The Synthesis of Some $N$-linked Carba-Sugars as Glycosidase Inhibitors

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The work described in this thesis was carried out by the author in the Department of Chemistry at The University of Western Australia under the supervision of Associate Professor Robert V. Stick. Unless duly referenced, the work described in this thesis is original.

Matthew J. McDonough

February 2002
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Summary

Complementing the massive structural diversity of carbohydrates is an equally diverse group of enzymes responsible for the hydrolysis of O-, N- and S-linked glycosides, named the glycosidases. Inhibitors of glycosidases have proven to be attractive compounds to glycobiologists, both as biological tools for studying the mechanism of action of the glycosidases, and for their potential as therapeutic drugs. A particularly well studied glycosidase inhibitor is acarbose (1), which exhibits pronounced inhibitory activity against the starch degrading enzyme α-amylase, leading to the use of acarbose in the clinical treatment of type II non-insulin dependant diabetes. Owing to the interesting biological activities of acarbose, the synthesis and biological testing of carba-saccharides related to it have attracted considerable attention. This thesis outlines the synthesis of a number of β-linked analogues of acarbose, aimed at inhibiting a variety of β-glycosidases.

The construction of the amino linkage, between the cyclitol and carbohydrate chain, presents itself as the most difficult feature of the construction of acarbose and its analogues. A novel intramolecular approach at constructing this linkage, by means of a palladium(0) catalysed rearrangement, of the amine (115), was attempted, yet was ultimately unsuccessful.
With this novel approach failing, the more traditional triflate displacement by an amine was explored. Thus, the alkylation of the amine (29) or the thiol (30) with the triflate (162) was the key step in the synthesis of the β-D-xylosidase inhibitors (24) and (25), respectively. The introduction of the amine or thiol functionality onto the cyclitol rings was effected by a 3,3-sigmatropic rearrangement of the trichloroacetimidate (151) or dithiocarbonate (157), respectively. The N-linked carba-disaccharide (24) proved to a potent inhibitor ($K_i$ 40 µM) of a β-D-xylosidase isolated from the bacterium, *Thermoanaerobacterium saccharolyticum*. A similar alkylation method was used to synthesise the 1,3-linked carba-disaccharide (27).
Two different routes for the synthesis of the carba-trisaccharide (26) were explored. The initial approach undertaken was to alkylate the amine (181) with the triflate (178), with the amine (181) itself being hopefully available from cellobiose. However, various endeavours at transforming cellobiose into the amine (181) proved fruitless.

Ultimately successful was the glycosynthase-catalysed reaction of α-D-glucopyranosyl fluoride (204) and the carba-disaccharide (20). Using this method, both the carba-trisaccharide (26) and carba-tetrasaccharide (219) were produced. In enzyme inhibition experiments (performed by Dr Jon Fairweather in the laboratory of Prof. Marc Claeysens), the carba-tetrasaccharide (219), in particular, proved to be a potent inhibitor of some cellulases, interestingly exhibiting far greater inhibition of these enzymes than the all β-linked analogue of acarbose.
Lastly, efforts towards the synthesis of the 1,3;1,4 linked carba-trisaccharide (28), a potential inhibitor of 1,3;1,4 β-glucan hydrolases, are outlined. The first approach involved the glycosylation of the carba-disaccharide (215). Several glycosylation methods with various donors, including glycosyl bromides, thioglycosides, trichloroacetimidates and ortho-esters were tried, however none of these afforded the desired glycosylated material. A sulfoxide donor did result in glycosylation, however an inseparable α/β mixture was formed. Initial undertakings towards the alternative approach of alkylating the carba-disaccharide amine (291) with the triflate (178) are also presented. Following the successful synthesis of the azide (287), attempts at subsequent reduction to afford the amine (291) failed, possibly owing to the lability of the protecting groups.
Acknowledgments

I would like to warmly thank my supervisor Associate Professor Bob Stick, for his encouragement, support and friendship over the years.

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A big thankyou goes to Dr Lindsay Byrne for the acquisition of many high field n.m.r. spectra.

Additionally I'd like to thank Brad, Grant, Matt, Hank and Sophia, for their friendship throughout the years.

