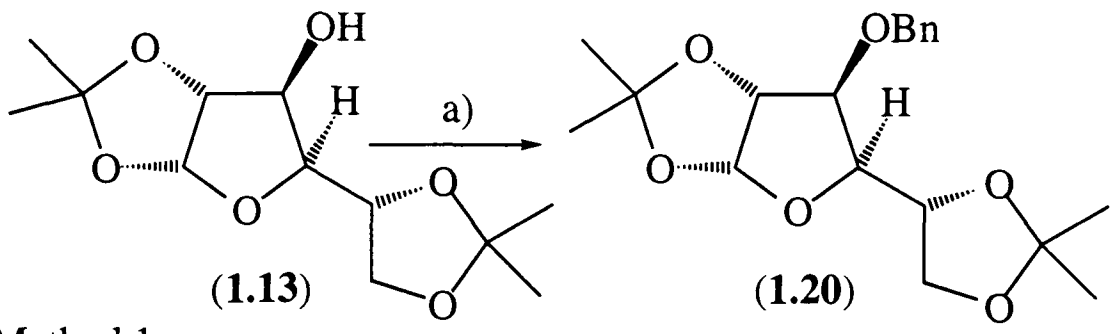
1.2.i Synthesis of DMDP (1.1) from diacetone-D-glucose (1.13)

The choice of starting material for the synthesis was diacetone-α-**D**-glucose (**1.13**). This is a commercially available form of **D**-glucose (**1.19**) and gives initial access to C-3.



(1.13)

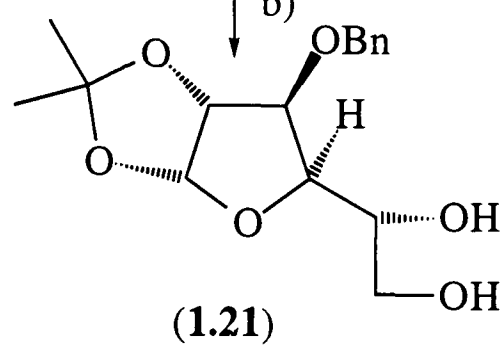
The free C-3 hydroxyl of diacetone glucose (**1.13**) was firstly protected as a benzyl ether. The C-3 carbon functionality is constant during the conversion to DMDP (**1.1**) hence this protection was chosen as it is stable to both acid and base conditions and can easily be removed at the end of the synthesis by hydrogenation. The primary acetonide was then selectively removed by heating to 55 °C in a mixture of acetic acid, water and 1,4-dioxane (Figure 1.14).



(1.13) **(1.20)**

Method 1:
a) NaH, BnBr, Bu_4NI, THF, 60 °C
b) AcOH, H_2O, dioxane, 55 °C

Method 2:
a) KOH, BnBr, THF
b) ion exchange resin (H^+), water, dioxane.



(1.21)

Figure 1.14

This gave the monoacetonide (**1.21**) in a yield of 60% from diacetone glucose (**1.13**). With larger scale reactions (greater than 10 g) a different method was used, due to the hazard and expense of using large quantities of sodium hydride. Powdered potassium hydroxide was stirred with diacetone glucose (**1.13**) and benzyl bromide at room temperature for 18 hours to form the benzyl ether. On the larger scales, it was also found necessary to change the conditions for removal of the primary acetonide. The length of time required to evaporate the acetic acid, was such that the secondary acetonide was being partially hydrolysed. Acidic ion exchange resin was used as an alternative method of hydrolysis.[34] When no diacetonide (**1.20**) was detected, the resin was filtered off, thus removing the acidic conditions quickly and halting any hydrolysis of the secondary acetonide. This gave the monoacetonide (**1.21**) in a yield of 65% from diacetone glucose (**1.13**). Using ion exchange resin for hydrolysis also has the advantage that the resin can be recycled.

With the C-5 and C-6 deprotected, a benzoyl ester (**1.22**) was formed selectively on the terminal carbon by stirring the diol (**1.21**) in dichloromethane with pyridine at -5 °C, then adding benzoyl chloride dropwise, whilst ensuring that the reaction temperature did not rise above 0 °C or selectivity was lost (Figure 1.15).

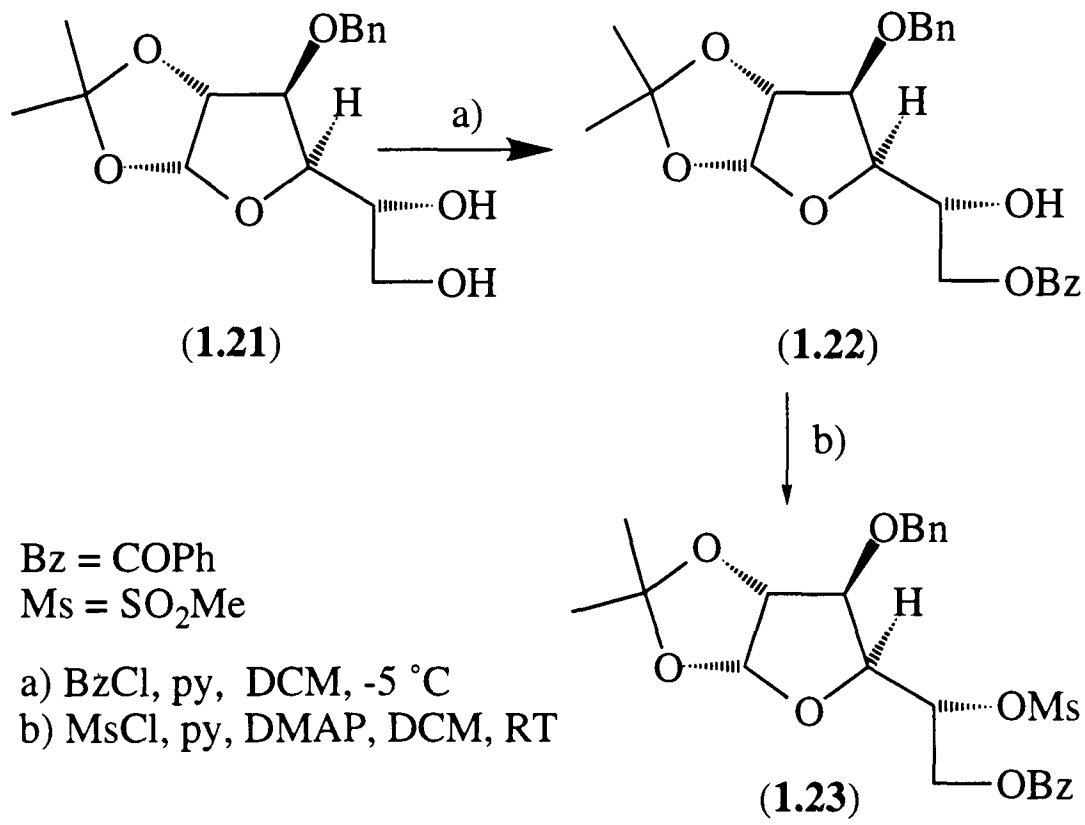


Figure 1.15

When all the starting material had been consumed, methanesulphonyl chloride was added along with a catalytic amount of dimethylaminopyridrine. The solution then was stirred at room temperature for 12 hours giving 6-*O*-benzoyl-3-*O*-benzyl-1,2-*O*-isopropylidene-5-methanesulphonyl-α-**D**-glucofuranose (**1.23**) in 65% yield from the monoacetonide (**1.21**).

The molecule was now set up to carry out the first of the inversions required at C-5. Treatment with sodium methoxide in DMF hydrolysed the benzoyl ester at C-6. The resulting anion then displaced the mesyl ester at C-5 with S_N2 inversion to form the epoxide (**1.24**) in quantitative yield with **L**-idose stereochemistry (Figure 1.16).

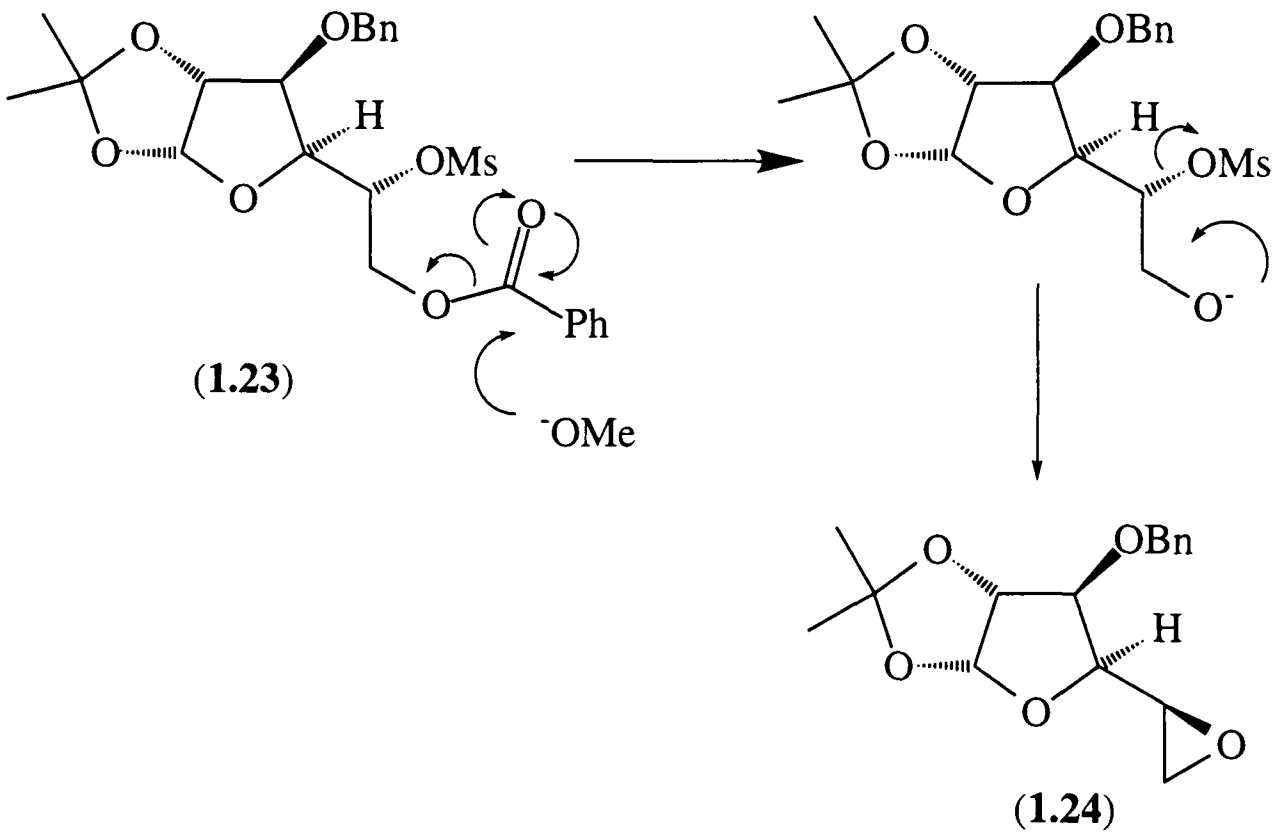
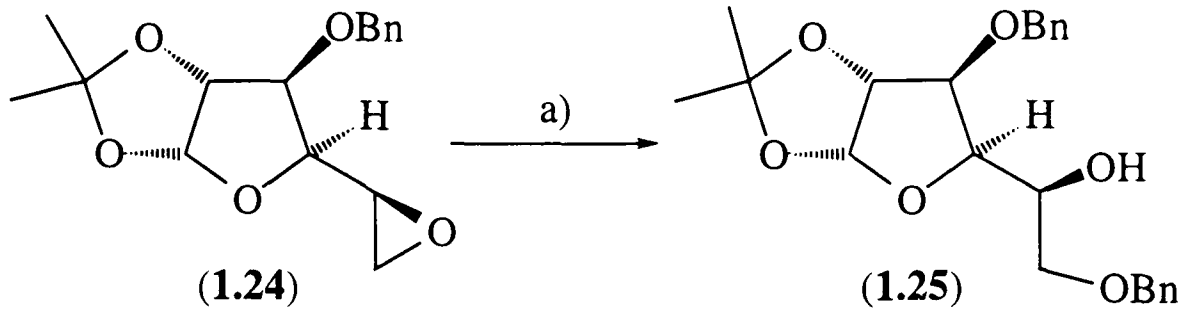


Figure 1.16

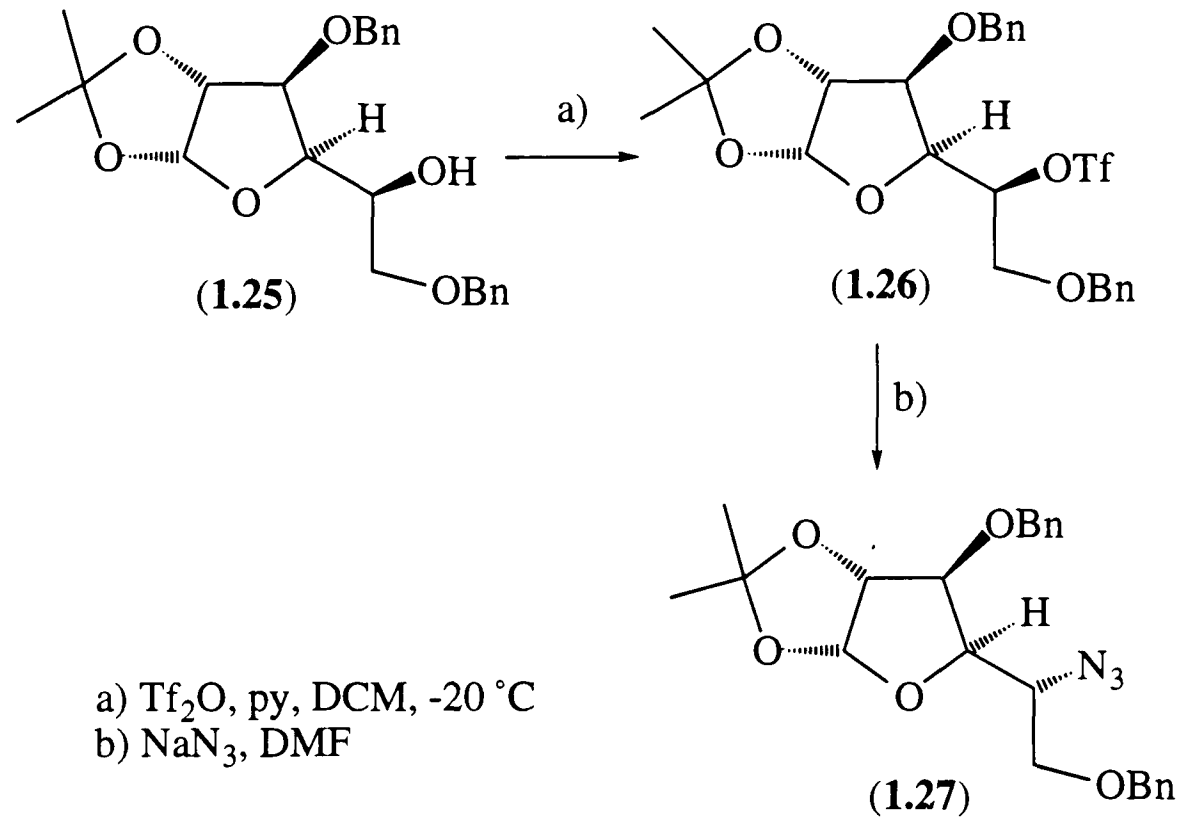
The epoxide (**1.24**) was opened with the sodium salt of benzyl alcohol in DMF. Attack of the benzyl oxide occurred at the primary carbon as this is the least hindered position (Figure 1.17). The opening proceeded in 60% yield giving an hydroxyl at C-5 and benzyl protection at C-6 (**1.25**).



a) NaOBn, DMF, 80 °C

Figure 1.17

With the C-5 being the only hydroxyl not protected, nitrogen could then be introduced. This was achieved using an azido group which with reduction would give a nucleophilic amine. Azide was chosen as it is a good nucleophile in DMF and as such the reaction occurs rapidly with S_N2 chemistry.



a) Tf_2O, py, DCM, -20 °C
b) NaN_3, DMF

Figure 1.18

The hydroxyl was first converted to the trifluoromethanesulphonate ester (triflate) (**1.26**) using triflic anhydride in dichloromethane to form a good leaving group. The triflate ester (**1.26**) formed was not isolated as it was found to be unstable with respect to

elimination. When treated with sodium azide in DMF, the azide displaced the triflate ester with inversion of configuration in a yield of 76% (Figure 1.18). This second inversion at C-5 reformed the original **D**-glucose stereochemistry.

Removal of the secondary acetonide was then achieved removed using acidic methanol. This was formed by reaction of acetyl chloride with anhydrous methanol to ensure an anhydrous solution of hydrochloric acid. During the hydrolysis the stereochemistry at C-1 was scrambled giving a mixture of two methyl glycosides (**28**α, **28**β), in the ratio 1/1.9 with a combined yield of 92%. The methyl glycosides were formed as opposed to the diols due to the anhydrous conditions (Figure 1.19).

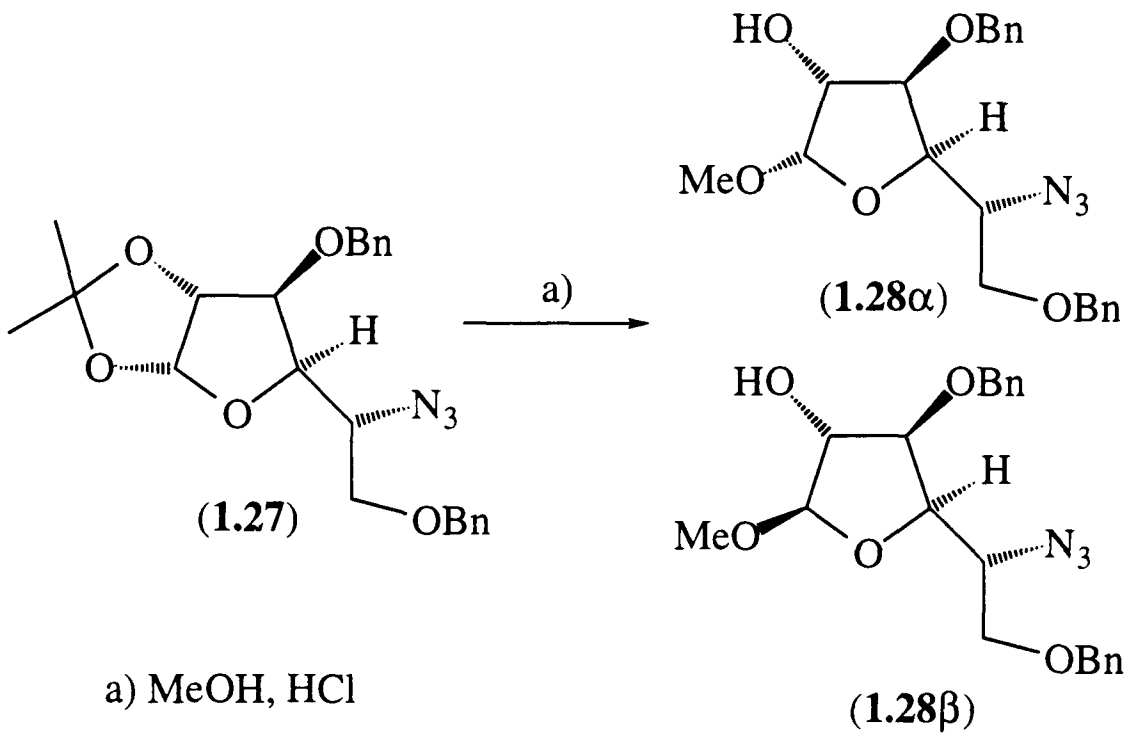


Figure 1.19

The two anomers were separated for characterisation, but as reduction of C-1 was later required, the stereochemistry would then be lost and thus further separation of the methyl glycosides was not necessary.

The system was now set up to cyclise the nitrogen onto C-2. This required inversion of configuration at C-2. The hydroxyl was converted to its triflate ester (**1.29**) by the action of triflic anhydride, to form a good leaving group. The azide at C-5 was then reduced to its amine forming a nucleophilic species which could cyclise by displacement of

the triflate ester at C-2. Reduction of a similar azide (**1.30**) in the synthesis of 6-deoxy-DMDP carried out by Hughes (Figure 1.20),[38] was achieved by hydrogenolysis. However, in this case it was not possible as the primary benzyl ether was removed preferentially.

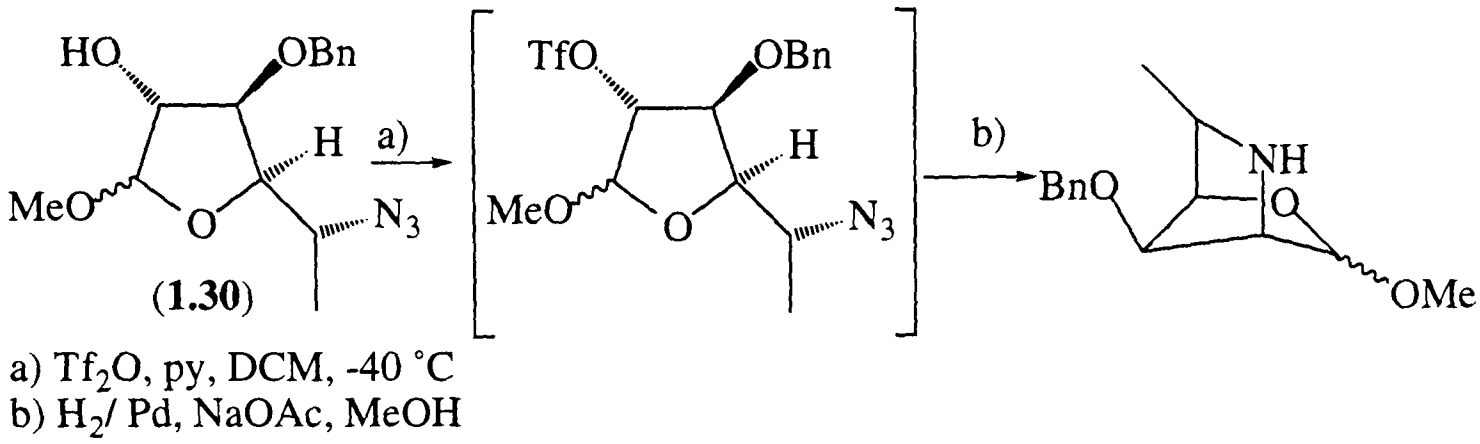


Figure 1.20

It was therefore necessary to reduce the azide (**1.29**) without the use of hydrogen. The chosen method was reduction with triphenyl phosphine.[39] The progress of this reaction was followed by monitoring the infra-red stretch of the azide at 2100 cm^{-1}.

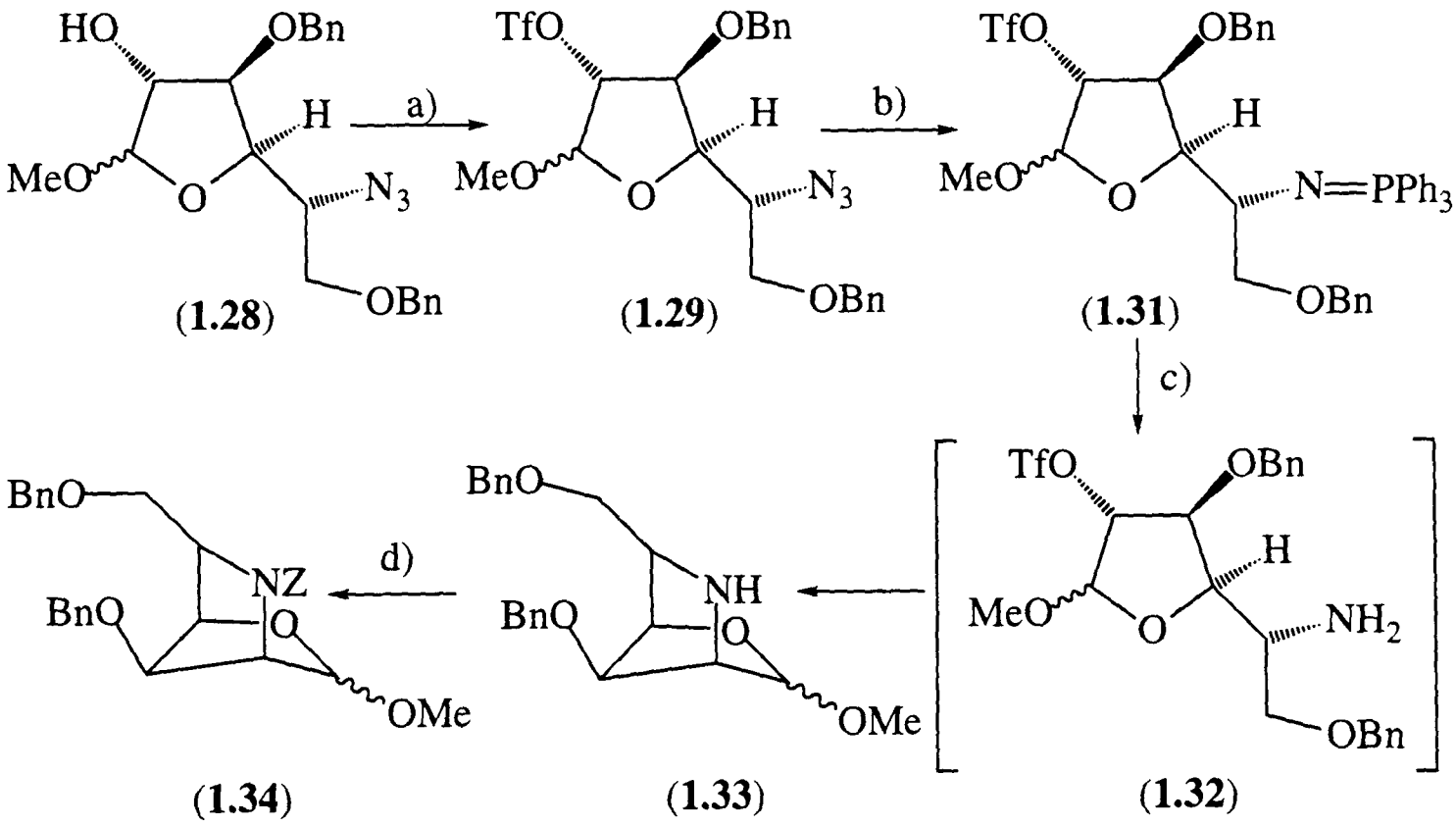


Figure 1.21

This reaction gave the aza-ylid (**1.31**), which when hydrolysed with potassium carbonate furnished the amine (**1.32**). This cyclised under the reaction conditions to form the bicyclic amine (**1.33**) with complete inversion at C-2. For the ease of handling and protection in subsequent steps, the amine was converted to the benzyloxycarbonyl (Z) derivative (**1.34**) (Figure 1.21). Over the four steps the bicycle was formed in 34% yield.

The final conversion required the reduction of the aldehyde to an hydroxyl. C-1 was firstly deprotected by stirring with trifluoroacetic acid in water, then the reduction was carried out using sodium borohydride in ethanol. This gave a 50% yield over the two steps, forming the protected DMDP (**1.35**) (Figure 1.22).

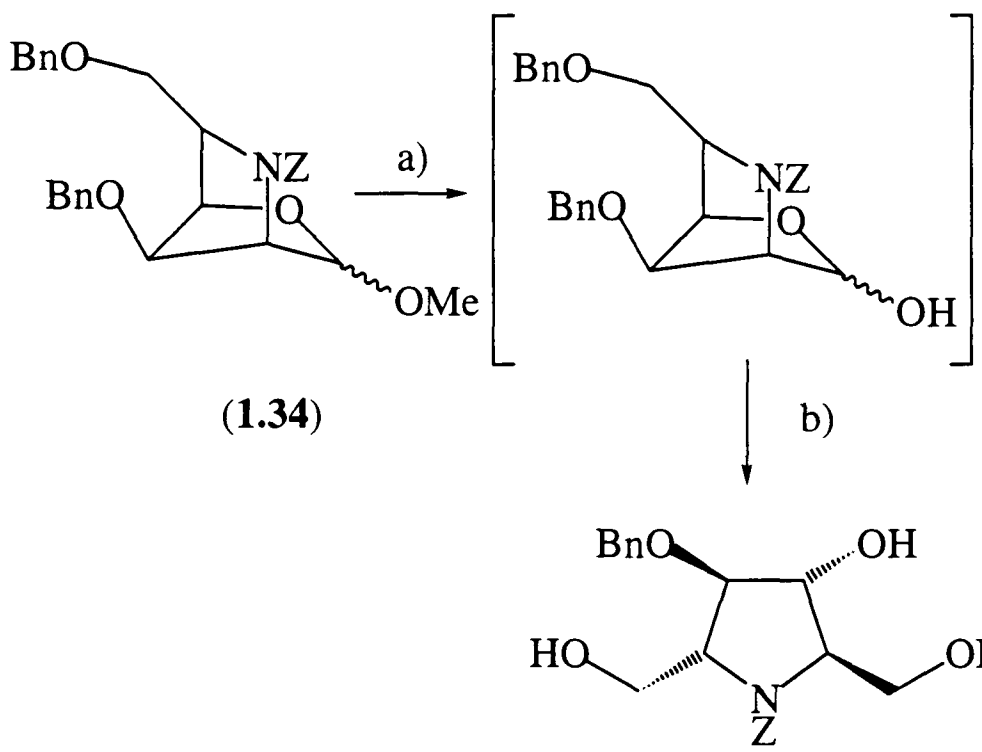
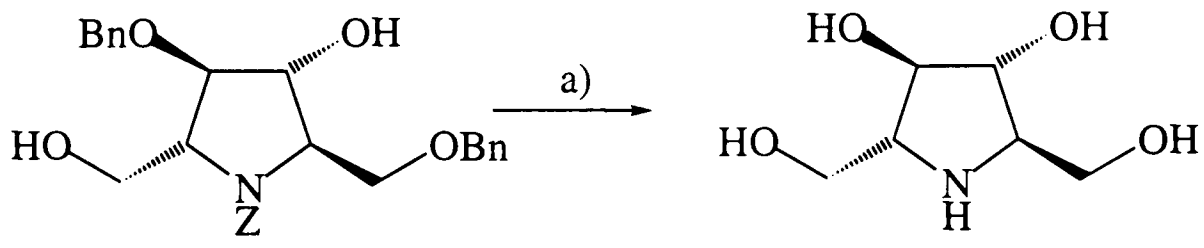


Figure 1.22

The final step of the synthesis was to removal the **Z** protecting group and the two benzyl ethers. This was carried out by hydrogenolysis in acidic ethanol using a catalytic amount of palladium black to give DMDP as the hydrochloride salt. The free amine was released by using basic ion exchange resin to give the deprotected DMDP (**1.1**) in a quantitative yield (Figure 1.23).



(1.35) **(1.1)**

a) H_2/ Pd, EtOH, HCl

Figure 1.23

This synthesis gives DMDP (**1.1**) in 16 steps from diacetone glucose (**1.13**). The overall yield is 3.4% averaging 70% per step. Although this is only a moderate yield, it gives access to compounds that are of use in the synthesis of further potential glycosidase inhibitors. Use of these are discussed in Chapter 2.

The stereochemical outcome of the synthesis was shown by analysis of the ^{13}C NMR spectra of the final product only half the signals that were initially expected appear in the spectra. This is due to the C_2 axis of symmetry which causes half the signals to coincide with each other (Figure 1.24). The proton spectrum does not show this simplification due to long range couplings.

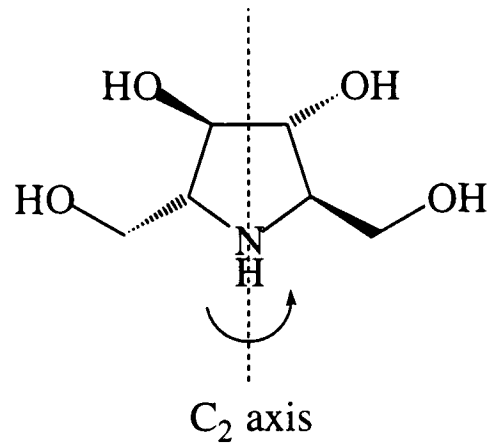


Figure 1.24

The synthesised compound was also found to be identical in all respects to the natural product supplied by R.J. Nash.[40] The proton nmr is shown in Appendix 1 along with that of the isolated natural product.

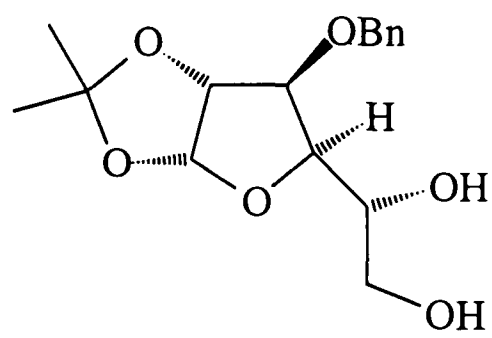
New enzymatic tests that have been carried out have indicated that DMDP has an inhibitory effect towards xylanase.[40] After 10 minutes pre-incubation at 30 °C, Oat spelt xylan in an acetate buffer (pH 4.6) was added as the substrate and incubated at 25 °C for a further 10 minutes. Xylose was detected by a modified neocuproine assay for reducing sugars and the DMDP was found to have a greater than 50% inhibitory effect.

1.3 General Experimental

Melting points were recorded on a Kofler hot block and are uncorrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on Varian Gemini 200 (at 200 MHz) or Bruker AC 200 (at 200 MHz) or Bruker AM 500 (at 500 MHz) spectrometers. ^{13}C Nuclear magnetic resonance spectra (δ_C) were recorded on Varian Gemini 200 (at 50 MHz) or Bruker AM 500 (at 125 MHz) spectrometers. Multiplicities were assigned using the DEPT sequence. All chemical shifts are quoted on the δ-scale using residual solvent as an internal standard. Infra red spectra were recorded in a Perkin-Elmer 1750 IR Fourier Transform spectrometer. Mass spectra were recorded on VG 20-250, ZAB 1F or VG bio-Q spectrometers using desorption chemical ionisation (DCI, NH_3), chemical ionisation (CI, NH_3), fast ion bombardment (FAB) or electrospray ionisation (ESI) as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalysis services of the Dyson Perrins laboratory. Thin layer chromatography was carried out on aluminium sheets coated with 60 F_{254} silica or glass plates coated with silica blend GF_{254}. Plates were developed using a spray of 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid or 0.5% ninhydrin in methanol. Flash column chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures; dichloromethane was refluxed over and distilled from calcium hydride; pyridine was distilled from calcium hydride and stored over potassium hydroxide; tetrahydrofuran was distilled, under nitrogen, from a solution dried in the presence of benzophenone; diethyl ether was distilled, under nitrogen, from a solution dried in the presence of benzophenone; *N,N*-dimethylformamide was distilled under reduced pressure from calcium hydride or purchased dry from the Aldrich Chemical Company in Sure-Seal™ bottles; hexane was distilled at 68 °C before use to remove involatile fractions.

1.4 Experimental

3-*O*-Benzyl-1, 2-*O*-isopropylidene-α-**D**-glucofuranose (**1.21**)



Method 1

A dispersion of 60% sodium hydride in oil (308 mg, 7.7 mmol) was stirred in tetrahydrofuran (10 ml). 1, 2: 5, 6-Di-*O*-isopropylidene-α-**D**-glucofuranose (**1.13**) (2.00 g, 7.7 mmol) and tetrabutyl ammonium iodide (550 mg, 1.5 mmol) was added to the suspension and stirred until the evolution of hydrogen ceased. Benzyl bromide (1.4 ml, 11.5 mol) was then added dropwise over 30 minutes and the mixture was heated to 60 °C. After 2 hours, tlc analysis (ethyl acetate/ hexane 1/1) showed no starting material (R_f 0.5) and one product (R_f 0.8). After cooling, methanol (2 ml) was added and stirred for 30 minutes. The suspension was filtered thorough Celite and the solvent was removed from the filtrate *in vacuo* to afford 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**1.20**) contaminated with benzyl alcohol (3.3 g). A small aliquot was purified by flash column chromatography (ethyl acetate/ hexane 1/3) to afford 3-*O*-benzyl-1, 2: 5, 6-di-*O*-isopropylidene-α-**D**-glucofuranose (**1.20**) as a clear oil.

The crude 3-*O*-benzyl-1, 2: 5, 6-di-*O*-isopropylidene-α-**D**-glucofuranose (**1.20**) was stirred at 55 °C in acetic acid (50 ml), water (50 ml) and 1,4-dioxane (50 ml) for 1 hour, when tlc analysis (ethyl acetate/ hexane 1/1) showed no starting material (R_f 0.8) and one product (R_f 0.3). The solvent was removed *in vacuo* and coevaporated with

toluene (3x 50 ml). The residue was purified by dry flash chromatography with solvent gradient (ethyl acetate/ hexane 1/4 to 1/1) to afford 3-*O*-benzyl-1,2-*O*-isopropylidene-α-**D**-glucofuranose (**1.21**) (1.4 g, 60%) as a clear oil.

Method 2

1, 2: 5, 6-Di-*O*-isopropylidene-α-**D**-glucofuranose (**1.13**) (40.10 g, 0.15 mol) and powdered potassium hydroxide (16.8 g, 0.3 mol) were stirred in tetrahydrofuran (160 ml). Benzyl bromide (35.6 ml, 0.3 mol) was then added dropwise over 30 minutes and the mixture stirred at room temperature. After 18 hours, tlc analysis (ethyl acetate/ hexane 1/1) showed no starting material (R_f 0.5) and one product (R_f 0.8). Methanol (20 ml) was then added and stirred for 30 minutes. The suspension was filtered thorough Celite and the solvent was removed from the filtrate *in vacuo* to afford 3-*O*-benzyl-1, 2: 5, 6-di-*O*-isopropylidene-α-**D**-glucofuranose (**1.20**) contaminated with benzyl alcohol.

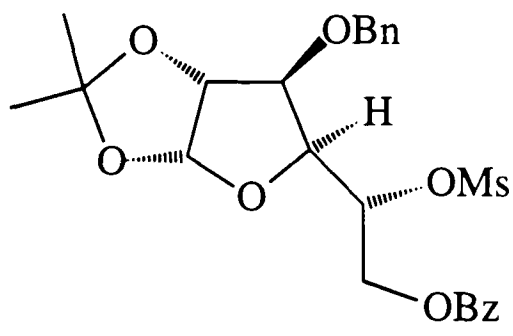
The crude 3-*O*-benzyl-1, 2: 5, 6-di-*O*-isopropylidene-α-**D**-glucofuranose (**1.20**) was stirred at 55 °C in water (50 ml), 1,4-dioxane (50 ml) and Amberlite acidic ion exchange resin (IR120, 10 g) for 1 hour, when tlc analysis (ethyl acetate/ hexane 1/1) showed no starting material (R_f 0.8) and one product (R_f 0.3). The mixture was filtered through Celite, the solvent was removed *in vacuo* and coevaporated with toluene (3x 50 ml). The residue was purified by dry flash chromatography with solvent gradient (ethyl acetate/ hexane 1/4 to 1/1) to afford 3-*O*-benzyl-1,2-*O*-isopropylidene-α-**D**-glucofuranose (**1.21**) (30.2 g, 65%) as a clear oil.

3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**1.20**):- $[\alpha]_D^{21}$ -25.0 (c 0.72, $CHCl_3$), [lit.[41] $[\alpha]_{578}^{22}$ -25.7 (c 1.0, $CHCl_3$)]. δ_H (500 MHz, $CDCl_3$); 1.32 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.51 (3H, s, CH_3), 4.02 (1H, dd $J_{6,5}$ 5.9 $J_{6,6'}$ 8.6 Hz, H-6), 4.04 (1H, d $J_{3,4}$ 3.1, H-3), 4.13 (1H, dd $J_{6',5}$ 6.2 $J_{6',6}$ 8.6 Hz, H-6'),

4.17 (1H, dd $J_{4,3}$ 3.1 $J_{4,5}$ 7.8 Hz, H-4), 4.39 (1H, dt $J_{5,4}$ 7.8 $J_{5,6}$ 6.1 Hz, H-5), 4.60 (1H, d $J_{2,1}$ 3.7 Hz, H-2), 4.65, 4.69 (2H, 2x d J_{AB} 11.8 Hz, $PhCH_2O$), 5.91 (1H, d $J_{1,2}$ 3.7 Hz, H-1), 7.27- 7.37 (5H, m, Ph). δ_C (50 MHz, $CDCl_3$); 25.4, 26.2, 26.7, 26.8 (4x q, 2x $C(\underline{C}H_3)_2$), 67.4, 72.3 (2x t, 2x CH_2), 72.5, 81.3, 91.6, 82.6 (4x d, C-2, C-3, C-4, C-5), 105.3 (d, C-1), 108.9, 111.8 (2x s, 2x $\underline{C}(Me)_2$), 127.6, 127.8, 128.4 (3x d, ArCH), 137.6 (s, ArC).

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-**D**-glucofuranose (**1.21**):- $[\alpha]_D^{21}$ -38.4 (c 1.25, $CHCl_3$), [lit.[42] $[\alpha]_D^{20}$ -50.8 (c 1.24, $CHCl_3$)]. ν_{MAX} (thin film); 3436 (br, s, OH) cm^{-1}. δ_H (200 MHz, $CDCl_3$); 1.32 (3H, s, CH_3), 1.49 (3H, s, CH_3), 2.66 (2H, br s, 2x OH), 3.68 (1H, dd $J_{6,5}$ 5.5 $J_{6,6'}$ 11.5 Hz, H-6), 3.80 (1H, dd $J_{6',5}$ 3.0 $J_{6',6}$ 11.5 Hz, H-6'), 4.03 (1H, dt $J_{5,4}$ 3.0 $J_{5,6'}$ 3.0 $J_{5,6}$ 5.5 Hz, H-5), 4.10 (1H, s, H-3), 4.15 (1H, d $J_{4,5}$ 3.0 Hz, H-4), 4.56, 4.73 (2H, 2x d J_{AB} 12.0 Hz, $PhCH_2O$), 4.62 (1H, d $J_{2,1}$ 4.0 Hz, H-2), 5.93 (1H, d $J_{1,2}$ 4.0 Hz, H-1), 7.35 (5H, s, Ar). δ_C (50 MHz, $CDCl_3$); 26.5, 27.0 (2x q, $C(CH_3)_2$), 64.5, 72.0 (2x t, 2x CH_2), 69.0, 80.0, 82.0, 82.5 (4x d, C-2, C-3, C-4, C-5), 105.0 (d, C-1), 112.0 (s, $C(Me)_2$), 128.0, 128.5, 129.0 (3x d, ArCH), 137.5 (s, ArC).

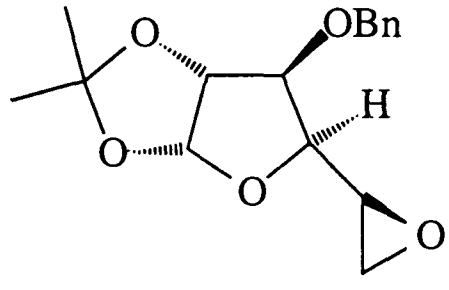
6-*O*-Benzoyl-3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-methanesulphonyl-α-**D**-glucofuranose (**1.23**)



Dry pyridine (48.5 ml, 0.6 mol) was added to a solution of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-**D**-glucofuranose (**1.21**) (30.86 g, 0.1 mol) in dichloromethane (450 ml). Benzoyl chloride (12.8 ml, 0.13 mol) was added dropwise over 30 minutes at -5 °C. The solution was stirred at -5 °C for 1.5 hours when tlc analysis (diethyl ether/ hexane 2/3) show no starting material (baseline) and one product (R_f 0.3). 4-(*N*,*N*-Dimethylamino)pyridine (0.61 g, 5.0 mmol) and methanesulphonyl chloride (11.6 ml, 0.15 mol) were added and the solution stirred at room temperature for 12 hours. Tlc analysis (ethyl acetate/ hexane 1/1) showed no monobenzoylated material (R_f 0.3) and one product (R_f 0.1). The solution was then stirred with methanol (50 ml) for 30 minutes and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate (500 ml), washed with dilute hydrochloric acid (2M, 250 ml), water (2x 250 ml), brine (250 ml), dried (magnesium sulphate) and the solvent removed *in vacuo*. Crystallisation from diethyl ether/ hexane afforded 6-*O*-benzoyl-3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-methanesulphonyl-α-**D**-glucofuranose (**1.23**) (31.6 g, 65%) as a white crystalline solid. M.p.(diethyl ether/ hexane); 93.5- 94.0 °C, [lit.[43] 89-90 °C]. $[\alpha]_D^{21}$ -10.3 (c 1.0, $CHCl_3$), [lit.[43] $[\alpha]_D^{20}$ -9.4 (c 3.3, $CHCl_3$)]. ν_{MAX} (KBr); 1724 (s, C=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 1.34 (3H, s, CH_3), 1.52 (3H, s, CH_3), 3.00 (3H, s, SCH_3), 4.17 (1H, d $J_{3,4}$ 3.1 Hz, H-3), 4.50 (1H, dd $J_{4,3}$ 3.1 $J_{4,5}$ 8.4 Hz, H-4), 4.53 (1H, dd $J_{6,5}$ 6.1 $J_{6,6'}$ 12.8 Hz, H-6), 4.63, 4.72 (2H, 2x d J_{AB} 11.0 Hz, $PhCH_2O$), 4.64 (1H, d $J_{2,1}$ 3.6 Hz, H-2), 4.95 (1H, dd $J_{6',5}$ 2.0 $J_{6',6}$ 12.8

Hz, H-6'), 5.44 (1H, ddd $J_{5,6'}$ 2.0 $J_{5,6}$ 6.1 $J_{5,4}$ 7.7 Hz, H-5), 5.94 (1H, d $J_{1,2}$ 3.6 Hz, H-1), 7.29- 7.59 (8H, m, Ar), 8.09, 8.10 (2H, 2x s, Ar). δ_C (50 MHz, $CDCl_3$); 26.5, 27.0 ($C(\underline{C}H_3)_2$), 39.5 (q, SCH_3), 64.0, 73.0 (2x t, CH_2), 75.5, 78.0, 82.0, 82.5 (4x d, C-2, C-3, C-4, C-5), 105.5 (d, C-1), 112.5 (s, $\underline{C}(Me)_2$), 129.0, 129.5, 130.0, 133.0 (4x d, ArCH), 137.5 (s, ArC), 166.5 (s, C=O).

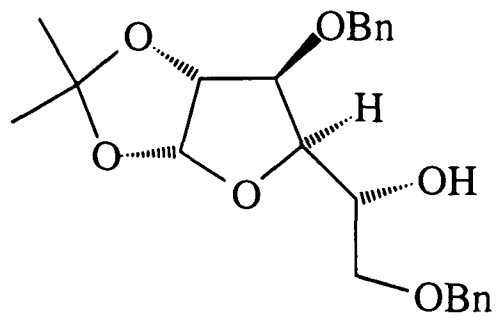
5, 6-Anhydro-3-*O*-benzyl-1, 2-*O*-isopropylidene-β-**L**-idofuranose (**1.24**)



To a solution of 6-*O*-benzoyl-3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-methane sulphonyl-α-**D**-glucofuranose (**1.23**) (29.20 g, 60 mmol) in *N,N*-dimethylformamide (100 ml) was added sodium methoxide (6.48 g, 120 mmol). The solution was stirred for 3 hours when tlc analysis (diethyl ether/ hexane 1/1) showed no starting material (R_f 0.2) and one product (R_f 0.3). The solvent was removed *in vacuo* and the residue redissolved in diethyl ether (100 ml). The solution was washed with dilute hydrochloric acid (2M, 50 ml), water (2x 100 ml), the aqueous layers were combined and washed with diethyl ether (2x 100 ml). The combined organic layers were washed with brine (100 ml), dried (magnesium sulphate) and the solvent removed *in vacuo* to afford 5,6-anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene-β-**L**-idofuranose (**1.24**) (17.5 g, quantitative) as a clear oil. $[\alpha]_D^{25}$ -73.0 (c 0.65, $CHCl_3$), [lit.[44] $[\alpha]_D^{20}$ -70.5 (c 2.60, $CHCl_3$)]. δ_H (200 MHz, $CDCl_3$); 1.32 (3H, s, CH_3), 1.45 (3H, s, CH_3), 2.54 (1H, dd $J_{6,5}$ 3.0 $J_{6,6'}$ 5.0 Hz, H-6), 2.76 (1H, t *J* 4.5 Hz, H-6'), 3.28 (1H, ddd $J_{5,6'}$ 4.5 $J_{5,6}$ 2.5 $J_{5,4}$ 6.5 Hz, H-5), 3.81 (1H, dd $J_{4,3}$ 3.5 $J_{4,5}$ 6.0 Hz, H-4), 3.98 (1H, d $J_{3,4}$ 3.5 Hz, H-3), 4.52, 4.75 (2H, 2x d J_{AB} 12.0 Hz,

$PhCH_2O$), 4.65 (1H, d $J_{2,1}$ 4.0 Hz, H-2), 6.00 (1H, d $J_{1,2}$ 4.0 Hz, H-1), 7.34 (5H, s, ArH). δ_C (50 MHz, $CDCl_3$); 26.3, 26.8 (2x q, $C(\underline{C}H_3)_2$), 43.1, 71.9 (2x t, 2x CH_2), 50.2, 82.1, 82.4, 82.6 (4x d, C-2, C-3, C-4, C-5), 105.4 (d, C-1), 111.9 (s, $\underline{C}(Me)_2$), 127.7, 128.0, 128.5 (3x d, ArCH), 137.3 (s, ArC).

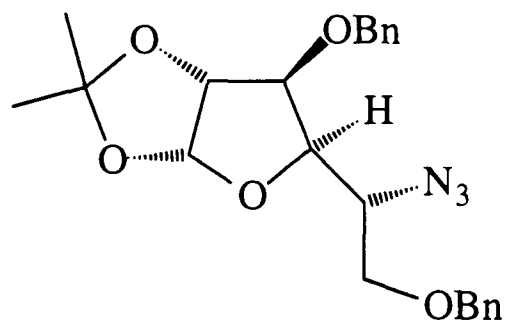
3, 6 Di-*O*-benzyl-1, 2-*O*-isopropylidene-β-**L**-idofuranose (**1.25**)



Benzyl alcohol (4.65 ml, 45 mmol) was added dropwise to a suspension of 60% sodium hydride in oil (1.6 g, 40 mmol) in *N,N*-dimethylformamide (80 ml), the mixture was stirred until the evolution of hydrogen ceased. To this solution 5,6-anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene-β-**L**-idofuranose (**1.24**) (11.5 g, 40 mmol) was added at room temperature. The mixture was stirred at 80 °C for 1 hour when tlc analysis (ether/ hexane 2/1) showed no starting material (R_f 0.5) and one product (R_f 0.2). After cooling, water (10 ml) was added and stirred for 10 minutes. The mixture was concentrated *in vacuo,* the residue was redissolved in diethyl ether (200 ml), washed with water (2x 100 ml), brine (100 ml), then dried (magnesium sulphate) and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (diethyl ether/ hexane 2/1). Recrystallisation (diethyl ether) afforded 3,6 di-*O*-benzyl-1,2-*O*-isopropylidene-β-**L**-idofuranose (**1.25**) (8.92 g, 60%) as a white crystalline solid. M.p.(diethyl ether); 73-74 °C, [lit. [44] 74 °C]. $[\alpha]_D^{21}$ -46.9 (c 1.1, $CHCl_3$), [lit.[44] $[\alpha]_D^{20}$ -48.9 (c 4.4, $CHCl_3$)]. ν_{MAX} (thin film); 3503 (br, OH) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 1.29 (3H, s, CH_3), 1.45 (3H, s, CH_3), 3.48 (1H, dd $J_{6,5}$ 9.7 $J_{6,6'}$ 17.6 Hz, H-6), 3.49 (1H, dd $J_{6',5}$ 9.7 $J_{6',6}$ 17.0 Hz, H-

6'), 3.91 (1H, d $J_{3,4}$ 3.6 Hz, H-3), 4.13 (1H, dd $J_{5,4}$ 5.3 $J_{5,6}$ 10.6 Hz, H-5), 4.24 (1H, dd $J_{4,3}$ 3.6 $J_{4,5}$ 5.2 Hz, H-4), 4.33, 4.61 (2H, 2x d J_{AB} 11.7 Hz, $PhCH_2O$), 4.44, 4.50 (2H, 2x d J_{AB} 12.0 Hz, $PhCH_2O$), 4.58 (1H, d $J_{2,1}$ 3.9 Hz, H-2), 5.95 (1H, d $J_{1,2}$ 3.9 Hz, H-1), 7.22- 7.32 (10H, m, ArH). δ_C (50 MHz, $CDCl_3$); 26.8, 26.4 (2x q, $C(\underline{C}H_3)_2$), 69.4, 79.8, 82.3, 82.9 (4x d, C-2, C-3, C-4, C-5), 104.9 (d, C-1), 70.7, 71.8, 73.4 (3x t, 3x CH_2), 111.9 (s, $\underline{C}(Me)_2$), 127.7-128.6 (6x d, ArCH), 136.8, 138.0 (2x s, 2x ArC).