Finally, thanks must go to my family, my brothers Andrew and Nicholas, and most of all, my Mum, Helen for supporting and having faith in me during the course of my studies.
Glossary

Abg  Agrobacterium faecalis β-glucosidase
AcCl  acetyl chloride
Ac2O  acetic anhydride
AcOH  acetic acid
AIBN  α,α'-azobisisobutyronitrile
BnBr  benzyl bromide
Bu2SnO  dibutyltin oxide
BzCl  benzoyl chloride
CSA  camphorsulfonic acid
d  day(s)
DCC  1,3-dicyclohexylcarbodiimide
DBU  1,8-diazabicyclo[5.4.0.]undec-7-ene
DDQ  2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD  diethyl azodicarboxylate
DMAP  4-N,N-dimethylaminopyridine
DMF  N,N-dimethylformamide
DMI  1,3-dimethyl-2-imidazolidinone
dppb  1,4-bis(diphenylphosphino)butane
Et3N  triethylamine
EtOAc  ethyl acetate
Et2O  diethyl ether
Et2O.BF3  boron trifluoride diethyl etherate
EtOH  ethanol
h  hour(s)
K_{i}  inhibition constant
K_{m}  Michaelis constant
MeCN  acetonitrile
MeOH  methanol
min  minute(s)
NaOMe  sodium methoxide
NBS  N-bromosuccinimide
NIS  N-iodosuccinimide
P  protecting group
PivCl  pivaloyl chloride
PMBCl  4-methoxybenzyl chloride
Pr_i2O  diisopropyl ether
rt  room temperature
TBAI  tetrabutyl ammonium iodide
TFA  
trifluoroacetic acid

Tf2O  
trifluoromethanesulfonic anhydride

TfOH  
trifluoromethanesulfonic acid

THF  
tetrahydrofuran

TMSOTf  
trimethylsilyl trifluoromethanesulfonate

TMU  
$N,N$-tetramethylurea

Functional Groups

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<td>TMS</td>
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Introduction
For much of the twentieth century, carbohydrates were thought to be primarily important both as a source of biological energy and as structural polymers in plants, fungi, insects and crustaceans. However, it is now well recognised that carbohydrates play an essential role in many other biological phenomena. Carbohydrates are involved in infection by a variety of pathogens, additionally, they play roles in metastasis, inflammation, cell-cell recognition and cellular development. This realisation has been hampered by both the massive structural complexity of carbohydrates, and the often minuscule amounts, usually attached to other biomolecules, of these biologically important carbohydrates present in the cell. However, recent advances in the synthesis and analysis of carbohydrates have, to some extent, helped overcome these hurdles.

Complementing the massive structural diversity of carbohydrates is an equally diverse group of enzymes responsible for the hydrolysis of O-, N- and S-linked glycosides, named the glycosidases. Glycosidases play an important role in a variety of biological processes, ranging from the simple hydrolysis of stored glycosides to the development of eukaryotic and prokaryotic cell walls, viral replication and the mediation of cell-cell interactions. Additionally, glycosidases are increasingly used in biotechnology roles, notably in the detergent and paper-pulping industry.

The glycosidases can be classified into sub-groups according to:

(i) substrate specificity
(ii) the stereochemistry of the glycosidic linkage cleaved (α or β)
(iii) the anomeric stereochemistry of the product versus that of the substrate (retaining or inverting)
(iv) whether glycosidic cleavage occurs at either the non-reducing end of an oligosaccharide chain (exo-) or at internal points within the chain (endo-)

Additionally, glycosidases may be classified into families, based on their sequence homology.
The Catalytic Mechanism of the Glycosidases

Owing to the importance of the glycosidases, their mechanism of action has been the subject of much study.\textsuperscript{11,12} The framework for the currently accepted mechanism of action of the glycosidases was originally proposed by Koshland in 1953 and, despite some adjustments, has stood the test of time.\textsuperscript{13}

Mechanistically, glycosidases operate by one of two routes, depending on whether the substrate undergoes inversion or retention of its original configuration during the hydrolytic process. Inverting glycosidases are thought to operate by a single displacement mechanism. The breaking of the glycosidic bond is accompanied by the general-base assisted nucleophilic attack of water at the anomeric carbon (Scheme 1).