5-Azido-5-deoxy-3,6-*O*-dibenzyl-1,2-*O*-isopropylidene-α-**D**-glucofuranose (**1.27**)



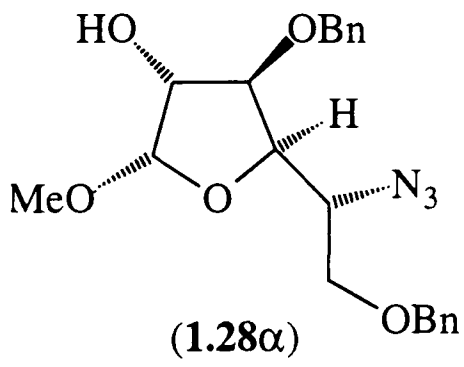
To a solution of 3,6 di-*O*-benzyl-1,2-*O*-isopropylidene-β-**L**-idofuranose (**1.25**) (8.92 g, 22.5 mmol) in dichloromethane (250 ml) was added pyridine (5.4 ml, 66.9 mmol), the solution was then cooled to -20 °C and triflic anhydride (5.3 ml, 31.5 mmol) was added dropwise over 5 minutes. After stirring for 30 minutes tlc analysis (ethyl acetate/ hexane 2/1) showed no starting material (R_f 0.2) and one product (R_f 0.5). Water (1 ml) was added and the solution was stirred for 10 minutes at room temperature. The solution was then filtered through a plug of silica and magnesium sulphate. The solvent was removed *in vacuo* to afford the crude triflate ester (**1.26**).

The crude triflate ester (**1.26**) was dissolved in *N*,*N*-dimethylformamide (100 ml) and to this was added sodium azide (2.89 g, 44.6 mmol). The solution was stirred for 12 hours when tlc analysis (ethyl acetate/ hexane 2/1) showed no triflate ester (R_f 0.5) and one product (R_f 0.7). The solvent was removed *in vacuo*. The residue was redissolved in dichloromethane (100 ml), washed with brine (2x 50 ml), water (50 ml) dried (magnesium

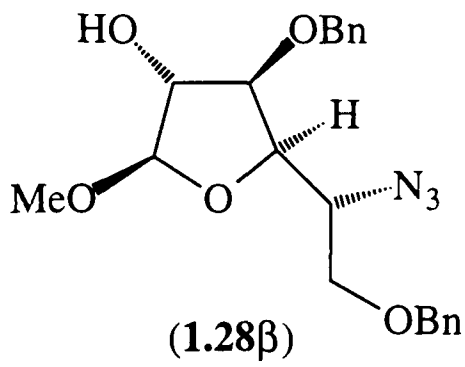
sulphate) and the solvent removed *in vacuo*, the residue was purified by flash column chromatography (diethyl ether/ hexane 1/3) to afford 5-azido-5-deoxy-3,6-*O*-dibenzyl-1,2-*O*-isopropylidene-α-**D**-glucofuranose (**1.27**) (7.24 g, 76%) as a white crystalline solid. M.p.(diethyl ether/ hexane); 68-69 °C, [lit.[44] 71-72 °C]. $[\alpha]_D^{22}$ -31.7 (c 3.9, $CHCl_3$), [lit.[44] $[\alpha]_D^{20}$ -36.9 (c 2.9, $CHCl_3$)]. ν_{MAX} (thin film); 2098 (N_3) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 1.33 (3H, s, CH_3), 1.48 (3H, s, CH_3), 3.65 (1H, dd $J_{6,5}$ 7.4 $J_{6,6'}$ 10.1 Hz, H-6), 3.92 (1H, dd $J_{6',5}$ 2.3 $J_{6',6}$ 10.1 Hz, H-6'), 4.02 (1H, ddd $J_{5,6'}$ 2.3 $J_{5,4}$ 7.4 $J_{5,6}$ 10.0 Hz, H-5), 4.06 (1H, dd $J_{4,3}$ 3.0 $J_{4,5}$ 7.3 Hz, H-4), 4.09 (1H, d $J_{3,4}$ 3.0 Hz, H-3), 4.62, 4.69 (2H, 2x d J_{AB} 11.4 Hz, $PhCH_2O$), 4.63 (1H, d $J_{2,1}$ 3.8 Hz, H-2), 5.90 (1H, d $J_{1,2}$ 3.7 Hz, H-1), 7.27- 7.40 (10H, m, ArH). δ_C (50 MHz, $CDCl_3$); 26.2, 26.7 (2x q, $C(\underline{C}H_3)_2$), 58.9, 78.5, 81.5, 81.6 (4x d, C-2, C-3, C-4, C-5), 71.0, 72.2, 73.4 (3x t, 3x CH_2), 105.3 (d, C-1), 111.9 (s, $\underline{C}(Me)_2$), 127.5, 127.6, 127.9, 128.0, 128.3, 128.5 (6x d, ArCH), 137.1, 138.2 (2x s, 2x ArC).

Methyl 5-azido-5-deoxy-3,6-di-*O*-benzyl-**D**-α-glucofuranoside (**1.28α**) and

methyl 5-azido-5-deoxy-3,6-di-*O*-benzyl-**D**-β-glucofuranoside (**1.28β**)



(**1.28α**)



(**1.28β**)

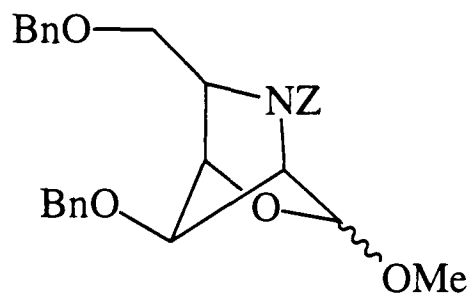
A solution of approximately 2M hydrochloric acid in methanol was prepared by addition of acetyl chloride (7.0 ml, 0.1 mol) to dry methanol (50 ml) with cooling. 5-Azido-5-deoxy-3,6-*O*-dibenzyl-1,2-*O*-isopropylidene-α-**D**-glucofuranose (**1.27**) (7.24 g, 17 mmol) was then added to the solution of hydrochloric acid in methanol. After

stirring at room temperature for 24 hours, tlc analysis (diethyl ether/ hexane 1/1) showed no starting material (R_f 0.7) and two products (R_f 0.2, 0.3). The solvent was removed *in vacuo* and the two products separated by flash column chromatography (solvent gradient diethyl ether /hexane 1/4 to 1/1) to afford methyl 5-azido-5-deoxy-3,6-di-*O*-benzyl-α-**D**-glucofuranoside (**1.28**α) (4.16 g, 62%) and methyl 5-azido-5-deoxy-3,6-di-*O*-benzyl-β-**D**-glucofuranoside (**1.28**β) (2.08 g, 30%).

Methyl 5-azido-5-deoxy-3,6-di-*O*-benzyl-α-**D**-glucofuranoside (**1.28**α):- $[\alpha]_D^{23}$ +38.1 (c 5.85, $CHCl_3$), [lit.[43] $[\alpha]_D^{20}$ +36.8 (c 0.95, $CHCl_3$)]. ν_{MAX} (thin film); 3525 (br s, OH), 2100 (m, N_3) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 3.49 (3H, s, CH_3), 3.70, (1H, dd $J_{6,5}$ 6.4 $J_{6,6'}$ 10.1 Hz, H-6), 3.92 (1H, dd $J_{6',5}$ 2.5 $J_{6',6}$ 10.1 Hz, H-6'), 4.02 (1H, dd $J_{3,4}$ 2.0 $J_{3,2}$ 9.4 Hz, H-3), 4.05 (1H, dd $J_{4,3}$ 2.0 $J_{4,5}$ 4.4 Hz, H-4), 4.17 (1H, dd $J_{2,1}$ 4.4 $J_{2,3}$ 9.1, H-2), (1H, dt $J_{5,6}$ 1.8 $J_{5,4}$ 4.6 Hz, H-5), 4.45, 4.66 (2H, 2x d J_{AB} 12.0 Hz, $PhCH_2O$), 4.66, 4.76 (2H, 2x d J_{AB} 11.5 Hz, $PhCH_2O$), 5.05 (1H, d $J_{1,2}$ 4.4 Hz, H-1), 7.31- 7.41 (10H, m, ArH). δ_C (50 MHz, $CDCl_3$); 56.0 (q, CH_3), 59.7, 75.9, 77.3, 83.5 (4x d, C-2, C-3, C-4, C-5), 70.9, 71.9, 73.4 (3x t, 3x CH_2), 102.6 (d, C-1), 127.6, 127.7, 128.0, 128.5 (4x d, ArCH), 137.6, 138.1 (2x s, 2x ArC).

Methyl 5-azido-5-deoxy-3,6-di-*O*-benzyl-β-**D**-glucofuranoside (**1.28**β):- $[\alpha]_D^{22}$ -44.3 (c 1.4, $CHCl_3$), [lit.[43] $[\alpha]_D^{20}$ -41.2 (c 1.25, $CHCl_3$)]. ν_{MAX} (thin film); 3525 (br s, OH), 2100 (m, N_3) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 3.35 (3H, s, CH_3), 3.69 (1H, dd $J_{6,5}$ 7.2 $J_{6,6'}$ 10.2 Hz, H-6), 3.92 (1H, dd $J_{6',5}$ 2.5 $J_{6',6}$ 9.8 Hz, H-6'), 3.93 (1H, d $J_{3,2}$ 5.1 Hz, H-3), 4.05 (1H, ddd $J_{5,6'}$ 2.5 $J_{5,6}$ 7.2 $J_{5,4}$ 9.7 Hz, H-5), 4.17 (1H, dd $J_{4,3}$ 5.0 $J_{4,5}$ 9.7 Hz, H-4), 4.21 (1H, d $J_{2,3}$ 3.4 Hz, H-2), 4.63 (2H, s, $PhCH_2O$), 4.64, 4.69 (2H, 2x d J_{AB} 11.8 Hz, $PhCH_2O$), 4.78 (1H, s, H-1), 7.28- 7.41 (10H, m, ArH). δ_C (50 MHz, $CDCl_3$); 55.91 (q, CH_3), 60.2, 78.1, 79.6, 82.6 (4x d, C-2, C-3, C-4, C-5), 71.0, 72.3, 73.4 (3x t, 3x CH_2), 110.1 (d, C-1), 127.6, 127.7, 127.9, 128.1, 128.4 (5x d, 5x ArCH), 137.5, 138.0 (2x s, 2x ArC).

N-Benzyloxycarbonyl (methyl 3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-manno furanoside (**1.34**)

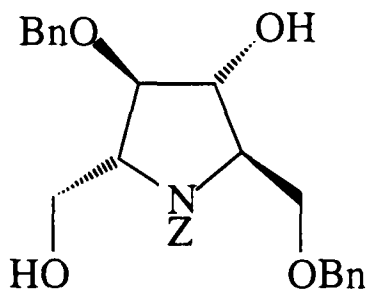


To a solution of methyl 5-azido-5-deoxy-3,6-di-*O*-benzyl-**D**-glucofuranoside (**1.28**) (6.25 g, 15.6 mmol) in dichloromethane (100 ml) was added dry pyridine (3.78 ml, 46.8 mmol) and the solution was cooled to -10 °C. Triflic anhydride (3.93 ml, 23.6 mmol) was added dropwise over 10 minutes and the solution stirred for a further 20 minutes when tlc analysis (diethyl ether/ hexane 2/1) showed no starting material (R_f 0.4) and one product (R_f 0.8). Water (1 ml) was added and the solution was stirred for 10 minutes at room temperature. The solution was then filtered through a plug of silica and magnesium sulphate to afford crude methyl 5-azido-5-deoxy-3,6-di-*O*-benzyl-2-*O*-trifluoromethanesulphonate-**D**-glucofuranoside (**1.29**).

The crude triflate ester (**1.29**) was dissolved in dichloromethane (100 ml) and triphenyl phosphine (8.17 g, 31.2 mmol) was added, the solution was heated to 50 °C for 1 hour when IR analysis showed no azide stretch (2100 cm^{-1}). The solution was cooled to room temperature and saturated aqueous potassium carbonate (20 ml) was added. This was vigorously stirred for a further 12 hours. The organic and aqueous layers were separated. The aqueous layer was washed with dichloromethane (2x 20 ml), the combined organic layers were washed with brine (30 ml), dried (magnesium sulphate) and the solvent removed *in vacuo*.

The crude amine (**1.33**) was stirred vigorously in a suspension of diethyl ether (100 ml) and saturated aqueous sodium bicarbonate (100 ml). To this suspension was added benzyl chloroformate (2.4 ml, 17.16 mmol) and stirred for 16 hours. The organic and aqueous layers were separated. The aqueous layer was washed with diethyl ether (2x 50 ml), the combined organic layers were washed with brine (50 ml), dried (magnesium sulphate) and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (diethyl ether/ hexane, 1/3) to afford *N*-benzyloxycarbonyl (methyl 3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannofuranoside) (**1.34**) (2.61 g, 34%) as a clear oil.. ν_{MAX} (thin film, $CHCl_3$); 1707 (C=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 3.35 (3H, s, CH_3), 3.40 (3H, s, CH_3), 3.81 (1H, dd *J* 3.8, 8.8 Hz), 3.86 (1H, dd *J* 3.7, 13.1 Hz), 3.9- 3.97 (2H, m), 4.00 (1H, d *J* 4.8 Hz), 4.24 (1H, s), 4.37 (1H, s), 4.38 (2H, d *J* 11.8 Hz), 4.42 (2H, d *J* 12.0 Hz), 4.50 (2H, d *J* 12.1 Hz), 4.51- 4.55 (2H, m), 4.56 (2H, s), 4.58 (1H, d *J* 2.5 Hz), 4.65 (1H, d *J* 11.8 Hz), 4.66 (1H, s), 5.24, 5.16 (2H, 2x d J_{AB} 12.4 Hz, $PhCH_2O$), 5.18, 5.22 (2H, 2x d J_{AB} 12.5 Hz, $PhCH_2O$), 7.27- 7.40 (30H, m). δ_C (50 MHz, $CDCl_3$); 55.3 + 55.4 (2q, 2x CH_3), 61.0 + 61.5, 63.5 + 63.7, 75.2 + 75.8, 79.0 + 79.7, (8x d, C-2, C-3, C-4, C-5), 67.3 + 67.4, 67.6 + 68.3, 72.5 + 72.6, 73.2 + 73.3 (8x t, 4x CH_2), 105.2 + 105.3 (2x d, C-1), 127.2, 127.6, 127.8, 128.2, 128.4, 128.7, 128.9, (7x d, ArCH), 136.6 + 136.8, 137.6, 138.6 + 138.7 (5x s, 3x ArC), 156.2 + 156.4 (2x s, C=O). m/z (CI, NH_3); 490 (75, MH^+), 91 (100%, Bn).

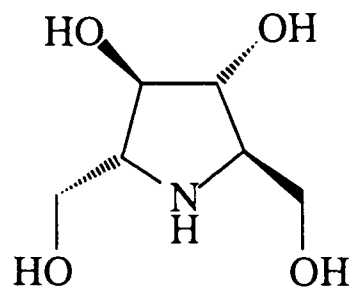
N-Benzyloxycarbonyl (3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol) (**1.35**)



N-Benzyloxycarbonyl (methyl 3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-manno furanoside) (**1.34**) (2.61 g, 5.3 mmol) was dissolved in a mixture of trifluoroacetic acid (40 ml) and water (40 ml) and stirred for 2 hours, when tlc analysis (diethyl ether/ hexane, 2/ 1) showed no starting material (R_f 0.6) and one product (R_f 0.4). The solvent was removed *in vacuo* and the residue was coevaporated with toluene (2x 10 ml). This was dissolved in ethanol (30 ml) and sodium borohydride (197 mg, 5.3 ml) was added. After 1 hour stirring, tlc analysis (diethyl ether/ hexane 1/1) showed no starting material (R_f 0.5) and one product (R_f 0.1). Ammonium chloride (600 mg) was added, the mixture stirred for 30 minutes and the solvent was removed *in vacuo*. The residue was redissolved in brine (50 ml) and extracted with dichloromethane (3x 20 ml), dried (magnesium sulphate) and evaporated to dryness. Purification by flash column chromatography (diethyl ether/ hexane 1/1) and recrystallisation (diethyl ether/ hexane) afforded *N*-benzyloxycarbonyl (3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol) (**1.35**) (1.16 g, 50%) as a white crystalline solid. M.p.(diethyl ether/ hexane); 98- 98.5 °C, [lit.[43] 96- 97 °C]. $[\alpha]_D^{25}$ -41.1 (c 0.7, $CHCl_3$), [lit.[43] $[\alpha]_D^{20}$ -36.6 (c 7.5, $CHCl_3$)]. Found C 70.16 H 6.42 N 2.71%, $C_{28}H_{31}O_6N$ requires C 70.42 H 6.54 N 2.93%. ν_{MAX} (thin film); 3372 (br, OH), 1680 (C=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$, 25 °C); 3.56 (1H, t *J* 9.7 Hz), 3.67 (1H, dd *J* 27, 11.3 Hz, H-6), 3.71 (1H, dd *J* 4.3, 9.1 Hz), 3.88 (1H, br s), 4.01 (1H, br, s), 4.31 (1H, dd J 3.1, 11.3 Hz), 4.39, 4.46 (2H, 2x d J_{AB} 12.0 Hz, $PhCH_2O$), 4.52, 4.57 (2H, 2x d J_{AB} 11.7 Hz, $PhCH_2O$), 4.45- 4.50 (2H, m), 5.16- 5.03 (2H, m). δ_H (500 MHz, CD_5CD_3, 90 °C); 3.40 (d *J* 10.0 Hz), 3.61 (t, *J* 7.6 Hz), 3.75 (s), 3.95 (s), 4.05 (br s), 4.15 (br d, 6.4 Hz),

4.30, 4.35 (2H, 2x d, J_{AB} 12.7 Hz, $PhCH_2O$), 4.40 (2H, s, $PhCH_2O$), 4.95, 5.09 (2H, 2x d, J_{AB} 12.3 Hz, $PhCH_2O$), 7.05- 2.20 (15H, m, Ar). δ_C (125 MHz, $CDCl_3$, non aromatic); 61.5 (t, $PhCH_2O$), 66.1, 66.7 (2x d, C-2, C-5), 67.2 (t, $PhCH_2O$), 68.8, 71.6 (2x t, C-1, C-6), 73.1 (t, $PhCH_2COO$), 74.1 (d, C-4), 86.9 (d, C-3), 155.0 (s, C=O). m/z (CI, NH_3); 478 (10, MH^+), 370 (100, M- OBn), 91 (70%, Bn).

2,5-Dideoxy-2,5-imino-**D**-mannitol (**1.1**)



N-Benzyloxycarbonyl-(3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol) (**1.35**) (10 mg, 0.021 mmol) was dissolved in a mixture of ethanol (5 ml) and concentrated hydrochloric acid (10 µL). To this was added a catalytic amount of palladium black (~10 mg). This suspension was stirred under an atmosphere of hydrogen for 18 hours when tlc analysis (diethyl ether/ hexane 1/1) showed no starting material (R_f 0.1) and one product (baseline). The palladium was filtered of through Celite and concentrated *in vacuo*. The free amine was formed by basic ion exchange chromatography (Amberlite CG-400,) to afford 2,5-dideoxy-2,5-imino-**D**-mannitol (**1.1**) as a hygroscopic white crystalline solid. $[\alpha]_D^{23}$+59.0 (c 0.1, H_2O), [lit.[1] $[\alpha]_D^{20}$ +56.4 (c 7.0, H_2O)]. δ_H (500 MHz, D_2O); 3.06 (2H, m, H-2, H-5), 3.62 (2H, dd *J* 6.3, 11.6 Hz, H-1 H-1'), 3.69 (2H, dd *J* 4.3, 11.6 Hz, H-6, H-6'), 3.81 (2H, m, H-3, H-4). δ_C (50 MHz, D_2O); 61.4 (d, C-2, C-5), 62.2 (t, C-1, C-6), 77.33 (d, C-3, C-4). m/z (CI, NH_3); 478 (MH^+).

1.5 References

1. A. Welter, J. Jadot, G. Dardenne, M. Marlier and J. Casimir, *Phytochemistry*, 1978, **15**, 747.
2. P.J. Card and W.D. Hitz, *J. Org. Chem.*, 1985, **50**, 891.
3. G.W.J. Fleet, S.J. Nicholas, P.W. Smith, S.V. Evans, L.E. Fellows and R.J. Nash, *Tetrahedron Lett.*, 1985, **26**, 3127.
4. L.E. Fellows, *New Scientist*, 1985, 5 August, **107**, 40.
5. A.D. Elbein, M. Mitchell, B.A. Sanford, L.E. Fellows and S.V. Evans, *J. Biol. Chem*, 1984, **20**, 12409.
6. P. Duesberg, *The Lancet*, 1992, **339**, 1547.
7. *Morbidity and Mortality Weekly Report*, 5 June 1981.
8. *Morbidity and Mortality Weekly Report*, 3 July 1981.
9. World Health Organisation, *AIDS*, 1995, **9**, 409.
10. World Health Organisation Figures, published bimonthly in *AIDS*.
11. E. Barré-Sinoussi, J.C. Chermonn, F. Rey, M-T. Nugeyre, S. Chamont, J. Gruest, C. Dauget, C. Axler-Blin, F. Vzinet-Brun, C. Roizioux, W. Rozenbaum and L. Montagnier, *Science*, 1983, **220**, 868.
12. M. Popvic, M.G. Sargadharan, E. Read and R.C. Gallo, *Science*, 1984, **224**, 497.
13. S. Wein-Hobsen, J.-P. Vartonian, M. Henry, N. Chenciner, R. Cheynier, S. Delassus, L.P. Martins, M. Sala, M.-T. Nugeyre, D. Guétard, D. Klatzmann, J.-C. Gluckman, W. Razenbam, E. Barré-Sinoussi and L. Montagnier, *Science*, 1991, **252**, 961.
14. A.J. Nahmias, J. Weiss, X. Yao, F. Lee, R. Kadsi, M. Schanfield, T. Matthews, D. Bolognesi, D. Durack, A. Motulsky, P. Kanki and M. Essex, *The Lancet*, 1986, **i**, 1279.

15. W.C. Soxinger, P.H. Levine, A.G. Dean, G. de Thé, G. Lange-Wantzin, J. Moghini, F. Laurent, M. Hoh, M.G. Sargadharon, R.C. Gallo, *Science*, 1985, **227**, 1036.

16. L. Chakrabarti, M. Gayader, M. Alizon, M.D. Daniel, R.C. Desrasiers, P. Tiallais and P. Sanigo, *Nature*, 1987, **328**, 543.

17. T. Huet, R. Cheynier, A. Meyerhams, G. Roelants and S. Wein-Hobson, *Nature*, 1990, **345**, 356. M. Peters, C. Hononé, T. Huet, L. Bedjabaga, S. Ossari, P. Bussi, R.W. Cooper and E. Delaporta, *AIDS*, 1989, **3**, 625.

18. C. Gilks, *Nature*, 1992, **355**, 305.

19. B. Goldberg, *The Lancet*, 1992, **339**, 1548.

20. R. Anderson, *Nature*, 1993, **363**, 393.

21. C.H. Fox and M. Fox-Cottler, *Immunology Today*, 1992, **13**, 353.

22. B.D. Schoub, "*Aids and HIV in Persective*", Cambridge University Press, 1994, p55-70 and references cited therein.

23. S. Wein-Hobsen, *Nature*, 1995, **373**, 102 and references cited therein.

24. M.A. Fiscil, D.D. Richman, M.H. Grieco, M.S. Gottlieb, P.A. Volberding, O.L. Laskin, J.M. Leedom, J.E. Groopman, D. Mildvan, R.T. Schooley, G.G. Jackson, D.T. Durack and D. King, *N. Engl. J. Med.*, 1987, **317**, 185.

25. Concorde co-ordinating committee, *The Lancet*, 1994, **343**, 871.

26. D.D. Richman, M.A. Fiscil, M.H. Grieco, M.S. Gottlieb, P.A. Volberding, O.L. Laskin, J.M. Leedom, J.E. Groopman, D. Mildvan, M.S. Hirsch, G.G. Jackson, D.T. Durack and S. Nusinoff-Lehrman, *N. Engl. J. Med.*, 1987, **317**, 192.

27. J.S. Allen, J.E. Coligan, F. Barin, M.F. McLance, J.G. Sodrowski, C.A. Rosen, W.A. Haseltine, T.H. Lee and M. Essex, *Science*, 1985, **228**, 1091.

28. H. Geyer, C. Holschbach, G. Hunsmann and J. Schneider, *J. Biol. Chem.*, 1988, **263**, 11760.

29. T.J. Matthews, W.G. Robey, S. Putney, L. Arthur, P. Nara, P. Fischinger, R.Gallo and A. Rankin, *Proc. Natl. Acad. Sci. USA*, 1987, **83**, 7023.

30. A.S. Tyms, E.M. Berrie, T.A. Ryder, R.J. Nash, M.P. Hegarty, D.L. Taylor, M.A. Mobberley, J.M. Davis, E.A. Bell, D.J. Jeffries, D. Taylor-Robinson and L.E. Fellows, *The Lancet*, 1987, **ii**, 1025.

31. R.J. Nash, L.E. Fellows, A.C. Plant, G.W.J. Fleet, A.E. Derome, P.D. Baird, M.P. Hegarty and A.M. Scofield, *Tetrahedron*, 1988, **44**, 5959.

32. I.C. di Bello, P. Darling, L.E. Fellows and B. Winchester, *FEBS Letters*, 1984, **176**, 61.

33. B. Winchester and G.W.J. Fleet, *Glycobiology*, 1992, **2**, 199.

34. K.H. Park, Y.J. Yoon and S.G. Lee, *Tetrahedron Lett.*, 1994, **35**, 9737.

35. R. Cusk, M. Hugemer and A. Vasella, *Helv. Chim. Acta*, 1988, **71**, 609.

36. P.W. Smith and G.W.J. Fleet, *Tetrahedron*, 1987, **43**, 971.

37. D.P. Dumas, T. Kajimoto, K.K.-C. Liu, C.-H. Wong, D.B. Berkowitz and S.J. Danishefsky, *Bio. Med. Chem. Lett.*, 1992, **2**, 33.

38. D.J. Hughes, D.Phil thesis, Oxford University, 1993.

39. W.S. Mungall, G.L. Greene, G.A. Heavner and R.L. Letsinger, *J. Org. Chem.*, 1975, **40**, 1659.

40. Isolated DMDP supplied by R.J. Nash, extracted from *Hyacinthoides non-scriptus*, enyzmatic assays carried out by R. Griffiths, University of Wales, Aberystwyth.

41. K. Freudenberg, H. Hochstetter and H.Engels, *Ber.*, 1925, **58**, 666.

42. P.M. Collins (Ed.), "Carbohydrates", Chapman and Hall, London, 1987, p 298.

43. N.G. Ramsden, D.Phil.thesis, Oxford University, 1990.

44. R.L. Whistler and R.E. Gramera, *J. Org. Chem.*, 1964, **29**, 2609.

CHAPTER 2

CHAPTER 2

Synthesis of Manno-Glucoside Analogues.

2.1 Introduction

In 1987 a new enzyme was isolated from the Golgi complex of rats' livers.[1] The oligosaccharide $Glc_1Man_9GlcNAcH_2$ was found to be trimmed to $Man_5GlcNAcH_2$ and $Man_6GlcNAcH_2$ despite the presence of DNJ (**1.7**), a known inhibitor of glucosidase II.[2] It was clear that there was a second enzymatic pathway by which glucose was cleaved. When incubated with DNJ (**1.7**) and EDTA, to stop the action of glucosidase II and mannosidase I, the oligosaccharide was trimmed to $Man_8GlcNAcH_2$ and one other compound. By a series of degradation reactions the released product was discovered to be Glcα1-3Man (**2.1**) and therefore the mystery enzyme causing its removal from $Glc_1Man_9GlcNAcH_2$ was an endo-α-**D**-mannosidase (Figure 2.1).

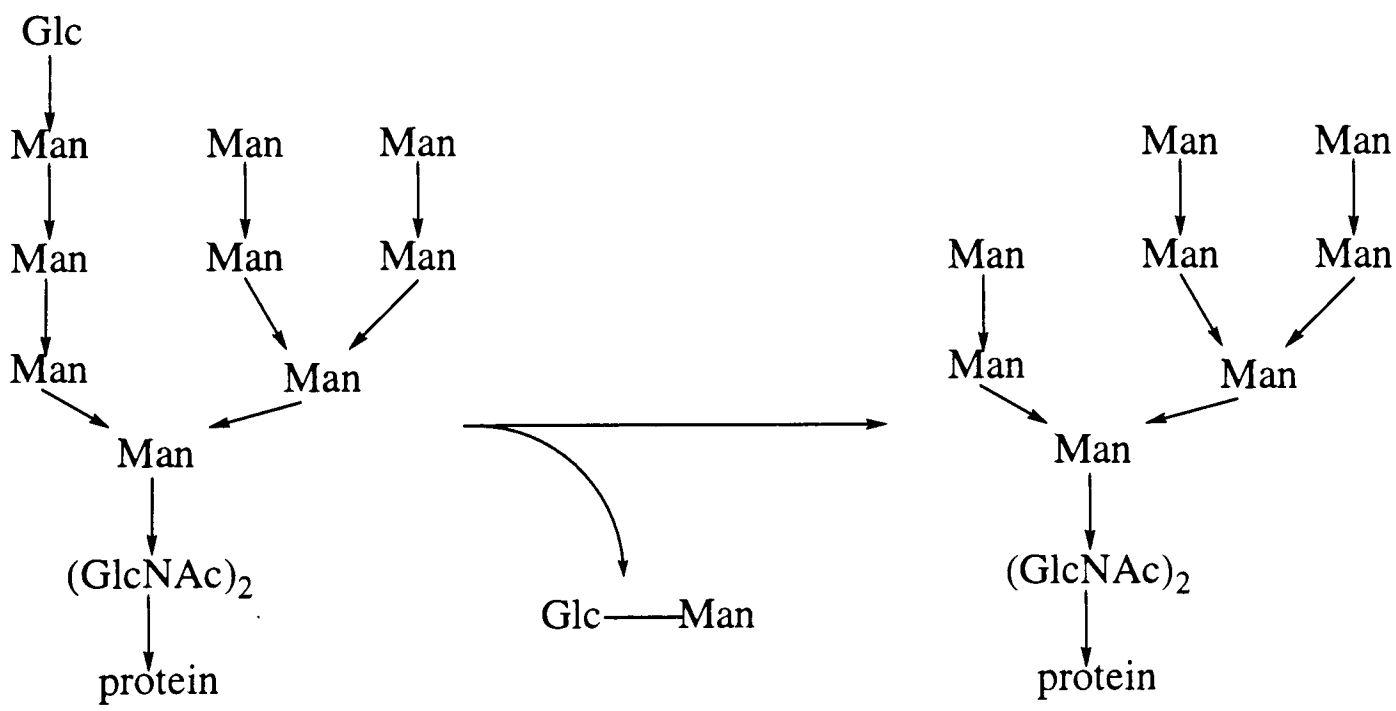
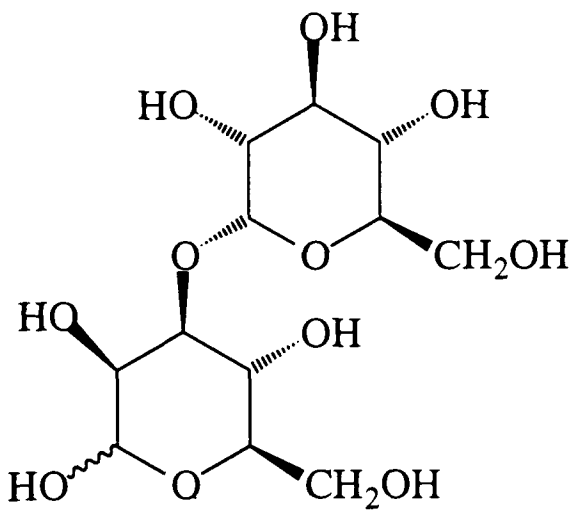
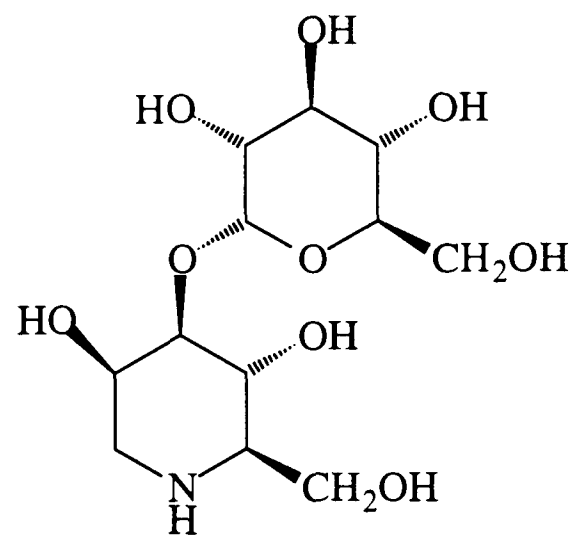


Figure 2.1 Action of endo-α-**D**-mannosidase.

As this endomannosidase was not inhibited by DNJ (**1.7**), this would suggest a mechanism whereby glycoprotein processing is not completely blocked by the inhibitor. As the complete blockage of gp120 formation is one aim of anti-HIV therapies, then an agent to block endomannosidase is required. Since endomannosidase cleaves a Glcα1-3Man (**2.1**) unit, it is postulated that such an inhibitor would be a disaccharide unit that consists of an α–glucose monosaccharide linked to a mannose analogue. A series of compounds that fitted this criterion have been tested against endomannosidase,[3] the most potent one found being 1,5-didexoy-3-*O*-(α-**D**-glucopyranosyl)-1,5-imino-**D**-mannitol (Glcα1-3DMJ) (**2.2**) (Figure 2.2).



Glcα1-3Man (**2.1**)



Glcα1-3DMJ (**2.2**)

Figure 2.2

A large number of structures have been investigated. These have included analogues of the mannose and glucose portions of the disaccharide.[4,5,6] The analogues of the mannose unit that have been trialed include many pyranose derivatives, but no furanose derivatives have been reported. It was therefore decided to synthesis glucosides of a mannofuranose analogue. The mannose analogue chosen was DMDP (**1.1**) itself a glycoprotein processing inhibitor.[2]

2.1.a Glycosylation methods

As the synthesis of each oligosaccharide is different, there is no universal reaction or set of reaction conditions for carrying out glycosylations. A good glycosylation method must have the following characteristics:-

- Formation of the glycosyl donor should occur in a high yielding sterically uniform step, producing a stable α- or β- anomeric centre.
- The donor should be able to be activated under mild conditions forming a highly activated species.
- The glycosidic bond formation should be catalysed and occur irreversibly with either complete steric inversion or retention at the anomeric centre.
- Existing glycosidic links should be unaffected by the formation of new ones.

2.1.b Enzymatic Methods of Glycosylations

Two types of enzymes can be used for glycosylations; glycosyl transferases or glycosidases (Figure 2.3). The glycosyl transferases use a diphosphate sugar as the donor to transfer the glycosyl to the acceptor, whereas the glycosidase method uses the enzyme in reverse.

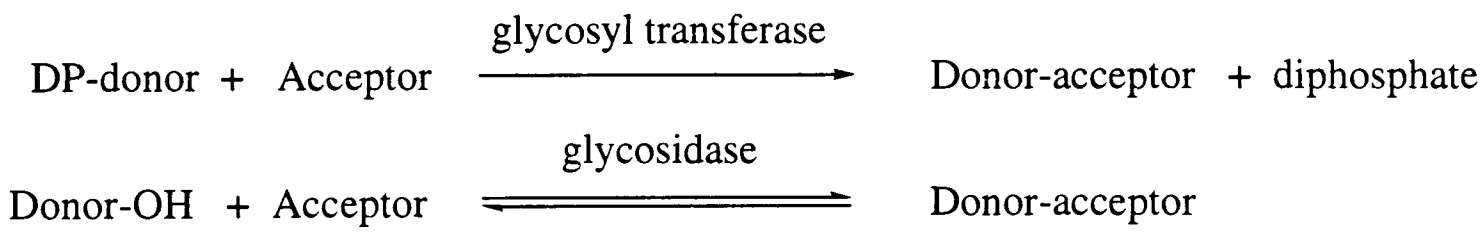


Figure 2.3

It has been found that if glycosidases are incubated with a donor and acceptor then if there is a high concentration of substrate compare to water then the enzyme will perform

the reverse reaction that occurs naturally.[7] The disadvantages of using enzymes are their expense, the requirement of having to find the right enzyme for the reaction that is being considered and the formation of a mixture of regioisomers. Taking this into account there are some glycosylations that are suited to the use of enzymes such as the formation of long repeating oligosaccharide chains.

2.1.c Chemical Methods of Glycosylations

Chemical glycosylations fall into two distinct types; one catalysed by acid and the other by base. Within both of these cases, there are further two classifications, where the glycosylating component (glycosyl donor) is reacted direct with a free hydroxyl (glycosyl acceptor) or an intermediate is formed which is subsequently activated (Figure 2.4).[8]

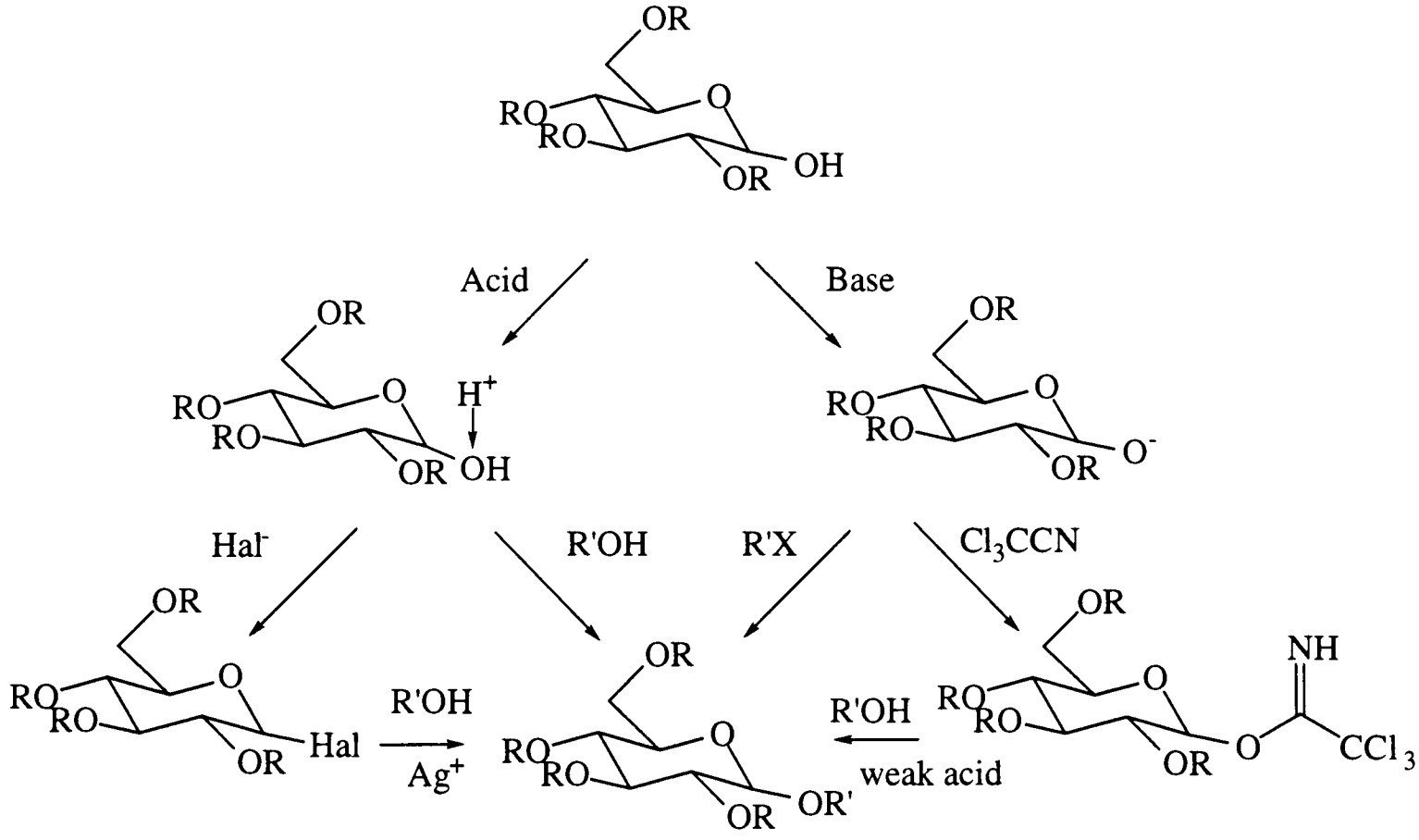


Figure 2.4

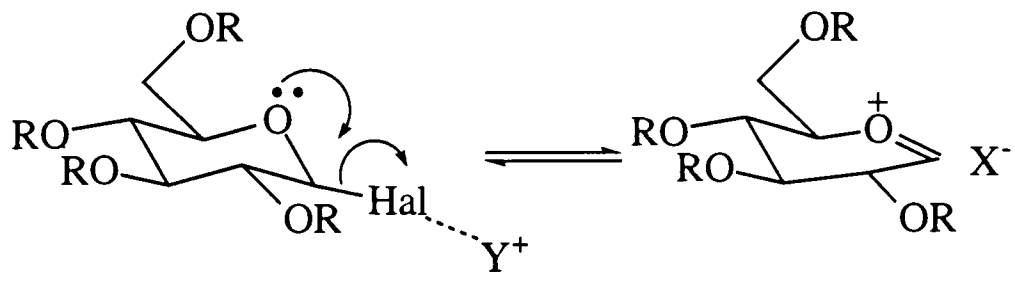
A variety of methods have been developed, the more widely used ones are discussed below:-

2.1.c.i Koenigs-Knorr Glycosylation

First reported in 1901,[9] with subsequent developments,[10] the Koenigs-Knorr method has become widely used in glycosylations. A glycosyl halide is used as the donor and reacted with the acceptor in the presence of a heavy metal salt.

The choice of halide has to take into account the fact that although the bromide is more reactive, it is less thermally stable than the chloride. Formation of each requires harsh conditions: the bromide is formed using hydrogen bromide in acetic acid[11] and the chloride is formed by the action of thionyl chloride and DMF.[12]

A number of catalysts for the reaction have been used including mercuric cyanide, mercuric bromide, silver perchlorate and silver triflate.[13] Silver triflate is the most commonly used, due to the toxicity of mercury salts and explosive nature of perchlorates. Although described as a catalyst, it is actually required in equimolar quantities during the reaction due to its hydrolysis by water released during the reaction. It is suggested that the catalyst acts by complexing with the halide and forming a close ion pair. The formation of this ion pair is aided by the use of a solvent of low polarity such as dichloromethane (Figure 2.5).[14]



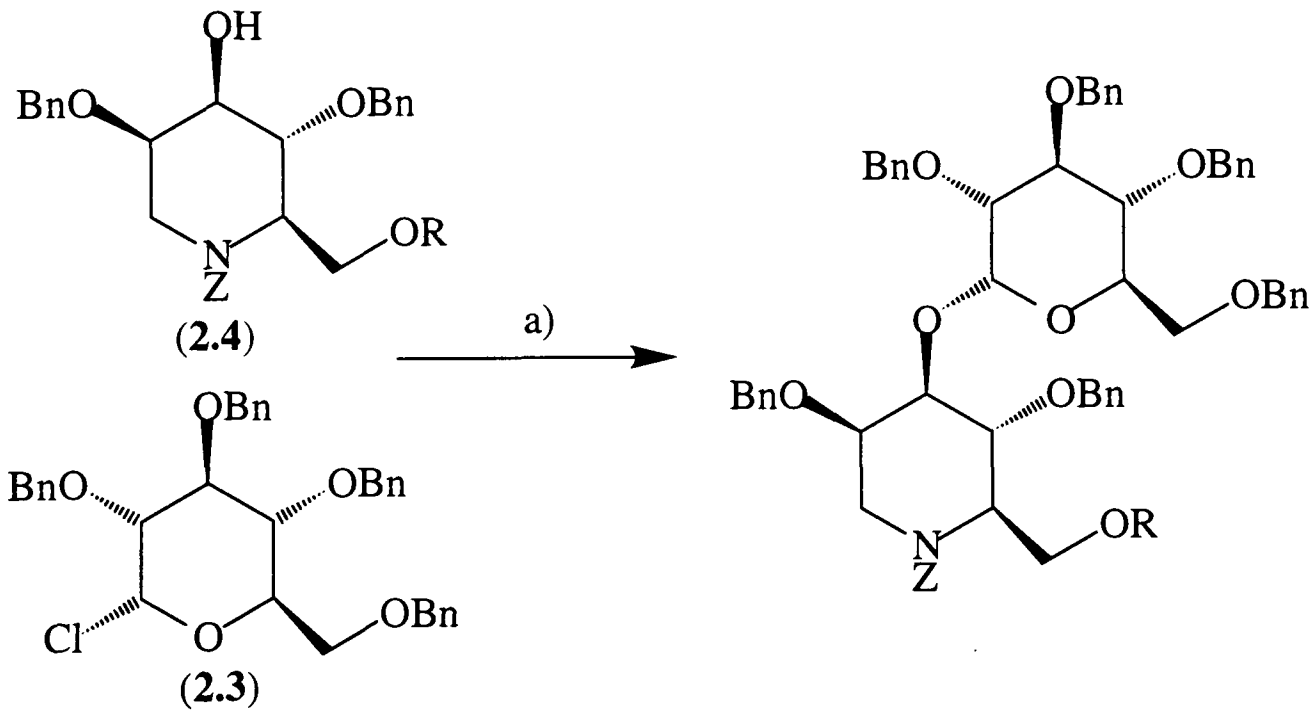
Y = Hg or Ag
X = CN, Br, ClO_4 or CF_3SO_3

Figure 2.5

The catalyst lowers the energy of activation thus increasing the rate of the reaction. As this is a reversible step both anomers exist *in situ*, with the catalyst increasing the rate of reaction of both anomers. It is the β-anomer that reacts quicker to form the thermodynamically more stable isomer.

Another main influence on the reaction is the nature of the glycosyl acceptor. For example, the reactivity of **D**-glucopyranose with acceptors is in the order water > methanol > ethanol > 6-OH » 3-OH > 2-OH > 4-OH groups of the **D**-glucose. The absolute reactivity is governed by the protecting groups used, with benzyl groups being more reactive than acetyl.[15] Since water is the best acceptor, the reaction must be carried out in strictly anhydrous conditions. To aid this powdered molecular sieves are added.

An example of the Koenigs-Knorr method was the synthesis of the endomannosidase inhibitor, Glcα1-3DMJ (**2.2**), by the action of silver triflate on the glucosyl chloride (**2.3**) in the presence of DMJ (**2.4**) (Figure 2.6).[5,16]



R = Ac [5]
= TBDMS [16]

a) AgOTf, trimethylpyridine, DCM, -78 °C

Figure 2.6

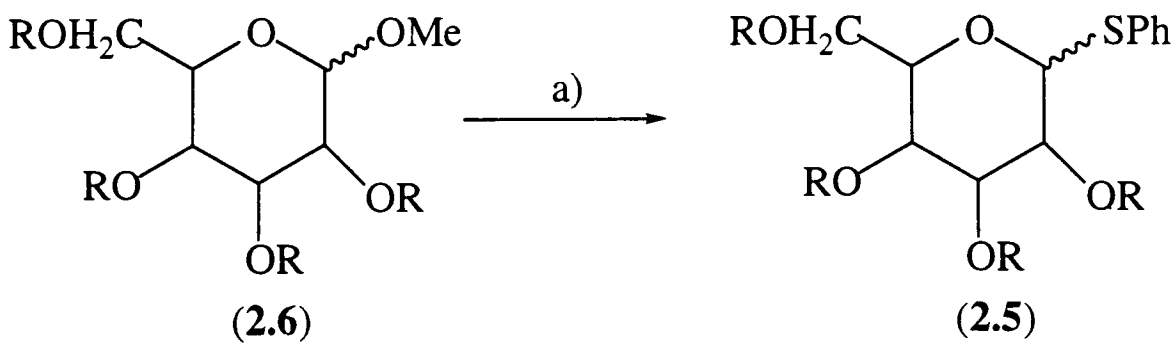
Although this method has been the most general and widely used for glycosylations, there are some considerable disadvantages:-

- Relatively harsh conditions are required to generate the glycosyl halide.
- The halides are thermally unstable with respect to the eliminated products.
- The halides are very sensitive to hydrolysis.
- The heavy metal salts are expensive.
- Large scale reactions are often dangerous (toxicity of heavy metal salts; explosive tendency of perchlorates).

A number of new glycosylation methods have been reported, which attempt to overcome these disadvantages.

2.1.c.ii Phenyl Thioglycoside Donors

In order to increase the stability of the donor and remove the requirement to use a heavy metal catalyst, a phenylthioglycoside was trialled as the donor.[17] The phenylthioglycoside (**2.5**) can be formed by the action of trimethylsilyl triflate and S-trimethylsilylthiophenol on methyl glycosides (**2.6**) (Figure 2.7).

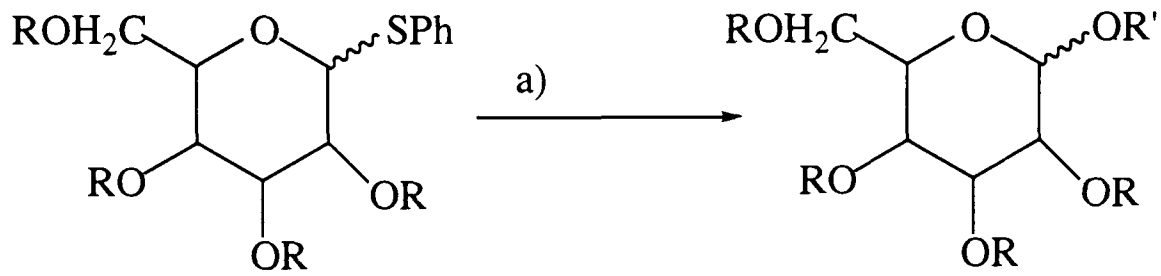


(2.6) **(2.5)**

a) $PhSSiMe_3$, $TfOSiMe_3$, DCM

Figure 2.7

The major problem with the formation of the phenylthioglycoside (**2.5**) is that the acidic conditions used are strong enough to remove acid labile protecting groups such as acetonides and silyl ethers. Once formed the phenylthioglycosides (**2.4**) are thermally stable and easier to handle than the glycosyl halides. The sulphur is oxidised, by treatment with *N*-bromosuccinimide in dichloromethane. The activated thioglycoside is a good leaving group, and with an acceptor present, it is displaced to produce α and β *O*-glycosides (**2.7**) in good yield (Figure 2.8).[17] Although this method provides access to *O*-glycosides, there is little stereochemical control and the result is commonly a mixture of α and β-anomers in approximately equal quantity.



(2.5) **(2.7)**

a) R'OH, NBS, DCM, 3A Molecular sieves

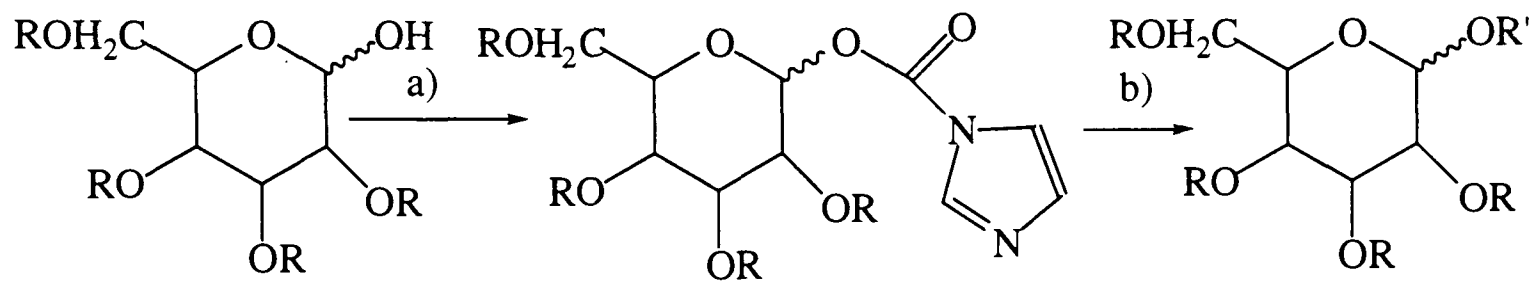
Figure 2.8

Another glycosylation using thiophenols activates the anomeric position by oxidising the thiophenol moiety with mCPBA to form the sulphoxide. This is then reacted with triflic anhydride and an acceptor to form the glycosidic link.[18] Once again the stereochemistry is only controlled by the substituents of the donor, *i.e.* if there is a group capable of neighbouring group participation then the β-anomer is formed.