\begin{center}
\includegraphics[width=\textwidth]{scheme1.png}
\end{center}

\textit{Scheme 1. Proposed mechanism of an inverting \(\beta\)-glycosidase}

Retaining glycosidases are believed to operate by a double-displacement mechanism. First, the aglycon is activated to leave by protonation of the exocyclic oxygen atom by a catalytic acid residue. A concerted attack by a carboxylate residue leads to a glycosyl-enzyme intermediate. A water molecule may then attack the glycosyl-enzyme intermediate, aided by the conjugate base of the catalytic acid [Scheme 2 (top)]. Alternatively, the transient formation of an ion-pair intermediate has long been popular, largely owing to the work of Phillips, published in 1967 [Scheme 2 (bottom)].\textsuperscript{14} Phillips postulated that a negatively charged active site carboxylate could sufficiently stabilise an oxocarbenium ion intermediate, based on x-ray structural studies on hen egg-white lysozyme. However, this mechanism has lately fallen out of favour; indeed, recent studies have shown evidence for the formation of a glycosyl-enzyme intermediate for hen egg-white lysozyme.\textsuperscript{15}
Scheme 2. Proposed mechanism of a retaining β-glycosidase.
a) Glycosyl-enzyme pathway. b) Ion-pair pathway

The proximity of the two catalytic acid residues seems to play a key role in determining whether a glycosidase is either a retainer or an inverter. For inverting glycosidases, the distance between the two residues is approximately 9.5 Å; for retaining glycosidases, at 5 Å, the distance is much smaller. The extra room in the active site for an inverting glycosidase allows a water molecule between the glycoside and the general acid-base.
Heightman and Vasella have further classified retaining glycosidases into *anti-* and *syn-*protonators.\textsuperscript{17} As shown below, *anti-*protonators have an acid residue that is *anti* to the C1-O5 bond, conversely, *syn-*protonators have an acid residue *syn* to the C1-O5 bond (Scheme 3). Heightman and Vasella came to this conclusion after a systematic study of all available crystal structures of glycosidases having bound substrates or inhibitors.

![Scheme 3. *Anti-*protonation (left) and *syn-*protonation (right)](image)

**Inhibitors of the Glycosidases**

Glycosidase inhibitors have proven to be attractive compounds to glycobiologists, both as biological tools for studying the mechanism of action of the glycosidases and for their potential as therapeutic drugs.\textsuperscript{18} Potential therapeutic applications of glycosidase inhibitors range from the treatment of AIDS, to cancer and metabolic disorders.\textsuperscript{19,20} Glycosidase inhibitors currently in therapeutic use include acarbose (1), Miglitol (2), Relenza (3) and Tamiflu (4). The anti-influenza drug Relenza (3) is a classic example of a rationally designed drug, being developed by computer-aided design following analysis of the x-ray crystal structure of viral neuraminidase.\textsuperscript{21}
Affinity labels have been useful for the labelling and identification of the catalytic residues of glycosidases. These inhibitors act by forming a stable covalent linkage originating from a reactive functional group, such as an epoxide, on the inhibitor, with a catalytic amino acid of the enzyme; isolation of the labelled enzyme can then allow identification of the catalytic nucleophile of the enzyme under study. Crucially, these inhibitors must also have a strong non-covalent interaction with the active site to avoid non-specific labelling with other amino acids of the enzyme under study. True mechanism based inhibitors are the 2- and 5-fluoro sugars, such as (5) and (6), developed by Withers and co-workers. Using these types of compounds, Withers and co-workers have been able to identify, by crystallography, the existence of a covalently bound enzyme intermediate implicated in the mechanism of retaining glycosidases. This was made possible by the electron-withdrawing fluorine substituents reducing the rate of release of the glycosyl unit, from the extremely stable glycosyl-enzyme intermediate.
Additionally, glycosylmethyltriazenes, conduritol epoxides, epoxyalkyl glycosides, bromoacetyl N-glycosides and glycosyl isothiocyanates, have found use as affinity labels of glycosidases. Some representative examples of these classes of compounds are shown below. However, these compounds often lack the specificity of the fluoro sugars developed by Withers.

Notable competitive inhibitors that mimic the substrate are S-linked oligosaccharides, which, being resistant to hydrolysis, have found widespread use as glycosidase inhibitors. For example, a crystal structure of the complex between the all S-linked pentasaccharide (7) and the cellulase EG1 showed how the enzyme distorts the substrate in the active site, leading to the leaving group (and thus the glycosidic bond) having an axial orientation.