The major disadvantage of these two methods using thiophenols is the lack of control to form the α-anomer, thus with only the anomeric centre influencing the outcome a mixture of anomers will always be formed. A further disadvantage of these methods is the production of the activated intermediate as two diastereomers which may have an effect on the stereochemical outcome.

2.1.c.iii (1-Imidazolylcarbonyl) Glycoside Donors

(1-Imidazolylcarbonyl) glycosides (IMG) (**2.8**) can be prepared by the reaction of carbonyldiimidazole with a free hydroxyl in ether. When treated with a glycosyl acceptor in the presence of zinc bromide a glycosidic link is formed (Figure 2.9).[19]



a) carbonyldiimidazole, ether
b) R'OH, $ZnBr_2$, DCM, reflux

Figure 2.9

This reaction gives a wide range of yields with carbohydrate acceptors, depending on the nature of the two carbohydrates. The stereochemical outcome of such glycosylations is however very dependent on the steric hindrance of the acceptor.

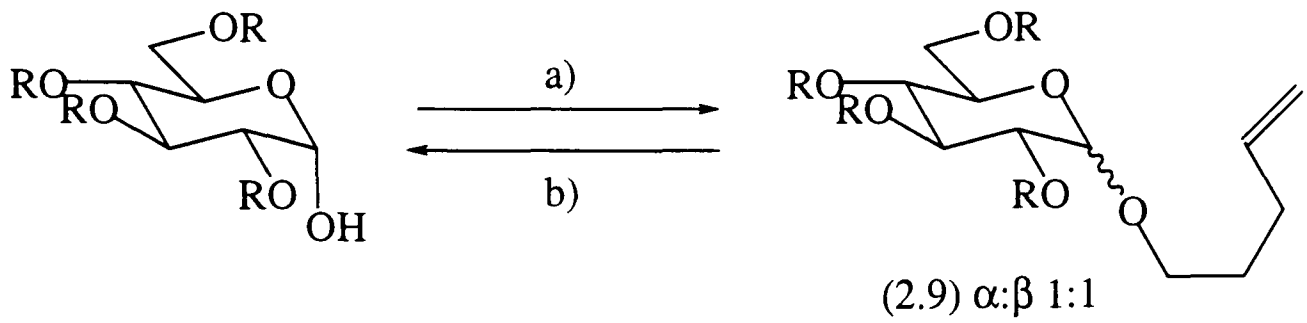
It was also noted that this reaction occurs in ether, thus making it possible to carry out the reaction in one pot by adding zinc bromide to the freshly prepared IMG (**2.8**). When the reactions were carried out in the more polar ether it was also observed that the percentage of α-anomer increased relative to the β-anomer.

Using an IMG (**2.8**) overcomes the problem of an unstable donor but does not remove the requirement of a heavy metal salt catalyst.

2.1.c.iv Pent-4-enyl Glycoside Donors

It was discovered that pent-4-enyl glycosides (**2.9**) undergo specific cleavage at the anomeric position with *N*-bromosuccinimide in aqueous acetonitrile.[20] The pent-4-enyl

glycosides can be formed by refluxing the hydroxyl carbohydrate in benzene with camphorsulphonic acid and pent-4-enyl alcohol *via* the Fischer glycosylation reaction[21] (Figure 2.10).

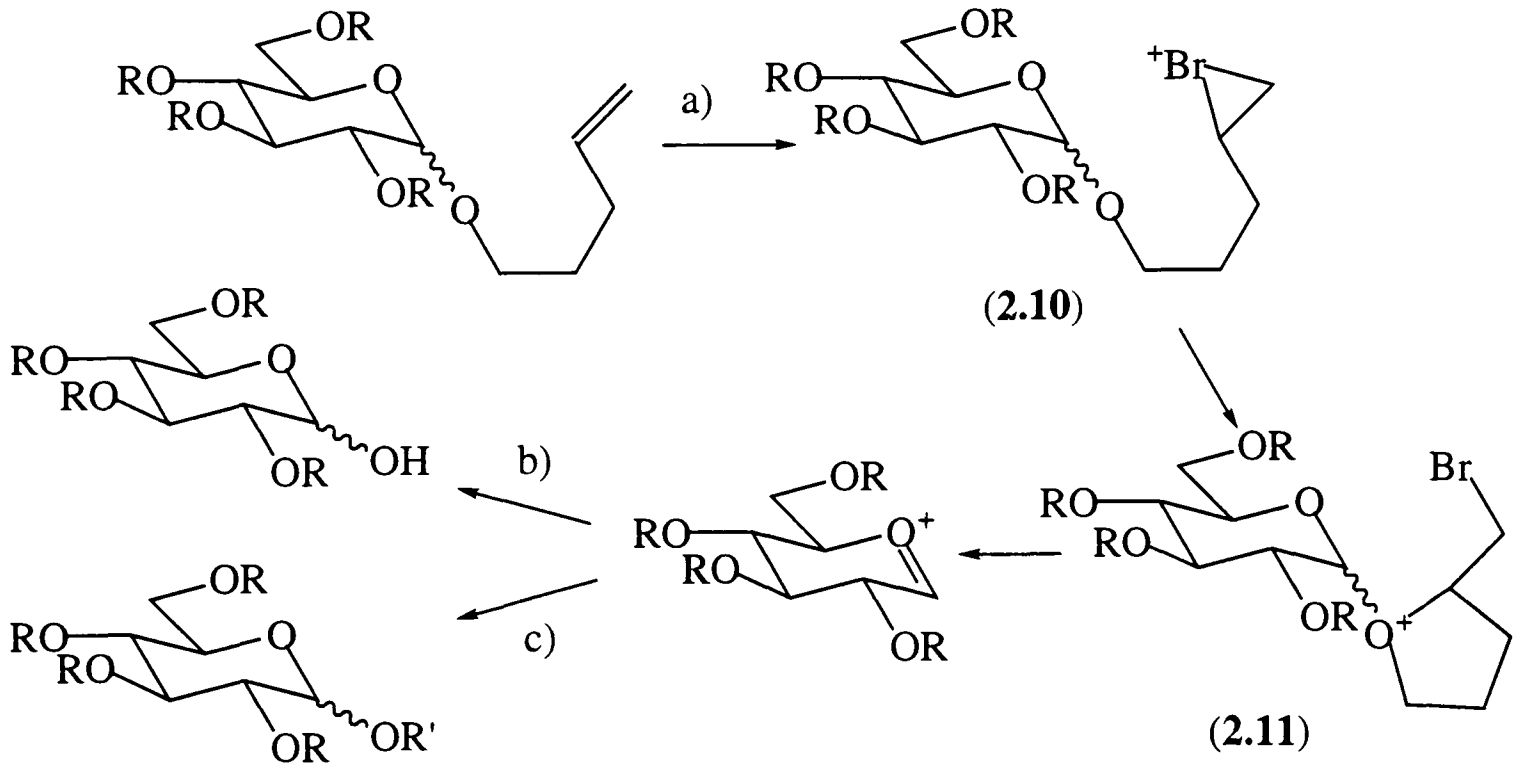


(2.9) α:β 1:1

a) CSA, C_6H_6, pent-4-enyl alcohol
b) NBS, CH_3CN / H_2O

Figure 2.10

Further investigation showed that if the water was replaced with an alcohol, the glycoside was formed.[22] The proposed mechanisms the formation of a cyclic bromonium ion (**2.10**) which is displaced by the anomeric oxygen to give a cyclic oxolanium ion (**2.11**). This eliminates to give an oxonium ion which can be trapped by water or an alcohol (Figure 2.11).



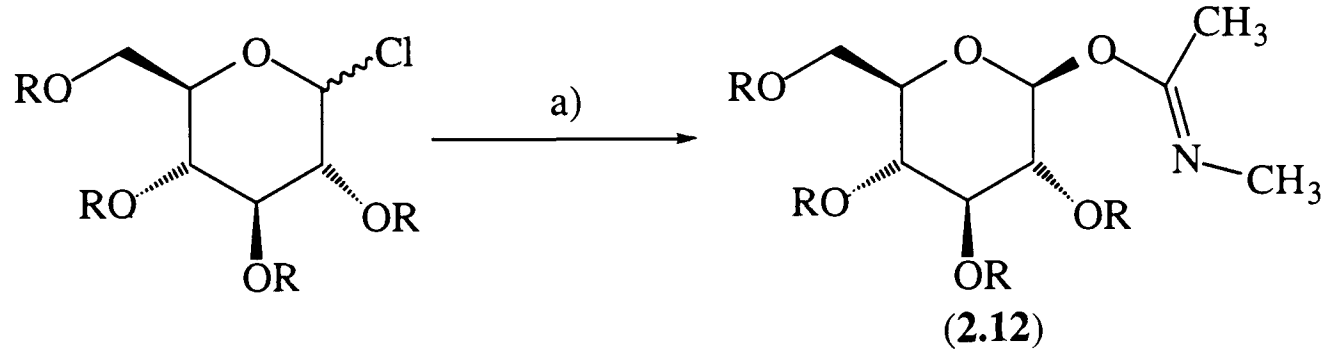
a) NBS
b) CH_3CN / H_2O
c) CH_3CN / R'OH

Figure 2.11

The anomeric ratio is dependent on the solvent used, acetonitrile favouring β-formation and ether favouring α-formation. With ether as the solvent it was also found that primary alcohols are much better stereoselective acceptors than secondary alcohols in the case of glucopyranose, this being reversed where mannopyranose is used. The advantages of this reaction include a stable donor and there is no requirement to use a heavy metal salt.

2.1.c.v Imidate Glycoside Donors

The reaction of a glycosyl chloride with a secondary amide in the presence of a silver salt was found to give an imidate (**2.12**) (Figure 2.12).



(2.12)

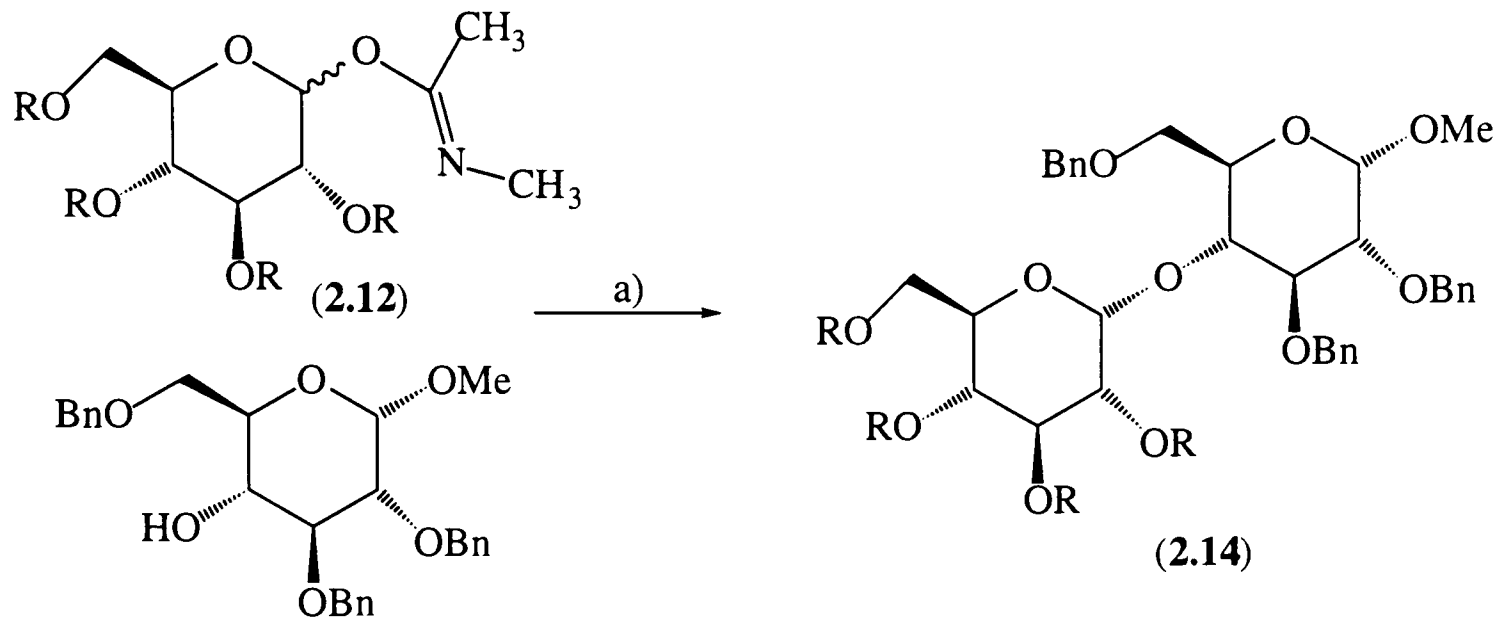
a) $CH_3CONHCH_3$, Ag_2O, $(i\text{-}Pr)_2EtN$, 3A moecular sieves

Figure 2.12

It was then found that treatment with an alcohol in benzene together with an equimolar quantity of *p*-toluenesulphonic acid gave the α-glycoside exclusively. When methyl 2,3,6-tri-*O*-benyzl-α-**D**-glucopyranoside (**2.13**) was used as the alcohol, the disaccharide (**2.14**) was formed in 85% yield as the α-anomer (Figure 2.13).[23]

This reaction was carried out on a number of substrates, all giving a very high ratio of α to β anomers. In most cases only traces of the β anomer were detected. If acetonitrile was used as the solvent higher quantities of the β anomer were formed. This synthesis represents an approach which can be used to produce a wide variety of disaccharides and

also oligosaccharides.[23] The disadvantage of this route is the need to form the thermally unstable halide, as in the Koenigs-Knorr method.



R= Bn
a) p-$MeC_6H_4SO_3H$, C_6H_6

Figure 2.13

2.1.c.vi Trichloroacetimidate Donors

The requirement of using glycosyl halides in Sinaÿ's synthesis of imidates was overcome in 1980 by Schmidt.[24] Electron deficient nitriles had previously been shown to undergo direct, reversible base-catalysed addition to alcohols, for example trichloroacetonitrile (**2.15**) adds to alcohols to give alkyloxytrichloroacetimidate (**2.16**) (Figure 2.14).[25]

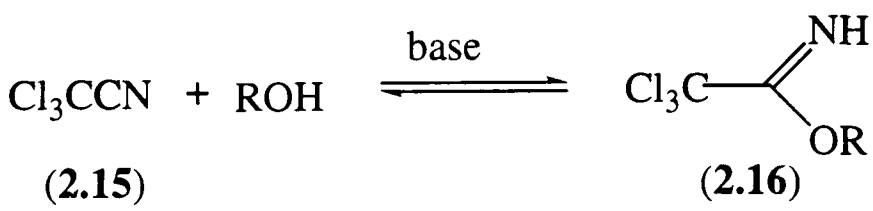


Figure 2.14

Formation of trichloroacetimidate derivatives of carbohydrates occurs smoothly. Depending on the base used and protecting groups, the α or β trichloroacetimidate can be formed selectively. Schmidt studied the formation of tetra-*O*-benzylglucosyloxy trichloroacetimidate (**2.17**).[26] He showed that when reacted with base the 1-oxides (**2.18**) were formed in 1/1 ratio (Figure 2.15).

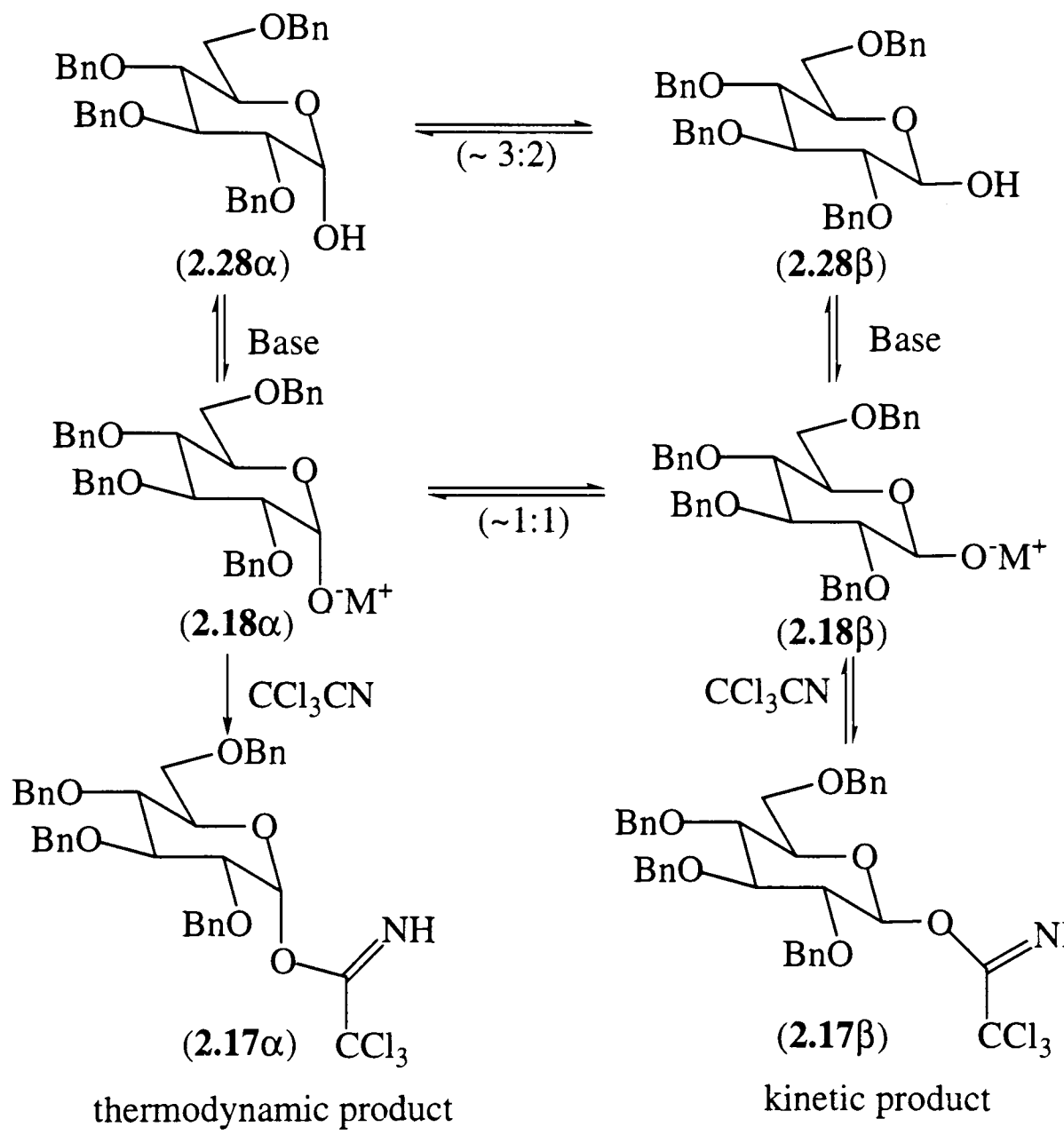
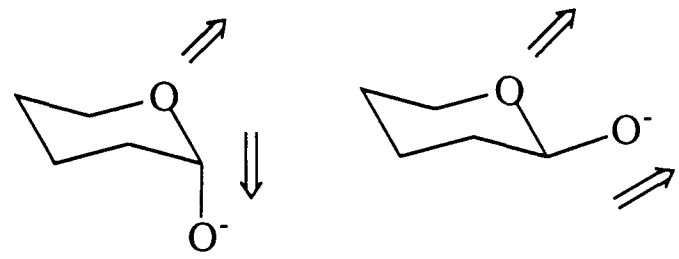


Figure 2.15

Of these two the β-1-oxide (**2.18β**) reacts faster to form the kinetic β-trichloroacetimidate (**2.17β**). The increased reactivity of the β-1-oxide (**2.18β**) is due to the repulsion of the two dipoles. This dipole-dipole interaction increases the nucelophility

of the oxygen thus increasing the rate of reaction of the β-1-oxide (**2.18**β) with respect to the α-1-oxide (**2.18**α) (Figure 2.16).

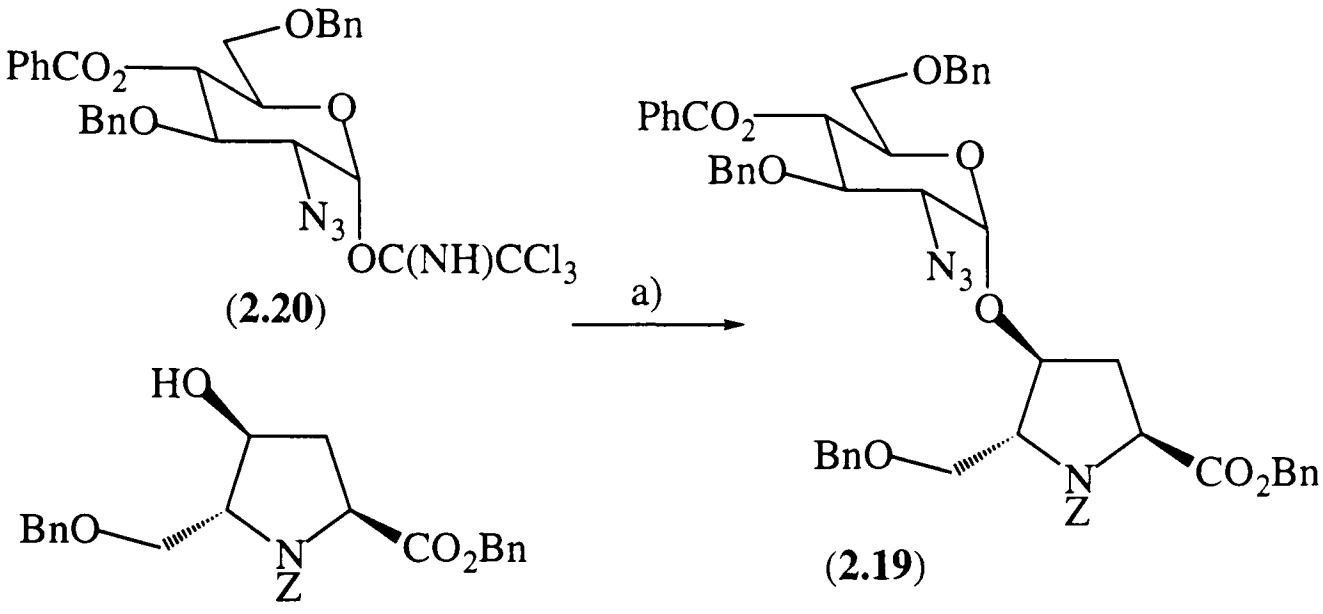


(**2.18**α) (**2.18**β)

Figure 2.16

As these reactions are all reversible, the kinetic product can be hydrolysed back to the 1-oxide (**2.18**). Epimerisation to the α-1-oxide (**2.18**α) occurs and then formation of the thermodynamic product can proceed. If a weak base is used, the hydrolysis of the β-trichloroacetimidate (**2.17**β) is slow with respect to its formation and as such the kinetic product can be isolated. If the β-anomer is treated is treated with a stronger base then hydrolysis is fast and the thermodynamic α-anomer is formed.

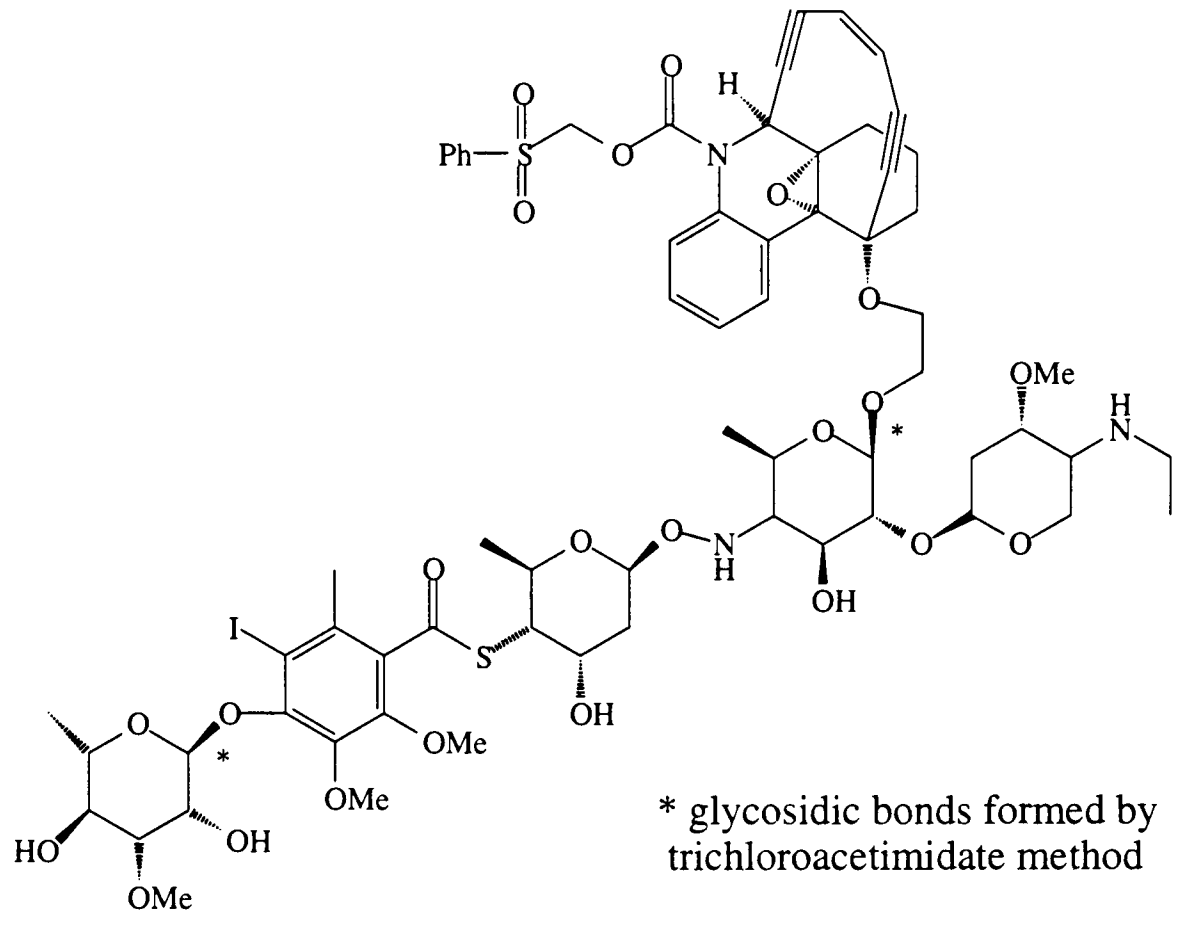
As well as being able to synthesis both anomers without the need of forming the glycosyl halides the advantage of using trichloroacetimidates is their chemical and thermal stability. Then when treated with a Lewis acid catalyst, they smoothly react to form the respective glycoside. Catalysts used include boron trifluoride etherate,[24] TMS triflate[27] and *p*-toluenesulphonic acid.[24] There are many examples of the usage of the trichloroacetimidate method in forming glycosyl links including the formation of oligosaccharides and other complex molecules. Barrett[28] utilised a glucosyl trichloroacetimidate in his synthesis of Bulgecin C (**2.19**). Using the α-anomer (**2.20**) with boron trifluoride etherate in dichloromethane, he formed the β-glycoside in 42% (Figure 2.17).



a) $BF_3 \cdot OEt_2$, DCM, -40 °C

Figure 2.17

Larger oligosaccharides have been also prepared. Vasella[29] performed two glycosylations with trichloroacetimidates during the total synthesis of Allasamidin, each of the glycosidic links were formed using TMS triflate as the catalyst. Other complex molecules synthesised by the use of trichloroacetimidates include Amphotericin B (**2.22**), synthesised by Nicolaou[30] using pyridinium *p*-toluene sulphonate as the catalyst forming the glycosyl bond in 40% yield. One of the most complex syntheses to employ the trichloroacetimidate method was the synthesis of Calicheomicin derivative (**2.23**). Danishefsky[31] used this approach to form the terminal **L**-mannose unit link to an aromatic residue. Using boron trifluoride etherate in dichloromethane formation of the bond occurred in 89% yield. A second glycosidic bond was formed in a synthesis by Nicolaou[32] between the oligosaccharide segment and the enediyne side chain, with boron trifluoride as the catalyst forming the bond in 70% yield.



(2.23)

This extensive use of the trichloroacetimidate method demonstrates its distinct advantages over the Koenigs-Knorr method.

2.1.d Solid Phase Glycosylations

A method by which solid phase technology could be employed to carry out glycosylations would be a large advantage. The field of oligosaccharides would be opened up to many researchers who do not have specialist knowledge of glycosylation methods as has happened with oligonucleotides and polypeptides. A solid phase method would have to be rapid, efficient and occur under the same reaction conditions for each glycosylation. The main problem that would occur would be the control of the stereochemistry of the growing oligomer.

Two methods have explored this possibility. The phenyl sulphoxide method previously discussed has been used with an insoluble resin to form Lewis^x blood antigen[33]

Another method using insoluble resin as the support is that using 1,2-dianhydrosugars (**2.24**), these being activated as the epoxide (**2.25**) (Figure 2.17).

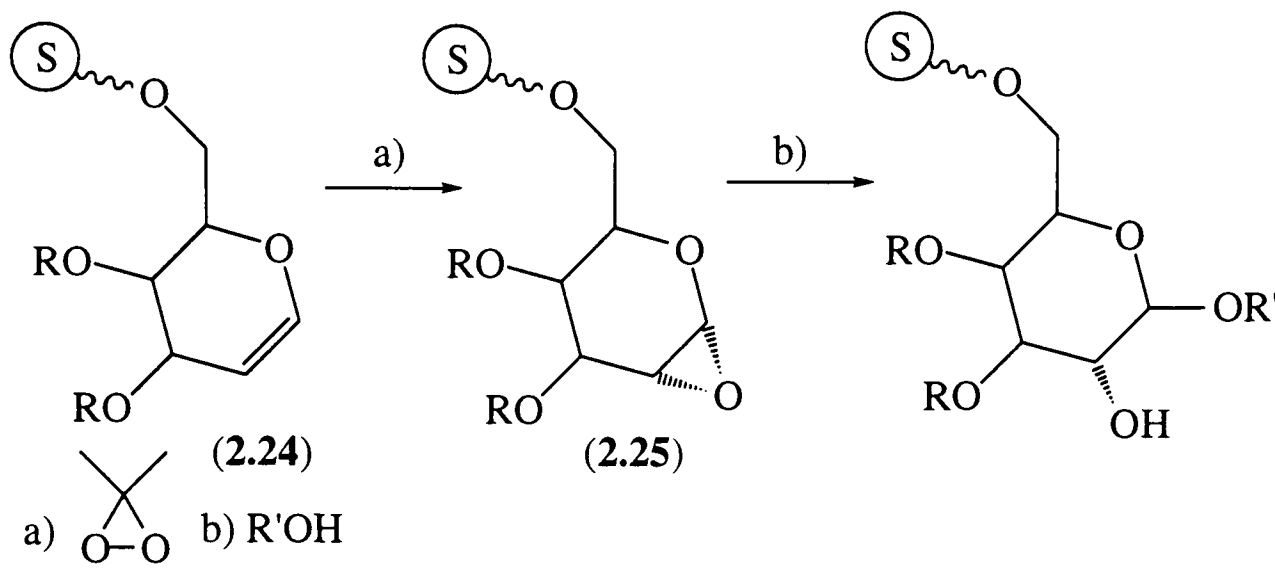


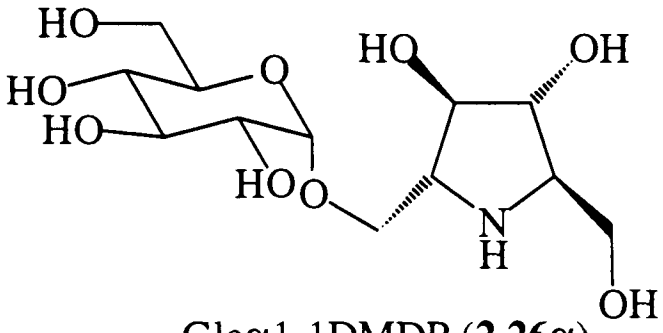
Figure 2.17

This methodology has also been used to synthesis a blood antigen this time Lewis[b].[34] Both of these methods still have a major problem with forming the α-anomer, the second method employing a different glycosylation where an α-link is required. For a general method of solid phase synthesis this unspecific stereochemical formation of the α-anomer would cause major problems as mixtures would be produced which would be inseparable by solid phase techniques.

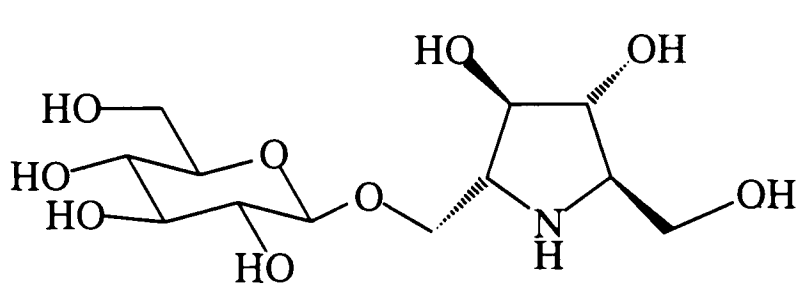
For the glycosylation of DMDP (**1.1**) the trichloroacetimidate method was chosen, as it has been extensively used and well documented as a flexible method of glycosidic bond formation.[38]

2.2 Results and Discussion

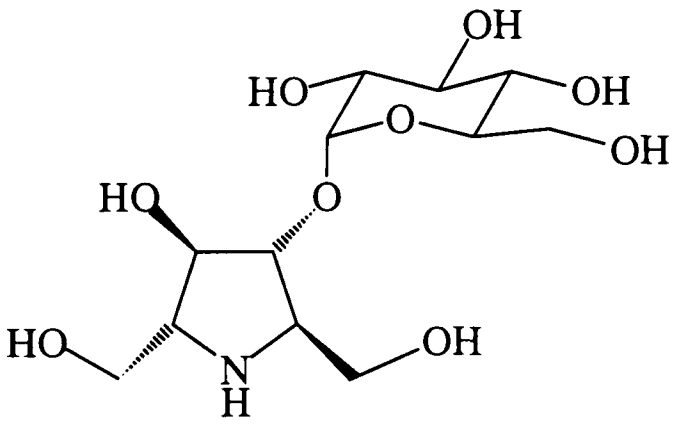
DMDP (**1.1**) has two secondary and two primary hydroxyls which can be used to form glycosyl links, but due to its symmetry only half the number of glycosides are found. Each of the hydroxyls can form either α or β-glycosides, and therefore four glycosides in total are expected (Figure 2.18).



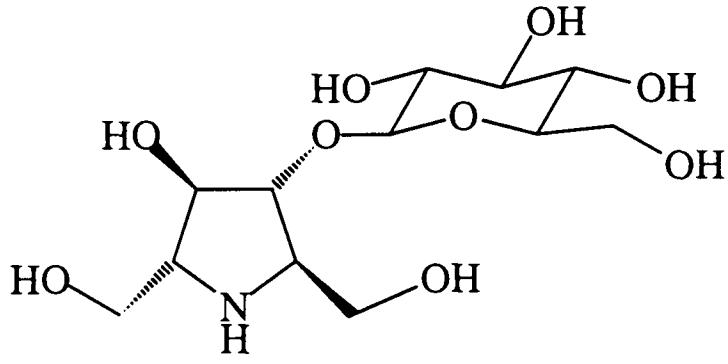
Glcα1-1DMDP (**2.26α**)



Glcβ1-1DMDP (**2.26β**)



Glcα1-3DMDP (**2.27α**)



Glcβ1-3DMDP (**2.27β**)

Figure 2.18

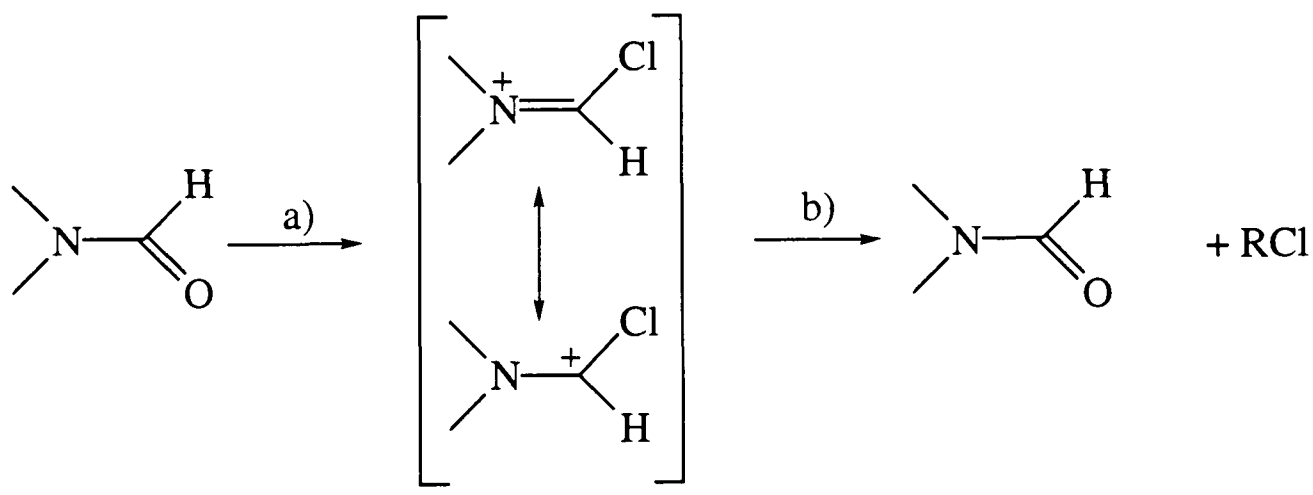
The disaccharide released by endomannosidase glycoprotein processing was identified as Glcα1-3Man (**2.1**),[1] as such the glycoside Glcα1-3DMDP (**2.27α**) is the closest of the four DMDP glucosides to the natural substrate, and as such it is postulated that it is the most likely glucoside to show inhibition of the enzyme.

2.2.a Study of the Glycosylation of Diacetone-D-glucose (1.13) as a Model System

A model system was investigated and first glycosylations carried out were performed on diacetone glucose (**1.13**), in order to distinguish between the different types of glycosylation and establish the techniques required for the diferent glycosylations.

2.2.a.i Koenigs-Knorr Glycosylation

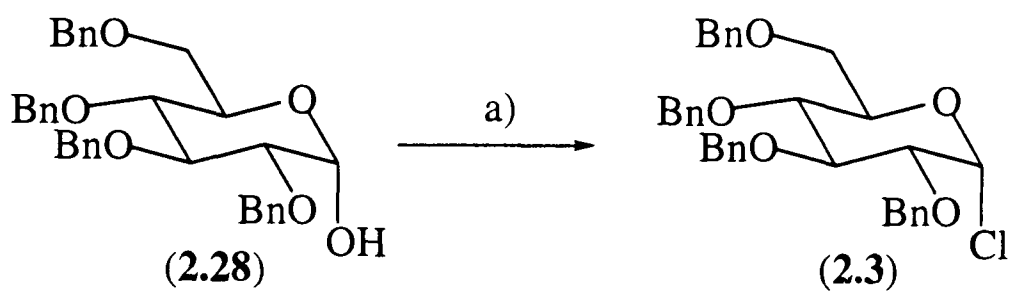
The Koenigs-Knorr method of glycosylation utilises a glycosyl halide as the glycosyl donor. For this synthesis, the chloride is the preferred halide due to its greater stability over the bromide. Tetra-*O*-benzylglucosyl chloride (**2.3**) was prepared as required by the reaction of tetra-*O*-benzyl-**D**-glucose (**2.28**) with *N,N*-dimethylchloroforminium chloride formed *in situ*. This highly reactive chlorinating agent was prepared from thionyl chloride and a catalytic amount of *N,N* dimethylformamide (Figure 2.19).[35]



a) $SOCl_2$
b) ROH

Figure 2.19

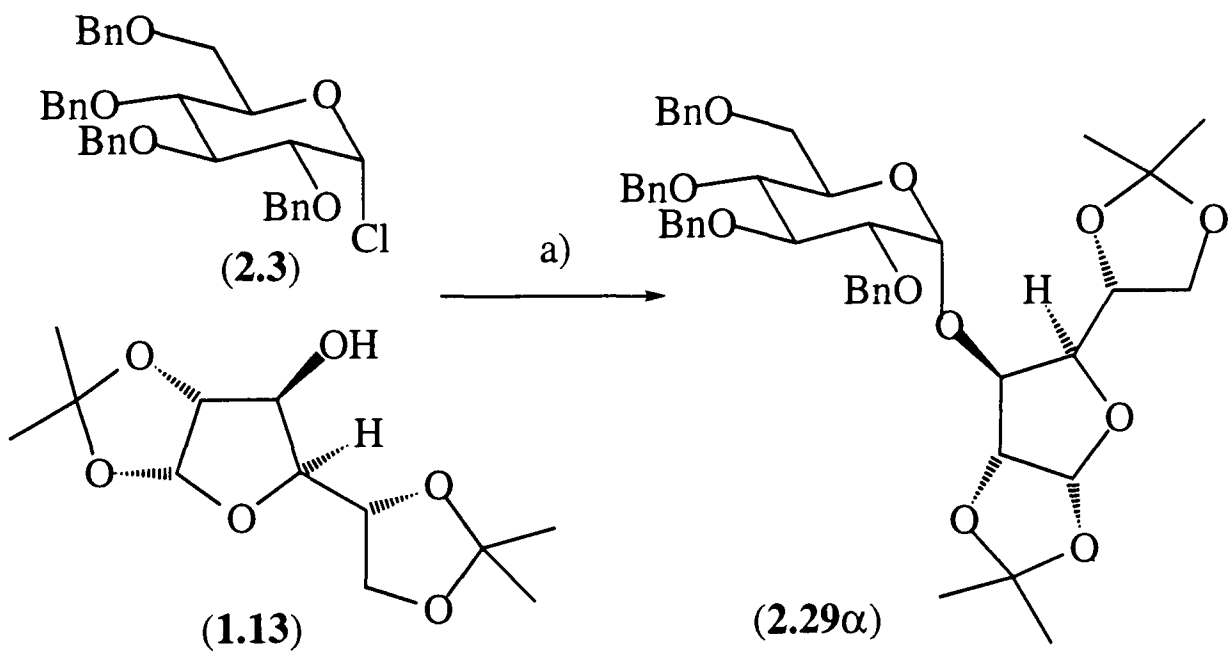
The chlorination occurred in 84% yield forming tetra-*O*-benzylglucosyl chloride (**2.3**) as the α-anomer (Figure 2.20).



a) $SOCl_2$, DMF

Figure 2.20

Glycosylation was effected by stirring a mixture of diacetone glucose (**1.13**) and tetra-*O*-benzyl glucosyl chloride (**2.3**) in dichloromethane and treating with silver triflate in the presence of 3A molecular sieves. 2,4,6-Trimethylpyridine was also added to the reaction mixture to act as an acid scavenger. This mixture was stirred in the dark at -20 °C for 24 hours then room temperature for a further 24 hours. Analysis of the products showed that a 54% yield of the two expected disaccharides was formed with an anomeric ratio of 1/1.3 α/β (Figure 2.21).

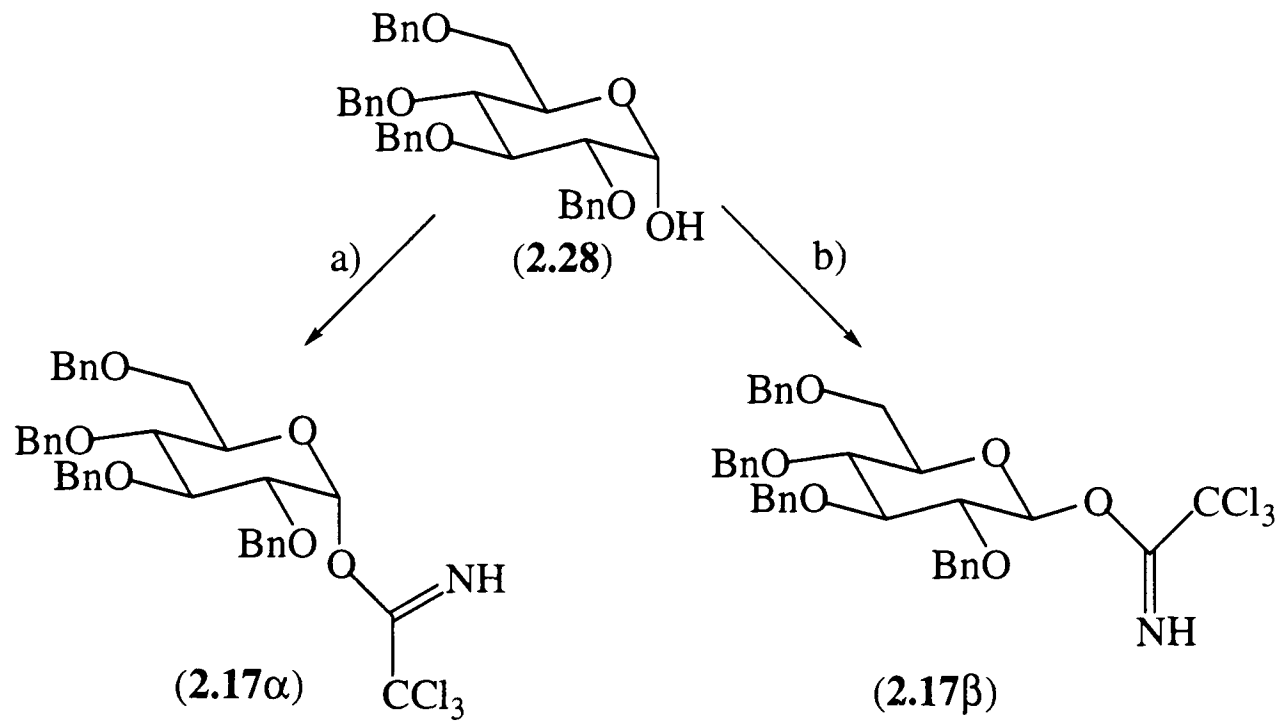


a) AgOTf, DCM, trimethylpyridine,
3A molecular sieves, -20 °C → RT

Figure 2.21

2.2.a.ii Glycosylation *via* a Trichloroacetimidate donor

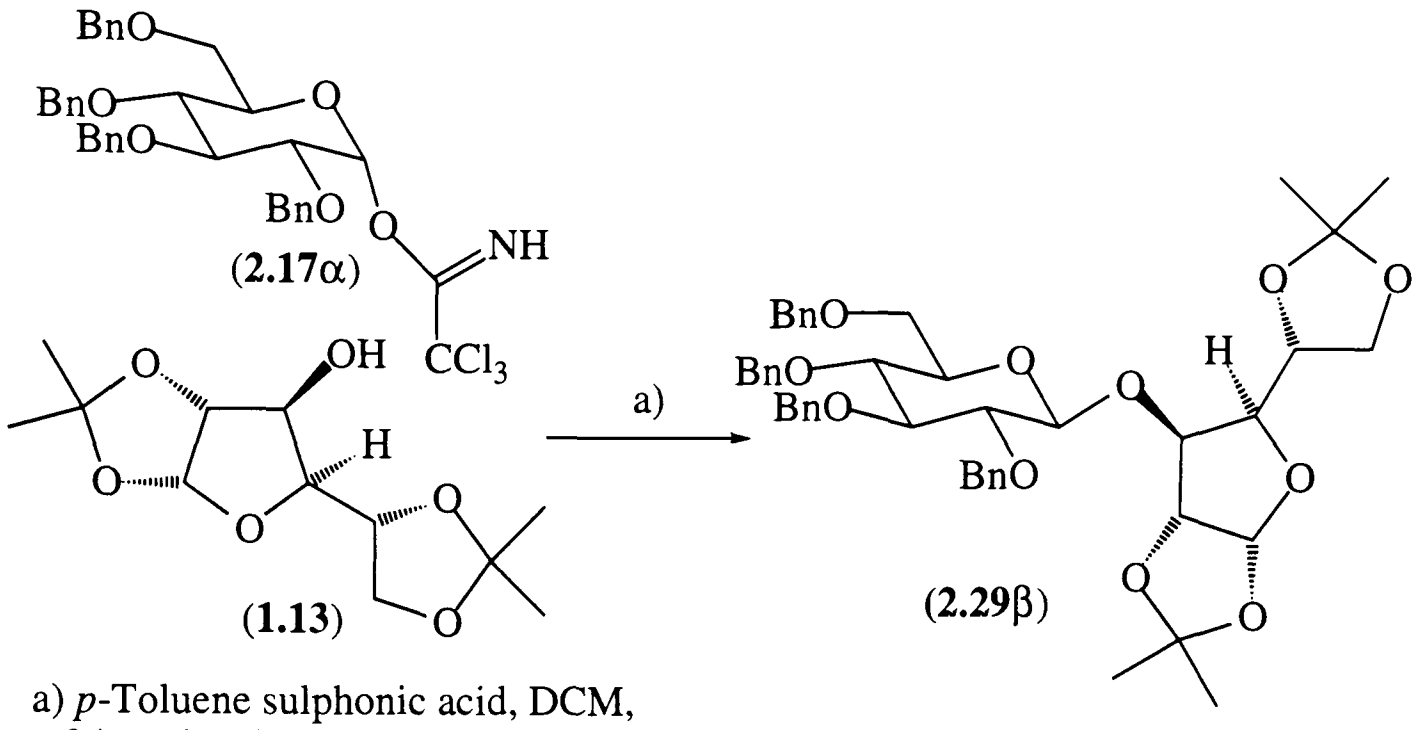
The diacetone glucose glucosides (**2.29**) were also prepared by use of trichloroacetimidates as the donor under three separate sets of reaction conditions. 2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.17**) was formed as both the α and β anomer (Figure 2.22)



a) NaH, Cl_3CCN, DCM
b) K_2CO_3, Cl_3CCN, DCM

Figure 2.22

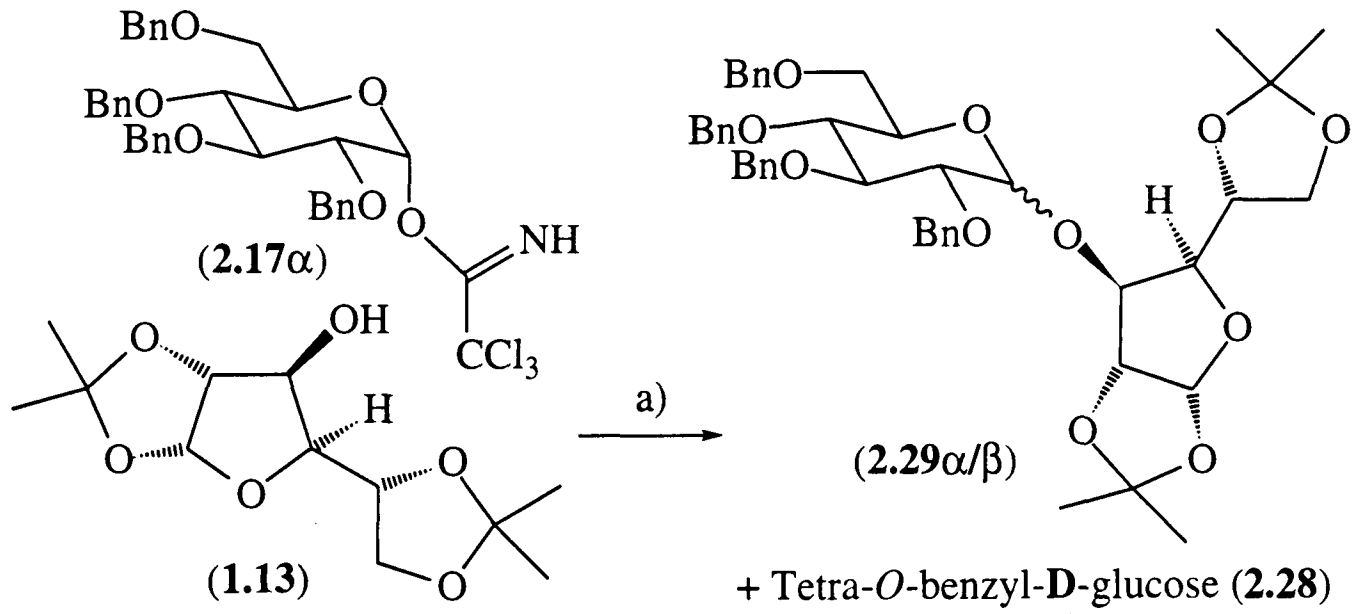
The benzyl protected α-imidate (**2.17α**) and diacetone glucose (**1.13**) was stirred in dichloromethane with 3A molecular sieves to ensure anhydrous conditions. *p*-Toluene sulphonic acid was added as the catalyst. After 24 hours isolation of the products showed that protected Glcβ1-3DAG (**2.29β**) was formed in 21% yield, with tetra-*O*-benzyl-**D**-glucose (**2.28**) and diacetone glucose (**1.13**) also recovered from the reaction. Taking the recovered starting material into account the disaccharide was formed in 63%, with no detection of the α-anomer (**2.29α**) (Figure 2.23).



a) *p*-Toluene sulphonic acid, DCM,
3A molecular sieves

Figure 2.23

A second catalyst was trialed with the benzyl protected α-imidate (**2.17α**) as the donor. Use of the more powerful Lewis acid catalyst, trimethylsilyl triflate in diethyl ether has previously been observed to favour formation of the thermodynamic α-anomer.[27] The benzyl protected α-imidate (**2.17α**) was reacted with diacetone glucose (**1.13**) in diethyl ether using TMS triflate as catalyst. After one hour all the donor had been consumed. Analysis of the products showed only 16% glycoside had been formed. The TMS triflate is more oxophilic and therefore seemed to be causing more hydrolysis of the acetimidate and the acceptor (**1.13**) than glycosylation (Figure 2.24). Nmr analysis of the glucoside formed showed that the α-anomer (**2.29α**) was favoured in a ratio of α/β 3/1.

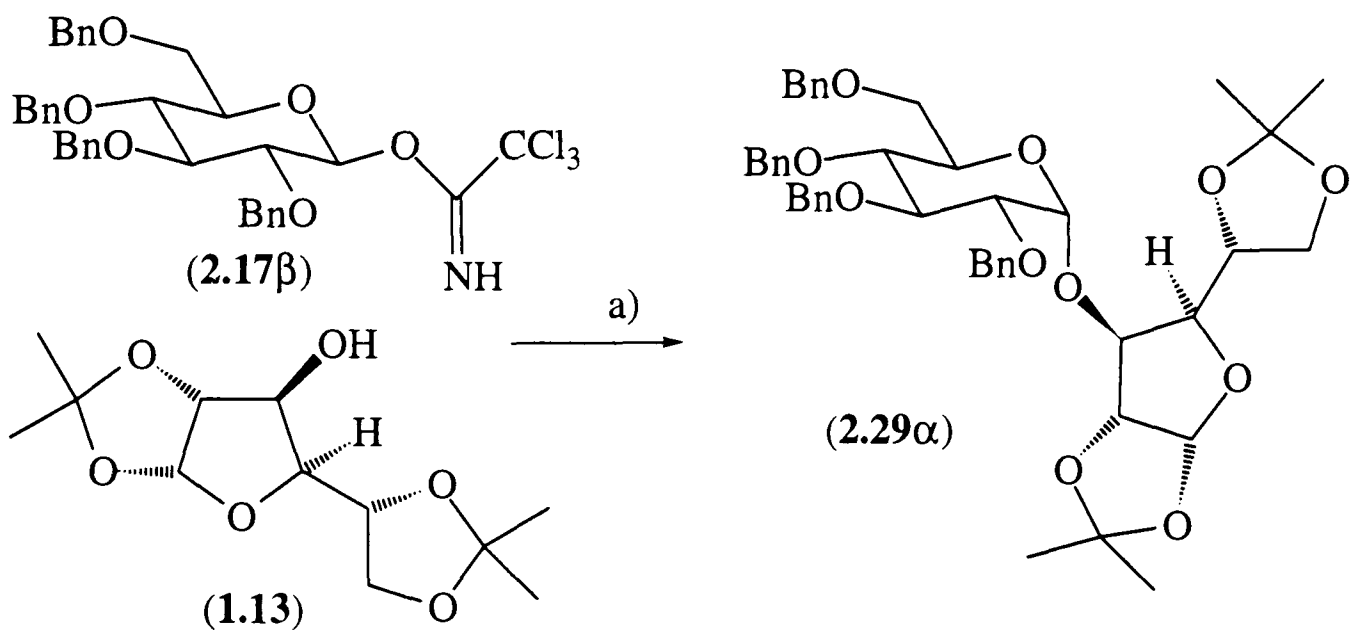


a) TMSOTf, diethyl ether
3A molecular sieves

+ Tetra-*O*-benzyl-**D**-glucose (**2.28**)
and **D**-glucose (**1.19**)

Figure 2.24

2,3,4,6-Tetra-*O*-benzyl-β-**D**-glucopyranosyl trichloroacetimidate (**2.17**β) was then used as the donor and boron trifluoride etherate as the catalyst. After 20 minutes at -20 °C, the protected disaccharide Glcα1-3DAG (**2.29**α) was found to be formed in 75% yield with little hydrolysis of the trichloroacetimidate to tetra-*O*-benzylglucose (**2.28**) and no trace of the β-anomer (**2.29**β) was observed (Figure 2.25).



a) $BF_3 \cdot EtO_2$, DCM, -20 °C
3A molecular sieves

Figure 2.25

2.2.a.iii Evaluation of the Glycosylations of Diacetone-D-Glucose (1.13)

The results from the glycosylations carried out on diacetone glucose (**1.13**) are summarised in table 2.1. It is seen that the classical Koenigs-Knorr method gives a moderate yield but a mixture of anomers in approximately equal ratio. The trichloroacetimidate donor with toluene sulphonic acid as the catalyst gives the β-anomer (**2.29**β) exclusively, although there is significant hydrolysis of the glycosyl donor before the glycosyl link is formed. Use of boron trifluoride etherate gives complete inversion of stereochemistry producing the α-glucoside (**2.29**α) in good yield from the benzyl

protected β-imidate (**2.17β**). If TMS triflate is used as the catalyst the reaction is rapid but there is competing hydrolysis of the acetimidate and diacetone glucose (**1.13**).

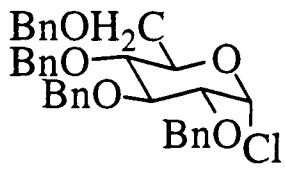
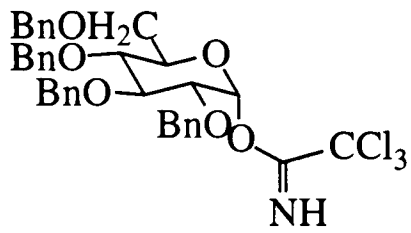
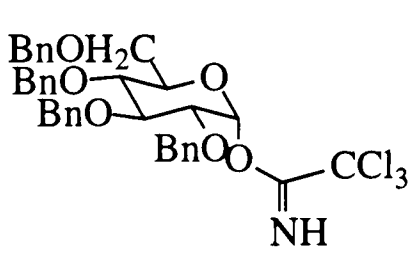
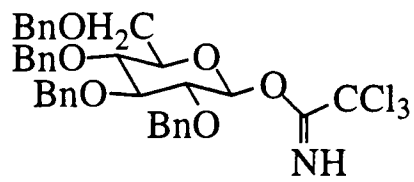
Donor	Catalyst	Yield	α/β ratio
 (**2.3**)	AgOTf	54%	1/1.3
 (**2.17α**)	TMSOTf	16%	3/1
 (**2.17α**)	*p*-Tol SO_2OH	21% (63%)	0/1
 (**2.17β**)	$BF_3 \cdot OEt_2$	75%	1/0

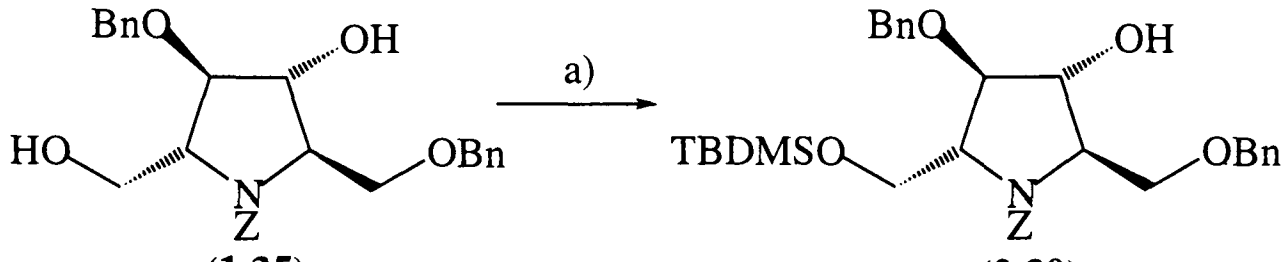
Table 2.1

2.2.b.i Glycosylation of the Secondary Hydroxyl of DMDP (1.1)

From the synthesis of DMDP (**1.1**), the partly protected derivative (**1.35**) was used during the glycosylations. This derivative (**1.35**) has protection at one primary and

one secondary hydroxyl thus giving access to a single primary and secondary hydroxyl. To gain access to the secondary hydroxyl, protection of the primary hydroxyl would also be essential.

Initial protection of the primary hydroxyl was achieved using *tert*-butyldimethyl silyl triflate to form the silyl ether (**2.30**). This was chosen as it could be formed selectively and could be removed by the small amount of acid used in the debenzylation step. The silyl ether was formed in 61% from the alcohol, thus allowing access to the C-3 hydroxyl (Figure 2.26).

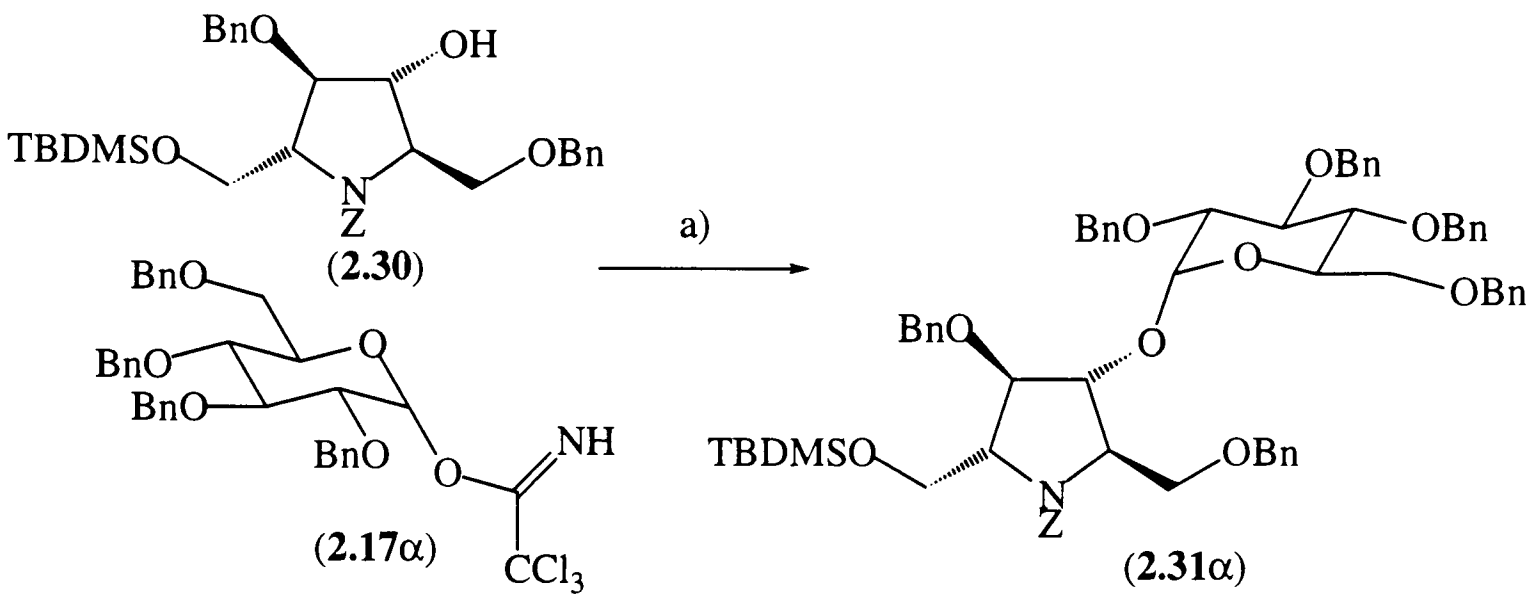


(1.35) **(2.30)**

a) TBDMSOTf, py, DCM, -20 °C

Figure 2.26

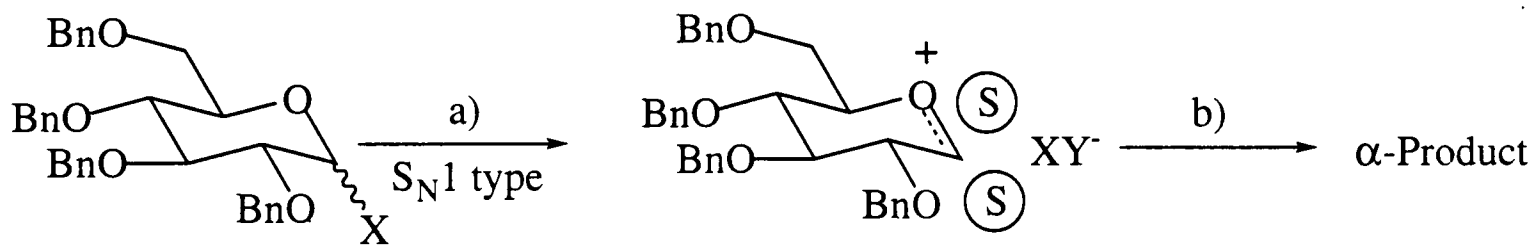
The benzyl protected α-imidate (**2.17α**) was used as the donor with the silyl protected DMDP (**2.30**) as the glycosyl acceptor. As anticipated boron trifluoride etherate could not be used as the catalyst for this particular reaction as it could lead to the hydrolysis of the silyl ether.[36] TMS triflate was used as an alternative catalyst, it is known to give high stereochemical yields. The α-glycoside is favoured when TMS triflate is used in diethyl ether.[27] The reaction was carried out at room temperature and after two hours all the silyl DMDP (**2.30**) had been consumed forming the protected glycoside Glcα1-3DMDP (**2.31α**) in 51% yield (Figure 2.27). This glycosylation gives the α-anomer (**2.31α**) in 50% yield and the β-anomer (**2.31β**) in 1% from the benzyl protected α-imidate (**2.17α**) thus leading to majority retention of configuration at the anomeric centre.



a) TMSOTf, Diethyl ether, 3A molecular sieves

Figure 2.27

This has been shown to be due to the participation of the solvent during the reaction forming close ion pairs between the catalyst and the oxonium ion.[37] The preferred attack is then from the lower face to give the more thermodynamically favoured α-anomer (Figure 2.28).

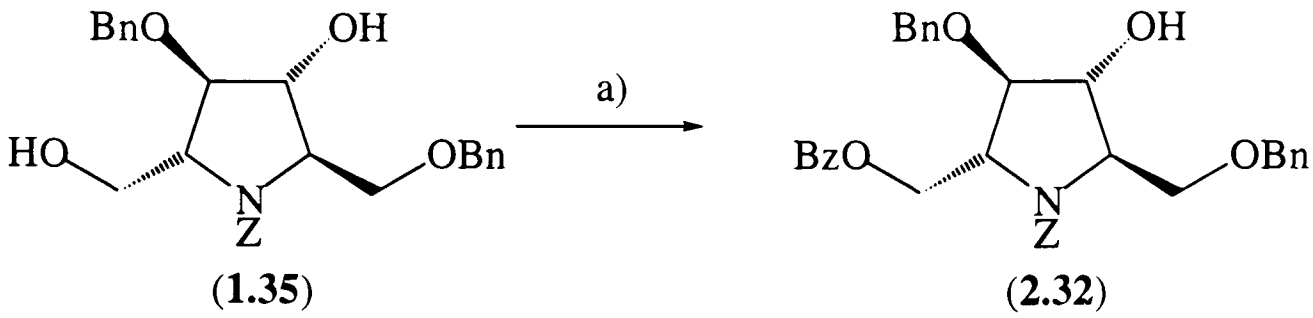


S = EtO_2

a) Promoter Y
b) Acceptor A-H

Figure 2.28

A second protecting group for the secondary hydroxyl of DMDP was tried. A benzoyl ester was formed selectivity by the treatment with benzoyl chloride, with the ester (**2.32**) being produced in 66% yield (Figure 2.29).

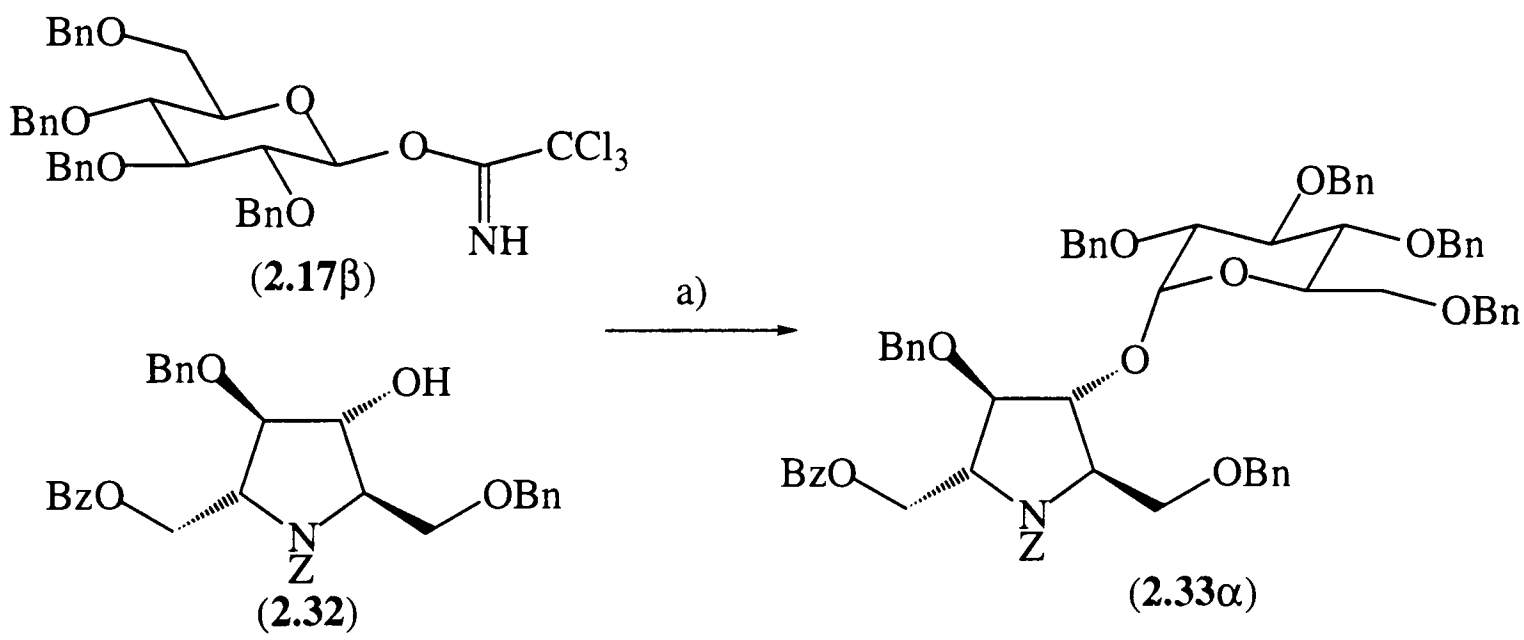


(1.35) **(2.32)**

a) BzCl, py, DCM, -10 °C

Figure 2.29

This alcohol (**2.32**) was mixed with benzyl protected β-imidate (**2.17**β) in dichloromethane using the milder boron trifluoride etherate as the catalyst thus increasing the tendency for S_N2 displacement of the imidate (**2.17**β) (Figure 2.30).[24]



(2.17β**)** **(2.32)** **(2.33**α**)**

a) $BF_3 \cdot O_2Et$, DCM, 3A molecular sieves, -20 °C

Figure 2.30

After stirring for two hours at -20 °C, the glucoside had formed in 75% yield. This glycosylation occurred with the predicted S_N2 inversion of configuration forming the α-glucoside (**2.33**α). Close association between the imidate and the boron could led to the observed displacement and stereochemical outcome. It is suggested that co-ordination to the nitrogen of the imidate weakens the C-O anomeric bond sufficiently to allow the acceptor to displace the trichloroacetimidate (Figure 2.31).

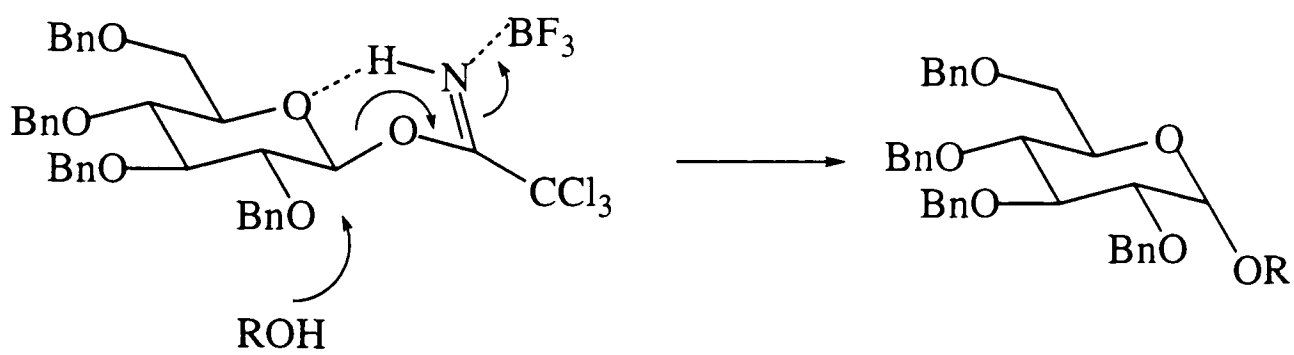


Figure 2.31

Synthesis of the β-anomers of the DMDP-glucosides made use of the widely found neighbouring group participation effect that occurs when acetyl protection is employed on the C-2 of the donor **D**-glucose (Figure 2.32). [38]

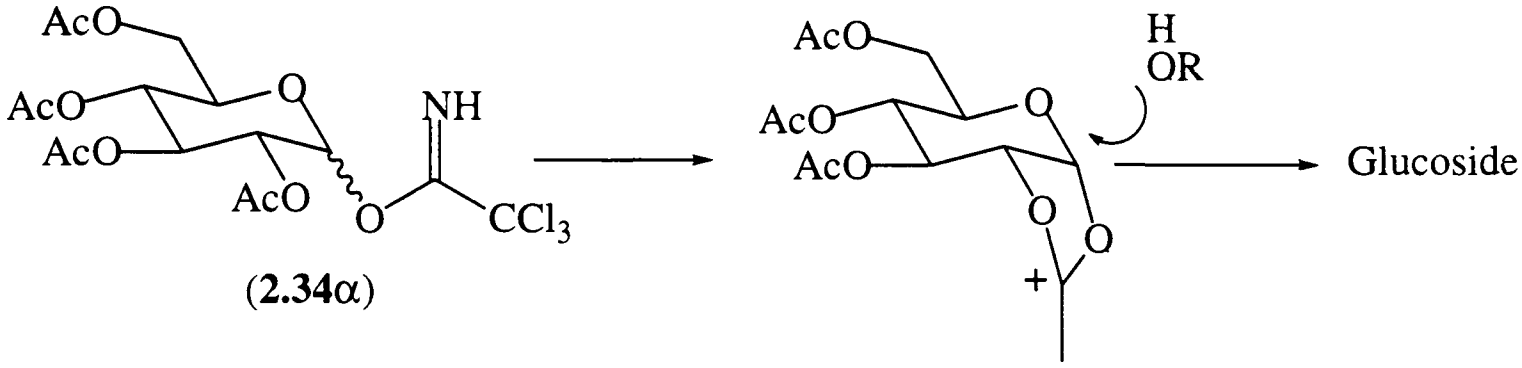
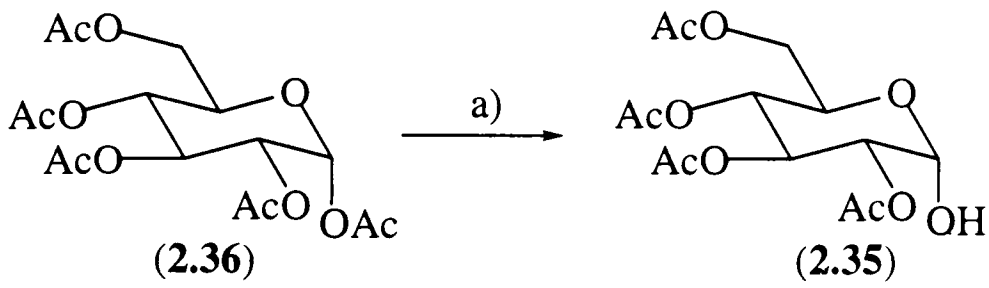


Figure 2.32

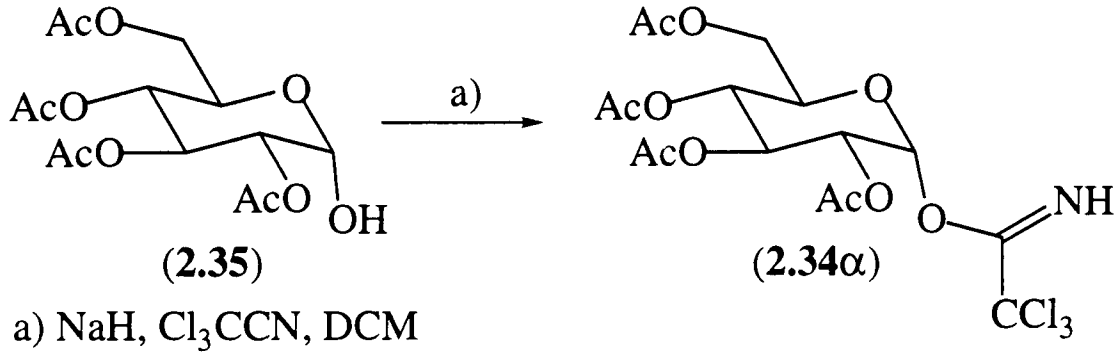
Tetra-*O*-acetylglucopyranosyl trichloroacetimidate (**2.34α**) is formed from tetra-*O*-acetyl-α-**D**-glucose (**2.35**) which in turn is prepared from commercially available penta-*O*-acetyl-α-**D**-glucose (**2.36**) by mild hydrolysis with ammonium carbonate in DMF (Figure 2.33).[39]



a) $(NH_4)_2CO_3$, DMF

Figure 2.33

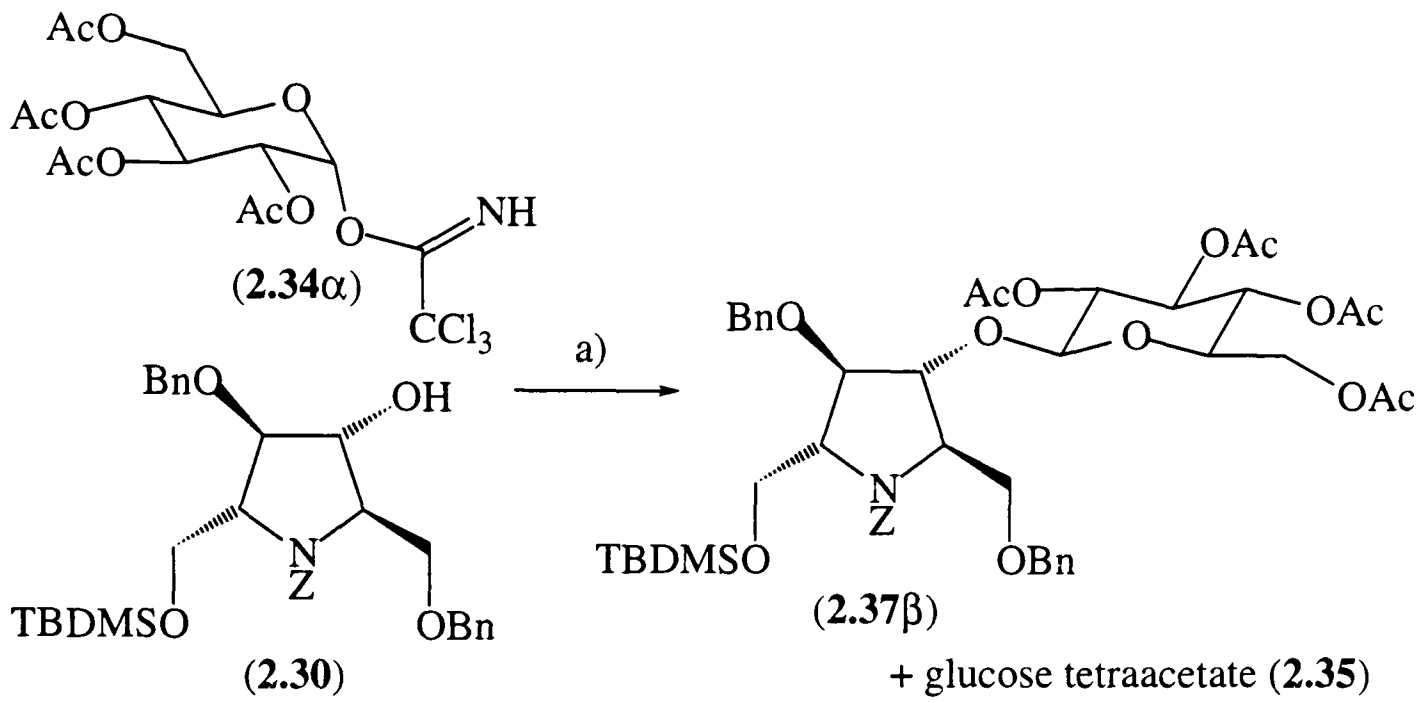
Hydrolysis occurs only at the most active centre and no removal of the other acetyl esters was detected. The acetyl protected imidate (**2.34**) was formed in an analogous fashion to the benzyl protected imidates (**2.17**). The 1-oxide was formed with sodium hydride then trichloroacetonitrile (**2.15**) acted as the electrophile giving the α-anomer (**2.34**α) (Figure 2.34).



a) NaH, Cl_3CCN, DCM

Figure 2.34

The silyl protected DMDP (**2.30**) was used as the acceptor for the glycosylation with the acetyl protected imidate (**2.34**α). A stronger Lewis acid was used as the catalyst due to electron withdrawing acetyl groups deactivating the system. A small quantity of glucoside was formed with the trichloroacetimidate being completely consumed during the reaction, the major product formed was tetra-*O*-acetyl glucose (**2.35**) (Figure 2.35).



a) TMSOTf, Diethyl ether, 3A molecular sieves

Figure 2.35

It has been observed in many glycosylation methods that when nitriles are used as the solvent then β-formation can be favoured.[40,41] It has been suggested that this is due to the participation of the solvent, with the solvent rapidly forms a complex with the glycosyl donor thus favouring attack for the top face leading to the β-anomer (Figure 2.36).[13]

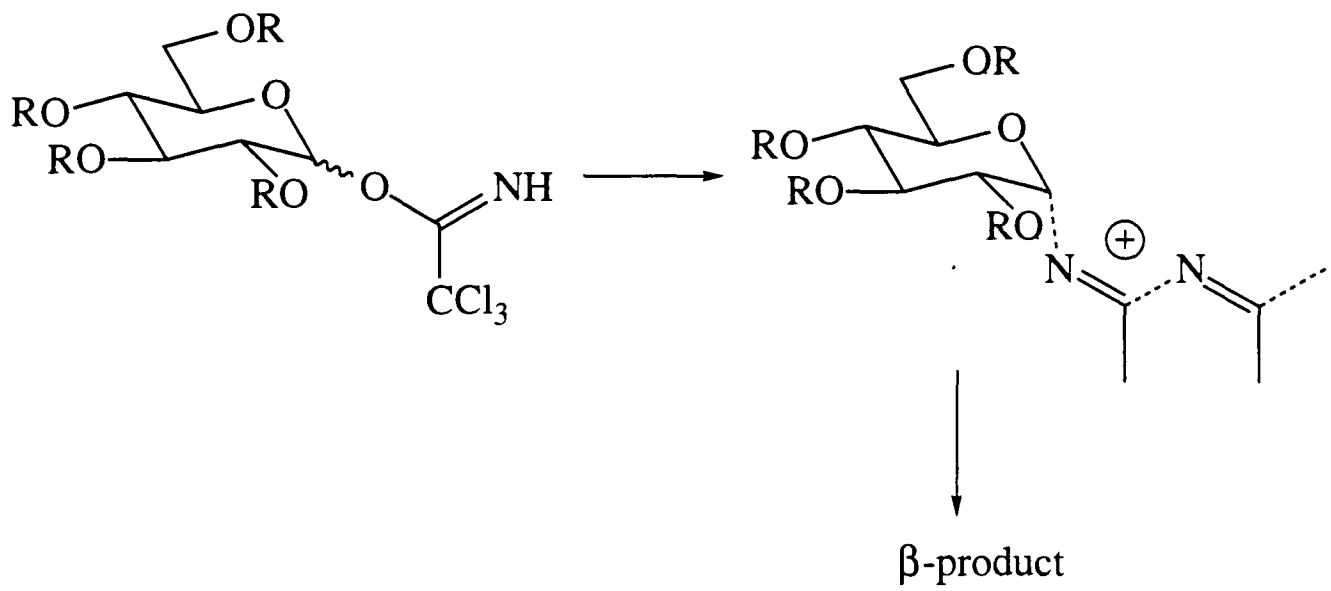


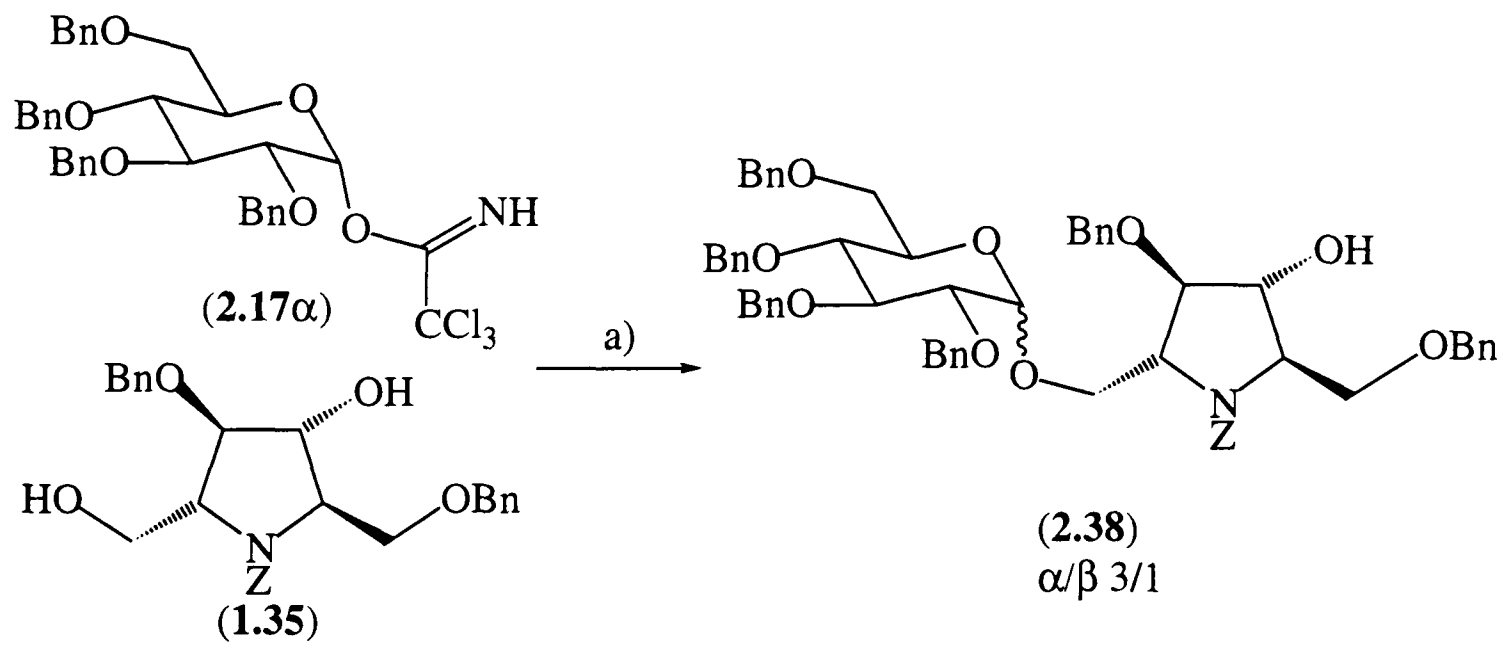
Figure 2.36

Glycosylation between the silyl protected DMDP (**2.30**) and acetyl protected imidate (**2.34**α) was carried out in acetonitrile at -40 °C. Exclusively β-product was observed although an even lower yield of 6% for the formation of the product was found.

2.2.b.ii Glycosylation of the Primary Hydroxyl of DMDP (1.1)

A similar strategy was employed to form the two primary glucosides. A differential in reactivity between the primary and secondary hydroxyls had been observe during the primary protection of the DMDP derivative (**1.35**) as the benzoyl ester (**2.32**) and the silyl ether (**2.30**). The glycosylations were carried in a similar manner to the secondary glycosylations but with both hydroxyls unprotected. From the previous observation of the selectivity it was envisaged that the reactions would give the required primary glucosides.

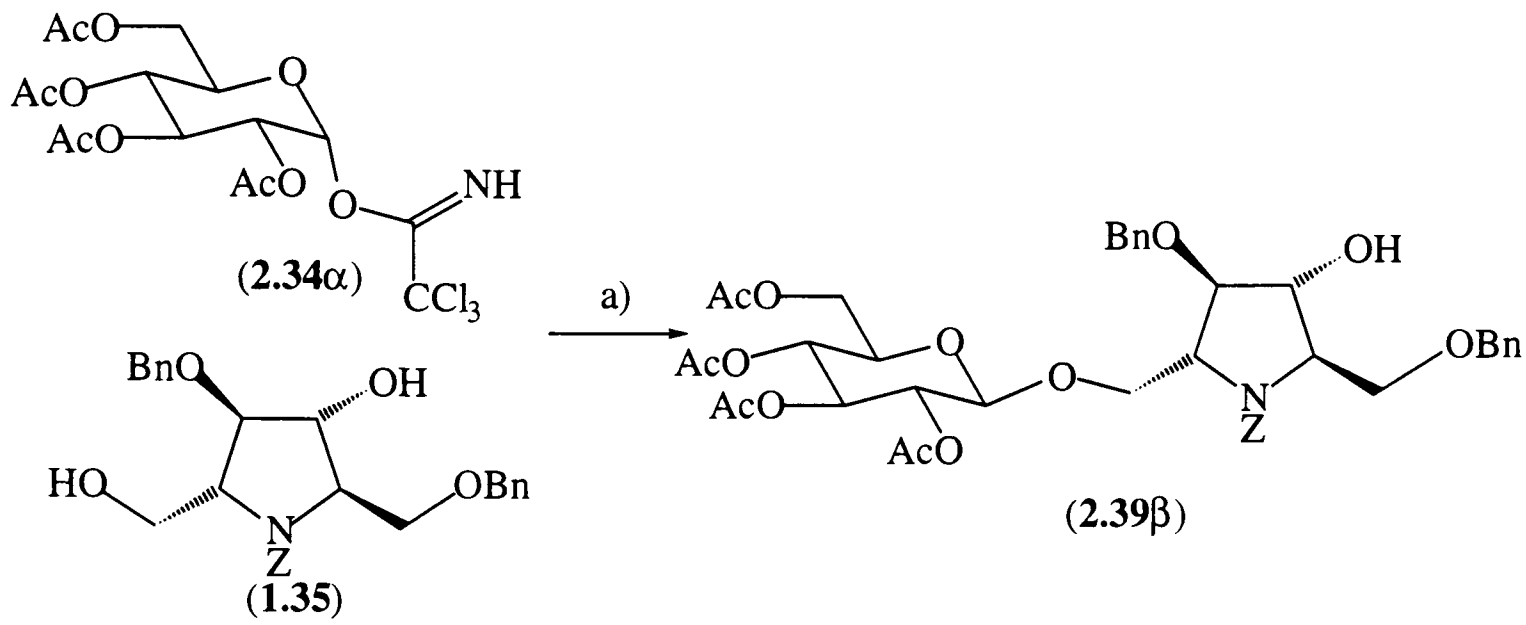
Treating dibenzyl DMDP (**1.35**) with benzyl protected imidate (**2.17**α) in diethyl ether with TMS triflate gave a complex mixture of compounds. As well as the expected primary glycoside and tetrabenzylglucose (**2.28**) from the hydrolysis of the imidate, NMR studies of the reaction products also show production of secondary glycosides and also small quantities of possible trisaccharides. Of the primary glycosides formed the two anomers occurred in a ratio of 3/1 α/β (Figure 2.37).



a) TMSOTf, Diethyl ether, 3A molecular sieves

Figure 2.37

β-Glucoside of DMDP linked at the primary hydroxyl was synthesised *via* the acetyl protected imidate (**2.34**α) and the dibenzyl DMDP (**1.35**) (Figure 2.38).



a) TMSOTf, Diethyl ether, 3A molecular sieves

Figure 2.38

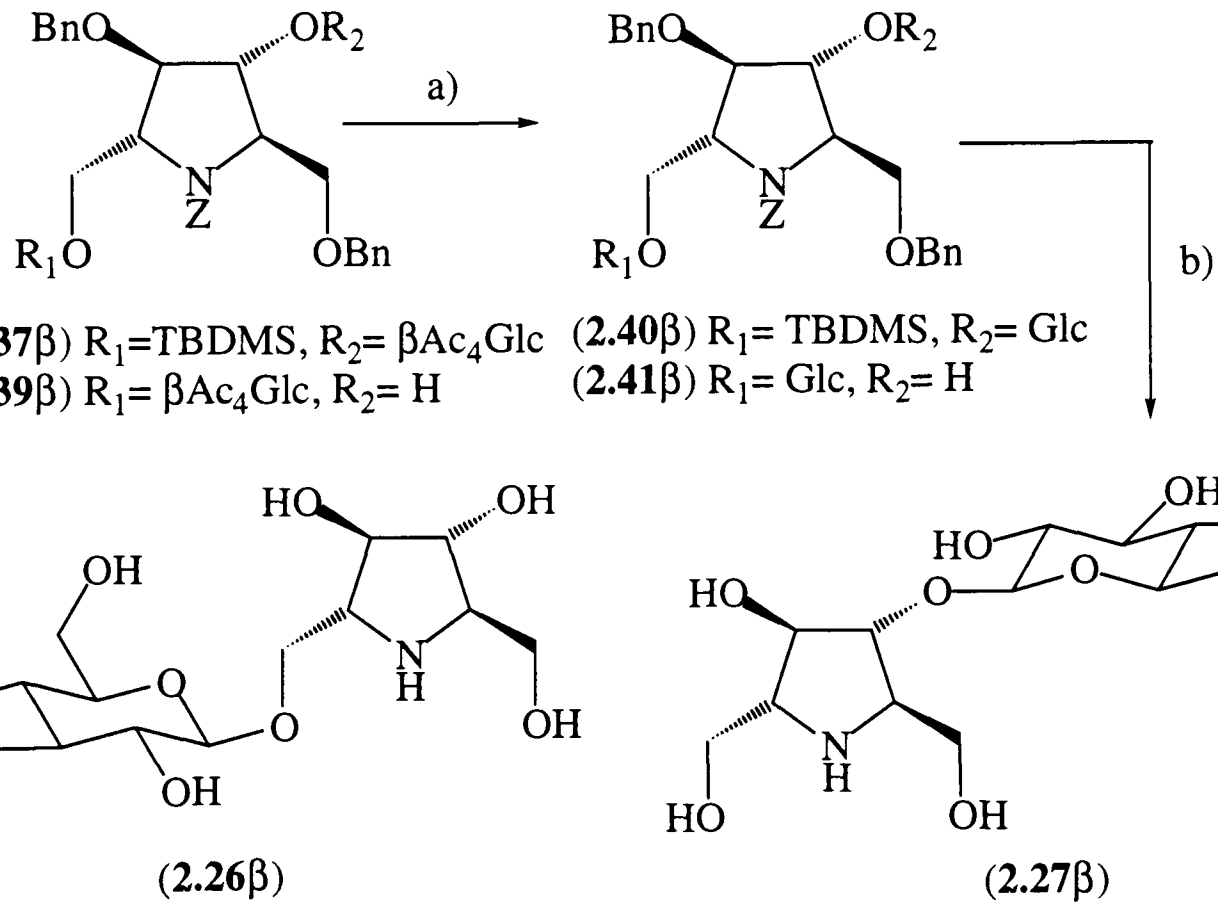
The major product from this reaction was the required glucose1-1DMDP derivative (**2.39**β). Also formed was the secondary β-glycoside in a ratio of 1/3 to the primary glycoside, with no α-products or trisaccharide observed. This could mainly be due to the lower reactivity of the acetyl protected imidate thus causing a greater selectivity between the primary and secondary positions.

During the glycosylation of both the β-anomers, no product was detected from the attack of the acceptor on the C-2 acetyl during neighbouring group participation.

2.2.c.i Deprotection of the β-glucosides

Both the β-glucosides (**2.37**β) and (**2.39**β) were deprotected in an analogous manner to each other, as they both had acetyl protection of the glucose and benzyl protection of the aglycone. A primary hydroxyl of (**2.37**β) also was protected as a silyl ether. It was envisaged that the silyl group would be removed during the hydrogenation of

the benzyl ethers due to the addition of a small quantity of concentrated acid to assist the benzyl cleavage. The first stage was the removal of the acetyl groups, this was achieved by basic hydrolysis using sodium methoxide in DMF. After approximately 2 hours cleavage was complete. The glucosides were recovered by dissolving in chloroform and washing with a small amount of dilute hydrochloric acid to neutralise the solution to give partly deprotected glucosides (**2.40**β) and (**2.41**β) respectively. These glucosides were then exposed to an atmosphere of hydrogen in the presence of a palladium catalyst and a small quantity of hydrochloric acid. After stirring overnight, no starting material was present, the catalyst was filtered off and solvent removed (Figure 2.39).



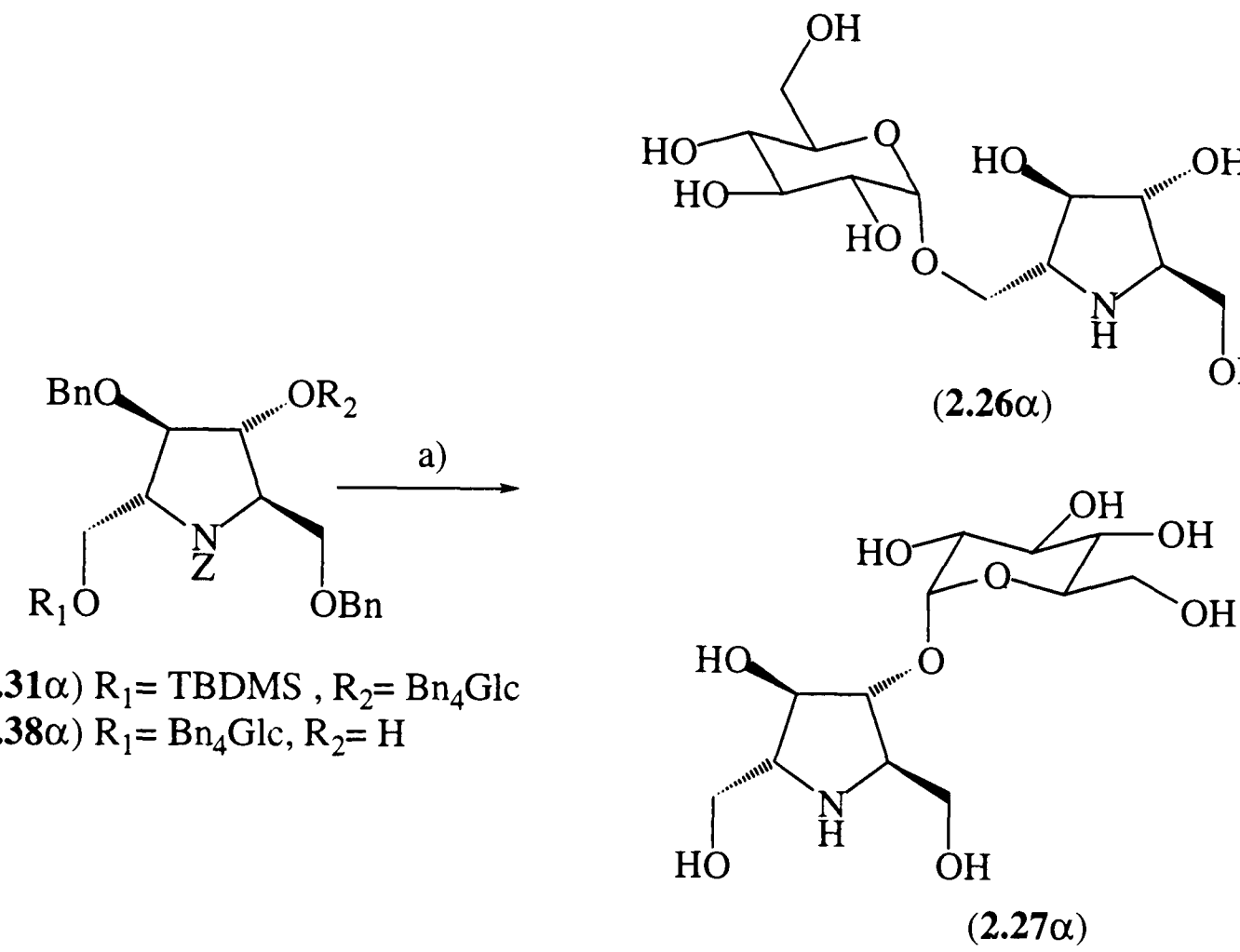
a) NaOMe, DMF
b) i. H_2/ Pd, EtOH, HCl ii. basic ion exchange

Figure 2.39

The glucosides were formed as their hydrochloride salts which were then passed down a basic ion exchange column to give the free bases.

2.2.c.ii Deprotection of the α-glucosides

The α-glucosides (**2.31α**) and (**2.38α**) were deprotected by hydrogenation. Again this was carried out with a small quantity of concentrated hydrochloric acid to give the glucosides which were isolated as their salts. Basic ion exchange chromatography gave the free base (Figure 2.40).

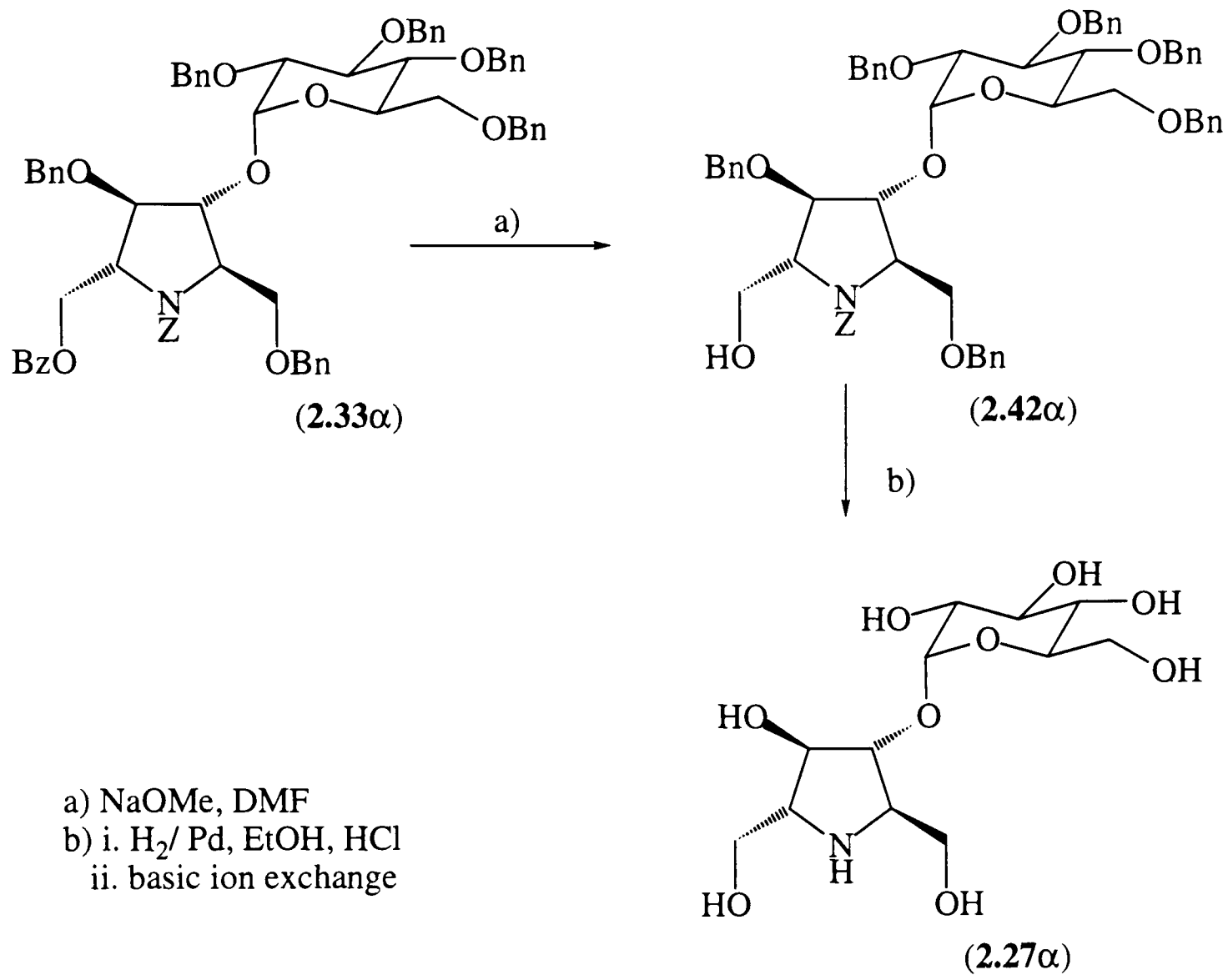


(**2.31α**) R_1= TBDMS , R_2= Bn_4Glc
(**2.38α**) R_1= Bn_4Glc, R_2= H

a) H_2/ Pd, EtOH, HCl

Figure 2.40

The remaining α-glucoside (**2.33α**) containing the primary benzoyl ester protection was subject to basic hydrolysis, then hydrogenation in an identical manner to the β-glucosides. Again the free amine was formed using basic ion exchange (Figure 2.41).



(**2.33**α) (**2.42**α) (**2.27**α)

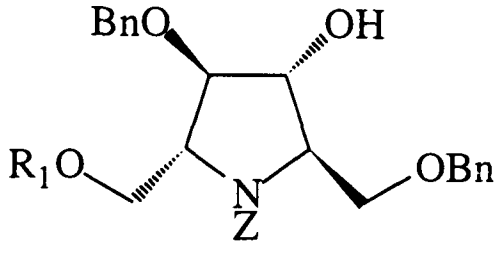
a) NaOMe, DMF
b) i. H_2/ Pd, EtOH, HCl
ii. basic ion exchange

Figure 2.41

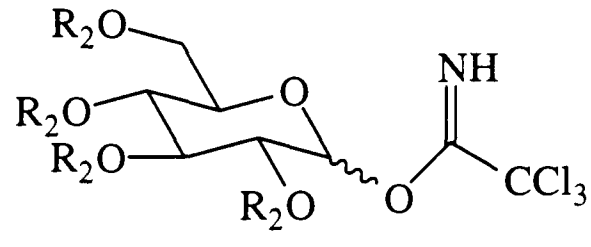
Structures of the final deprotected glucosides were determined by COSY, NOESY, HMQC and HMBC performed by M. Wormald.[42]

2.2.d Review of Glycosylation of DMDP (1.1)

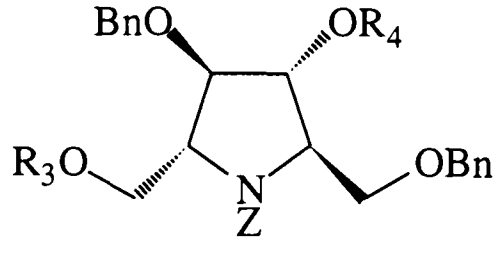
Table 2.2 shows a summary of the glycosylation reactions carried out.



(**2.30**) R_1 = TBDMS
(**2.32**) R_1 = Bz
(**1.35**) R_1 = H



(**2.17α**) R_2 = Bn
(**2.17β**) R_2 = Bn
(**2.34α**) R_2 = Ac



(**2.31α**) R_3 = TBDMS, R_4 = Bn_4Glc
(**2.33α**) R_3 = Bz, R_4 = Bn_4Glc
(**2.37β**) R_3 = TBDMS, R_4 = Ac_4Glc
(**2.38α**) R_3 = Bn_4Glc, R_4 = H
(**2.39β**) R_3 = Ac_4Glc, R_4 = H

Glycosyl donor	Glycosyl acceptor	Glycoside	Catalyst	Solvent	Yield
(**2.17α**)	(**2.30**)	(**2.31α**)	TMSOTf	Et_2O	51
(**2.17β**)	(**2.32**)	(**2.33α**)	$BF_3{\cdot}OEt_2$	DCM	75
(**2.34α**)	(**2.30**)	(**2.37β**)	TMSOTf	Et_2O	12
(**2.34α**)	(**2.30**)	(**2.37β**)	TMSOTf	MeCN	6
(**2.17α**)	(**1.35**)	(**2.38α**)	TMSOTf	Et_2O	13 (20*)
(**2.34α**)	(**1.35**)	(**2.39β**)	TMSOTf	Et_2O	18 (53*)

* Yields based on recovered starting material

Table 2.2

Although use of boron trifluoride etherate gave the best yield of glycoside, it was found necessary to use the stronger Lewis acid TMS triflate for the glycosylations carried out where acetyl protection was used. Only low yields where formed during these glycosylations due to the reduced reactivity of the glycosyl donor and the competing hydrolysis reactions.

Diethyl ether proved to be a good solvent for forming both α and β-glycosides, even though it has been shown to favour α-glycoside formation with non-participating groups at C-2.[38] Using acetyl protecting groups it was found that the glycosylation proceeded with 100% formation of the β-product as predicted.

The formation of the α-anomer using boron trifluoride etherate in dichloromethane is suggested to be due to the reaction occurring with S_N2 chemistry such that the β-donor used favours the formation of the α-glycoside. This catalyst can only be used when there are no silyl ethers present and when the donor does not have reduced reactivity due to acetyl protection.

Although access to all glucosides of DMDP was achieved, a wide range of yields was observed reflecting the problems of glycosylation reactions. The major problems encounter were :-

- The high sensitivity to water.
- The dependence of the reactivity on the nature of solvent and concentration.
- Specific nature of each type of glycosidic link.

2.2.e Biological activity of the glucosides of DMDP

The results of tests to determine the enzymic inhibition properties of the four glucosides against a wide range of enzymes are shown in table 2.3.[43] They were performed using *para*-nitrophenylpyranosides as substrates. The assays were pre-incubated for

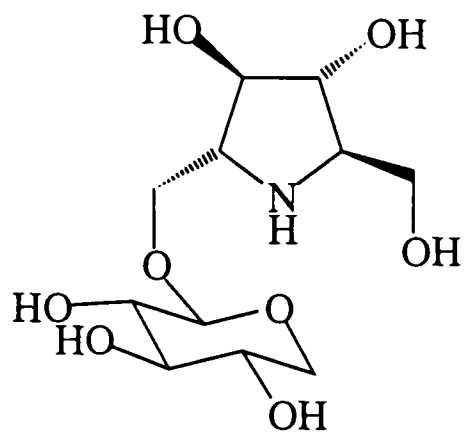
10 minutes at 30 °C. The assays were stopped after 10 minutes by addition of glycine buffer and the absorbence at 405 nm measured.

It is noted that whereas DMDP is a strong inhibitor of several glycosidases, the DMDP glucosides do not show the same inhibitory properties. A recent report has shown that the β-**L**-xylopyranoside of DMDP (**2.43**) to also have a dramatically lower inhibition than DMDP (**1.1**). It was found to have K_i values of 1.38 mM against α-glucosidase and 6.89 mM against β-glucosidase.[44]

Enzyme	DMDP (**1.1**)†	Glcα1-1 (**2.26α**)	Glcβ1-1 (**2.26β**)	Glcα1-3 (**2.27α**)	Glcβ1-3 (**2.27β**)
α–glucosidase (Brewers yeast)	I	I	X	X	I
β–glucosidase (Almond)	I	I	X	X	I
α–galactosidase (green coffee bean)	I	i	X		i
α–galactosidase (E. coli)	I	I	X	X	i
β–galactosidase (E. coli)	X		X		
β–*N*-acetylglucosaminidase (Bovine)	X	X	X	X	X
Cellulase (A. niger)*	X	X	X	X	X
Pectinase (T. viride)*	X	X	X	X	X
Xylanase (A. niger)*	I	i	X	X	i
α–glucosidase (Rabbit gut mucous)	I	I	X		I
β–glucosidase (Rabbit gut mucous)	I	I	X		I
β–galactosidase (Rabbit gut mucous)			60%		
β–glucosidase (Rabbit liver)	I	i	X		i
β–galactosidase (Rabbit liver)	I	I	42%		I

X = no inhibition detected, I = 50% inhibition or greater, i = less than 50% inhibition
Percentage inhibition at 409μM of DMDP†, 205μM of glucoside except at 154 μM where stated (*).

Table 2.3



(2.43)

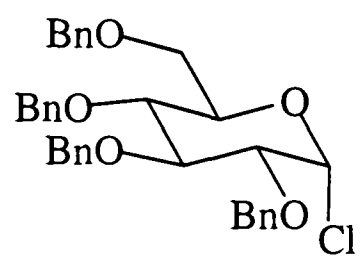
The two glucosides (**2.26**α) and (**2.27**β) were found to be unstable with respect to their monosaccharide components particularly under acidic conditions, therefore the enzymatic asseys were carried out with a small contamination of DMDP (**1.1**) present. Although the glycosidase inhibitor was present it was noted that the inhibition of the glucosides was orders of magnitude smaller then that reported for DMDP (**1.1**).

The blocking of either the primary or secondary positions of DMDP (**1.1**) with a glycoside appears to remove the exoglycosidase inhibitory properties shown by DMDP (**1.1**). This could be due to the need for both the hydroxyls for binding and recognition or the extra steric bulk of the glycoside may be interfering with the adoption of the required configuration to match the transition state. Either of these would dramatically effect the activity of the molecule.

Assays were also carried out against β-endomannosidase and glucosyl transferase,[45] and once again no strong inhibitory properties were found for any of the DMDP glucosides.

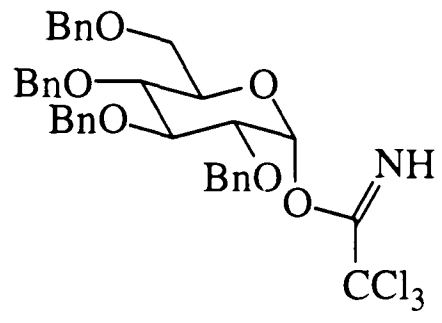
2.3 Experimental

2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucopyranosyl chloride (**2.3**)



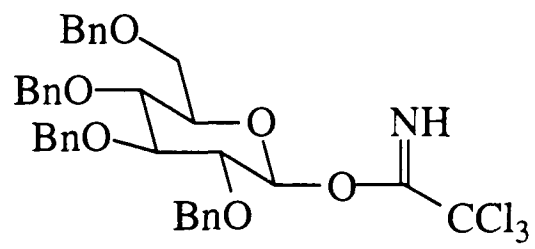
2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucose (**2.28**) (500 mg, 1.0 mmol) was added to a freshly prepared solution of *N,N*-dimethylchloroforminium chloride (**2.29**) (*N,N*-dimethylformamide 0.32 ml, thionyl chloride 2.0 ml) and stirred at room temperature. After 3 hours tlc analysis (diethyl ether/ hexane 1/ 3) showed no starting material (R_f 0.1) and one product (R_f 0.3). The solvent was removed *in vacuo* and the residue was coevaporated with toluene (2x 5 ml). The residue was purified by flash column chromatography (diethyl ether/ hexane 1/ 4) to afford 2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl chloride (**2.3**) (453 mg, 84%) as a clear oil. $[\alpha]_D^{25}$ +63.5 (c 4.6, $CHCl_3$), [lit.[46] $[\alpha]_D^{20}$ +62 (c 1.0, $CHCl_3$)]. δ_H (200 MHz, $CDCl_3$); 3.6 -3.9 (4H, m, H-6, H-2, H-3), 4.0 -4.2 (2H, m, H-4, H-5), 4.55, 4.90 (2H, 2x d J_{AB} 10.6 Hz, $PhCH_2$), 4.51, 4.64 (2H, 2x d J_{AB} 12.1 Hz, $PhCH_2$), 4.77 (2H, s, $PhCH_2$), 4.88, 5.04 (2H, 2x d J_{AB} 10.8 Hz, $PhCH_2$), 6.12 (1H, d $J_{1,2}$ 3.6 Hz, H-1). δ_C (50 MHz, $CDCl_3$, non aromatic); 67.5 (t, C-6), 72.5, 74.2, 75.0, 75.9 (4x t, 4x $PhCH_2$), 74.1, 76.0, 79.8, 80.2 (4x d, C-2, C-3, C-4, C-5), 98.2 (d, C-1).

2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.17**α)



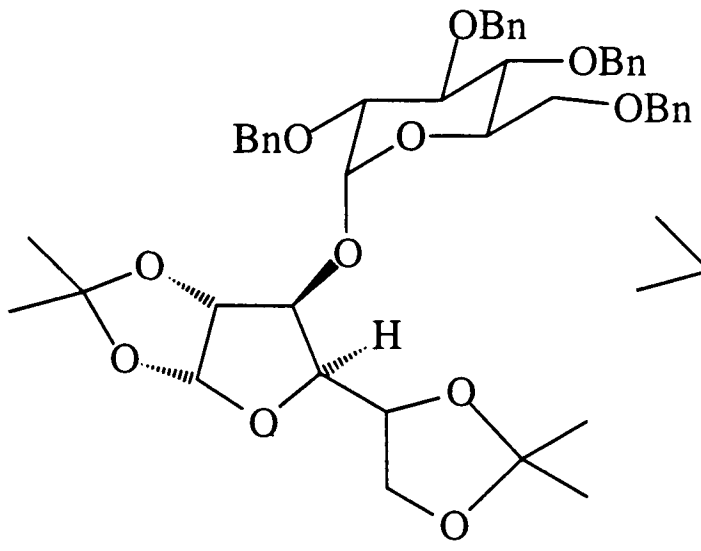
To a solution of 2,3,4,6-tetra-*O*-benzyl-α-**D**-glucose (**2.28**) (500 mg, 0.97 mmol) in dichloromethane (5 ml) was added sodium hydride (24 mg, 0.97 mmol) and trichloroacetonitrile (0.34 ml, 3.4 mmol). After stirring for 1 hour, tlc analysis (diethyl ether/ hexane, 2/3) showed no starting material (R_f 0.1) and one product (R_f 0.5), the mixture was filtered through Celite and washed with dichloromethane (10 ml), the filtrate was concentrated *in vacuo* and the oily residue purified by flash column chromatography (diethyl ether/ hexane, 2/3) to afford 2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.17**α) (379 mg, 57%) as a white crystalline solid. M.p.; 76-77 °C, [lit.[47] 77 °C]. $[\alpha]_D^{23}$ +60.4 (c 0.9, $CHCl_3$), [lit.[47] $[\alpha]_D^{20}$ +61.5 (c 1.0, $CHCl_3$)]. ν_{MAX} (KBr); 3318 (NH), 1675 (C=N) cm^{-1}. δ_H (500 MHz, CD_3CN); 3.64 (1H, dd $J_{3,4}$ 4.3, $J_{3,2}$ 9.8 Hz, H-3), 3.65 (1H, m, H-5), 3.71 (1H, dd $J_{4,3}$ 4.3, $J_{4,5}$ 11.1 Hz, H-4), 3.76 (1H, dd $J_{2,1}$ 3.4, $J_{2,3}$ 9.6 Hz, H-2), 3.95, 3.93 (2H, m, H-6, H-6'), 4.48, 4.53 (2H, 2x d J_{AB} 11.9 Hz, CH_2Ph), 4.58, 4.83 (2H, 2x d J_{AB} 10.9 Hz, CH_2Ph), 4.68, 4.74 (2H, 2x d J_{AB} 11.5 Hz, CH_2Ph), 4.80, 4.88 (2H, 2x d J_{AB} 11.1 Hz, CH_2Ph), 6.52 (1H, d $J_{1,2}$ 3.4, Hz H-1), 7.22- 7.38 (20H, m, 4x Ph), 8.96 (1H, s, NH). δ_C (50 MHz, CD_3CN, non aromatic); 68.5, 72.5, 72.7, 74.8, 75.0 (5x t, 4x C$\underline{H}_2$Ph, C-6), 73.1, 79.6, 81.2, 77.5 (4x d, C-2, C-3, C-4, C-5), 94.1 (d, C-1), 160.4 (s, C=N).

2,3,4,6-Tetra-*O*-benzyl-β-**D**-glucopyranosyl trichloroacetimidate (**2.17**β)

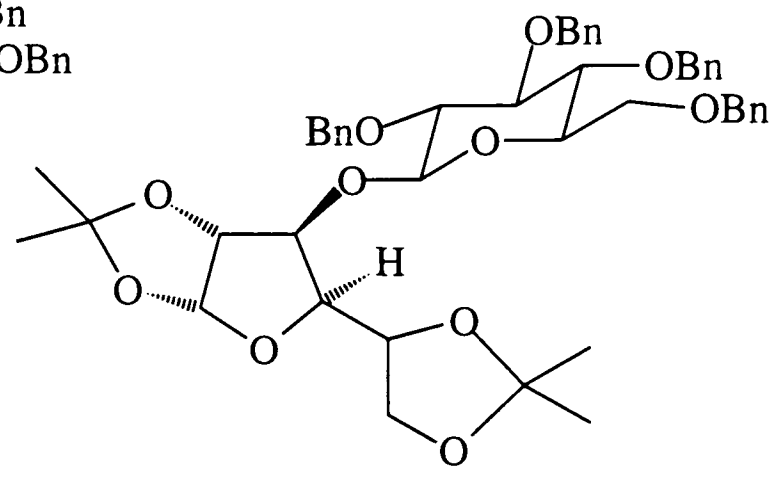


To a solution of 2,3,4,6-tetra-*O*-benzyl-**D**-glucose (**2.28**) (500 mg, 0.97 mmol) in dichloromethane (5 ml) was added potassium carbonate (0.5 g) and trichloroacetonitrile (0.5 ml, 0.49 mmol). The suspension was vigorously stirred under nitrogen for 5 hours at room temperature when tlc analysis (diethyl ether/ hexane 2/3) showed no starting material (R_f 0.1) and one product (R_f 0.4). The mixture was filtered through Celite and washed with dichloromethane (10 ml), the filtrate was concentrated *in vacuo* and the oily residue crystallised from diethyl ether/ hexane (1/1, 5 ml) to afford 2,3,4,6-tetra-*O*-benzyl-β-**D**-glucopyranosyl trichloroacetimidate (**2.17**β) (343 mg, 52%) as a white crystalline solid. M.p.; 73-74 °C, [lit.[26] 72-73 °C]. $[\alpha]_D^{22}$ +26.5 (c 1.0, $CHCl_3$), [lit.[26] $[\alpha]_D^{20}$ +22.0 (c 1.0, $CHCl_3$)]. ν_{MAX} (KBr); 3337 (w br, NH), 1673 (s, C=N). δ_H (500 MHz, $CDCl_3$); 3.71- 3.72 (2H, m), 3.81- 3.84 (5H, m), 4.61, 4.68 (2H, 2x d J_{AB} 12.2 Hz, $\underline{C}H_2Ph$), 4.66, 4.98 (2H, 2x d J_{AB} 10.9 Hz, $\underline{C}H_2Ph$), 4.83, 5.01 (2H, 2x d J_{AB} 10.9 Hz, $\underline{C}H_2Ph$), 4.88, 4.90 (2H, 2x d J_{AB} 2.5 Hz, $C\underline{H}_2Ph$), 5.89 (1H, d *J* 7.2 Hz, H-1), 7.23- 7.40 (20H, m, 4x Ph), 8.77 (1H, s, NH). δ_C (50 MHz, $CDCl_3$, non aromatic); 68.2, 73.4, 75.0, 75.7, (4x t, 4x $\underline{C}H_2Ph$), 75.9, 77.1, 81.0, 84.6 (4x d, C-2, C-3, C-4, C-5), 98.4 (d, C-1), 161.2 (s, C=N).

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-**D**-glucopyranosyl)-1,2: 5,6-di-*O*-isopropylidene-α -**D**-glucofuranose (**2.29**)



(**2.29α**)



(**2.29β**)

Method 1

A mixture of 1,2:5,6 di-*O*-isopropylidene-α-**D**-glucofuranose (**1.13**) (104 mg, 0.40 mmol) and 2,3,4,6-tetra-*O*-benzyl-**D**-glucopyranosyl chloride (**2.3**) (111 mg, 0.20 mmol) was coevaporated with toluene (2x 2 ml). This mixture was then dissolved in dry dichloromethane (3 ml) with powdered 3A molecular sieves (300 mg) and stirred for 20 minutes. To this was added 2,4,6-trimethylpyridine (50 µl, 0.40 mmol) and the solution cooled to -20 °C. Silver triflate (102 mg, 0.40 mmol) was added in the dark and the solution stirred for 24 hours at -20 °C and then warmed to room temperature for a further 24 hours when tlc analysis (diethyl ether /hexane, 1/1, 2 elutions) showed no 1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**1.13**) (R_f 0.1), and two products (R_f 0.45, 0.50). The solution was stirred in the light for 1 hour. It was then diluted with dichloromethane (10 ml) and the molecular sieves filtered off through Celite. The residue was washed with dilute hydrochloric acid (3 ml), water (2x 5 ml), brine (5 ml) and dried (magnesium sulphate). The products were evaporated onto silica and purified by flash column chromatography (diethyl ether /hexane 1/3 to 1/1) to afford 3-*O*-(2,3,4,6-tetra-*O*-

benzyl-α-**D**-glucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**2.29**α) (36 mg, 23%) as a white crystalline solid and 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-**D**-glucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**2.29**β) (46 mg, 31%) as a white crystalline solid.

Method 2.

A mixture of 1,2: 5,6 di-*O*-isopropylidene-α-**D**-glucofuranose (**1.13**) (50 mg, 0.19 mmol) and 2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.17**α) (100 mg, 0.15 mmol) was stirred in dry dichloromethane (5 ml) with powdered 3A molecular sieves for 30 minutes. *p*-Toluene sulphonic acid (5 mg, 0.03 mmol) was then added and the mixture was stirred at room temperature. After 24 hours tlc analysis (diethyl ether / hexane 1/2, 2 elutions) showed no imidate (R_f 0.6) and one major product (R_f 0.45). The mixture was diluted with dichloromethane (20 ml) and filtered through Celite. The filtrate was washed with saturated aqueous sodium bicarbonate (10 ml), water (10 ml), brine (5 ml) and dried (magnesium sulphate). Purification by flash column chromatography (diethyl ether/ hexane 1/1) afforded 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-**D**-glucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**2.29**β) (32 mg, 21%, 63% based on recovered starting material) as a white crystalline solid.

Method 3.

A mixture of 1,2: 5,6 di-*O*-isopropylidene-α-**D**-glucofuranose (**1.13**) (95 mg, 0.35 mmol) and 2,3,4,6-tetra-*O*-benzyl-β-**D**-glucopyranosyl trichloroacetimidate (**2.17**β) (311 mg, 0.45 mmol) was stirred in dry dichloromethane (3 ml) with powdered 3A molecular sieves for 30 minutes. The solution was cooled to -20 °C and boron trifluoride etherate (50 μl, 0.45 mmol) was then added to the mixture and stirred at -20 °C. After 90

minutes tlc analysis (diethyl ether/ hexane 1/2, 2 elutions) showed no imidate (R_f 0.6) and a major product (R_f 0.5). The mixture was diluted with dichloromethane (20 ml), warmed to room temperature and filtered through Celite. The filtrate was washed with saturated aqueous sodium bicarbonate (10 ml), water (10 ml), brine (5 ml) and dried (magnesium sulphate). Purification by flash column chromatography (diethyl ether/ hexane 1/1) afforded 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**2.29**α) (204 mg, 75%) as a white crystalline solid.

Method 4.

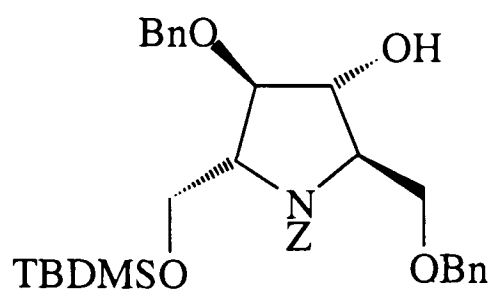
A mixture of 1,2: 5,6 di-*O*-isopropylidene-α-**D**-glucofuranose (**1.13**) (8 mg, 0.03 mmol) and 2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.17**α) (20 mg, 0.03 mmol) was stirred in dry diethyl ether (2 ml) with powdered 3A molecular sieves for 30 minutes. Trimethylsilyl triflate (19 μl, 0.01 mmol) was then added to the mixture and stirred at room temperature. After 60 minutes tlc analysis (diethyl ether/ hexane 1/2, 2 elutions) showed no alcohol (R_f 0.1) and a major product (R_f 0.5). To the solution was added saturated aqueous sodium bicarbonate solution (0.5 ml). The mixture was diluted with diethyl ether (20 ml) and filtered through Celite. The organic layer was separated and washed with water (5 ml), brine (5 ml) and dried (sodium sulphate). Purification by flash column chromatography (diethyl ether/ hexane 1/1) afforded 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**2.29**α) (2 mg, 16%) as a white crystalline solid.

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**2.29**α) :- M.p. 87-90 °C, [lit.[23] 90-91°C]. $[\alpha]_D^{25}$ +41.9 (c 1.4, $CHCl_3$) [lit.[23] $[\alpha]_D^{20}$ +46.0 (c 2.0, $CHCl_3$)]. δ_H (500 MHz, $CDCl_3$); 1.24, 1.26, 1.42, 1.56 (12H, 4x s, 2x $C(CH_3)_2$), 3.57 (1H, dd $J_{6,5}$ 3.6 $J_{6,6'}$ 9.8 Hz, H_G-6), 3.62 (1H, t *J* 9.5 Hz, H_G-6'),

3.71 (1H, s, H_G-3), 3.72 (1H, d $J_{4,5}$ 3.3 Hz, H_G-4), 3.80 (1H, dt $J_{5,4}$ 3.0 $J_{5,6}$ 3.0 $J_{5,6'}$ 10.0 Hz, H_G-5), 3.95 (1H, t J 6.4 Hz, H_D-6), 4.05 (1H, s, H_D-3), 4.06 (1H, d $J_{4,5}$ 5.0 Hz, H_D-4), 4.14 (1H, dd $J_{6',5}$ 2.7, $J_{6',6}$ 8.0 Hz, H_D-6'), 4.24 (1H, d $J_{2,1}$ 3.2 Hz, H_G-2), 4.47, 4.84 (2H, 2x d J_{AB} 10.8 Hz, $PhCH_2O$), 4.49 (1H, ddd $J_{5,6}$ 2.8 $J_{5,4}$ 5.5 $J_{5,6'}$ 9.1 Hz, H_D-5), 4.50, 4.63 (2H, 2x d J_{AB} 12.1 Hz, $PhCH_2O$), 4.68 (1H, d $J_{2,1}$ 3.6 Hz, H_D-2), 4.71 (2H, 2x d J_{AB} 11.8 Hz, $PhCH_2O$), 4.82, 4.97 (2H, 2x d J_{AB} 10.9 Hz, $PhCH_2O$), 5.26 (1H, d $J_{1,2}$ 3.5 Hz, H_G-1), 5.89 (1H, $J_{1,2}$ 3.6 Hz, H_D-1), 7.13-7.15 (2H, m, ArH), 7.27- 7.36 (18H, m, ArH). δ_C (125 MHz, $CDCl_3$, non aromatic); 25.46, 26.12, 26.77, 26.98 (4x q, 4x CH_3), 67.03, 68.57 (2x t, 2x C-6), 71.17, 72.33, 77.65, 79.94, 80.61, 81.17, 81.48, 83.68 (8x d, 2x C-2, C-3, C-4, C-5), 73.03, 73.54, 75.30, 75.62 (4x t, 4x $Ph\underline{C}H_2$), 97.93, 105.17 (2x d, 2x C-1).

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-**D**-glucopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose (**2.29**β) :- M.p. (diethyl ether/ hexane); 112-114 °C, [lit.[23] 118-119 °C]. $[\alpha]_D^{25}$ +4.0 (c 0.6, $CHCl_3$), [lit.[23] $[\alpha]_D^{20}$ +1.0 (c 1.0, $CHCl_3$)]. δ_H (500 MHz, $CDCl_3$); 1.25, 1.32, 1.44, 1.50 (12H, 4x s, 4x CH_3), 3.42 (1H, m), 3.45 (1H, m), 3.65 (1H, m), 3.66 (1H, d J 3.3 Hz), 3.72 (2H, d J 3.2 Hz), 4.09 (2H, d J 6.36 Hz), 4.32 (1H, d J 3.2 Hz), 4.40 (1H, dd J 3.7, 4.5 Hz), 4.45- 4.50 (2H, m), 4.51 (1H, d J 3.3 Hz), 4.58, 4.63 (2H, 2x d J_{AB} 12.1 Hz, $PhCH_2$), 4.75 (2H, s, $PhCH_2$), 4.60, 4.83 (2H, 2x d J_{AB} 10.8 Hz, $PhCH_2$), 4.85, 4.93 (2H, 2x d J_{AB} 11.0 Hz, $PhCH_2$), 5.78 (1H, d J 3.8 Hz) 7.20- 7.22 (2H, m, Ph), 7.27- 7.38 (18H, m, Ph). δ_C (50 MHz, $CDCl_3$, non aromatic); 25.3, 26.1, 26.5, 26.7 (4x q, 4x CH_3), 64.4, 65.8 (2x t, 2x C-6), 68.5, 73.0, 77.7, 80.2, 80.4, 82.0, 82.6, 84.6 (8x d, 2x C-2, C-3, C-4, C-5), 73.6, 75.1, 75.3, 75.6 (4x t, 4x $Ph\underline{C}H_2$), 101.3, 105.1 (2x d, 2x C-1).

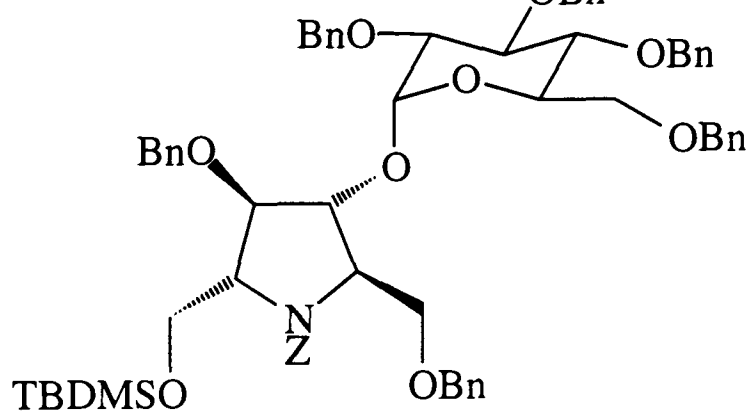
N-Benzyloxycarbonyl-1-*O*-tertbutyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.30**)



To a solution of *N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**1.35**) (200 mg, 0.42 mmol) in dichloromethane (50 ml) at -20 °C was added dry pyridine (0.07 ml, 0.9 mmol) and *tert*-butyldimethylsilyl triflate (0.13 ml, 0.6 mmol). After one hour tlc analysis(diethyl ether/ hexane 2/1) showed no starting material (R_f 0.2) and one product (R_f 0.8). Water (5 ml) was added and stirred for 10 minutes, then the solution was washed with dilute hydrochloric acid (2M, 10 ml), water (2x 10 ml) and brine (10 ml). The organic layers were dried (magnesium sulphate) and concentrated *in vacuo*. The mixture was purified by flash column chromatography (diethyl ether/ hexane, 2/1) to afford *N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.30**) (152 mg, 61%) as a clear oil. $[\alpha]_D^{25}$ -44.5 (c 0.75, $CHCl_3$). Found C 69.16 H 7.34 N 2.16%, $C_{34}H_{45}NO_6Si$ requires C 69.00 H 7.66 N 2.37%. ν_{MAX} (thin film); 3402 (OH), 1703 (C=O) cm^{-1}. δ_H (500 MHz, $C_6D_5CD_3$, 30 °C); -0.09 (2H, d *J* 9.3 Hz, $(CH_3)_2$ minor rotamer), 0.02 (4H, s, $(CH_3)_2$ major rotamer), 0.86 (4H, s, $C(CH_3)_3$ minor rotamer), 0.89 (8H, s, $C(CH_3)_3$ major rotamer), 3.40 (0.3H, d *J* 8.7 Hz), 3.49 (0.6H, dd, *J* 1.7, 10.2 Hz), 3.51 (0.3H, dd *J* 1.7, 10.2 Hz), 3.63 (0.6H, dd *J* 8.7, 11.6 Hz), 3.70 (0.3H, dd *J* 8.7, 11.6 Hz), 3.75 (0.6H, dd *J* 4.4, 8.7 Hz), 3.89 (1H, s), 3.98 (1H, m), 4.08 (1H, d *J* 3.6 Hz),4.2- 4.4 (3H, m), 4.45 (0.6H, d *J* 11.6 Hz), 4.50 (0.3H, d *J* 4.4 Hz), 4.52 (0.3H, d *J* 10.2 Hz), 4.53 (0.6H, d *J* 11.6 Hz) 4.92, 5.16 (2H, 2x d *J* 11.6 Hz), 4.99, 5.08 (2H, 2x d *J* 13.0 Hz), 7.00- 7.25 (15H, m, Ph). δ_H (500 MHz, $C_6D_5CD_3$,; 90 °C); 0.00 (6H, s, 2x CH_3), 0.86 (9H, s, 3x CH_3), 3.63 (1H, dd $J_{1,1'}$ 2.9,

$J_{1,2}$ 10.1 Hz, H-1), 3.65 (1H, t *J* 9.8 Hz, H-5), 3.92 (1H, s, H-3), 4.01 (1H, br s, OH), 4.19 (1H, dd $J_{1',1}$ 4.3, $J_{1',2}$ 10.1 Hz, H-1'), 4.3-4.4 (5H, m, $PhCH_2$, H-2, H-6, H-6'), 4.43 (1H, d $J_{4,5}$ 9.8 Hz, H-4), 4.80, 5.10 (2H, 2x d J_{AB} 13.05 Hz, $PhCH_2$), 4.99 (2H, s, $PhCH_2OCO$), 7.00-7.24 (15H, m, 3x Ph). δ_C (50 MHz, $CDCl_3$, non aromatic, 30 °C); -5.0 (q, CH_3), 17.6 (s, $\underline{C}(CH_3)_3$), 26.3 (q, CH_3), 61.5, 68.3, 75.0, 86.1 (4x t, C-2, C-3, C-4, C-5), 65.9, 67.2, 69.5, 71.1, 73.6 (5x d, 3x CH_2, 2x $PhCH_2OCO$ rotamers), 155.8 (s, C=O). m/z (DCI, NH_3); 592 (10, MH^+), 91 (100%, $PhCH_2$).

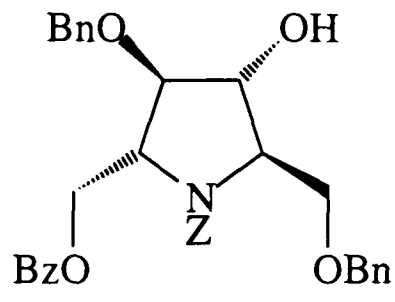
4-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.31**α)



To solution of *N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.30**) (104 mg, 0.17 mmol) in diethyl ether (5 ml) was added 2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.17**α) (137 mg, 0.2 mmol) and stirred for 30 minutes with powdered 3A molecular sieves under nitrogen. To this was then added trimethylsilyl triflate (10 μL, 0.05 mmol) and the mixture stirred at room temperature. After 2 hours, tlc analysis (diethyl ether/ hexane, 1/2, 2 elutions) showed no alcohol (R_f 0.7) and a major product (R_f 0.3) an excess of solid sodium bicarbonate was added and stirred for 15 minutes. The mixture was filtered through Celite and diluted with diethyl ether (30 ml). This was washed with saturated aqueous

sodium bicarbonate solution (2x 10 ml), then water (10 ml). The organic layer was dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by column chromatography (diether ether/ hexane 1/3, normal pressure) to afford 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.31**α) (95 mg, 51%) as a clear oil. $[\alpha]_D^{25}$ +12.5 (c 0.85, $CHCl_3$). Found C 73.41 H 7.08 N 0.98%, $C_{68}H_{79}O_{11}NSi$ requires C 73.29 H 7.14 N 1.26%. ν_{MAX} (thin film); 1703 (C=O) cm^{-1}. δ_H (500 MHz, $C_6D_5CD_3$, 90 °C); 0.00 (s, $Si(CH_3)_2$), 0.89 (s, $C(CH_3)_3$), 3.46 (dd, *J* 3.4, 9.7 Hz), 3.56 (dd, *J* 1.6, 10.9 Hz), 3.64 (td, *J* 3.4, 10.9 Hz), 3.70 (d, *J* 9.4 Hz), 3.91 (dt, *J* 2.8, 9.7 Hz), 4.01 (t, *J* 9.1 Hz), 4.15 (br s), 4.32, 4.41 (2x d, J_{AB} 12.2 Hz), 4.35 (s), 4.48, 4.51 (2x d, J_{AB} 10.0 Hz), 4.53, 4.58 (2x d, J_{AB} 10.9 Hz), 4.67 (s), 4.72 (d, *J* 11.6 Hz), 4.80 (d, *J* 10.9 Hz), 4.88 (d, *J* 10.9 Hz), 4.89 (d, *J* 12.5 Hz), 4.99 (d, *J* 3.4 Hz), 5.11 (d, *J* 12.5 Hz), 7.0- 7.3 (m, PhH). δ_C (125 MHz, $CDCl_3$, non aromatic) -5.39, -5.28, -5.16, -5.00 (4x q, $Si(CH_3)_2$), 18.17, 18.08 (2x s, $\underline{C}(CH_3)_3$), 25.86, 25.97 (2x q, 3x $C(\underline{C}H_3)$), 60.15, 61.08, 67.00, 67.29, 67.62, 67.78, 68.54, 70.95, 72.51, 72.64, 72.94, 73.07, 73.35, 74.80, 75.08, 75.63 (16x t, 10x CH_2), 63.84, 64.56, 65.78, 66.07, 70.68, 78.06, 78.66, 78.77, 79.65, 81.58, 81.96, 87.16, 84.79, 95.54, 96.68 (15x d, 9x CH), 154.38 (s, C=O).

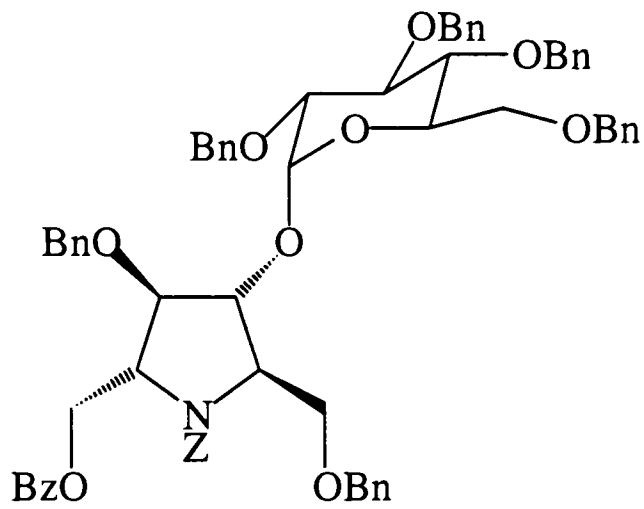
N-Benzyloxycarbonyl-1-*O*-benzoyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.32**)



To a solution of *N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**1.35**) (890 mg, 1.86 mmol) in dichloromethane (50 ml) at -10 °C was added dry

pyridine (0.45 ml, 5.6 mmol) and benzoyl chloride (0.24 ml, 2.0 mmol). After one hour tlc analysis (diethyl ether/ hexane 2/1) showed no alcohol (R_f 0.2) and one product (R_f 0.5). Water (5 ml) was added and stirred for 10 minutes, then the solution was washed with dilute hydrochloric acid (2M, 10 ml), water (2x 10 ml) and brine (10 ml). The organic layers were dried (magnesium sulphate) and concentrated *in vacuo*. The mixture was purified by flash column chromatography (diethyl ether/ hexane, 2/1) to afford *N*-benzyloxycarbonyl-1-*O*-benzoyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.32**) (721 mg, 66%) as a clear oil. $[\alpha]_D^{22}$ -18.8 (c 0.95, $CHCl_3$). Found C 72.34 H 5.88 N 2.41%, $C_{35}H_{35}NO_7$ requires C 72.27 H 6.06 N 2.41%. ν_{MAX} (thin film); 3444 (OH), 1723 (PhC=O), 1704 (NC=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 2.35 (1H, br d $J_{OH,H}$ 28 Hz, OH), 3.62 (0.5H, m), 3.78 (0.5H, dd *J* 4.09, 8.88 Hz), 4.02 (1H, m), 4.07 (1H, m), 4.14 (0.5H, dd *J* 4.1, 8.6 Hz), 4.21 (0.5H, dd *J* 3.0, 8.0 Hz), 4.33 (0.5H, dd *J*, 3.5, 8.6 Hz), 4.30, 4.46 (1H, dd *J* 12.0, 28.0 Hz), 4.50- 4.65 (5.5H, m), 4.71(0.5H, dd *J* 3.8, 10.5 Hz), 5.11, 4.17 (2H, 2x d J_{AB} 12.3 Hz, $PhCH_2O$), 7.24- 7.98 (20H, m, ArCH). δ_C (50 MHz, $CDCl_3$, non aromatic); 62.3 (t, CH_2), 62.7, 63.1 (2x d, CH), 65.7, 65.9 (2x d, CH), 67.3, 67.4 (2x t, CH_2), 68.4, 68.7 (2x t, CH_2), 71.3 (t, CH_2), 73.3, 73.4 (2x CH_2), 75.6, 76.3 (2x d, CH), 84.1, 84.5 (2x d, CH), 154.7, 154.8 (N$\underline{C}$OCH_2), 166.5 (s, Ph$\underline{C}$=O). m/z (CI, NH_3); 582 (40, MH^+), 474 (25, MH^+ - $PhCH_2O$), 108 (60, $PhCH_2O$), 91 (100%, $PhCH_2$).

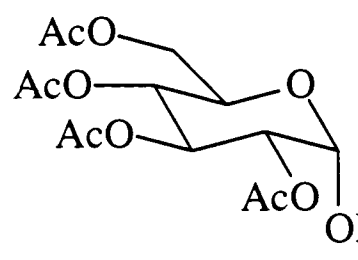
4-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-benzoyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.33**α)



To a solution of *N*-benzyloxycarbonyl-1-*O*-benzoyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.32**) (221 mg, 0.42 mmol) in dichloromethane (4 ml) was added 2,3,4,6-tetra-*O*-benzyl-β-**D**-glucopyranosyl trichloroacetimidate (**2.17**β) (215 mg, 0.31 mmol) and the mixture was stirred for 30 minutes with powdered 3A molecular sieves under nitrogen. This was cooled to -20 °C and then boron trifluoride etherate (52.7 μL, 0.40 mmol) was added. After 2 hours, tlc analysis (diethyl ether/ hexane, 1/2, 2 elutions) showed no alcohol (R_f 0.7) and a major product (R_f 0.33). An excess of solid sodium bicarbonate was added and stirred for 15 minutes. The mixture was filtered through Celite and diluted to 30 ml with dichloromethane. This was washed with saturated aqueous sodium bicarbonate solution (2x 10 ml), then water (10 ml). The organic layer was dried (sodium sulphate) and concentrated in vacuo. Purification by column chromatography (diether ether/ hexane 1/3, normal pressure) afforded 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-benzoyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.33**α) (239 mg, 75%) as a clear oil. $[\alpha]_D^{20}$ -3.3 (c 0.9, $CHCl_3$). Found C 75.05 H 6.30 N 1.27%, $C_{69}H_{69}O_{12}N$ requires C 75.31 H 6.66 N 1.61%. ν_{MAX} (thin film); 1729 (PhC=O), 1704 (NC=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$, non aromatic protons); 3.39 (1H, t *J* 11.2 Hz), 3.49 (1H, dd *J* 3.4, 9.6 Hz), 3.56 (1H, m), 3.58 (1H,

m), 3.71 (1H, dd *J* 2.8, 6.6 Hz), 3.79 (1H, dd, *J* 4.5, 8.6 Hz), 3.95 (1H, t 8.2 Hz), 4.14 (1H, d 9.6 Hz), 4.29, 4.32 (2H, 2x d, *J* 12.5 Hz), 4.38, 4.41 (2H, 2 x d *J* 0.4 Hz), 4.43 (1H, m), 4.45 (1H, m), 4.49- 4.65 (7H, m), 4.79- 4.89 (5H, m), 5.08- 5.25 (2H, m). δ_C (125 MHz, $CDCl_3$, non aromatic); 62.7, 62.9, 63.0, 67.3, 70.8 (5x t, CH_2), 63.9, 67.2, 67.8, 68.9 (4x d, CH_2), 73.1, 73.4, 74.8, 75.1, 75.6 (5x CH_2), 78.6, 81.8, 82.0, 84.7, 102.8 (5x d, CH), 154.3 (s, NC=O), 165.7 (s, PhC=O). m/z (ESI); 1104.7 (100%, MH^+).

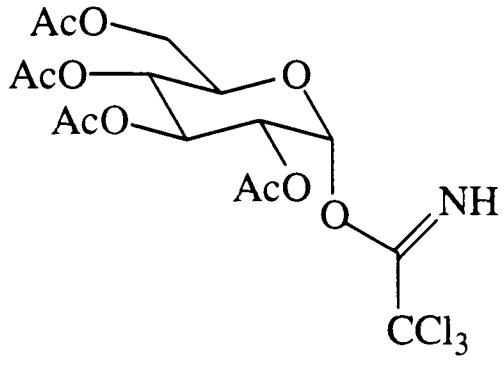
2,3,4,6-Tetra-*O*-acetyl-α-**D**-glucose (**2.35**)



To a solution of 1,2,3,4,6-penta-*O*-acetyl-**D**-glucose (**2.36**) (1.0 g, 2.5 mmol) in *N,N*-dimethylformamide (6 ml) was added freshly powdered ammonium carbonate (0.5 g, 6.0 mmol). After 4 hours stirring at room temperature, tlc analysis (ethyl acetate/ hexane, 2/1) show one major product (R_f0.6) and some starting material (R_f0.4). This mixture was diluted with chloroform (20 ml) and added dropwise to 2M hydrochloric acid (20 ml) at 0 °C. The organic layer was separated and washed with sodium bicarbonate(20 ml) and water (20 ml). The organic layer was evaporated *in vacuo* and coevaporated with butanol-water. The residue was dissolved in dichloromethane (20 ml), dried (sodium sulphate) and then evaporated *in vacuo*. Purification by flash column chromatography (ethyl acetate/ hexane, 1/2) afforded 2,3,4,6-tetra-*O*-acetyl-α-**D**-glucose (**2.35**) (633 mg, 75%, 80% based on recovered starting material) as a clear oil. $[\alpha]_D^{25}$ +79.8 (c 1.4, $CHCl_3$), [lit.[39] $[\alpha]_D^{23}$ +71.6 (c 0.55, $CHCl_3$)]. δ_H (500 MHz, $CDCl_3$); 2.02 (3H, s, CH_3), 2.04 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.10 (3H, s, CH_3), 3.32 (d, *J* 3.7 Hz, exchanges D_2O, OH),

4.14 (dd, *J* 11.8, 1.8 Hz, H-6), 4.16- 4.27 (2H, m, H-5, H-6), 4.90 (1H, dd $J_{2,1}$ 3.6, $J_{2,3}$ 10.2 Hz, H-2), 5.09 (1H, t *J* 9.6 Hz, H-4), 5.47 (1H, t *J* 3.7 Hz, exchanges D_2O d $J_{1,2}$ 3.7 Hz, H-1), 5.54 (1H, t *J* 9.7 Hz, H-3). δ_C (50 MHz, $CDCl_3$); 20.8 (q, 4x CH_3), 61.9 (t, C-5), 67.2, 68.4, 69.8, 71.1 (4x d, C-2, C-3, C-4, C-5), 90.2 (d, C-1), 170.0, 170.4, 170.5, 170.6 (4x s, 4x C=O).

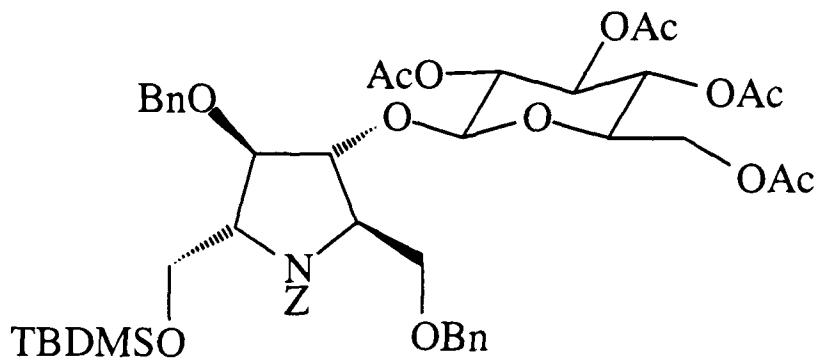
2,3,4,6-Tetra-*O*-acetyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.34α**)



To a solution of 2,3,4,6-tetra-*O*-acetyl-α-**D**-glucose (**2.35**) (541 mg, 1.6 mmol) in dichloromethane (15 ml) was added sodium hydride (60 mg, 1.5 mmol) and trichloroacetonitrile (0.56 ml, 5.64 mmol). After stirring for 1 hour, tlc analysis (diethyl ether) showed no starting material (R_f 0.3) and one product (R_f 0.5), the mixture was filtered through Celite and washed with dichloromethane (10 ml), the filtrate was concentrated *in vacuo* and the oily residue purified by flash chromatography (diethyl ether) to afford 2,3,4,6-tetra-*O*-acetyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.34α**) (430 mg, 55%) as a clear oil. $[\alpha]_D^{25}$ +80.0 (c 1.4, $CHCl_3$), [lit.[45] $[\alpha]_{578}^{23}$ +103 (c 1.2, $CHCl_3$)]. ν_{MAX} (thin film); 3322 (m, NH), 1756 (s, C=O), 1679 (s, C=N) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 2.00 (3H, s, CH_3), 2.02 (3H, s, CH_3), 2.04 (3H, s, CH_3), 2.06 (3H, s, CH_3), 4.12 (1H, dd $J_{6,5}$ 2.1 $J_{6,6'}$ 12.4 Hz, H-6), 4.20 (1H, ddd $J_{5,4}$ 10.3 $J_{5,6}$ 2.1 $J_{5,6'}$ 4.1 Hz, H-5), 4.26 (1H, dd $J_{6',5}$ 4.2 $J_{6',6}$ 12.4 Hz, H-4), 5.12 (1H, dd $J_{2,1}$ 3.7 $J_{2,3}$ 10.2 Hz, H-2), 5.16 (1H, t *J* 9.8 Hz, H-4), 5.55 (1H, t *J* 9.8 Hz, H-3), 6.55 (1H, d $J_{1,2}$ 3.7 Hz, H-1),

8.69 (1H, s, NH). δ_C (50 MHz, $CDCl_3$); 20.3, 20.4, 20.5 (3x q, 4x CH_3), 61.3 (t, C-6), 67.7, 69.6, 69.8, 70.0 (4x d, C-2, C-3, C-4, C-5), 90.7 (s, CCl_3), 92.9 (d, C-1), 160.9 (C=N), 169.8, 170.1, 170, 3, 170.8 (4x s, 4x C=O).

4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.37**β)



Method 1

To solution of *N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.30**) (61 mg, 0.1 mmol) in diethyl ether (4 ml) was added 2,3,4,6-tetra-*O*-acetyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.34**α) (197 mg, 0.4 mmol). To this was added trimethylsilyl triflate (6 µl, 0.03 mmol) and the mixture stirred at room temperature. After 2 hours, tlc analysis (diethyl ether/ hexane, 1/1, 2 elutions) showed no acetimidate (R_f 0.3) and a major product (R_f 0.4). The mixture was diluted with diethyl ether (25 ml). This was washed with saturated aqueous sodium bicarbonate solution (2x 10 ml), then water (10 ml). The organic layer was dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by column chromatography (diether ether/ hexane 1/3, normal pressure) to afford 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.37**β) (11 mg, 12%) as a clear oil.

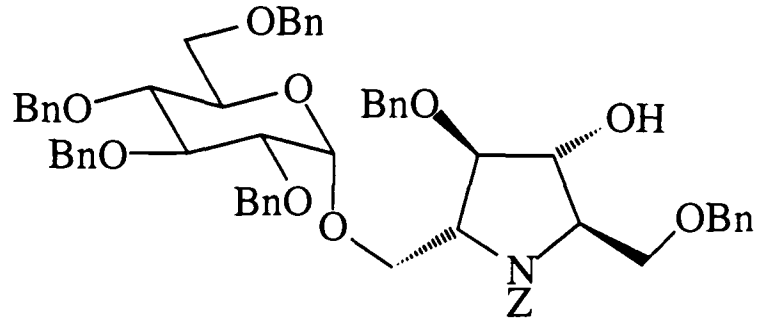
Method 2

To solution of *N*-benzyloxycarbonyl-1-*O*-*tert*- butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.30**) (94 mg, 0.16 mmol) in acetonitrile (2 ml) was added 2,3,4,6-tetra-*O*-acetyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.34**α) (148 mg, 0.3 mmol). The mixture was cooled to -40 °C and to this was added trimethylsilyl triflate (60 µl, 0.16 mmol) and the mixture stirred at room temperature. After 2 hours, tlc analysis (diethyl ether/ hexane, 1/1, 2 elutions) showed no acetimidate (R_f 0.3) and a major product (R_f 0.4). Water (0.5 ml) was added and the solvent was removed *in vacuo*. The residue was dissolved in diethyl ether (30 ml). This was washed with saturated aqueous sodium bicarbonate solution (2x 10 ml), then water (10 ml). The organic layer was dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by column chromatography (diether ether/ hexane 1/3, normal pressure) to afford 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.37**β) (9 mg, 6%) as a clear oil.

ν_{MAX} (thin film); 1750 (MeC=O), 1705 (NC=O) cm^{-1}. δ_H(500 MHz, $CDCl_3$); -0.10 (3H, s, CH_3), 0.03, 0.06 (3H, 2x s, CH_3), 0.80, 0.86 (9H, 2x s, 3x CH_3), 2.05, 2.07 (12H, 2x s, 4x $COCH_3$), 3.35 (2H, m), 3.59 (1H, dt J_d 3.1, J_t 9.1 Hz), 3.60 (1H, m), 3.72 (1H, dd *J* 4.3, 9.1 Hz), 3.92 (2H, m), 4.03 (1H, dd *J* 4.3, 8.9 Hz), 4.06 (1H, dd *J* 4.3, 10.3 Hz), 4.11 (1H, d *J* 4.8 Hz), 4.17 (1H, dd *J* 4.3, 10.3 Hz), 4.23 (1H, dd *J* 4.7, 9.4 Hz), 4.40, 4.49 (2H, 2x d *J* 12.1 Hz), 4.48, 4.62 (2H, 2x d *J* 12.0 Hz), 4.45, 4.61 (2H, 2x d *J* 12.0), 5.00, 5.23 (1H, 2x d *J* 12.1 Hz), 5.05, 5.15 (1H, 2x d 12.1 Hz), 5.46 (1H, d *J* 7.24 Hz, H_G-1), 7.23- 7.39 (15H, m, Ph). δ_C (125 MHz, $CDCl_3$, non aromatic); -5.68, -5.52, -5.28 (3x q, $Si(CH_3)_2$), 18.10 (s, $\underline{C}(Me)_3$), 21.06 (q, $C(\underline{C}H_3)_3$), 25.74, 25.81 (2x q, 4x OCH_3), 60.27, 61.20 (2x d, 2x CH), 63.01, 63.32, 65.40, 65.75 (4x t, 4x CH_2),

67.05, 67.21, 67.36, 68.35 (4x d, 4x CH), 71.23, 73.16 (2x t, 2x CH_2), 76.28, 82.31, 82.77 (3x d, 3x CH), 154.14, 154.21 (2x s, NC=O), 169.64, 172.50 (2x s, 4x C=OMe). m/z (ESI); 944 (5, M+Na^+), 921 (10, M^+), 862 (30, M-OAc), 547 (100%, M-Glc$(Ac)_4$).

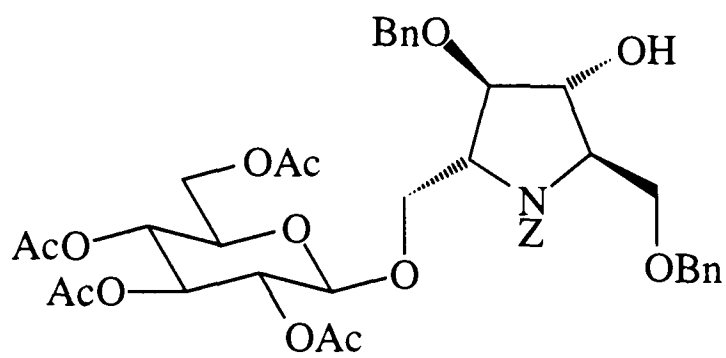
1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.38α**)



To solution of *N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**1.35**) (100 mg, 0.2 mmol) in diethyl ether (2 ml) was added 2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.17α**) (219 mg, 0.3 mmol) and powdered 3A molecular sieves. To this was added trimethylsilyl triflate (18 µl, 0.09 mmol) and the mixture stirred at room temperature. After 90 minutes, tlc analysis (diethyl ether/ hexane 1/1, 2 elutions) showed no alcohol (R_f 0.05) and a major product (R_f 0.4) and remaining acetimidate (R_f 0.5). The mixture was diluted with diethyl ether (30 ml). This was washed with saturated aqueous sodium bicarbonate solution (2x 10 ml), then water (10 ml). The organic layer was dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by column chromatography (diether ether/ hexane 1/3, normal pressure) to afford 1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.38α**) (28 mg, 13%, 20% based on recovered acetimidate) as a clear oil. $[\alpha]_D^{22}$ +25.8 (c 0.5, $CHCl_3$). Found C 74.49 H 6.79 N 1.37%, $C_{62}H_{65}O_{11}N$ requires C 74.45 H 6.55 N 1.40%. ν_{MAX} (thin film); 3400 (m, OH), 1703 (s, C=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 3.37 (1H, t *J* 9.2

Hz, 3.52 (1H, td J_d 3.1 J_t 7.3 Hz), 3.69 (2H, m), 3.93 (1H, dd *J* 3.6, 9.5 Hz), 4.19 (1H, dd *J* 3.2, 6.8 Hz), 4.26 (2H, m), 4.34 (1H, t *J* 2.6 Hz), 4.38 (2H, m), 4.41- 4.57 (5H, m), 4.62 (1H, dd *J* 6.9, 1.7 Hz), 4.68- 4.88 (6H, m), 4,93, 4.88 (2H, 2x d *J* 13.9 Hz), 5.03, 5.05 (2H, 2x d *J* 10.8 Hz), 5.19, 5.21 (2H, 2x d *J* 12.1 Hz). δ_C (125 MHz, CD_3CN, non aromatic); 62.8, 62.9, 67.6, 67.9, 68.3, 71.3, 73.4, 73.8, 74.6, 75.3 (10x t, 10x CH_2), 65.2, 65.6, 71.4, 77.7, 77.8, 79.8, 82.4, 83.8, 99.5 (9x d, 9x CH), 155.4 (s, C=O). m/z (FAB); 1022 (40, MNa^+), 1000 (100%, MH^+).

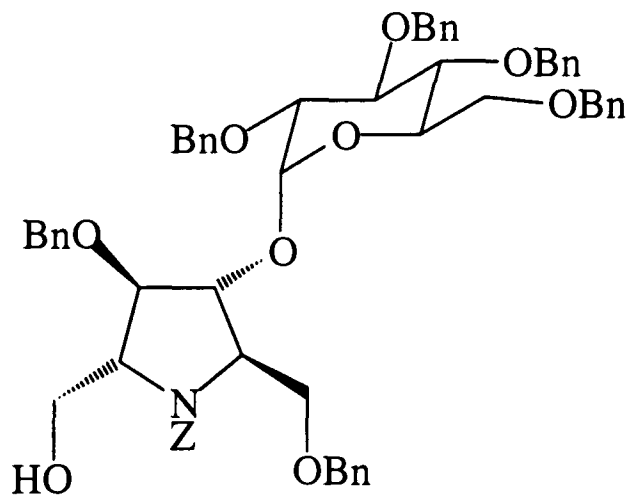
1-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.39**β)



To solution of *N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**1.35**) (100 mg, 0.2 mmol) in diethyl ether (5 ml) was added 2,3,4,6-tetra-*O*-acetyl-α-**D**-glucopyranosyl trichloroacetimidate (**2.34**α) (106 mg, 0.2 mmol). To this was added trimethylsilyl triflate (22 µl, 0.1 mmol) and the mixture stirred at room temperature. After 2 hours, tlc analysis (diethyl ether, 2 elutions) showed no alcohol (R_f 0.3) and a major product (R_f 0.4) and remaining trichloroacetimidate (R_f 0.5). The mixture was diluted to 30 ml with diethyl ether. This was washed with saturated aqueous sodium bicarbonate solution (2x 10 ml), then water (10 ml). The organic layer was dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by column chromatography (diether ether/ hexane 1/1, normal pressure) to afford 1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.39**β)

(31 mg, 18%, 53% based on recovered acetimidate) as a clear oil. $[\alpha]_D^{22}$ -25.8 (c 1.1, $CHCl_3$). ν_{MAX} (thin film); 3452 (m, OH), 1755 (s, C=O), 1703 (s, NC=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 2.00 (3H, s, CH_3), 2.01 (3H, s, CH_3), 2.03 (3H, s, CH_3), 2.04 (3H, s, CH_3), 3.40 (1H, m), 3.59 (1H, t *J* 9.5 Hz), 3.59 (1H, dd *J* 4.3, 9.0 Hz), 3.69 (1H, dd *J* 3.0, 9.7 Hz), 3.93 (1H, dd *J* 2.7, 7.7 Hz), 3.97 (1H, s), 4.03 (1H, t *J* 6.1 Hz), 4.04 (1H, d *J* 7.5 Hz), 4.10- 4.21 (1H, m), 4.25 (1H, dd *J* 2.3, 12.3 Hz), 4.40 (1H, d *J* 12.1 Hz), 4.45 (2H, s), 4.48- 4.55 (2H, m), 4.63 (1H, d *J* 11.9 Hz), 4.85- 5.21 (4H, m), 5.95 (1H, d *J* 3.6 Hz), 7.28- 7.40 (15H. m, Ph). δ_C (125 MHz, $CDCl_3$, non aromatic); 20.57, 20.67 (2x q, 4x CH_3), 61.41, 67.03, 67.17, 68.02, 68.17, 68.44 (6x t, 6x CH_2), 63.45, 65.62, 70.90, 71.06, 71.90, 73.03, 73.20, 84.82, 100.56 (9x d, 9x CH), 137.68, 138.29 (2x s, NC=O), 169.30, 169.37, 170.22, 170.62 (4x s, 4x C=OCH_3). m/z (DCI, NH_3); 808 (70, MH^+), 642 (65), 520 (75), 91 (100%, $PhCH_2$).

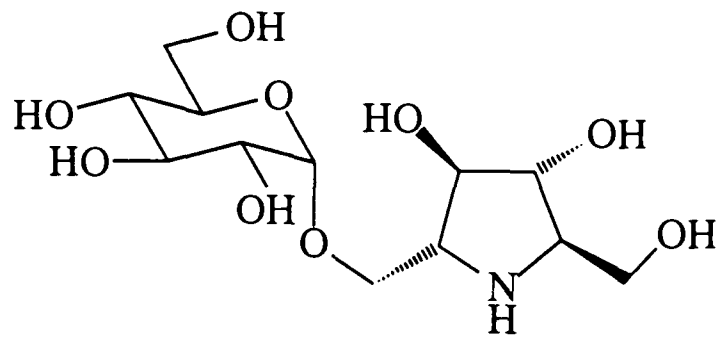
3-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1,4-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.42**α)



To a solution of 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-benzoyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.33**α) (239 mg, 0.2 mmol) in *N,N*-dimethylformamide (20 ml) was added sodium methoxide (22 mg, 0.4 mmol). After stirring for 90 minutes tlc analysis (diethyl ether/

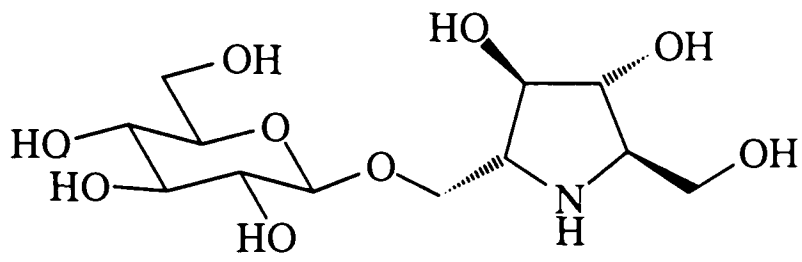
hexane 1/2, 2 elutions) showed no starting material (R_f 0.3) and one major product (0.06). The solvent was removed *in vacuo* and the residue redissolved in diethyl ether (20 ml). This was washed with dilute hydrochloric acid (~ 2M, 5 ml), water (10 ml) brine (10 ml) and dried (sodium sulphate). Purification by column chromatography (ether/ hexane 1/5 to 1/1) to afford 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1,4-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.42**α) (129 mg, 65%). $[\alpha]_D^{25}$ +32.6 (c 1.05, $CHCl_3$). Found C 74.31 H 6.36 N 1.37%, $C_{62}H_{65}O_{11}N$ requires C 74.55 H 6.55 N 1.40%. ν_{MAX} (thin film); 3357 (OH), 1810 (C=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 3.344 (1H, dd *J* 10.8, 17 Hz), 3.388 (1H, dd *J* 10.8, 9.1 Hz), 3.547 (1H, dd *J* 3.6, 9.7 Hz), 3.661 (1H, dt *J* 4.2, 7.7 Hz), 3.720 (1H, t *J* 7.2 Hz), 3.748 (1H, td *J* 4.7, 11.6), 3.908 (1H, dd *J* 8.3, 17.0 Hz), 4.019 (1H, dd *J* 3.3, 7.3 Hz), 4.098 (1H, t *J* 4.3 Hz), 4.194 (1H, dd *J* 4.3, 10.6 Hz), 4.292 (1H, dd *J* 12.1 Hz), 4.345 (1H, dd *J* 12.6 Hz), 4.429 (1H, dd *J* 7.3, 10.8), 4.464 + 4.475 (2H, 2x d *J* 5.3 Hz), 4.538 + 4.493 (2H, 2x d *J* 11.8 Hz), 4.691 (1H, dd *J* 11.5, 11.7 Hz), 4.748 (1H, dd *J* 3.6, 8.0 Hz), 4.790 (1H, t *J* 10.4 Hz), 4.831 + 4.879 (2H, 2x d *J* 10.9 Hz), 4.831 + 4.927 (2H, 2x d *J* 10.8 Hz), 5.045 + 5.213 (2H, 2x d *J* 12.2 Hz), 5.088 + 5.166 (2H, 2x d *J* 12.2 Hz), 6.461 (1H, d *J* 8.1 Hz), 7.11- 7.47 (35 H, m). δ_C (125 MHz, $CDCl_3$, non aromatic); 64.6, 66.8, 71.4, 77.7, 79.1, 79.7, 82.4, 83.6, 96.5 (9x d, 9x CH), 62.0, 62.9, 67.8, 68.0, 69.0, 73.4, 73.8, 74.3, 75.6, 76.2 (10x t, 10 CH_2), 155.0, 155.5 (2x s, C=O). m/z (FAB Na^+); 1022 (MNa^+), 91 (Bn).

1-*O*-(α-**D**-Glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.26**α)



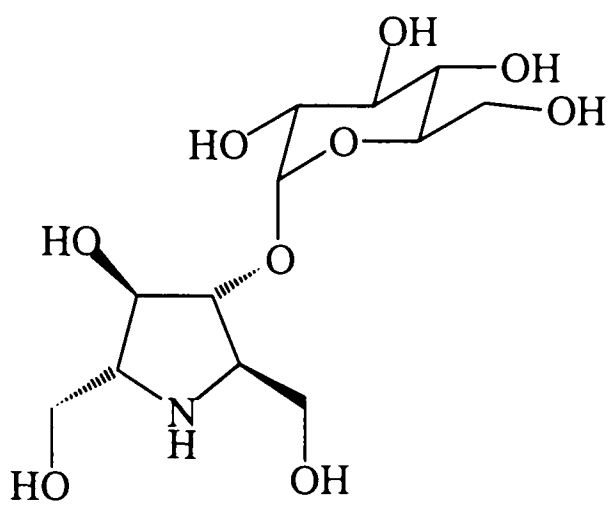
To a suspension of palladium black (~10 mg) in ethanol (4 ml) was added 1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.38**α) (28 mg, 0.03 mmol) was added with concentrated hydrochloric acid (10 μl) this solution was stirred under hydrogen for 18 hours when tlc analysis (chloroform/ methane/ acetic acid/ water 60/ 30/ 3/ 5) showed a single product (R_f 0.2). The palladium was filtered off through Celite and the filtrate evaporated to dryness *in vacuo*. Purification by basic ion exchange (Amberlite CG400) afforded 1-*O*-(α-**D**-glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.26**α) (8.9 mg, quantitative) as a white hygroscopic solid. δ_H (500 MHz, D_2O); 3.53 (1H, m, H_G-4), 3.65 (2H, m, H_D-6, H_G-5), 3.68 (1H, m, H_G-3), 3.72 (1H, m, H_D-1), 3.79 (1H, H_G-3), 3.80 (1H, m, H_D-1'), 3.90 (1H, m, H_D-1), 3.92 (1H, m, H_G-6), 4.14 (1H, m, H_D-3), 4.16 (1H, m, H_G-6'), 4.19 (1H, m, H_D-5), 4.20 (1H, m, H_D-4), 4.30 (1H, m, H_D-2), 5.06 (1H, $J_{1,2}$ 4.0 Hz, H_G-1); m/z (ESI); 326 (100%, MH^+).

1-*O*-(β-**D**-Glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.26**β)



To a solution of 1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.39**β) (31 mg, 0.03 mmol) in *N,N*-dimethylformamide (10 ml) was added sodium methoxide (5 mg, 0.1 mmol). After stirring for 90 minutes tlc analysis (diethyl ether, 2 elutions) showed no starting material (R_f 0.4) and one major product (0.06). The solvent was removed *in vacuo* and the residue redissolved in chloroform (20 ml). This was washed with dilute hydrochloric acid (~ 2M, 1 ml), water (5 ml), brine (5 ml) and dried (sodium sulphate). The solvent was removed *in vacuo*. The residue was redissolved in ethanol (5 ml) with a catalytic amount of palladium black (~ 10 mg) and concentrated hydrochloric acid (10 µl). This solution was stirred under hydrogen for 48 hours when tlc analysis (chloroform/ methanol/ acetic acid/ water, 60/ 30/ 3/ 5) showed a single product (R_f 0.05). The palladium was filtered off through Celite and the solution evaporated to dryness under reduced pressure. The crude product was purified by basic ion exchange column chromatography (Amberlite CG400) to afford 1-*O*-(β-**D**-glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.26**β) (8 mg, 66%) as a white hygroscopic solid. δ_H (500 MHz, D_2O); 3.41 (1H, dd *J* 7.3, 11.6 Hz, H_G-2), 3.43 (1H, m, H_D-2), 3.46 (1H, m, H_D-5), 3.49 (1H, m, H_G-4), 3.57 (1H, m, H_D-6), 3.58 (1H, m, H_G-5), 3.59 (1H, dd *J* 10.2, 12.3 Hz, H_G-3), 3.80 (1H, m, H_G-6), 3.81 (1H, m, H_D-6'), 3.89 (1H, dd *J* 4.4, 13.1 Hz, H_D-1), 4.05 (1H, m, H_G-6'), 4.07 (1H, dd *J* 8.0, 11.3 Hz, H_D-3), 4.14 (1H, dd *J* 5.1, 11.2 Hz, H_D-4), 4.19 (1H, dd *J* 3.6, 10.9 Hz, H_D-1'), 4.59 (1H, d *J* 8.0 Hz, H_G-1); m/z (ESI); 326 (100%, MH^+).

3-*O*-(α-**D**-Glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.27**α)



Method 1.

To a suspension of palladium black (~10 mg) in ethanol (4 ml) was added 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1,4-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.42**α) (121 mg, 0.12 mmol) was added with concentrated hydrochloric acid (10 μl) this solution was stirred under hydrogen for 18 hours when tlc analysis (chloroform/ methane/ acetic acid/ water 60/ 30/ 3/ 5) showed a single product (R_f 0.2). The palladium was filtered off through Celite and the filtrate was concentrated *in vacuo*. Purification by basic ion exchange (Amberlite CG400) afforded 3-*O*-(α-**D**-glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.27**α) (39 mg, quantitative) as a white solid.

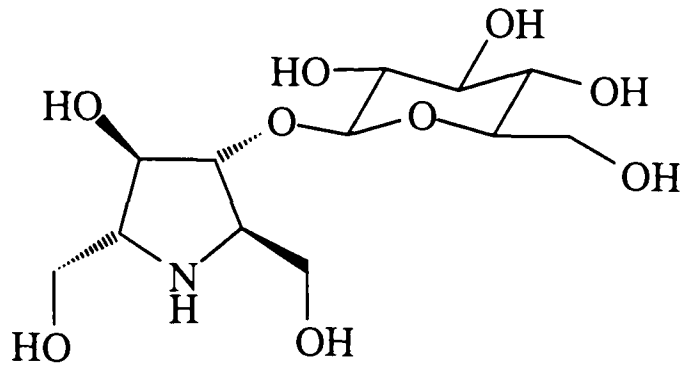
Method 2

To a suspension of palladium black (~5 mg) in ethanol (6 ml) was added 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-6-*O*-*tert*-butyldimethylsilyl-1,4-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.31**α) (80 mg, 0.072 mmol) and concentrated hydrochloric acid (30 μl) was added. This solution was

stirred under hydrogen for 26 hours when tlc analysis (chloroform/ methanol/ acetic acid/ water, 60/ 30/ 3/ 5) showed a single product (R_f 0.2). The palladium was filtered off through Celite and the solution evaporated to dryness *in vacuo*. The crude product was purified by basic ion exchange column chromatography (Amberlite CG400) to afford 3-*O*-(α-**D**-glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (22 mg, 96%) (**2.27**α) as a white solid.

M.p. (water); 117- 118 °C. $[\alpha]_D^{25}$ + 81.5 (c 0.65, MeOH). Found C 44.45 H 7.13 N 4.09%, $C_{12}H_{23}O_9N$ requires C 44.31 H 7.13 N 4.31%. δ_H (500 MHz, D_2O, pH 9.7); 3.14 (1H, m, H_D-5), 3.29 (1H, m, H_D-2), 3.48 (1H, t, *J* 9.8 Hz, H_G-4), 3.62 (1H, dd $J_{2,1}$ 4.0, $J_{2,3}$ 9.8 Hz, H_G-2), 3.71 (1H, m, H_D-6), 3.74 (H, m, H_D-1), 3.75 (1H, m, H_G-5), 3.76 (1H, t *J* 9.8 Hz, H_G-3), 3.79 (H, m, H_D-1'), 3.81 (1H, dd *J* 11.6, 6.2 Hz, H_D-6'), 3.83 (1H, dd $J_{6,5}$ 4.9, $J_{6,6'}$ 12.5 Hz, H_G-6), 3.92 (1H, dd $J_{6',5}$ 2.2, $J_{6',6}$ 12.5 Hz, H_G-6'), 4.04 (1H, t *J* 5.4 Hz, H_D-3), 4.12 (1H, dd $J_{3,4}$ 5.4, $J_{4,5}$ 7.0 Hz, H_D-4), 5.25 (1H, d $J_{1,2}$ 4.0 Hz, H_G-1). δ_C (125 MHz, D_2O, pH 9.7); 63.7 (t, C_G-6), 64.6 (d, C_D-2), 64.9 (t, C_G-5), 65.3 (t, C_G-2), 66.0 (d, C_D-5), 72.8 (d, C_G-4), 74.5 (d, C_G-2), 75.5 (d, C_G-5), 76.2 (d, C_G-3), 80.7 (d, C_D-4), 87.7 (d, C_D-3), 101.1 (d, C_G-1). m/z (ESI, CV =45V); 326 (100%, MH+); (ESI, CV =60V); 326 (80, MH+), 164 (100%, DMDP).

3-*O*-(β-**D**-Glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.27**β)



To a solution of 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-**D**-glucopyranosyl)-*N*-benzyloxycarbonyl-1-*O*-*tert*-butyldimethylsilyl-3,6-di-*O*-benzyl-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.37**β) (56 mg, 0.2 mmol) in *N,N*-dimethylformamide (10 ml) was added sodium methoxide (5 mg, 0.1 mmol). After stirring for 100 minutes tlc analysis (diethyl ether, 2 elutions) showed no starting material (R_f 0.4) and one major product (R_f 0.06). The solvent was removed *in vacuo* and the residue redissolved in chloroform (20 ml). This was washed with dilute hydrochloric acid (~ 2M, 1 ml), water (5 ml), brine (5 ml) and dried (sodium sulphate). The solvent was removed *in vacuo* and residue was redissolved in ethanol (5 ml) with a catalytic amount of palladium black (~10 mg) and concentrated hydrochloric acid (10 μl). This solution was stirred under hydrogen for 48 hours when tlc analysis (chloroform/ methanol/ acetic acid/ water, 60/ 30/ 3/ 5) showed a single product (R_f 0.05). The palladium was filtered off through Celite and the solution evaporated to dryness *in vacuo*. The crude product was purified by basic ion exchange column chromatography (Amberlite CG400) to afford 3-*O*-(β-**D**-glucopyranosyl)-2,5-dideoxy-2,5-imino-**D**-mannitol (**2.27**β) (15 mg, 75%) as a white solid. δ_H (500 MHz, D_2O); 3.20 (1H, dd $J_{2,1}$ 8.1, $J_{2,3}$ 9.1 Hz, H_D-2), 3.35 (1H, m, H_D-5), 3.44 (1H, dd $J_{3,2}$ 9.0 $J_{3,4}$ 6.4 Hz, H_D-3), 3.49 (1H, dd *J* 3.8, 9.7 Hz, H_G-4), 3.61 (1H, dd $J_{2,1}$ 8.1 $J_{2,3}$ 11.6 Hz, H_G-2), 3.68 (1H, m, H_D-1), 3.69 (1H, m, H_G-5), 3.72 (1H, m, H_D-6), 3.75 (1H, m, H_G-5), 3.79 (1H, m, H_D-6'), 3.82 (1H, dd $J_{1',2}$ 1.9 $J_{1,1'}$ 5.5 Hz, H_D-1'), 3.86 (1H, m, H_G-6), 3.94

(1H, m, H_G-6'), 4.09 (1H, t *J* 6.4 Hz, H_D-4), 4.60 (1H, d $J_{1,2}$ 8.1 Hz, H_G-1); m/z (ESI); 326 (100%, MH^+).

2.4 References

1. W. A. Lubas and R. G Spiro, *J. Biol. Chem.*, 1987, **262**, 3775.
2. I.C. di Bello, P. Darling, L.E. Fellows, B. Winchester, *FEBS Lett.*, 1984, **176**, 61.
3. S. Hiraizium, U. Spohr and R. G. Spiro, *J. Biol. Chem.*, 1993, **268**, 9927.
4. U. Spohr, M. Bach and R. G. Spiro, *Can. J. Chem.*, 1993, **71**, 1919.
5. U. Spohr, M. Bach and R. G. Spiro, *Can. J. Chem.*, 1993, **71**, 1928.
6. U. Spohr, M. Bach and R. G. Spiro, *Can. J. Chem.*, 1993, **71**, 1943.
7. G. Vic and D.H.G. Crout, *Tetrahedron Asym.*, 1994, **5**, 2513.
8. R. R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 1986, **25**, 212.
9. W. Koenigs and E. Knorr, *Ber.*, 1901, **34**, 957.
10. H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, 1982, **21**, 155 and references cited therein.
11. W.N. Haworth, J. Jackson and F. Smith, *J. Chem. Soc.*, 1940, 620.
12. V.D. Grob, T.G. Squires and J.R. Vercellotti, *Carbohydr. Res.*, 1978, **67**, 163.
13. H. Paulsen and O.Lockhoff, *Tetrahedron Lett.*, 1978, 4027.
14. R.U. Lemieux, K.B. Hendricks, R.V. Stick and K. James, *J. Am. Chem. Soc.*, 1975, **97**, 4056.
15. P. Sinaÿ, *Pure Appl. Chem.*, 1978, **50**, 1437.
16. H. Ardon, T.D. Butters, F.M. Platt, M.R. Wormald, R.A. Dwek, G.W.J. Fleet and G.S. Jacob, *Tetrahedron Asym.*, 1993, **4**, 2011.
17. K.C. Nicolaou, S.P. Seitz and D.P. Papahatjis, *J. Am. Chem. Soc.*, 1983, **105**, 2430.
18. D. Kahne, S. Walker, Y. Cheng and D.Van Engen, *J. Am. Chem. Soc.*, 1989, **111**, 6881.
19. M.J. Ford and S.V. Ley, *Synlett*, 1990, **5**, 255.
20. D.R. Mootoo, V. Date and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1988, **110**, 2662.

21. B. Capon, *Chem. Rev.*, 1969, **69**, 407.

22. B. Fraser-Reid, P. Konradsson, D.R. Mootoo and U. Udodong, *J. Chem. Soc., Chem. Commun.*, 1988, 823.

23. J.-R. Pougny, J.-C. Jacquinet, M. Nassr, D. Duchet, M.-L. Milat and P. Sinaÿ, *J. Am. Chem. Soc.*, 1977, **99**, 6762.

24. R.R. Schmidt and J. Michel, *Angew. Chem. Int. Ed. Engl.*, 1980, **19**, 731.

25. D.C. Neilson in "*The Chemistry of Amidines and Imidates*", S. Patai (Ed.), Wiley, New York, 1975, p 394.

26. R.R. Schmidt and J. Michel, *Tetrahedron Lett.*, 1984, **25**, 821.

27. B. Wegmann and R.R. Schmidt, *J. Carbohydr. Chem.*, 1987, **6**, 357.

28. A.G.M. Barrett and D. Pilipauskas, *J. Org. Chem.*, 1990, **55**, 5194.

29. J.L. Maloisel, A. Vasella, B.M. Trost, D.L. van Vranken, *J. Chem. Soc., Chem. Comm.*, 1991, 1099.

30. K.C. Nicolaou, R.A. Daines, Y. Ogawa and T.K. Chakraborty, *J. Am. Chem. Soc.*, 1988, **110**, 4696.

31. R.L. Halcomb, S.H. Bayer and S.J. Danishefsky, *Angew. Chem. Int. Ed. Engl.*, 1992, **31**, 338.

32. K.C. Nicolaou, E.P. Schneiner, Y. Iwabuchi and T. Suzuki, *Angew. Chem. Int. Ed. Engl.*, 1992, **31**, 340.

33. L. Yan, C.M. Taylor, R. Goodnow, and D. Kahne, *J. Am. Chem. Soc.*, 1994, **166**, 6953.

34. S.J. Danishefsky, Royal Chemistry of Chemistry, Carbohydrate Group Autumn meeting, Oxford, Sept. 1995.

35. M. Fieser and L.F. Fieser, *Reagents for Organic Synthesis*, 1971, **1**, 286.

36. D.R. Kelly, S.M. Roberts and R.F. Newton, *Synth. Commun.*, 1979, **9**, 295.

37. G. Wulff and G. Röhle, *Angew. Chem. Int. Ed. Engl.*, 1974, **13**, 157.

38. R.R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 1983, **22**, 776 and references cited therein.

39. M. Mikamo, *Carbohydr. Res.*, 1989, **191**, 150.

40. R.R. Schmidt, M. Behrendt and A. Toepfer, *Synlett*, 1990, 694.

41. R.R. Schmidt and E. Rücker, *Tetrahedron Lett.*, 1980, **21**, 1421.

42. NMR studies carried out by M. Wormald, Glycobiology, Oxford using a Varian Unity Plus 500.

43. Enzyme inhibition studies were performed by R.J. Nash, IGER, Aberystwyth.

44. G. Mikkelsen, T.V. Christensen, M. Bols, I. Lundt and M.R, Sierks, *Tetrahedon Lett.*, 1995, **36**, 6541.

45. Endomannosidase and Glycosyl transferase assays carried out by F.M. Platt and T.D. Butters, Glycobiology, Oxford.

46. V.D. Grob, T.B. Squires and J.R. Vercellotti, *Carbohydr. Res.*, 1969, **10**, 595.

47. R.R Schmidt and M. Stumpp, *Liebigs. Ann. Chem.*, 1983, 1249.

CHAPTER 3

CHAPTER 3

Natural Product Isolation and Identification

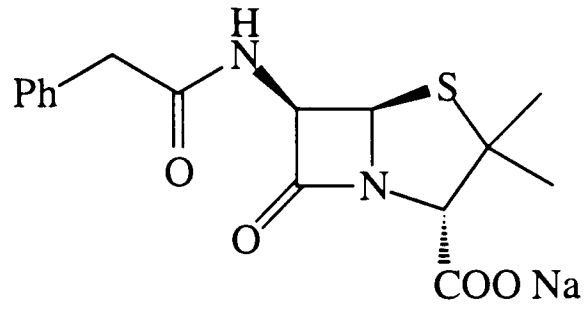
3.1 Introduction

There are tens of thousands of natural products which have been isolated from within organisms or as compounds secreted by organisms. Natural products range from highly toxic materials used by organisms as defences, or for reducing the growth of competing organisms, to volatile compounds that are used as scents. Much of this work produces compounds of ecological but little commercial interest, but occasionally a compound is found that is of commercial use as a therapeutic agent or pesticide.

For many years people have taken herbal remedies for their ailments, with alternative medicines becoming increasing popular again in recent years in developed countries.[1] The usefulness of natural products has been recognised by the scientific community for a long time, but now is also being recognised generally by the public and industry.[2,3]

Many of today's drugs were initially found as natural products. Most of these have then been manipulated to give a greater specificity or a stronger activity. One of the most famous of these natural products is penicillin (**3.1**), found accidentally by Fleming,[4] when he left a set of *Streptomyces sp.* bacterial culture plates, over a period of time. Re-examining the plates he found one that had a mould growing at one side, and in an area around the mould the bacteria had failed to develop fully. Fleming suggested that this was due to a compound released by the mould which he named penicillin. Some years later, Chain and Florey,[5] grew the mould *Penicillium pyocyoneu* in a culture broth. The liquor was then acidified and the penicillin extracted into organic solvents. This is the method by which the first penicillins were commercially made. At present there are a wide range of

penicillins available, although only one has been found as a natural product, the remainder have been chemically altered to change their effect.



Penicillin G (**3.1**)

Because of the commercial success of compounds, such as the penicillins, there is a constant search for new natural products and for synthetic analogues which may be beneficial. When searching for new compounds in organisms, all compounds found are compared to known structures, as analogues of previous isolated natural products may occur in other unrelated organisms. One example of this, is the compound 6-deoxynojirimycin (DNJ) (**1.7**), this was first formed by the reduction of the natural product nojirimycin (**3.2**)[6] (Figure 3.1). Then in 1976 it was isolated from the mulberry[7] and in 1980 from bacterial culture.[8]

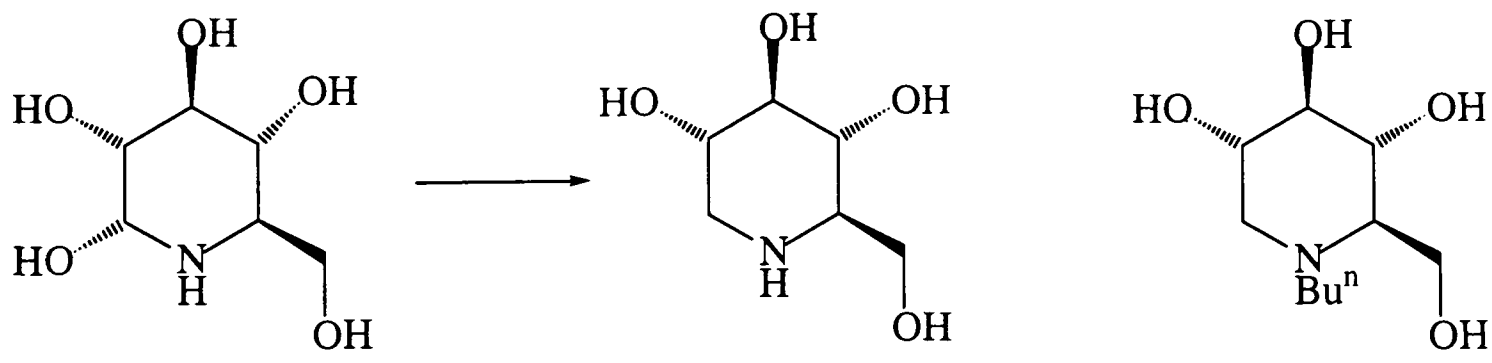


Figure 3.1

DNJ has been further manipulated by the addition of a *N*-butyl group to the nitrogen,[9] which increases the *in vitro* effect of the molecule, eliminating HIV from infected cell cultures. Further *in vivo* trails are still continuing with this molecule (**3.3**).

3.2 Results and Discussion

3.2.i Isolation of Disaccharide Natural Products.

The plant that was studied was the *Nephthytis poisonii* N.E.Br.(Araceae) (Figure 3.2). This is a native plant of the Cameroon. It is found on the western flank of Mount Cameroon in the Etinde reserve part of the South West Province.[10] Its natural habitat is the lowland rainforests at an altitude of 200 meters. It is a terrestrial herb approximately 50 cm high, found on the forest floor with hastate leaves, green spathe, white spadix and bright orange fruits. Other members of the Araceae family have been shown to contain alkaloids that inhibit glycosides[11] in particular *Aglaonema spp.* contains DMDP (**1.1**).[12]

In order to extract alkaloids from the *N. poisonii* it was first necessary to break open the cell walls and dissolve the organic materials into organic solvent. The extraction was carried out into a 50% aqueous ethanol solution this dissolves the alkaloids and amino acids but large oligosaccharides and proteins are insoluble. The extraction was kept at 5 °C for 2 hours with occasional stirring. If kept at a higher temperatures then the enzymes that occur in the plant could act upon the alkaloids and cause them to be hydrolysed. The emulsion was then filtered off in order to remove all the insoluble material such as cellulose and protein. This was then absorbed onto an acidic ion exchange resin. The basic alkaloids bind to the resin and their acidic counter ions and neutral compounds are washed through with ethanol. The alkaloids were then removed from the resin by elution with 2M sodium hydroxide leaving a solution of alkaloids and other basic material from the plant. As the alkaloids are only weakly basic they can be separated from the strongly basic fractions by passing the mixture down a weakly acidic ion exchange column. At this stage a small aliquot was removed and dried to carry out a quantification of the mixture. Samples were derivatised with Sigma-Sil-A, this was analysed by GCMS giving a series of peaks. The areas of the individual peaks and their retention times were noted. Then a sample of synthetic DMDP glucoside was derivatised and analysed.

Figure 3.2 *Nephthytis poisonii*[13]

This gave the retention size and peak sequence for the DMDP glucoside which was compared to the natural material to give the quantity of glucoside present in the plant.

The remainder of the ion exchanged sample was dried and passed down an alumina column, first eluting with acetone, then adding increasing amounts of water to deactivate the alumina thus release different polarities of material. Each fraction was reduced in volume and analysed by GCMS (Original traces shown in Appendix 2).

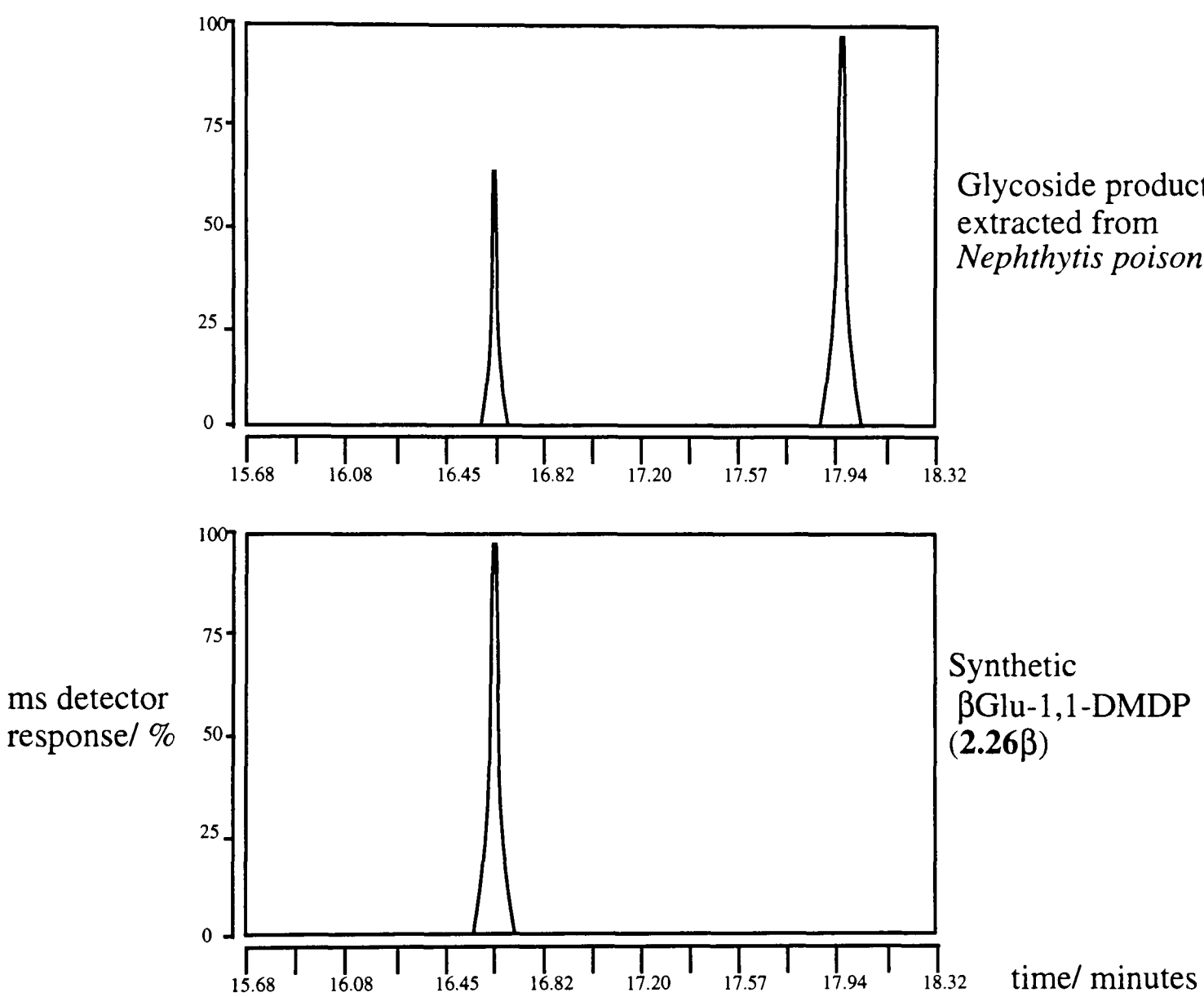
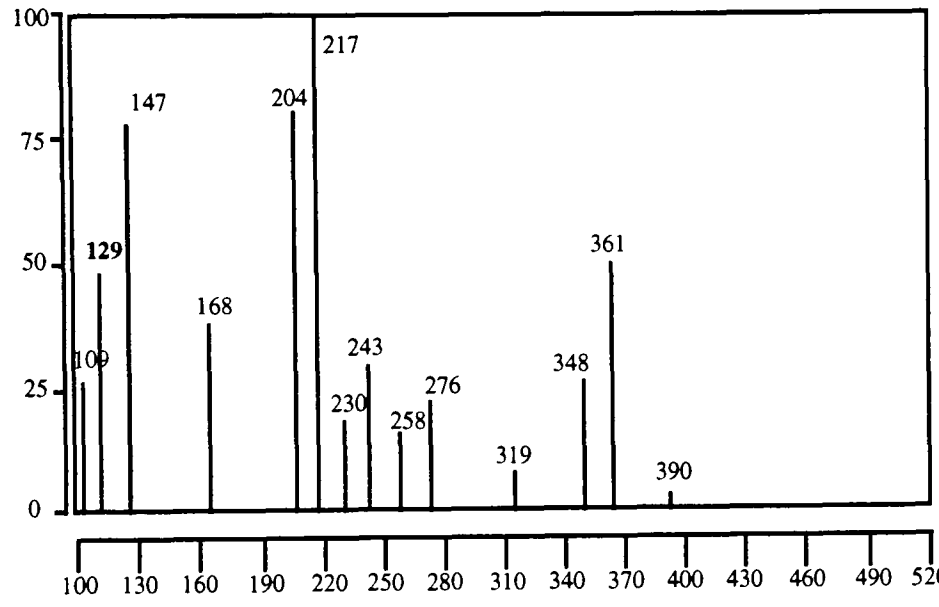


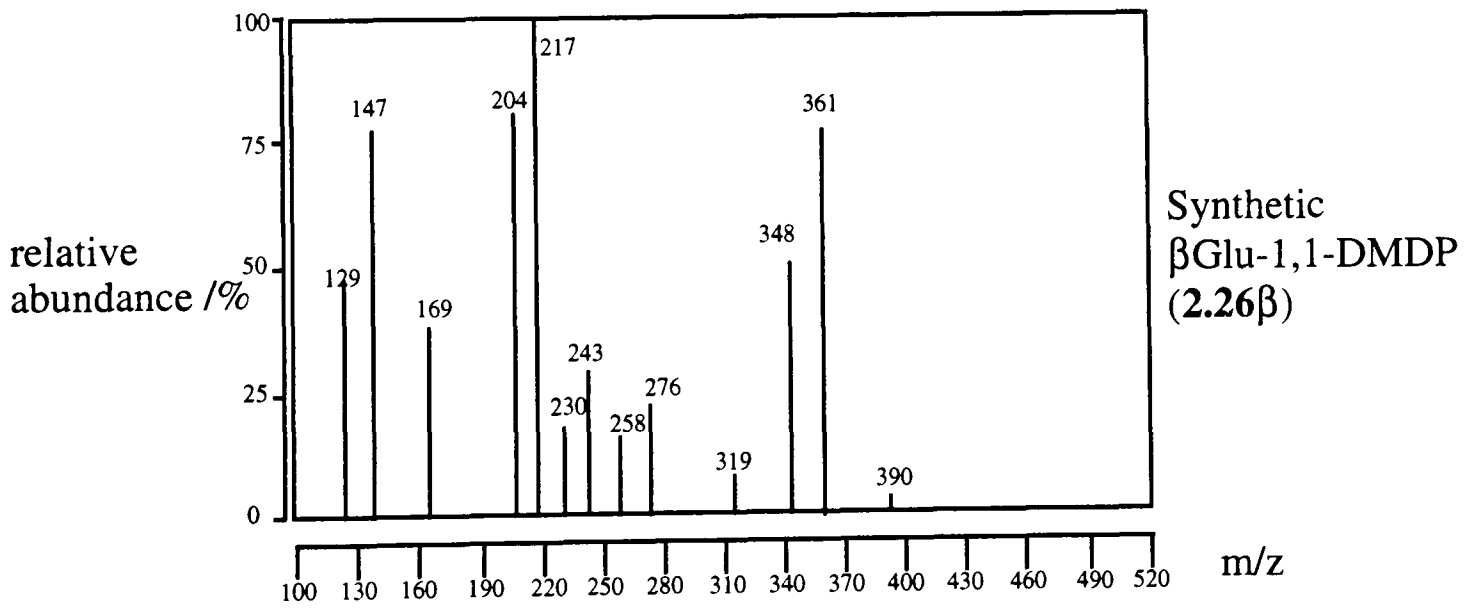
Figure 3.3

Comparison of the two GCMS traces shows a common peak at 16.62 minutes from the trace of the synthetic product this is suggested to be Glcβ1-1DMDP (**2.26**β) (Figure 3.3). The peak at 17.94 minutes is due to another unknown glucoside. Comparison between the two mass spectra of the peaks at 16.62 shows a similar

distribution of peaks (Figure 3.4). From the two sets of traces it indicates that the synthetic product matches the natural isolate thus suggesting a common structure. Evidence from the nmr of the natural product was inconclusive due occurrence of the unknown glycoside although it can be quite clearly seen that the anomer proton corresponds to a β-glucose anomer when compared to a mixture of α and β-synthetic glycoside and **D**-glucose (Appendix 3).



Glycoside product extracted from *Nephthytis poisonii*



Synthetic βGlu-1,1-DMDP (**2.26**β)

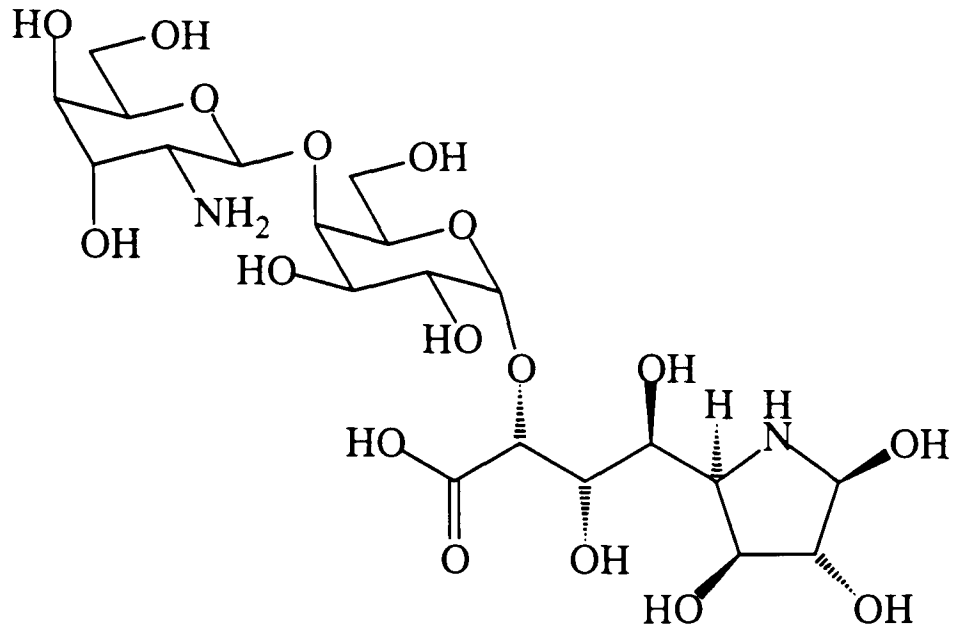
Figure 3.4

From the quantification it was shown that the *N. poisonii* contains 0.004% DMDP glucoside in the leaves and 0.006% in the fruit.

Another plant that was investigated was *Hyacinthoides non-scriptus* (L.) chouard ex Rothm more commonly know as the Bluebell or the wild hyacinth (Figure 3.5). It is 20- 50 cm tall, with green foliage. Its flower is 1.5- 2 cm long violet-blue occasionally pink or white and droops when fully open. It is found extensively throughout Britain except Orkney and Shetland, and through western Europe from Spain to the Netherlands. It prefers shaded areas, particularly in woodlands and hedgebanks often being a dominant ground species in woods on light acid soils. It is generally not eaten by native mammals or insects. It as been noted that cattle which graze of the newly grown leaves of *H. non-scriptus* become lethargic and extremely exhausted.[14] Some other species of the Hyacinthaceae family (*Hyacinthoides spp.*, *Boweia spp.* and *Urginea spp.*) have been found to contain glycosides which have been shown to be very similar to the cardiac glycosides of *Digitalis spp*[15,16] particularly *H. sibirica* and *U. maritima.*

Extraction of material from the bluebell was carried out by Watson and Nash[12] in a similar manner to that for *N. poisonii*. Comparison of the GCMS traces from the bluebell and that of the synthetic glycosides showed that although the bluebell does contain large quantities of DMDP (**1.1**), no DMDP glucoside was found. However, it was noted that the decomposition products formed from the glucosides (**2.26**α) and (**2.27**β) had very similar spectra to compounds found in the *H. non-scriptus*. A further extraction of the *H. non-scriptus* is in progress where strongly acidic ion exchange resin is not used, so that decomposition of these compounds is less likely.

A recently reported acaricide,[17] Gualamycin (**3.4**) has a similar pyrrolidine ring within its structure and also includes a disaccharide portion to molecule.



(3.4)

It was isolated from a culture broth of *Streptomyces sp.* NK11687 and further represents the wide ranging occurrence of glycosides of amino sugars.

Figure 3.5 *Hyacinthoides non-scriptus*[13]

3.3 Experimental

3.3.i Extraction from *Nephthytis poisonii* leaves

Fresh leaves of *Nephthytis poisonii* (103.896 g, fresh weight) were homogenised and extracted for 2 hours with aqueous ethanol (50%, 3 L) at 5 °C. This mixture was filtered through Celite and the filtrate absorbed on a 'Dowex 50' column charged with 1M hydrochloric acid and eluted with ethanol (500 ml) then 2M ammonium hydroxide (1 L). This solution was then concentrated to 8.0 ml *in vacuo*. The solution was passed down a weakly acidic ion exchange column (Amberlite CG50) and eluted with water (50 ml). A small aliquot (100 µl) was removed for quantification. The remaining solution was concentrated to 1.0 ml *in vacuo*. The residue was purified on an alumina column eluting with a solvent gradient of 100% acetone to 20% acetone/ water. Analysis of each fraction by GCMS (Sigma-Sil-A (TMSCl/ hexamethyldisilazane/ pyridine 1/3/9)), showed DMDP-glucoside to be present in the 50% acetone/ water fraction. This was concentrated and freeze dried to give the DMDP glycosides (9.3 mg).

3.3.ii Extraction from *Nephthytis poisonii* fruit

Fresh fruit of *Nephthytis poisonii* (17.0334 g, fresh weight) was homogenised and extracted for 2 hours with aqueous ethanol (50%, 1.5 L) at 5 °C. This mixture was filtered and the filtrate passed down a 'Dowex 50' column charged with 1M hydrochloric acid and eluted with ethanol (500 ml) then 2M ammonium hydroxide (1 L). This solution was then concentrated to 6.0 ml *in vacuo*. The remaining solution was passed down a weakly acidic ion exchange column (amberlire CG50) and eluted with water (50 ml). A small aliquot (100 µl) was removed for quantification. The remaining was concentrated to 1.0 ml *in*

vacuo. The residue was purified on an alumina column eluting with a solvent gradient of 100% acetone to 20% acetone/ water. Analysis of each fraction by GCMS (Sigma-Sil-A (TMSCl/ hexamethyldisilazane/ pyridine 1/3/9)), showed DMDP-glucoside present in the 50% acetone/ water fraction. This was concentrated and freeze dried to give the DMDP glucoside (0.3 mg).

3.4 References

1. "The Alternative Way; Complementary Medicine", *The Sunday Times (London)*, 2 July 1995.
2. N. Hawkes, "A love trap for insects", *The Times (London)*, 20 February 1995.
3. D. Attewell, "Plant Power", *Company Magazine (London)*, September 1995, p17.
4. A. Fleming, *Brit. J. Exp. Path*, 1929, **10**, 226.
5. E.Chain, H.W. Florey, A.D. Gardner, N.G. Heatly, M.A. Jennings, J.O. Ewing and A.G. Sanders, *The Lancet*, 1940, **ii**, 226.
6. S. Inouye, T. Tsurouka, T. Ito and T. Niida, *Tetrahedron* , 1968, **24**, 2125.
7. M. Yagi, T. Kuono, Y. Aoyagi and H. Murai, *Nippon Nogei Kagaku Kaishi*, 1976, **50**, 571.
8. S. Murao and S. Miyata, *Agric. Biol. Chem.*, 1980, **44**, 219.
9. G.W.J. Fleet, A. Karpas, R.A. Dwek, L.E. Fellows, A.S. Syms, S. Petursson, S.K. Namgoong, N.G. Ramsden, P.W. Smith, J.C. Son, F.X. Wilson, D.R. Witty, G.S. Jacob and T.W. Rademacher, *FEBS lett.*, 1988, **237**, 128.
10. *Nephthytis poisonii* specimen collected by T. Upson, 20 June 1990.
11. J.V. Dring, G.C. Kite, R.J. Nash and T. Reynolds, *Bot. J. Linn. Soc.*, 1995, **117**, 1.
12. A.A. Watson and R.J. Nash, unpublished results.
13. Reproduced with permission of the Royal Botanical Gardens, Kew, London.
14. R.H.C. Thrusby-Pelham, *The Veterinary Record*, 1967, **80**, 709.
15. C. Tamm, *Fortschr. Chem. Org. Naturstoffe*, 1956, **13**, 137.
16. A. Stoll, *Chem. Ind.*, 1959, **14**, 1558.
17. K. Tsuchiya, S. Kobayashi, T. Harada, T. Kurokawa, T. Nakagawa, N. Schimada and K. Kobayashi, *J. Antibiotics*, 1995, **48**, 626.

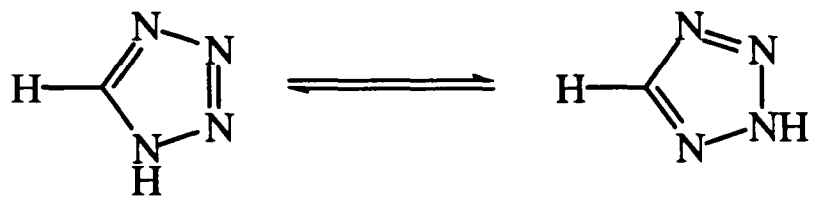
CHAPTER 4

CHAPTER 4

Investigations into Synthesis of a Tetrazole Precursor

4.1 Introduction

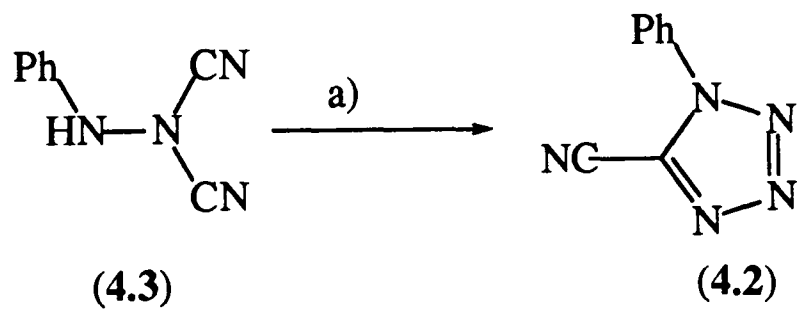
The aim of this work was to investigate the synthesis of an intermediate that could be used to form a number substituted tetrazoles. Unsubstituted tetrazole (**4.1**) is a five membered aromatic system with four nitrogen atoms and one carbon, it can exist in two tautomeric forms (Figure 4.1).



(4.1)

Figure 4.1

The first tetrazole was reported by Bladin,[1] where he suggested the structure (**4.2**) for the product formed by the reaction of nitrous acid on dicyanophenylhydrazine (**4.3**) (Figure 4.2). A large number of substituted tetrazoles have now been reported with their physical properties having been extensively studied.[2]

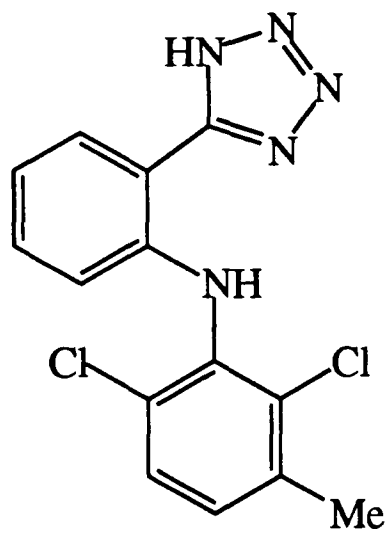


(4.3) **(4.2)**

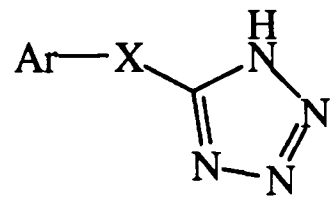
a) nitrous acid

Figure 4.2

The most common tetrazole derivatives are where they are linked into another molecule by the carbon in the ring. These 5-substituted tetrazoles have a similar acidity to carboxylic acid derivatives[3] but show more metabiological stability,[4] hence they would be expected to show similar biological activity to substituted carboxylic acids. Various studies have shown that when tetrazoles replace carboxylic acids in known biological agents the activity has increased, for example, the diphenylamine structure (**4.4**) have shown anti-inflammatory properties against oedema.[5]

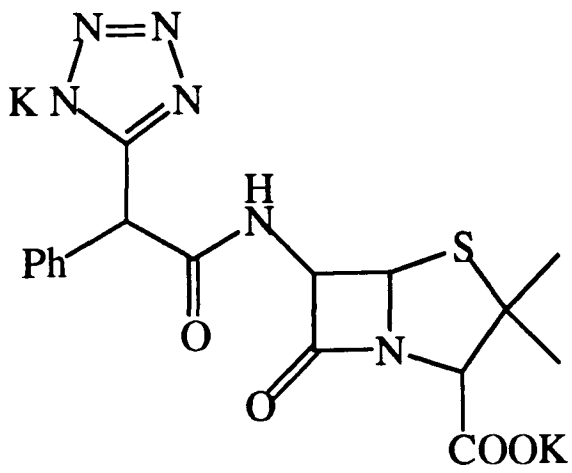


(**4.4**)

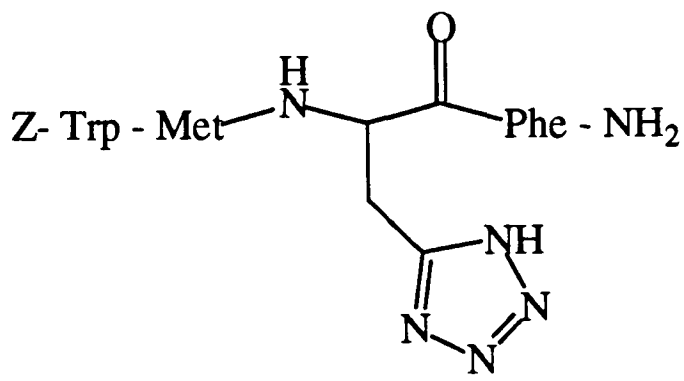


X = O, S

(**4.5**)



(**4.6**)

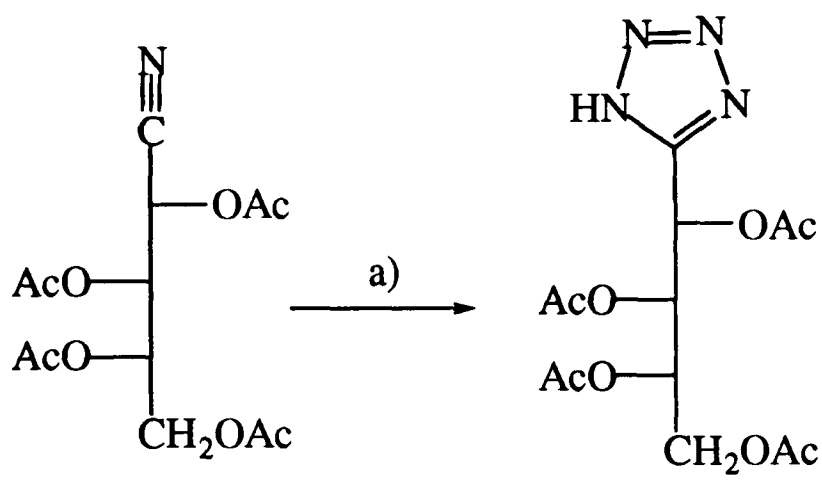


(**4.7**)

Tetrazole derivatives (**4.5**) have also been shown to reduce the production of cholesterol in rats.[6] By replacement of the carboxylic function in penicillins, tetrazoles can also be used as antibiotics,[7] for example the benzyl penicillin derivative (**4.6**). Tetrazoles

have also been incorporated into amino acids, then into peptides. The tetrapeptide (**4.7**)[8] has been synthesised and found to be equally active at stimulating gastric acid secretion as the acid analogue.

The most general route to 5-substituted tetrazoles involves the 1,3 dipolar cycloaddition of the appropriate nitrile and an inorganic azide ion.[9] Using this method the first polyhydoxylated tetrazoles were synthesised.[10] Taking tetra-*O*-acetyl-**L**-arabinonitrile (**4.8**) and condensing it with ammonium azide in DMF the tetrazole (**4.9**) was formed (Figure 4.3).

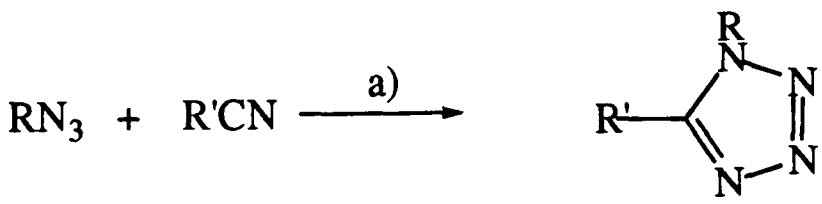


(4.8) **(4.9)**

a) NH_4N_3, DMF

Figure 4.3

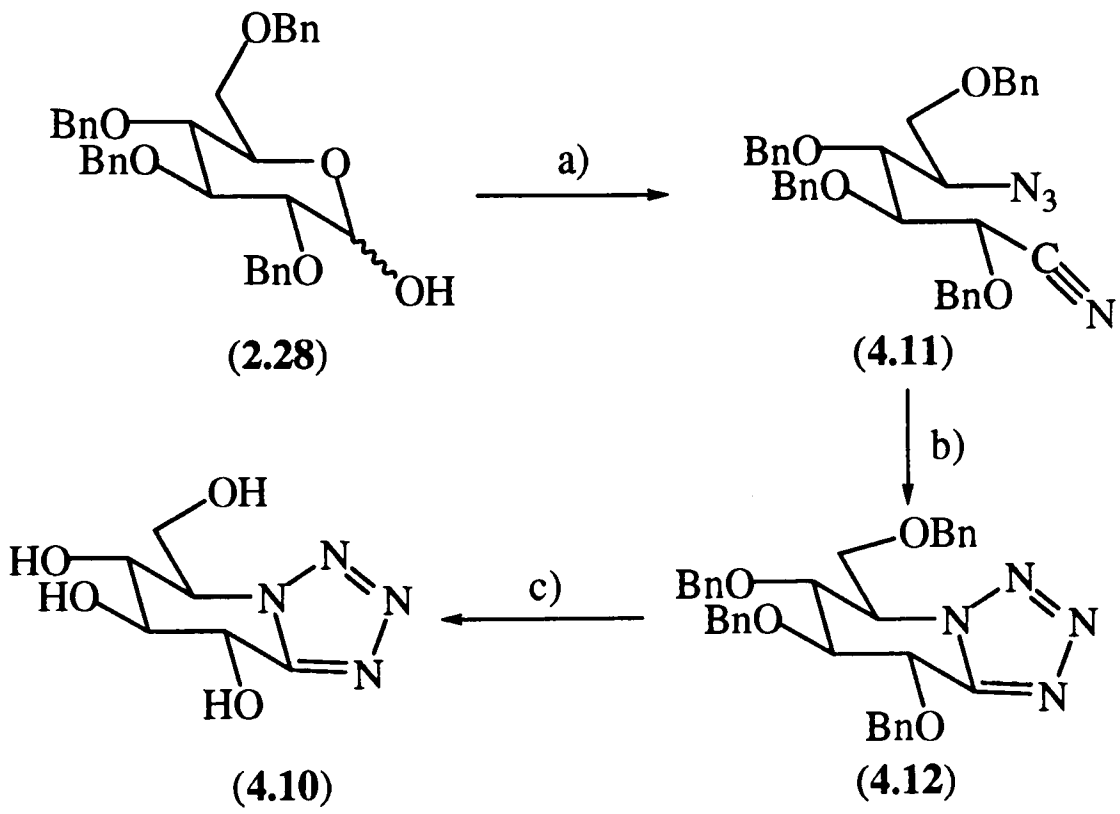
A more complex tetrazole is formed by use of an organic azide, this produces a 1,5 disubstituted tetrazole which exists as a single tautomer (Figure 4.4).[11]



a) Cl_3SO_3H

Figure 4.4

When the azide and nitrile are part of the same molecule then an intramolecular cyclisation can occur to form a fused bicyclic tetrazole. An example of this is the glucotetrazole (**4.10**) which has been synthesised by Vasella from tetra-*O*-benzyl-**D**-glucose (**2.28**) (Figure 4.5).[12]



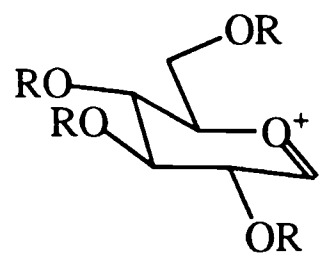
(2.28) **(4.11)**

(4.10) **(4.12)**

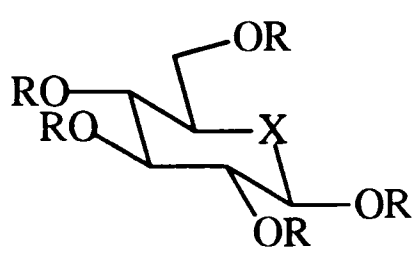
a) i. NH_2OH ii. PPh_3, CBr_4 iii. PPh_3, Br_2 iv. NaN_3
b) DMSO, Δ
c) H_2/ Pd/ C

Figure 4.5

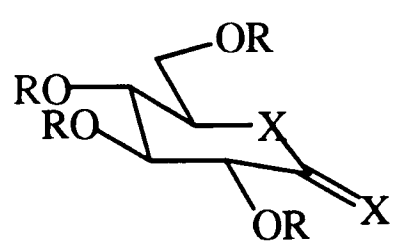
The oxime prepared from tetra-*O*-benzyl-**D**-glucose (**2.28**) is dehydrated with triphenylphosphine and carbon tetrabromide to form the nitrile. The remaining hydroxyl is transformed into a bromide and displaced with azide to give the azidonitrile (**4.11**) which on heating cyclises to the tetrazole (**4.12**). Once deprotected this tetrazole (**4.10**) was shown to be a β-glucosidase inhibitor. It is a non-basic analogue of DNJ (**1.7**) with a sp^2 centre at C-1 which is postulated to be a better mimic of the transition state of glycoside hydrolysis then the analogue with a sp^3 centre at C-1 (Figure 4.6).[13]



Proposed hydrolysis intermediate



X=O Substrate
X=N sp^3 transistion state analogues

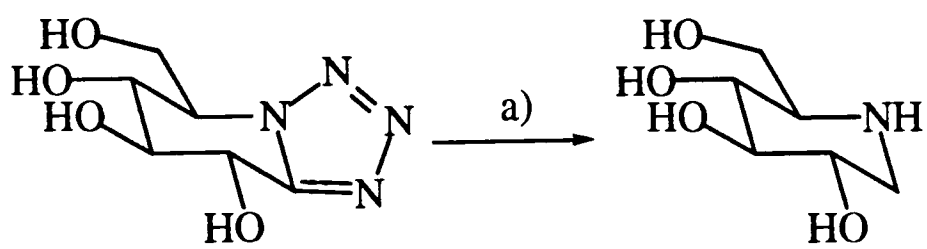


X= O, N sp^2 transistion state analogues.

Figure 4.6

If this functionality could be introduced into the end of a peptide chain then it may be of use in investigating the enzymes responsible for transferring the oligosaccharide chain on a protein during glycoprotein biosynthesis. In order to achieve this a carboxylate group or an amino group must be added onto the compound in place of one of the hydroxyls.

The glucotetrazole synthesised by Vasella, is a β-glucosidase inhibitor and can reduced with lithium aluminium hydride to DNJ (**1.7**), another glucosidase inhibitor (Figure 4.7).

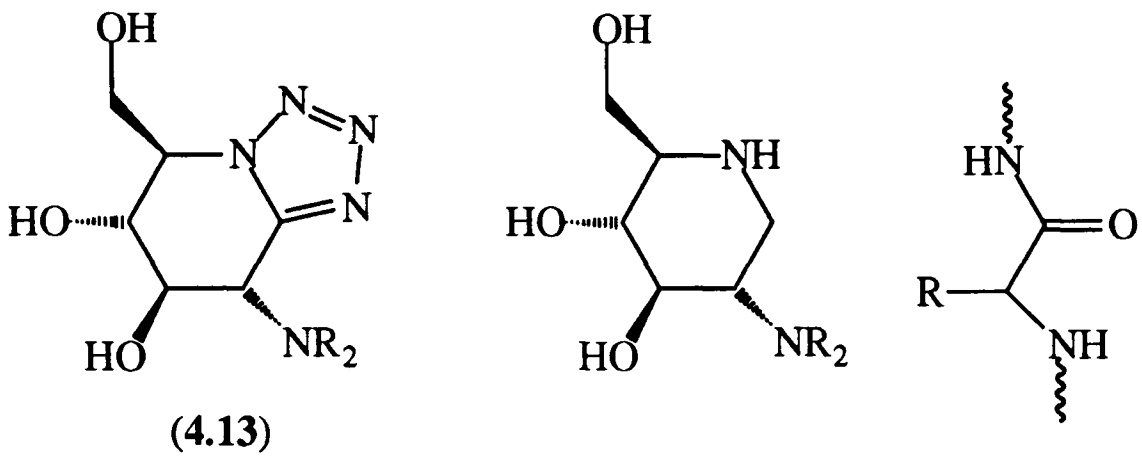


(4.10) **(1.7)**

a) $LiAlH_4$, Et_2O

Figure 4.7

Synthesising analogues of glucotetrazole with a nitrogen functionality at C-2 would allow the tetrazole to become an amine acid analogue, thus having a sp^2 centre at the C-1 position. Also it may be incorporated into a peptide sequence thus giving the possibility of producing an amino acid analogue that had a rigid structure, placing constraints on the folding of the peptide chain. Reduction with lithium aluminium hydride should give an analogue of 5-amino-DNJ another amino acid analogue (Figure 4.8).



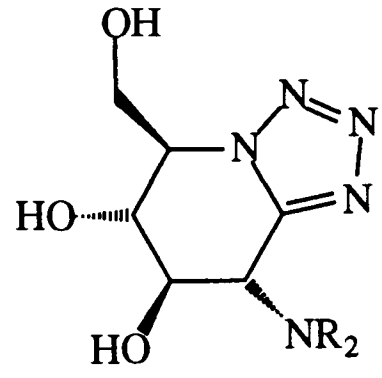
(4.13)

Figure 4.8

4.2 Results and Discussion

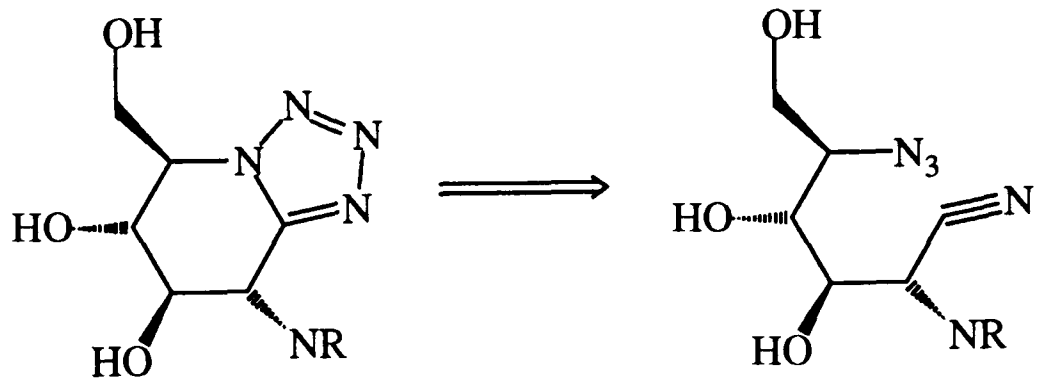
4.2.i Synthesis of 2,5-diazido-2,5-dideoxy-D-gluco-1,4-lactone (4.13)

The tetrazole analogue that was to be considered was the 2-aza-**D**-glucotetrazole (**4.13**).



(4.13)

It was envisaged that the tetrazole could be formed by the heating of the 5-azido-5-deoxygluconononitrile derivative in an analogous fashion to that of Vasella (Figure 4.9).[12]

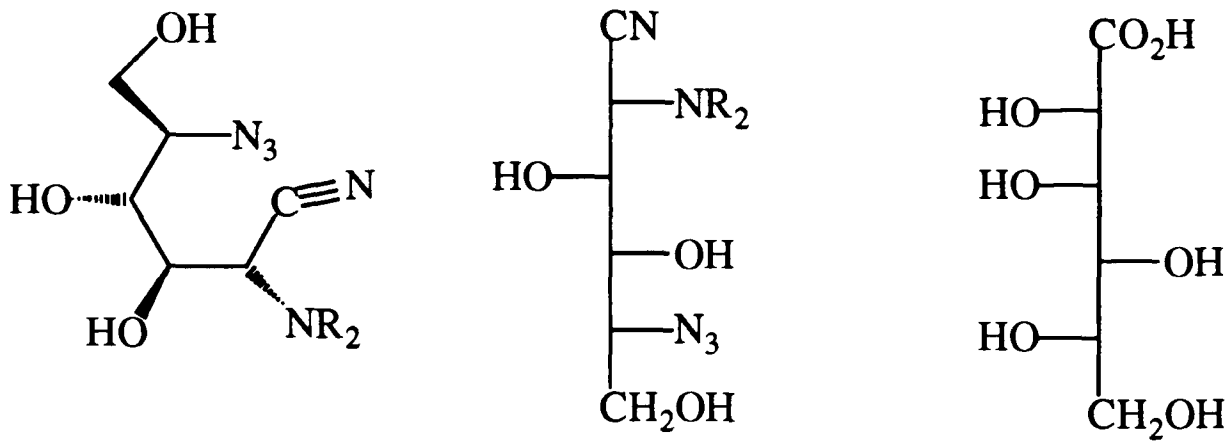


(4.13) **(4.14)**

Figure 4.9

A common method for introducing nitrogen into a carbohydrate framework is by displacement of a triflate ester formed with triflic anhydride. The displacement is normally carried out with sodium azide, this being a very strong nuecleophilic species in DMF, thus causing a rapid smooth S_N2 reaction. The 5-azido-**D**-gluconitrile (**4.14**) contains three

separate nitrogen containing groups, therefore to finish with **D**-glucose stereochemistry it is necessary to start with epimeric centres at C-5 and C-2, i.e. **L**-gulose (**4.15**) and an acid at C-1 (Figure 4.10).

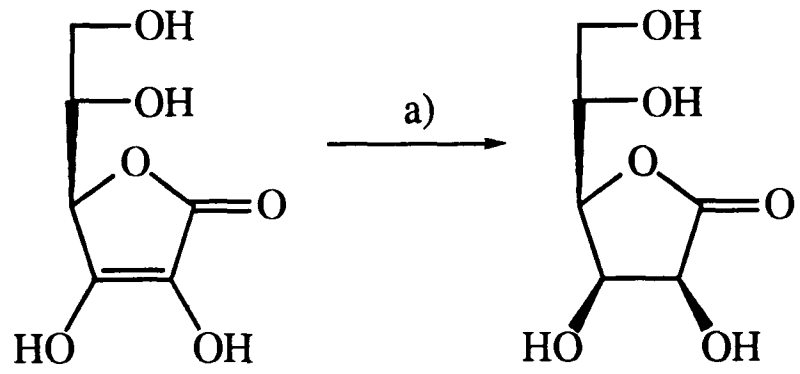


(4.14) **(4.15)**

D-Glucose **L**-Gulose

Figure 4.10

L-Gulono-1,4-lactone (**4.16**) was used as the starting material for this synthesis, as it is ready available it being formed by hydrogenation of **L**-asorbic acid (**4.17**) (Figure 4.11).[14]



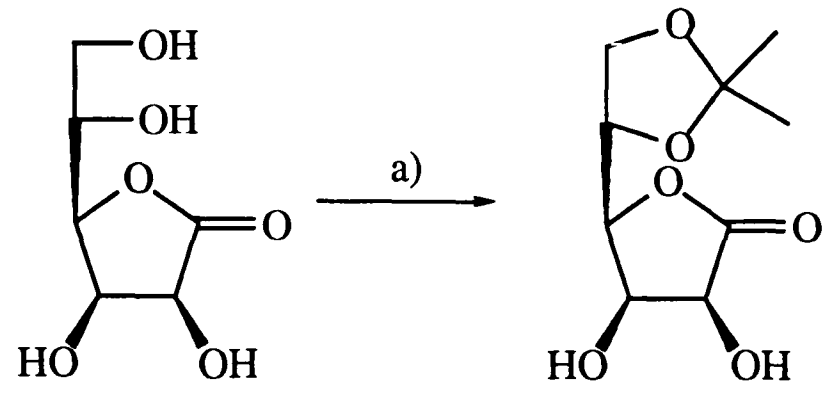
(4.17) **(4.16)**

a) H_2/ Pd/ C

Figure 4.11

The first step of the synthesis was to protect the C-5 and C-6 hydroxyls as an acetonide. This was selectively carried out by a literature method using methoxypropene in

DMF with toluene sulphonic acid.[14] The acetonide was formed in 65% yield with no secondary acetonide observed (Figure 4.12).

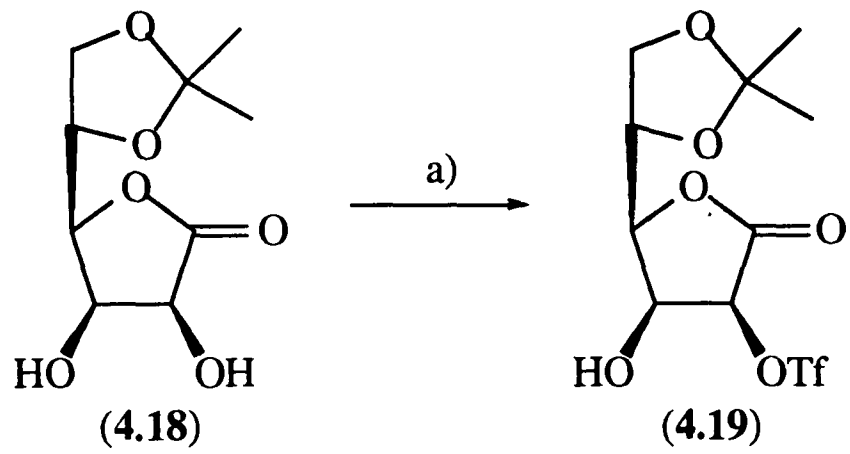


(4.16) **(4.18)**

a) 2-methoxypropene, *p*-toluene sulphonic acid, DMF.

Figure 4.12

The C-2 hydroxyl is converted into its triflate ester (**4.19**) by reaction with triflate anhydride at -40 °C. This is formed selectively over the C-3 hydroxyl due to a mixture of steric and electronic effects (Figure 4.13).

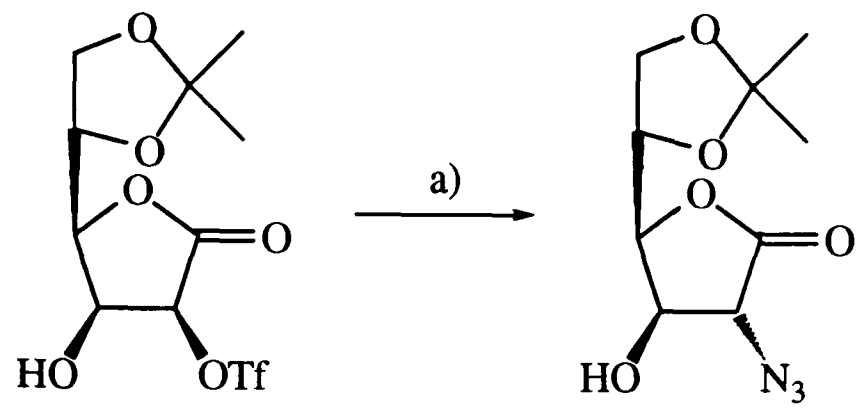


(4.18) **(4.19)**

a) Tf_2O, py, THF, -40 °C

Figure 4.13

A displacement reaction on the triflate ester (**4.19**) with sodium azide was attempted with the aim of producing inversion of configuration at C-2 (Figure 4.14).



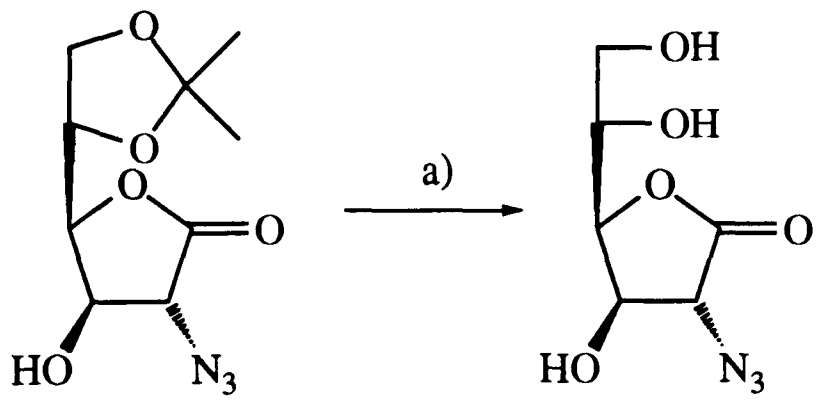
(4.19) **(4.20)**

a) NaN_3, DMF

Figure 4.14

NOe studies carried out on the triflate (**4.19**) shows a small enhancement of H-3 and H-4 when the H-2 proton is irradiated in d_6-benzene whereas the azide (**4.20**) showed no enhancement. This suggests that the azide displacement of the triflate group had occurred with an overall inversion of configuration, to give **L**-idose stereochemistry.

The acetonide was removed by acid hydrolysis to gain access to the C-5 hydroxyl in order to introduce the second nitrogen (Figure 4.15).



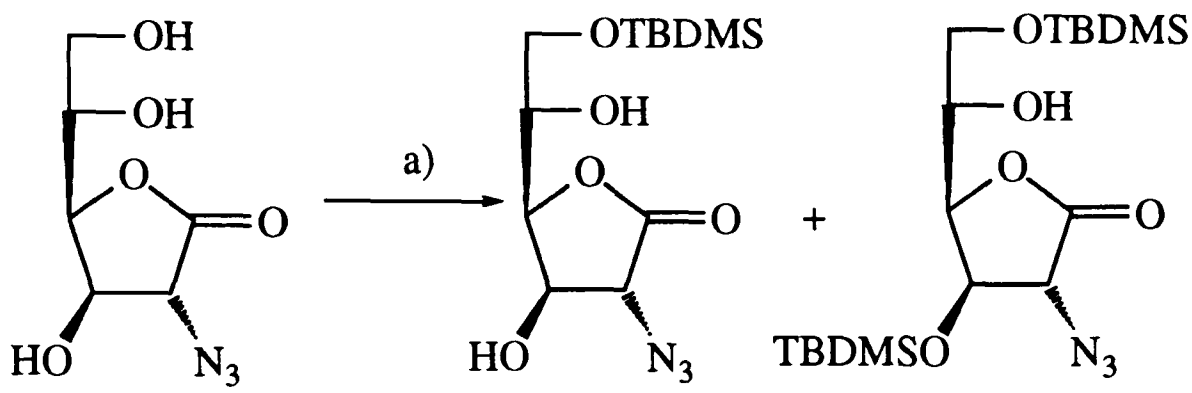
(4.20) **(4.21)**

a) 80% AcOH/ H_2O

Figure 4.15

A silyl ether was then formed between *tert*-butyldimethyl silyl chloride and the primary hydroxyl. Two products were found to have been formed, the C-6 mono-silyl ether (**4.22**) and a compound that contained two TBDMS ethers. It was hypothesised that

the disilyl compound was 2-azido-2-deoxy-3,6-di-*O*-(*tert*-butyldimethyl)silyl-**L**-ido-1,4-lactone (**4.23**) (Figure 4.16).

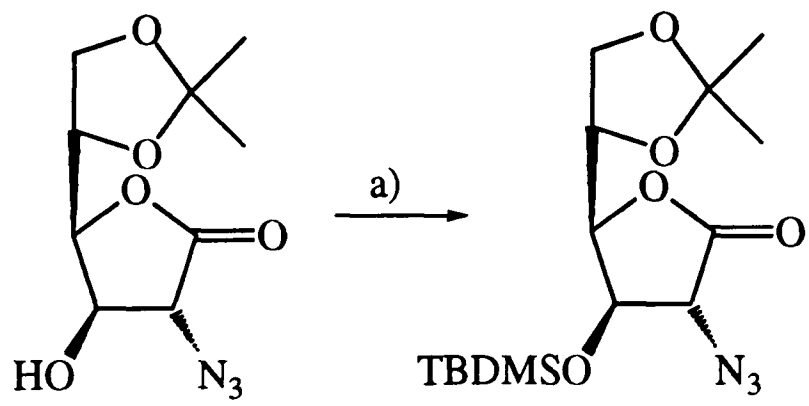


(4.21) **(4.22)** **(4.23)**

a) TBDMSCl, imidazole, DMF

Figure 4.16

The monosilylated compound was purified and reacted further with TBDMSCl. This formed the disilyl compound (**4.23**) exclusively. In order to confirm the regiochemistry of the second silyl ether group, the primary acetonide (**4.20**) was treated with TBDMS triflate to form the fully protected compound (**4.24**) (Figure 4.17).



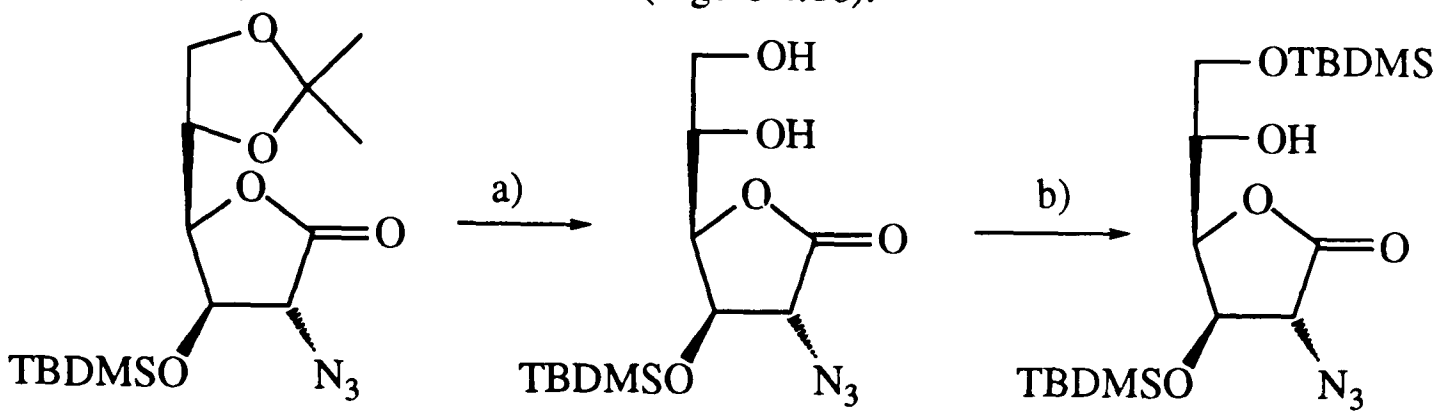
(4.20) **(4.24)**

a) TBDMSOTf, py, DCM

Figure 4.17

The acetonide was then selectively removed with 80% acetic acid at room temperature. The resulting diol (**4.25**) was reacted with TBDMSCl in DMF at -40 °C. This resulted in the formation of a disilyl compound which was identical in all respects to the

one that had previously been formed thus confirming the regiochemistry of the disilyl compound (**4.23**) formed from the triol (Figure 4.18).

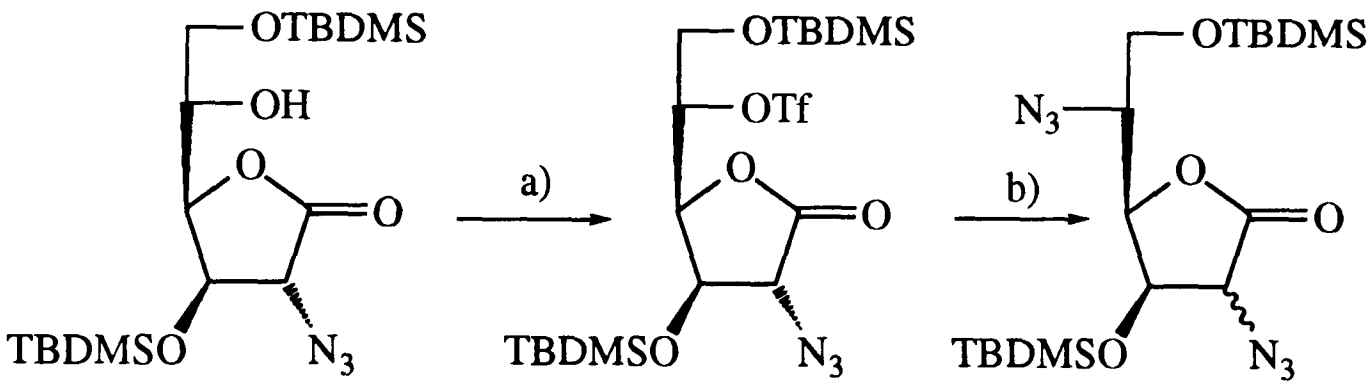


(4.24) **(4.25)** **(4.23)**

a) 80% AcOH/ H_2O
b) TBDMSCl, imidazole, DMF, -40°C

Figure 4.18

Treatment with triflic anhydride converted the remaining hydroxyl into its triflate ester (**4.26**). This was not isolated and was displaced with sodium azide to give the diazido compound (**4.27**) (Figure 4.19).



(4.23) **(4.26)** **(4.27)**

a) Tf_2O, py, DCM
b) NaN_3, DMF

Figure 4.19

The stereochemistry was investigated by means of a second nOe experiment. Irradiation of the H-2 proton at 2.9 ppm in d_6-benzene, afforded an nOe enhancement of 9.2% for the C-3 proton and 7.1% for the C-4 proton (Figure 4.20). This suggests that an inversion of the C-2 azide had occurred during this reaction, by means of direct azide

nucleophilic attack or by the action of the azide as a base to remove the C-2 proton (Figure 4.21).

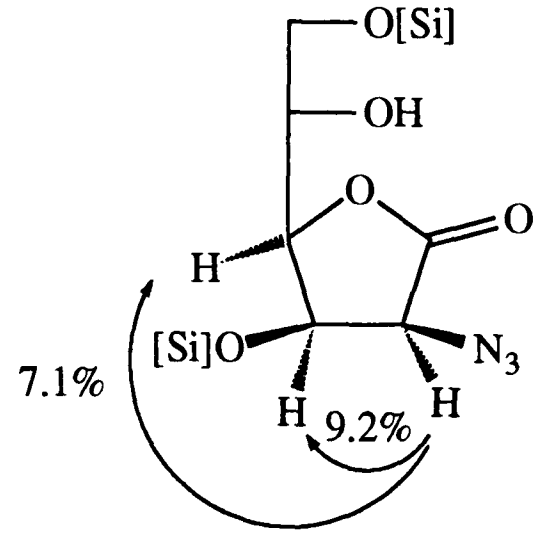


Figure 4.20

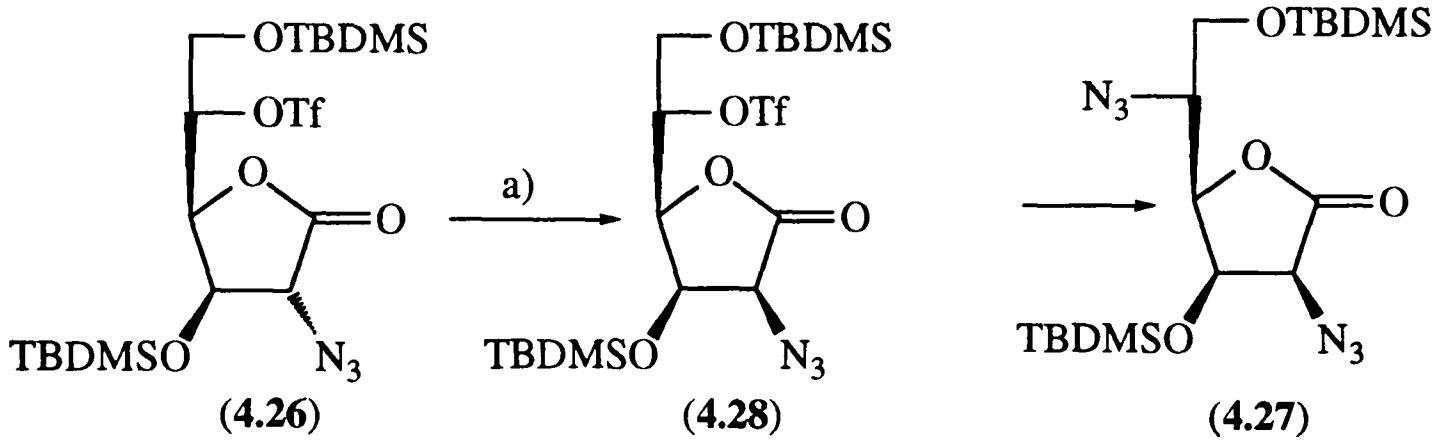
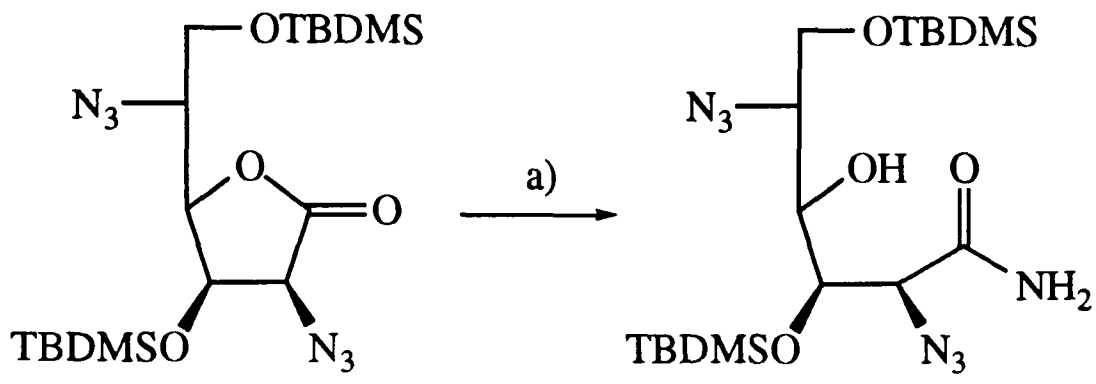


Figure 4.21

If the synthesis were to be continued with the opposite stereochemistry at C-2 (**4.27**) then the tetrazole analogue with **D**-manno stereochemistry would be formed. As there are many mannosidase enzymes which found in plants, a mannosidase inhibitor would also be of interest as a possible fungicide, as such the synthesis was continued.

4.2.ii Preliminary Studies into Tetrazole formation.

The diazido lactone (**4.27**) was dissolved in a saturated solution of ammonia in methanol. This caused the lactone to be opened producing the primary amide (**4.29**) (Figure 4.22).

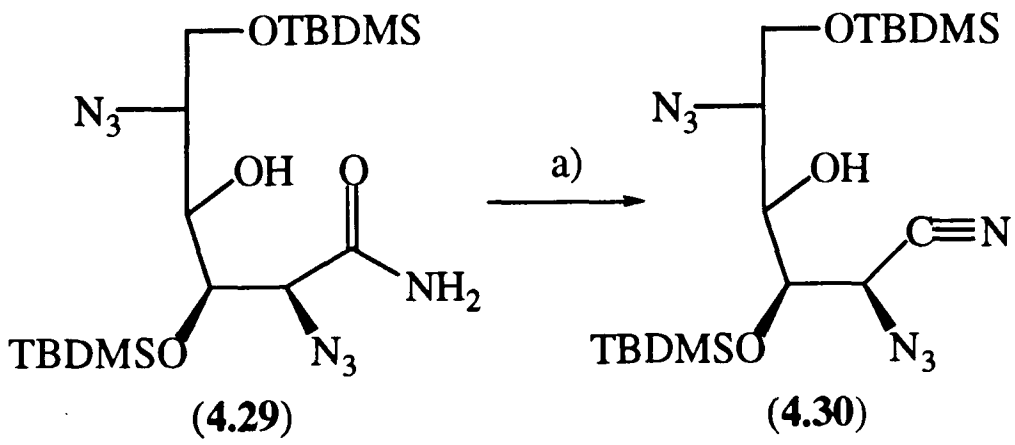


(4.27) **(4.29)**

a) NH_3/ CH_3OH

Figure 4.22

Primary amides when reacted with trifluoroacetic anhydride exist in an equilibrium with the nitrile, this being effectively a dehydration.[15] Excess anhydride causes the equilibrium to favour the formation of the nitrile. The primary amide (**4.29**) when reacted with an excess of trifluoroacetic anhydride forms the nitrile (**4.30**) which can be isolated in 80% yield (Figure 4.23).



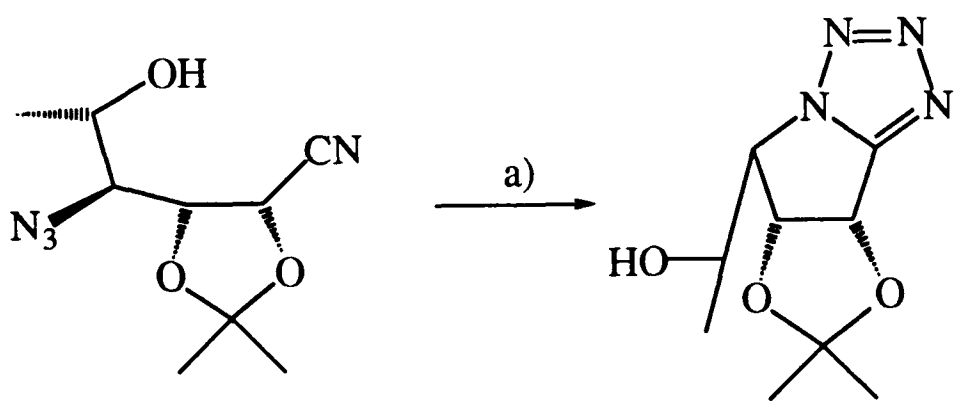
(4.29) **(4.30)**

a) $(F_3CCO)_2O$, py

Figure 4.23

This compound was set up to undergo an intramolcular 1,3 dipolar cyclisation to form the tetrazole. Following the method reported by Carpenter,[11] the cyclisation was attempted by reaction with chlorosulphonic acid. As opposed to the tetrazole this gave an inseparable complex mixture of compound with no evidence of a tetrazole being formed.

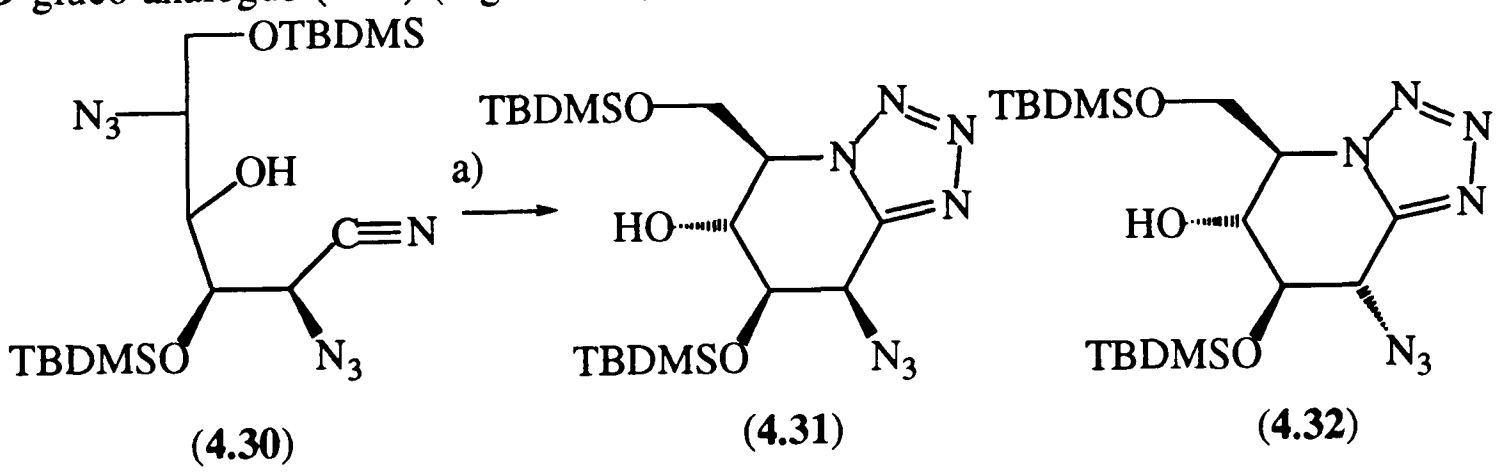
Recent work by Davis,[16] has shown that 5-azido-1-nitrile derivatives of carbohydrates when heated in dimethyl sulphoxide undergo cyclisation of give tetrazoles in high yield (Figure 4.24).



a) DMSO, 100 °C, 60 hours

Figure 4.24

The attempt to cyclise the diazido nitrile (**4.30**) was carried out by Davis.[17] The product formed was found to be the **D**-mannotetrazole (**4.31**) by comparison to the **D**-gluco analogue (**4.32**) (Figure 4.25).



(4.30) **(4.31)** **(4.32)**

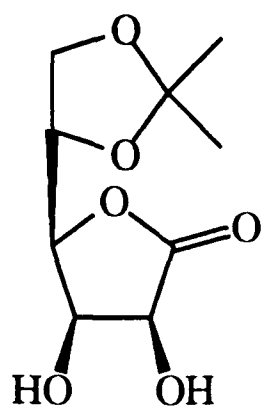
a) DMSO, Δ

Figure 4.25

This shows that the diazidolactone (**4.27**) can be used as a precursor to tetrazole formation. Reduction of the azide at C-2 of the tetrazole should allow the incorporation of the tetrazole at the end of a peptide chain. If the tetrazole ring is reduced with lithium aluminium hydride then this should allow DMJ to be incorporated into the middle of a peptide chain.

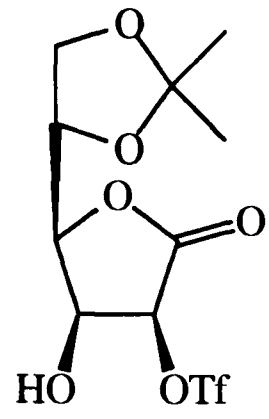
4.3 Experimental

5,6 *O*-Isopropylidene-**L**-gulono-1,4-lactone (**4.18**)



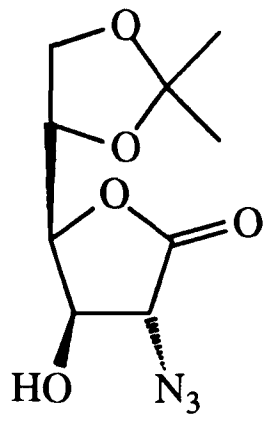
A solution of **L**-gulono-1,4-lactone (**4.16**) (10 g, 56.0 mmol) in *N,N*-dimethylformamide (50 ml) was cooled to 10 °C. *p*-Toluenesulphonic acid (190 mg, 1.0 mmol) was added portionwise with stirring followed by the addition of 2-methoxypropene (6.4 ml, 67 mmol) dropwise over 5 minutes and the mixture was stirred at room temperature for 24 hours. Tlc (ethyl acetate) analysis showed one product (R_f 0.4). Sodium carbonate (10 g) was added and the suspension stirred for 2 hours. The sodium carbonate was removed by filtering through Celite and the filtrate was concentrated *in vacuo*. Crystallisation from hot ethanol afforded 5,6-*O*-isopropylidene-**L**-gulono-1,4-lactone (**4.18**) (10.37 g, 85%) as a white crystalline solid. M.p.(ethanol); 164-167 °C, [lit.[14] 167-168 °C]. $[\alpha]_D^{25}$ +34.5 (c 1.0, MeOH), [lit.[14] $[\alpha]_D^{25}$ +38.3 (c 0.7, MeOH)]. ν_{MAX} (KBr); 3520 (OH, m), 1762 (C=O, s) cm^{-1}. δ_H (200 MHz, CD_3CN); 1.20 (3H, s, Me), 1.26 (3H, s, Me), 3.76 (1H, q $J_{6,5}$ 6.0 $J_{6,6'}$ 9.0 Hz, H-6), 4.08 (1H, q $J_{6',6}$ 9.0 $J_{6',5}$ 5.7 Hz, H-6'), 4.31- 4.36 (3H, m, H-3, H-4, H-5), 4.50 (1H, d $J_{2,3}$ 4.5 Hz, H-2). δ_C (50 MHz, CD_3CN); 24.5, 25.9 (2x q, C($\underline{C}H_3)_2$), 64.7 (t, C-6), 69.3, 70.3, 75.1, 81.4 (4x d, C-2, C-3, C-4, C-5), 117.6 (s, $\underline{C}(Me)_2$), 175.7 (s, C-1).

5,6-*O*-Isopropylidene-2-*O*-trifluoromethanesulphonate-**L**-gulono-1,4-lactone (**4.19**)



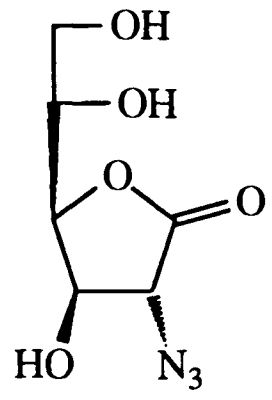
A solution of 5,6-*O*-isopropylidene-**L**-gulono-1,4-lactone (**4.18**) (4.90 g, 22.6 mmol) was stirred in tetrahydrofuran (200 ml) at -40 °C. To this was added pyridine (4.58 ml, 56.7 mmol), followed by triflic anhydride (5.70 ml, 33.9 mmol) dropwise over 5 minutes. After 90 minutes, tlc (ethyl acetate/ hexane 1/1) showed no starting material (baseline) and one product (R_f 0.4). Water (1 ml) was added and the mixture allowed to warm up to room temperature. The solvent was removed *in vacuo* and the residue was redissolved in ethyl acetate (100 ml) and washed with dilute hydrochloric acid (1M, 20 ml) followed by water (2x 20 ml) then brine (20 ml). The organic layer was dried (magnesium sulphate) and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/ hexane 1/1) to afford 5,6-*O*-isopropylidene-2-*O*-trifluoromethanesulphonate-**L**-gulono-1,4-lactone (**4.19**) (7.69 g, 97%) as a white crystalline solid. M.p.(ethyl acetate/ hexane); 104-105 °C. $[\alpha]_D^{25}$ + 14.0 (c 1.0, $CHCl_3$). Found C 34.12 H 3.52% $C_{10}H_{13}O_8SF_3$ requires C 34.39 H 3.46%. ν_{MAX} (film, $CHCl_3$); 3750 (OH, m), 1780 (s, C=O) cm^{-1}. δ_H (200 MHz, $CDCl_3$); 1.42 (3H, s, CH_3), 1.49 (3H, s, CH_3), 4.09 (2H, dd $J_{6,5}$ 6.0 Hz, H-6,6'), 4.50 (1H, t J 3.5 Hz, H-3), 4.56 (1H, t $J_{5,6}$ 6.0 Hz, H-5), 4.79 (1H, dd $J_{4,5}$ 6.0 $J_{4,3}$ 3.5 Hz, H-4), 5.33 (1H, d $J_{2,3}$ 6.0 Hz, H-2). δ_C (50 MHz, CD_3CN); 25.55, 26.86 (2x q, $C(\underline{C}H_3)_2$), 65.46 (t, C-6), 69.18, 75.42, 81.42, 83.04 (4x d, C-2, C-3, C-4, C-5), 110.93 (s, $\underline{C}(Me)_2$), 171.73 (s, C-1). m/z (CI, NH_3); 368 (100, MNH_4^+), 351 (20%, M^+).

2-Azido-2-deoxy-5,6-*O*-isopropylidene-**L**-ido-1,4-lactone (**4.20**)



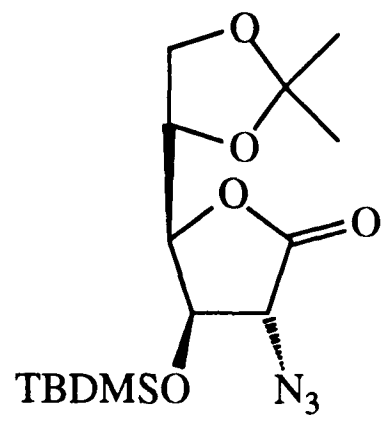
5,6-*O*-Isopropylidene-2-*O*-trifluoromethanesulphonate-**L**-gulono-1,4-lactone (**4.19**) (7.69 g, 22.0 mmol) was dissolved in *N,N*-dimethylformamide (80 ml) and stirred with sodium azide (2.5 g, 37.4 mmol). After 5 hours tlc analysis (ethyl acetate/ hexane 1/1) showed no starting material (R_f 0.4) and one product (R_f 0.1). The solvent was removed *in vacuo*, and the residue redissolved in ethyl acetate (50 ml) and washed with water (2x 10 ml) and brine (20 ml). The organic layer was dried (magnesium sulphate) and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/ hexane 1/1) to afford 2-azido-2-deoxy-5,6-*O*-isopropylidene-**L**-ido-1,4-lactone (**4.20**) (3.94 g, 75%) as a white crystalline solid. M.p.(ethyl acetate/ hexane); 70-72 °C. $[\alpha]_D^{25}$; +171.4 (c 1.0, $CHCl_3$). Found C 44.49 H 5.20 N 17.16% $C_9H_{13}O_5N_3$ C 44.45 H 5.39 N 17.28%. ν_{MAX} (film); 3431 (m, OH), 2115 (s, N_3), 1785 (m, C=O) cm^{-1}. δ_H (500 MHz, C_6D_6); 1.17 (3H, s, CH_3), 1.24 (3H, s, CH_3), 3.53 (1H, dd $J_{4,5}$ 1.5 $J_{4,3}$ 7.6 Hz, H-4), 3.60 (1H, dd $J_{6,6'}$ 8.3 $J_{6,5}$ 7.2 Hz, H-6), 3.65 (1H, dd $J_{6',6}$ 8.3 $J_{6',5}$ 7.2 Hz, H-6'), 3.83 (1H, dd $J_{3,4}$ 7.6 $J_{3,2}$ 8.5 Hz, H-3), 3.99 (1H, td $J_{5,6}$ 7.2 $J_{5,4}$ 1.5 Hz, H-5), 4.31 (1H, d $J_{2,3}$ 8.5 Hz, H-2). δ_C (50 MHz, $CHCl_3$); 25.31, 25.45 (2x q, $C(\underline{C}H_3)_2$), 63.10, 72.62, 73.12 (3x d, C-3, C-4, C-5), 65.09 (t, C-6), 77.55 (d, C-2), 110.94 (s, $\underline{C}(Me)_2$), 171.80 (s, C-1). m/z (CI, NH_3); 261 (100, MNH_4^+), 244 (50%, MH^+).

2-Azido-2-deoxy-**L**-ido-1,4-lactone (**4.21**)



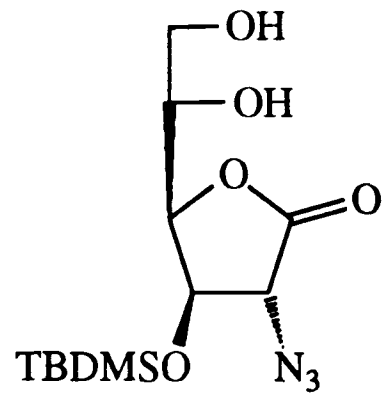
2-Azido-2-deoxy-5,6-*O*-isopropylidene-**L**-ido-1,4-lactone (**4.20**) (6.36 g, 26 mmol) was dissolved in 80% acetic acid (150 ml) and stirred at room temperature. After 22 hours tlc analysis (ethyl acetate) showed no starting material (R_f 0.8) and one product (R_f 0.2). The solvent was removed *in vacuo* and the residue purified by flash column chromatography (ethyl acetate) to afford 2-azido-2-deoxy-**L**-ido-1,4-lactone (**4.21**) (4.75 g, 89%) as a white crystalline solid. M.p.(ethyl acetate); 106-107 °C. $[\alpha]_D^{25}$ +12.5 (c 0.75, CH_3CN). Found C 35.62 H 4.31 N 20.73% $C_6H_9O_5N_3$ requires C 35.47 H 4.47 N 20.68%. ν_{MAX} (thin film); 3413 (m, OH), 2120 (s, N_3), 1778 (s, C=O) cm^{-1}. δ_H (500 MHz, CD_3CN); 3.51 (1H, d $J_{6,5}$ 6.8 Hz, H-6), 3.52 (1H, d $J_{6,5}$ 6.8 Hz, H-6'), 4.00 (1H, td $J_{5,6}$ 6.9 $J_{5,4}$ 1.3 Hz, H-5), 4.42 (1H, t J 8.5 Hz, H-3), 4.55 (1H, dd $J_{4,5}$ 1.4 $J_{4,3}$ 7.8 Hz, H-4), 4.68 (1H, d $J_{2,3}$ 8.8 Hz, H-2). δ_C (128 MHz, CD_3CN); 62.21 (t, C-6), 62.91, 68.26, 72.04, 78.92 (4x d, C-2, C-3, C-4, C-5), 172.47 (s, C-1). m/z (CI, NH_3); 221 (100, MNH_4^+), 203 (30%, M^+).

2-Azido-3-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-5,6-*O*-isopropylidene-**L**-ido-1,4-lactone **(4.24)**



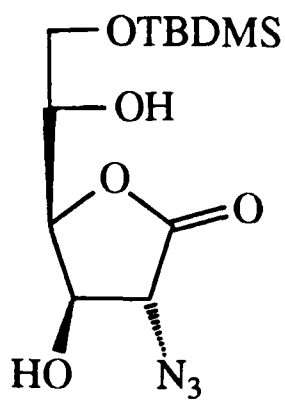
A solution of 2-azido-2-deoxy-5,6-*O*-isopropylidene-**L**-ido-1,4-lactone (**4.20**) (100 mg, 0.4 mmol) in dichloromethane (10 ml) was cooled to 0 °C. To this was added pyridine (1.2 mmol, 1.0 ml) followed by *tert*-butyldimethylsilyl triflate (0.6 mmol, 0.15 ml). After 2 hours, tlc analysis (ethyl acetate/ hexane 1/2) showed no starting material (R_f 0.1) and one product (R_f 0.7). The reaction was allowed to warm up to room temperature, then washed with dilute hydrochloric acid (1M, 2 ml), water (2x 2 ml) and brine (2 ml). The organic layer was dried (magnesium sulphate) and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/ hexane 1/ 3) to afford 2-azido-3-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-5,6-*O*-isopropylidene-**L**-ido-1,4-lactone (**4.23**) (125 mg, 85%) as a clear oil. Found C 50.32 H 7.92 N 12.02% $C_{15}H_{27}O_5N_3Si$ requires C 50.40 H 7.61 N 11.75%. ν_{MAX} (thin film); 2113 (s, N_3), 1795 (m, C=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 0.12 (3H, s, CH_3), 0.18 (3H, s, CH_3), 0.92 (9H, s, $C(CH_3)_3$), 1.36 (6H, s, $Si(CH_3)_2$), 3.90 (1H, t *J* 8.1 Hz, H-6), 4.09 (1H, dd $J_{6',6}$ 8.1 $J_{6',5}$ 6.8 Hz, H-6'), 4.32 (1H, dd $J_{5,4}$ 1.1 $J_{5,6}$ 7.7 Hz, H-5), 4.38 (1H, t *J* 8.7 Hz, H-3), 4.42 (1H, td $J_{4,5}$ 1.1 $J_{4,3}$ 7.9 Hz, H-4), 4.52 (1H, d $J_{2,3}$ 8.7 Hz, H-2). δ_C (50 MHz, $CDCl_3$); -5.27, -4.90, -4.58 (3x q, 4x CH_3), 17.73 (s, $SiC(Me)_3$), 25.41 (q, $C(\underline{C}H_3)_3$), 63.00, 72.37, 73.75, 76.84 (4x d, C-2, C-3, C-4, C-5), 65.29 (t, C-6), 110.55 (s, $\underline{C}(Me)_2$), 170.64 (s, C-1). m/z (CI, NH_3); 375 (100%, M^+).

2-Azido-3-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-**L**-ido-1,4-lactone (**4.25**)

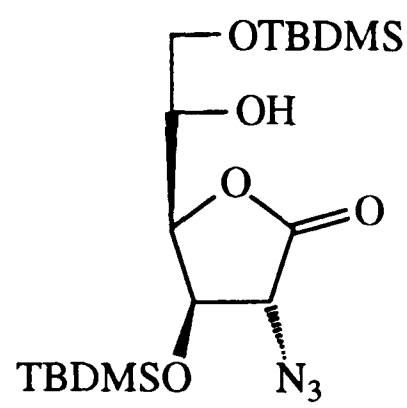


2-Azido-3-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-5,6-*O*-isopropylidene-**L**-ido-1,4-lactone (**4.24**) (102 mg, 0.28 mmol) was stirred in 80% acetic acid (50 ml). After 5 hours, tlc analysis (ethyl acetate/ hexane 1/ 1) showed no starting material (R_f 0.9) and one product (R_f 0.6). The solvent was removed *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate/ hexane 1/ 1) to afford 2-azido-3-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-**L**-ido-1,4-lactone (**4.25**) (80 mg, 90%) as a clear oil. Found C 45.18 H 7.66 N 13.36% $C_{12}H_{23}O_5N_3Si$ requires C 45.26 H 7.60 N 13.20%. ν_{MAX} (thin film); 3474 (m, OH), 2118 (s, N_3), 1791 (C=O) cm^{-1}. δ_H (200 MHz, $CDCl_3$). δ_C (50 MHz, $CDCl_3$); -5.27, -4.91 (2x q, 2x $Si(CH_3)$), 17.79 (s, $Si\underline{C}(Me)_3$), 24.51 (q, $C(\underline{C}H_3)_3$), 63.12 (t, C-6), 63.33. 68.96, 73.75, 79.17 (4x d, C-2, C-3, C-4, C-5), 172.10 (s, C-1). m/z (CI, NH_3); 335 (100%, MNH_4^+).

2-Azido-6-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-**L**-ido-1,4-lactone (**4.22**) and 2-azido-3,6-di-*O*-(*tert*-butyldimethyl) silyl-2-deoxy-**L**-ido-1,4-lactone (**4.23**)



(4.22)



(4.23)

Method 1

To a solution of 2-azido-2-deoxy-**L**-ido-1,4-lactone (**4.21**) (3.32 g, 16 mmol) in *N,N*-dimethylformamide (10 ml) at -30 °C was added imidazole (5.44 g, 80 mmol) and *tert*-butyldimethylsilyl chloride (6.00 g, 40 mmol). After stirring for 3 hours tlc analysis (ethyl acetate/ hexane 1/ 2) showed no starting material (baseline), a major product (R_f 0.6) and a minor product (R_f 0.3). The solvent was removed *in vacuo* and the residue redissolved in ethyl acetate (50 ml). This was washed with water (2x 20 ml), brine (20 ml) and dried (magnesium sulphate). The solvent was removed *in vacuo* and the residue purified by flash column chromatography (ethyl acetate/ hexane 1/ 4) to afforded 2-azido-2-deoxy-3,6-di-*O*-(*tert*-butyldimethyl)silyl-**L**-ido-1,4-lactone (**4.23**) (4.34 g, 62%) as a clear oil. Further elution (ethyl acetate/ hexane 1/ 2) afforded 2-azido-6-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-**L**-ido-1,4-lactone (**4.22**) (1.04 g, 20%) as a white crystalline solid.

Method 2 :- 2-Azido-3,6-di-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-**L**-ido-1,4-lactone (**4.23**)

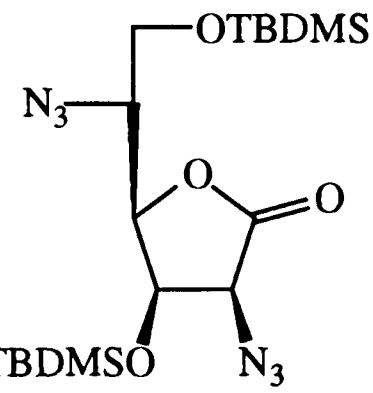
2-Azido-3-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-**L**-ido-1,4-lactone (**4.25**) (80 mg, 0.25 mmol) was dissolved in *N,N*-dimethylformamide (20 ml) at -40 °C. To this solution was added imidazole (39 mg, 0.6 mmol) and *tert*-butyldimethyl silyl chloride (45 mg, 0.30 mmol). After stirring for 18 hours, tlc analysis (ethyl acetate/ hexane 1/ 3) showed no starting material (R_f 0.1) and one product (R_f 0.6). The solvent was removed *in vacuo* and the residue was redissolved in ethyl acetate (20 ml), washed with water (2x 10 ml), brine (10 ml) and dried (magnesium sulphate). The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (ethyl acetate/ hexane 1/ 5) to afford 2-azido-2-deoxy-3,6-di-*O*-(*tert*-butyldimethyl)silyl-**L**-ido-1,4-lactone (**4.23**) (79 mg, 73%) as a clear oil.

2-Azido-6-*O*-(*tert*-butyldimethyl)silyl-2-deoxy-**L**-ido-1,4-lactone (**4.22**) :- M.p.(ethyl acetate/ hexane); 57-60 °C. $[\alpha]_D^{25}$ +154.6 (c 0.8, $CHCl_3$). Found C 45.51 H 7.35 N 13.18% $C_{12}H_{23}N_3O_5Si$ requires C 45.51 H 7.30 N 13.24%. ν_{MAX} (thin film); 3441 (m, OH), 2118 (s, N_3), 1779 (s, C=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 0.10 (6H, s, 2x CH_3), 0.90 (9H, s, $C(CH_3)_3$), 3.72 (1H, dd $J_{6,5}$ 9.98 $J_{6,6'}$ 19.0 Hz, H-6), 3.73 (1H, dd $J_{6',5}$ 9.9 $J_{6',6}$ 20.0 Hz, H-6'), 4.14 (1H, dt $J_{5,6}$ 6.1 $J_{5,4}$ 2.2 Hz, H-5), 4.44 (1H, t J 7.3 Hz, H-3), 4.53 (1H, dd $J_{4,3}$ 7.3 $J_{4,5}$ 2.2 Hz, H-4), 4.54 (1H, d $J_{2,3}$ 7.3 Hz, H-2), δ_C (50 MHz, $CDCl_3$); -5.70 (q, 2x CH_3), 18.07 (s, $SiC(Me)_3$), 25.67 (q, $C(CH_3)_3$), 62.98 (t, C-6), 63.43, 69.30, 72.99, 79.13 (4x d, C-2, C-3, C-4, C-5), 171.87 (s, C-1). m/z (CI, NH_3); 335 (40%, MNH_4^+), 317 (30%, M^+).

2-Azido-2-deoxy-3,6-di-*O*-(*tert*-butyldimethyl)silyl-**L**-ido-1,4-lactone (**4.23**) :- $[\alpha]_D^{25}$ +85.3 (c 0.75, $CHCl_3$). Found C 49.99 H 8.72 N 9.50% $C_{18}H_{37}O_5N_3Si_2$ requires C 50.08 H 8.64 N 9.73%. ν_{MAX} (thin film); 3605 (w, OH), 2118 (s, N_3), 1793 (m, C=O) cm^{-1}. δ_H (500 MHz, $CDCl_3$); 0.09 (6H, s, 2x CH_3), 0.15 (3H, s, CH_3), 0.19 (3H, s, CH_3),

0.90 (9H, s, $C(CH_3)_3$), 0.94 (9H, s, $C(CH_3)_3$), 3.68 (1H, dd $J_{6,5}$ 9.8 $J_{6,6'}$ 19.0 Hz, H-6), 3.69 (1H, dd $J_{6',5}$ 9.8 $J_{6',6}$ 19.0 Hz, H-6'), 4.06 (1H, t *J* 7.1 Hz, H-5), 4.42 (1H, t *J* 7.1 Hz, H-4), 4.43 (1H, t *J* 2.8 Hz, H-3), 4.62 (1H, d $J_{2,3}$ 2.8, H-2). δ_C (50 MHz, $CDCl_3$); - 5.66, - 5.26, - 4.95 (3x q, 6x CH_3), 17.81, 18.03 (2x s, 2x $Si\underline{C}(Me)_3$), 25.51, 25.67 (2x q, 2x $Si(\underline{C}H_3)_2$), 62.86 (t, C-6), 63.35, 68.65, 73.74, 77.90 (4x d, C-2, C-3, C-4, C-5), 171.87 (s, C-1). m/z (CI, NH_3); 449 (50%, MNH_4^+), 432 (100%, M^+).

2,5-Diazido-3,6-di-*O*-(*tert*-butyldimethyl)silyl-2,5-dideoxy-**D**-manno-1,4-lactone (**4.27**)



A solution of 2-azido-2-deoxy-3,6-di-*O*-(*tert*-butyldimethyl)silyl-**L**-ido-1,4-lactone (**4.23**) (1.55 g, 3.6 mmol) in dichloromethane (100 ml) was cooled to -20 °C. To this was added pyridine (0.58 ml, 7.2 mmol) and triflic anhydride (1.03 ml, 6.1 mmol). After 30 minutes tlc analysis (ethyl acetate /hexane 1/5) showed no starting material (R_f 0.4) and one product (R_f 0.7). Water (1 ml) was added and the mixture allowed to warm up to room temperature. The organic layer was separated and washed with dilute hydrochloric acid (1M, 20 ml), water (2x 20 ml) and brine (10 ml) then dried (magnesium sulphate). The solvent was removed *in vacuo* to afford crude 2-azido-2-deoxy-3,6-di-*O*-(*tert*-butyldimethyl)silyl-5-*O*-trifluoromethanesuphonate-**L**-ido-1,4-lactone (**4.26**) (1.83 g) as an oil.

The crude triflate ester (**4.26**) (1.83 g) was dissolved in *N,N*-dimethylformamide (80 ml) and sodium azide (0.38 g, 5.5 mmol) added. The solution was stirred for 3 hours when tlc analysis (ethyl acetate /hexane 1/5) showed no triflate (R_f 0.7) and one product

(R_f 0.5). The solvent was removed *in vacuo* and the residue redissolved in ethyl acetate (30 ml). This was washed with water (2x 20 ml), brine (10 ml) and then dried (magnesium sulphate). The solvent was removed *in vacuo* and the residue purified by flash column chromatography (ethyl acetate /hexane 1/ 5). Recrystallisation (ethyl acetate/ hexane) afforded 2,5-diazido-3,6-di-*O*-(*tert*-butyldimethyl)silyl-2,5-dideoxy-**D**-manno-1,4-lactone (**4.27**) (849 mg, 56%) as a white crystalline solid. M.p.(ethyl acetate/ hexane); 48-49 °C. $[\alpha]_D^{25}$ -46.4 (c 0.95, $CHCl_3$). Found C 47.53 H 8.20 N 18.12% $C_{18}H_{36}O_4N_6Si_2$ requires C 47.34 H 7.95 N 18.40%. ν_{MAX} (KBr):-2123 (s, N_3), 2100 (s, N_3), 1788 (C=O) cm^{-1}. δ_H (200 MHz, $CDCl_3$): -0.12 (6H, s, 2x CH_3), 0.21 (3H, s, CH_3), 0.25 (3H, s, CH_3), 0.92 (9H, s, $C(CH_3)_3$), 0.95 (9H, s, $C(CH_3)_3$), 3.71 (1H, dq $J_{5,6'}$ 2.4 $J_{5,6}$ 5.5 $J_{5,4}$ 10.0 Hz, H-5), 3.93 (1H, dd $J_{6,5}$ 5.5 $J_{6,6'}$ 10.9 Hz, H-6), 4.11 (1H, dd $J_{6',5}$ 2.4 $J_{6',6}$ 10.9 Hz, H-6'), 4.12 (1H, d $J_{2,3}$ 4.2 Hz, H-2), 4.21 (1H, dd $J_{4,3}$ 2.5 $J_{4,5}$ 10.0 Hz, H-4), 5.05 (1H, dd $J_{3,2}$ 4.2 $J_{3,4}$ 2.5 Hz, H-3). δ_C (50 MHz, $CDCl_3$); -5.83, -5.57, -5.27, 4.57 (4x q, CH_3), 14.20, 18.07 (2x s, 2x $SiC(Me)_3$), 25.62, 25.71 (2x q, 2x $SiC(CH_3)_3$), 58.10, 62.02, 71.42, 78.42 (4x d, C-2, C-3 ,C-4, C-5), 63.27 (t, C-6), 170.83 (s, C-1). m/z (FAB^+); 457 (100, MH+), 399 (50%, M-2(N_2)).

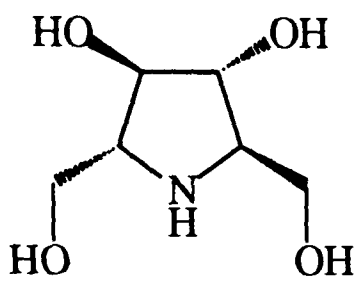
4.4 References

1. J.A. Bladin, *Ber.*, 1885, **18**, 1544.
2. F.R. Benson, *Chem. Rev.*,1947, **41**, 1, R.N. Bulter, *Advances in Heterocyclic Chemistry*, 1977, **21**, 323 and references cited therein.
3. R.M. Herbst, *Essays in Biochemistry*, Wiley, New York, 1956, p141
4. D.W. Esplin and D.M. Woodbury, *J. Pharmacol. Exp. Therap.*, 1956, **118**, 129.
5. P.F. Juby, T.W. Hudyma and M. Brown, *J. Med. Chem.*, 1968, **11**, 111, P.F. Juby and T.W. Hudyma, *J. Med. Chem.*, 1969, **12**, 396.
6. R.L. Buchanan, V. Sprancmanis and R.A. Partyka, *J. Med. Chem.*, 1969, **12**, 1001, R.L.Buchanan and V.Sprancmanis, *J. Med. Chem.*, 1973, **16**, 174.
7. J.M. Essery, *J. Med. Chem.*, 1969, **12**, 703.
8. J.S. Morley, *J. Chem. Soc.*, **C**, 1969, 809.
9. P.K. Kadadu, *Synthesis*, 1973, 71.
10. A.M. Seldes, E.G. Gros, I.M.E. Thiel and J.O. Defferrari, *Carbohydr. Res.*, 1975, **39**, 47.
11. W.R. Carpenter, *J. Org. Chem.*, 1962, **27**, 2085.
12. P. Ermert and A. Vasella, *Helv. Chim. Acta.*, 1991, **74**, 2043
13. M.K. Tong, G. Papandreou and B. Ganem, *J. Am. Chem. Soc.*, 1990, **112**, 6137.
14. C. Hubschwerlen, *Synthesis*, 1986, 962.
15. D. Davidson and H. Skovronek, *J. Am. Chem. Soc.*, 1958, **80**, 376.
16. T.W Brandstetter, B. Davis, D. Hyett, C. Smith, L. Hackett, B.G. Winchester, G.W.J. Fleet, *Tetrahedron Lett.*, 1995, **36,** in press.
17. B.J. Davis, unpublished results.

APPENDIX

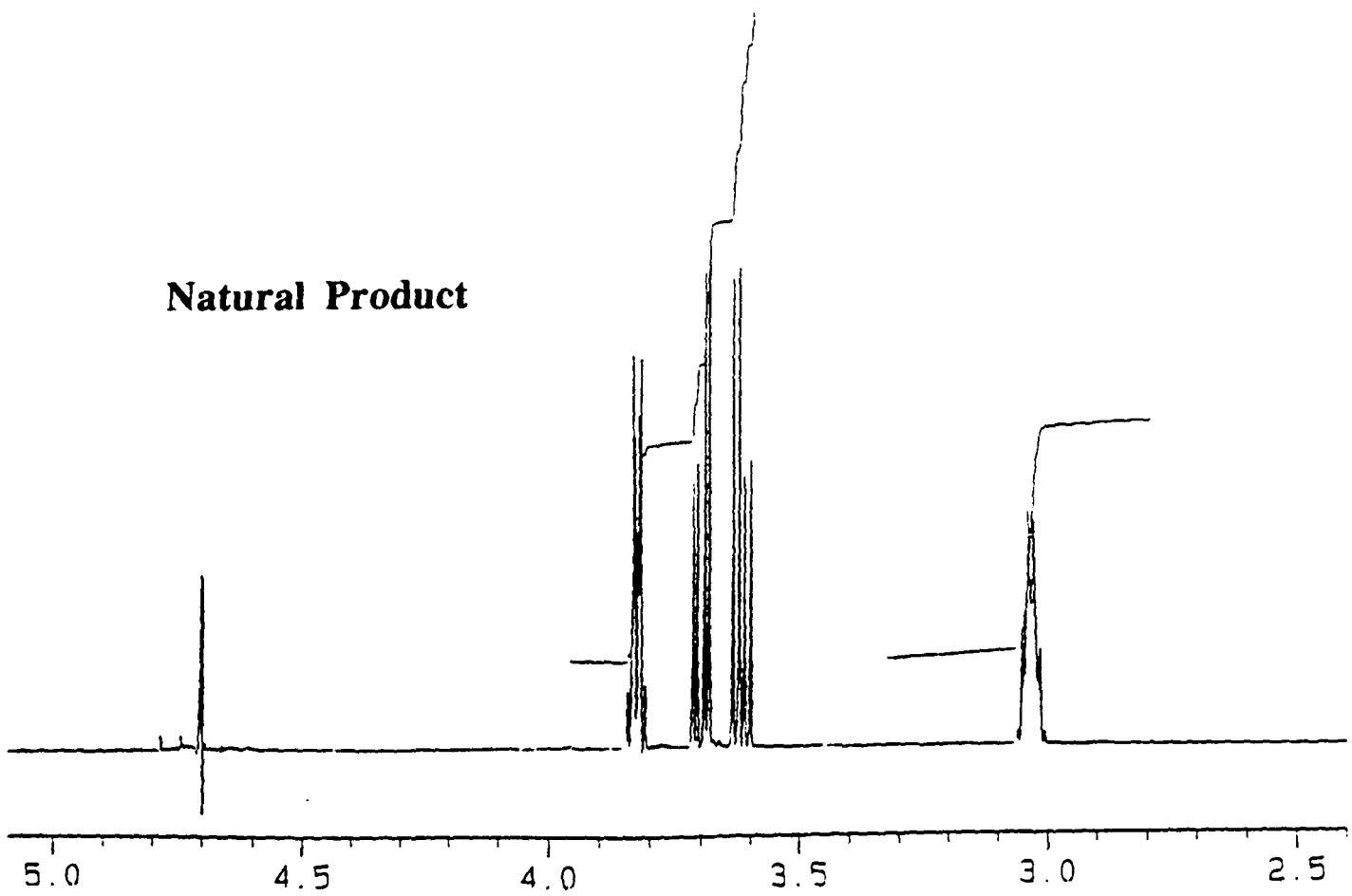
Appendix 1

Nmr of 2,5-dideoxy-2,5-imino-D-mannitol (1,1)



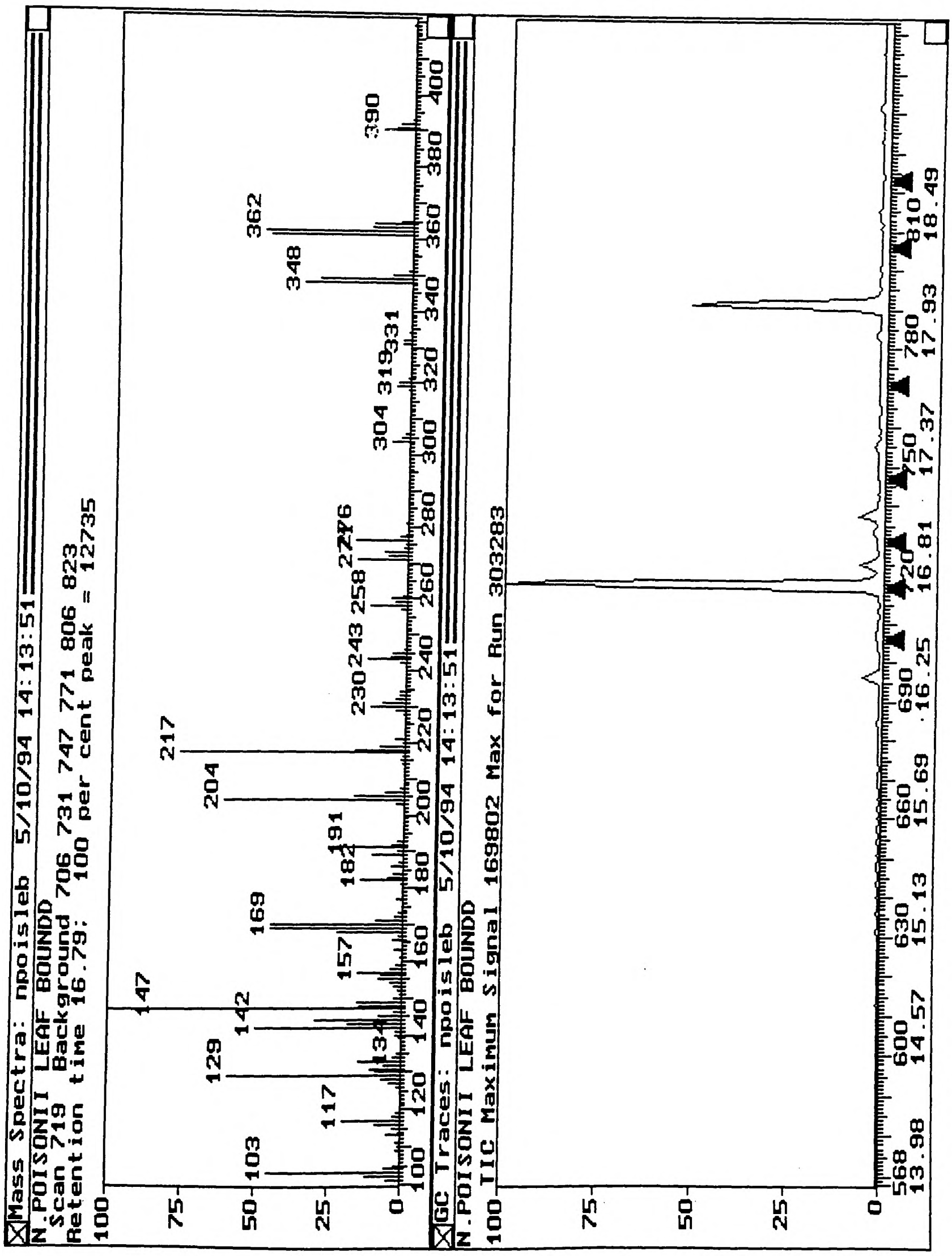
Synthetic Product

Natural Product

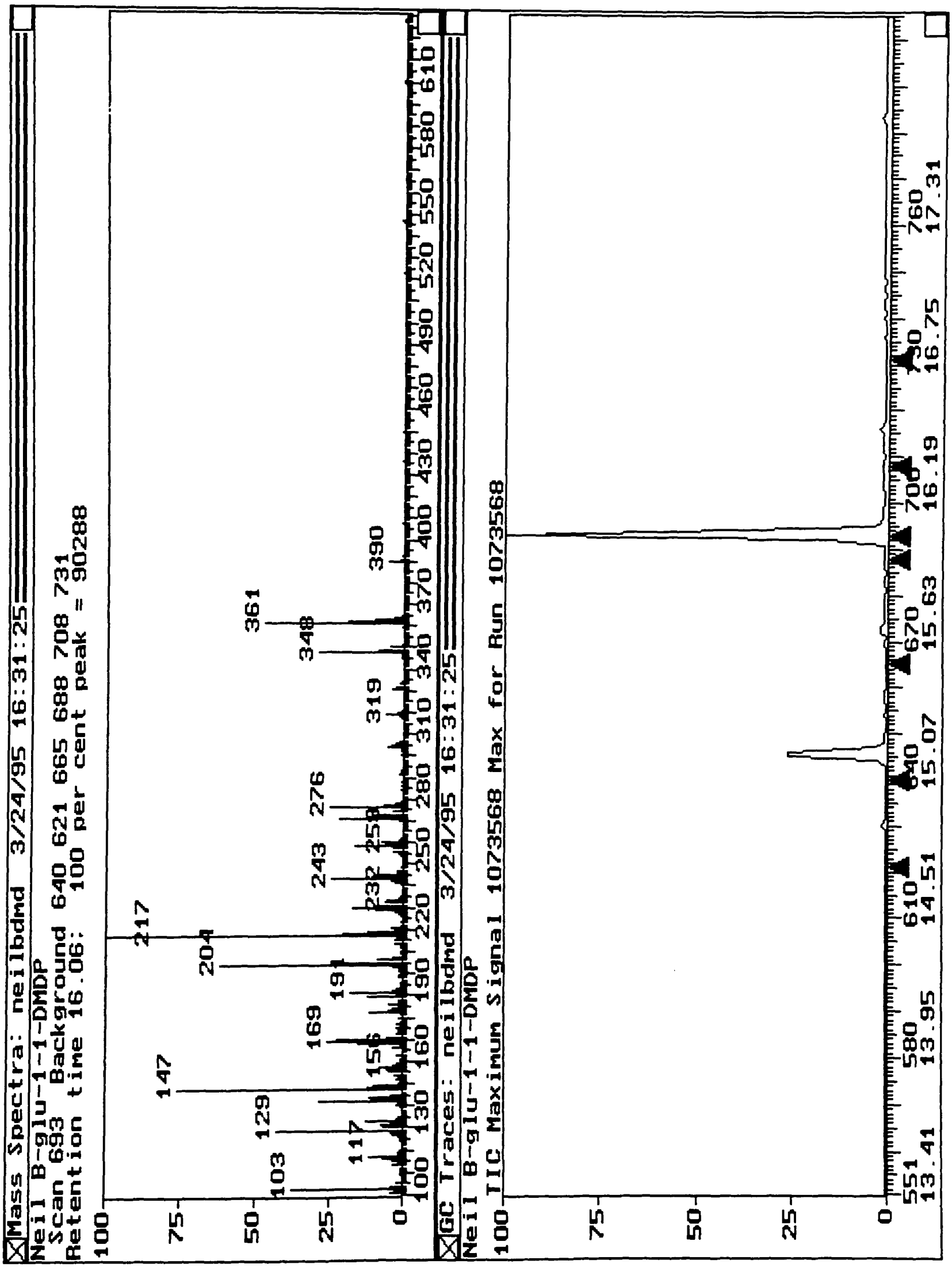


Appendix 2

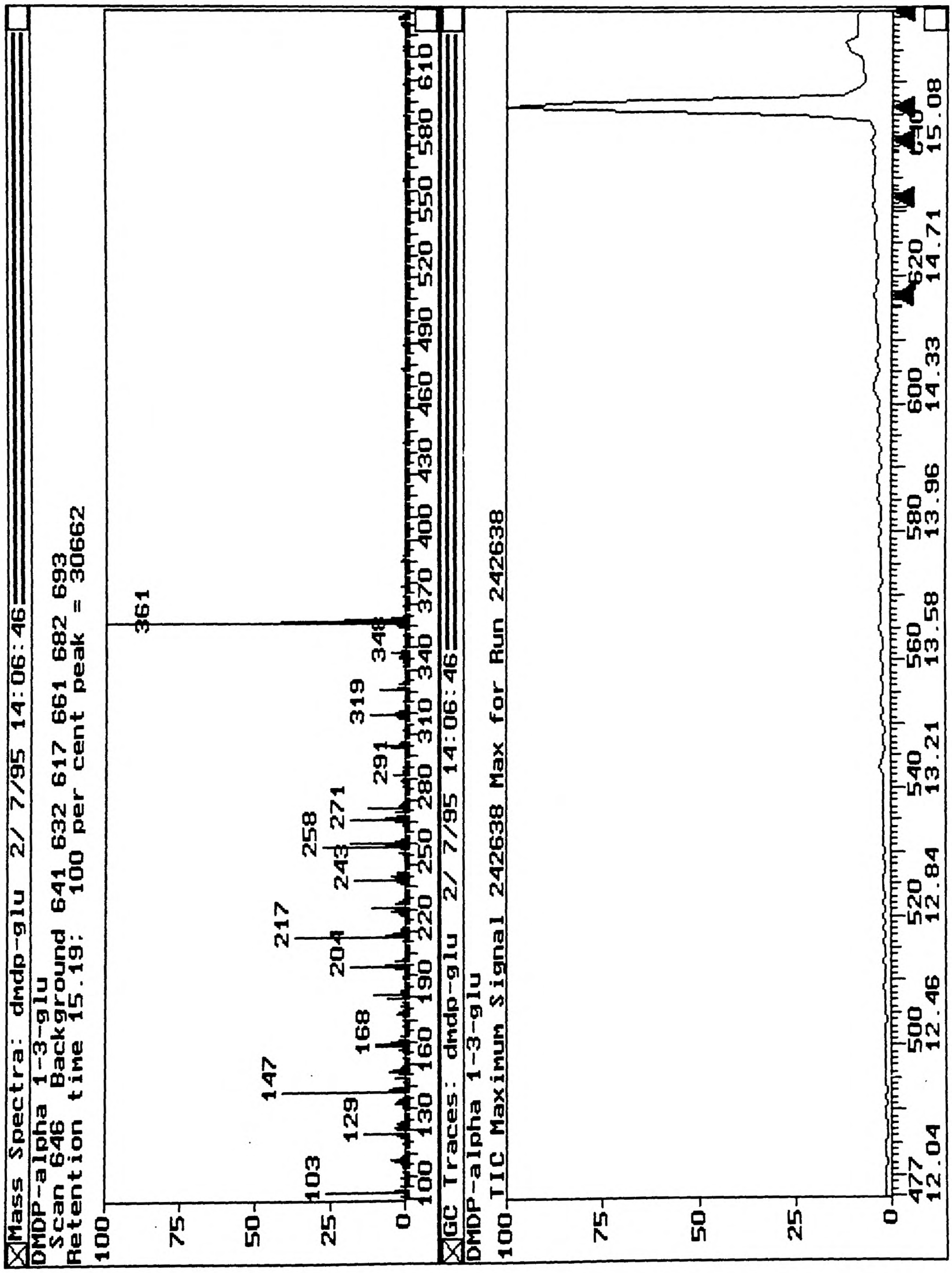
GCMS Trace of *Nephthytis poisonii*



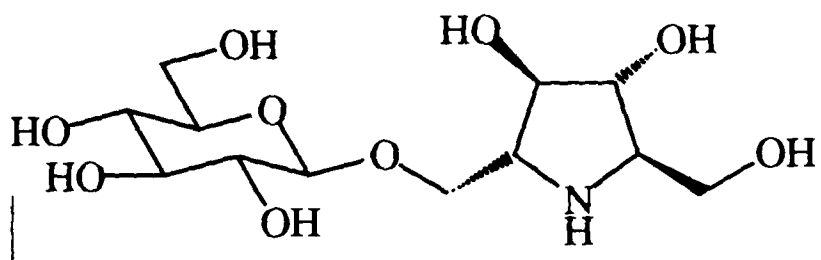
GCMS trace of Glcβ1-1DMDP



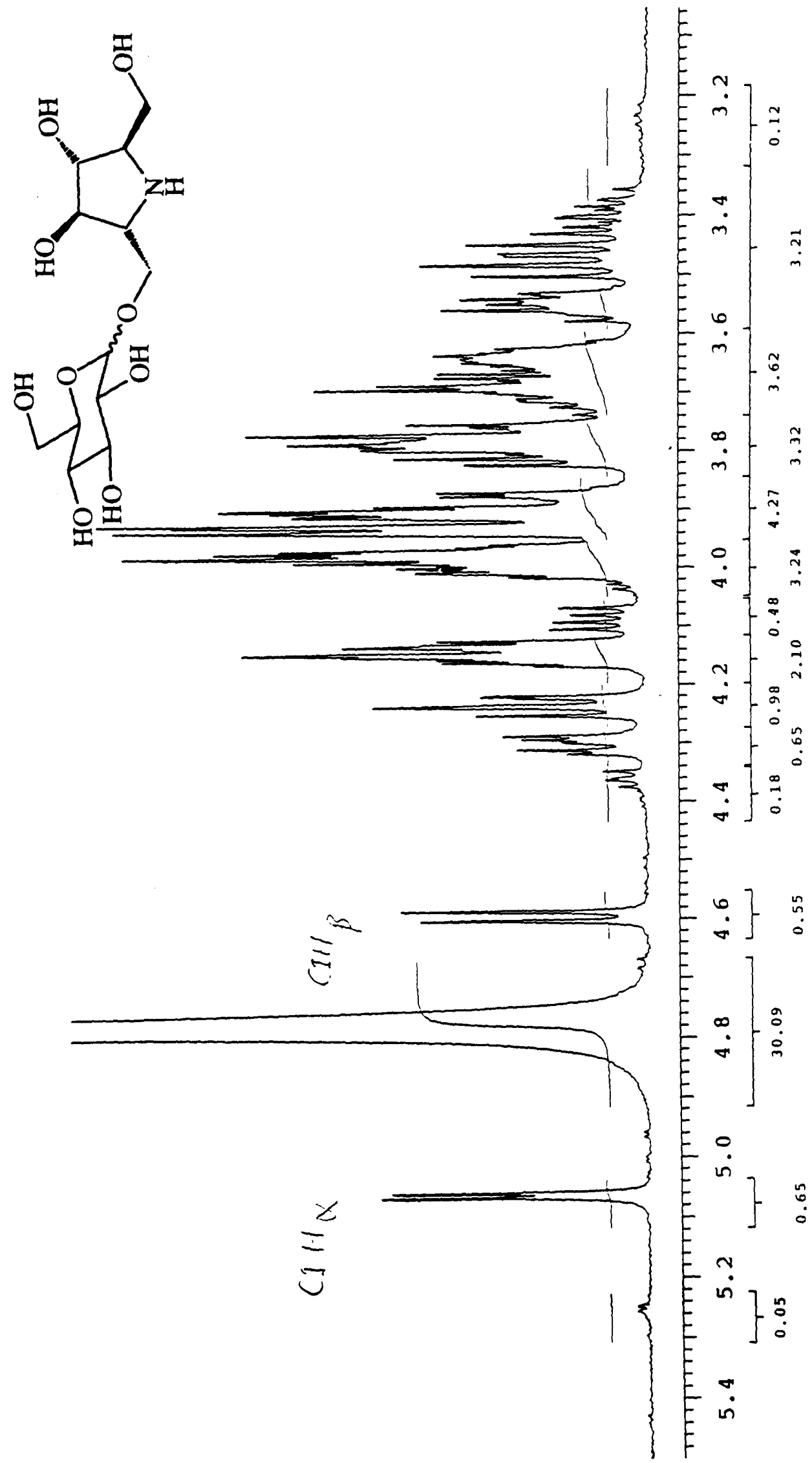
GCMS Trace of Glcα1-3DMDP



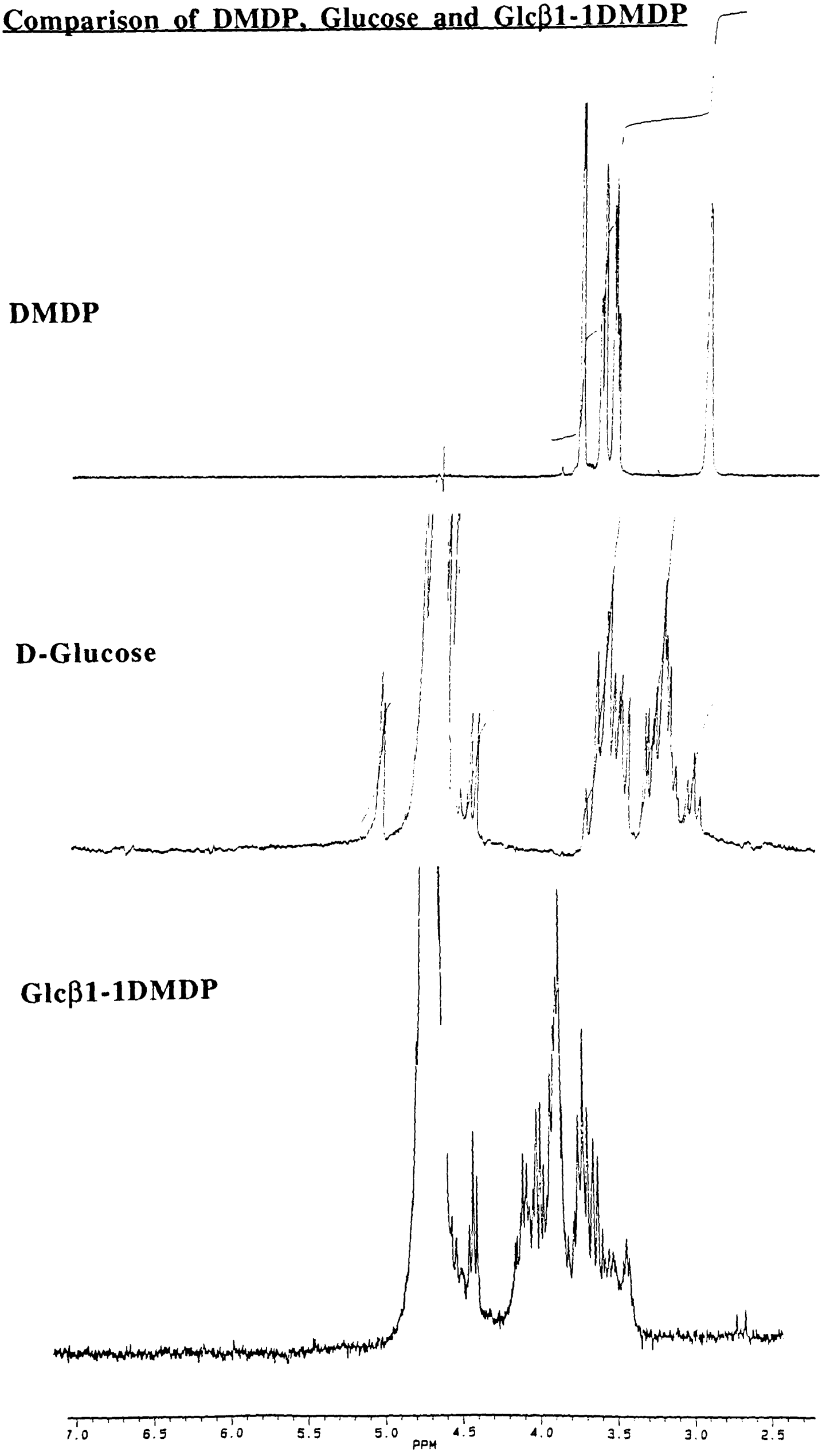
Appendix 3 Nmr of Isolated Natrual Product and Synthetic Glcβ1-1DMDP



Nmr of α and β glucose1-1DMDP



Comparison of DMDP, Glucose and Glcβ1-1DMDP



Appendix 4

NOe of 3,6-di-*O*-(*tert*-butyldimethyl)silyl-2,5-diazido-2,5-dideoxy-D-manno-1,4-lactone (4.28)