Transition-state based analogues act by mimicking the transition state in the glycosidase catalysed hydrolysis of the natural substrate and are the most extensively studied class of glycosidase inhibitors. Four factors have been identified as contributing to the transition-state character of a potential inhibitor: charge, trigonal anomeric centre, half-chair conformation and proper relative configuration. Some examples of glycosidase
inhibitors, which possess features that satisfy some of the criteria outlined above, include D-gluconolactone (8), isofagomine (9), salacinol (10), 1-deoxynojirimycin (11) and mannonojiricetetrazole (12).

The sugar-shaped nitrogen heterocycles, isofagomine (9) and 1-deoxynojirimycin (11), are two examples of aza-sugar inhibitors. The strong inhibition displayed by this class of compounds is believed to arise largely from the interaction between an active site carboxylate residue and a protonated nitrogen on the the inhibitor (Scheme 4).


**Acarbose**

In the 1970's, inhibitory activities against α-glucosidases were found in almost all the genera of the microbial order Actinomycetales. Two different groups of active substances were identified as contributing to this inhibitory activity. The first group had a polypeptide character, whilst the second, more studied group were complex oligosaccharides. This last group of inhibitors, called the amylostatins, has the general structure:

![Chemical structure of acarbose](image)

Of this group of compounds, the one studied most extensively was the carbacetrasaccharide (1), given the generic name acarbose. Acarbose was shown to exhibit pronounced inhibitory activity against the starch degrading enzyme, α-amylase, leading to its clinical use for the treatment of type II non-insulin dependant diabetes.

The cyclitol unit of the amylostatins is the structural element thought to be crucial for the inhibitory activity of acarbose. The substituents on this cyclitol unit are arranged in a stereochemically similar fashion to those of an α-D-glucopyranose unit. Also critical to the inhibitory activity of acarbose against enzymes that contain multiple binding sites, such as α-amylase, are the attached α-D-glucopyranose units, as shown by the relatively weak inhibitory activity of valienamine (13) itself for α-amylase.
Analogues of Acarbose

As mentioned previously, acarbose is but one of a series of compounds, termed the amylostatins. Since the original discovery of the amylostatins, a number of other classes of similar compounds, all known as carba-sugars, has been discovered from microbial sources. These compounds all contain a modified sugar residue linked in an α(1→4) fashion to D-glucose residues.

The adiposins were originally isolated from *Streptococcus calvus*. The adiposins differ from the amylostatins (of which acarbose is a member) by having a 4-amino-4-deoxy-D-glucose residue instead of a 4-amino-4,6-dideoxy-D-glucose residue. The trestatins were originally isolated from culture broths of *Streptomyces dimorphogenes* and are characterised by the presence of a trehalose unit. These molecules display strong inhibition against porcine pancreatic α-amylase. Interestingly, the trestatins may contain up to three carba-disaccharide residues per molecule. The oligostatins were originally isolated from the cultural filtrate of *Streptomyces myxogenes*. Curiously, these statins lack the double bond of the amylostatins, yet still exhibit strong inhibitory activity against intestinal α-glucosidase and sucrase. Series NS-504 contains an epoxide functionality instead of the double bond of the amylostatins. These epoxides were originally isolated from *Streptomyces flavocromogenus* and are reported to exhibit activity against α-amylase. The general structures of these classes of carba-sugars are shown below (Scheme 5).
Scheme 5

R = H acarbose, amylostatis and homologues
R = OH adiposins
The evaluation of the inhibitory activity of some semi-synthetic derivatives of acarbose has resulted in some useful information on the structural features of acarbose crucial to its inhibitory action. The acid-catalysed methanolysis of acarbose afforded methyl acarvisoin (14). The α-anomer of methyl acarviosin (14a) displayed strong activity against sucrase when compared to acarbose, but lower activity towards α-amylase. The strong inhibition of methyl acarviosin (14a) for sucrase is not surprising considering the similarities in size between (14a) and the natural substrate of sucrase, sucrose.

\[ \text{(14a) } \alpha- \]
\[ \text{(14b) } \beta- \]

The hydrogenation of the double bond of acarbose furnished the two diastereoisomers (15) and (16), having the D-gluco and L-ido configuration, respectively. Relative to acarbose, these compounds displayed reduced activity against α-amylase, suggesting the double bond of acarbose (or the ring distortion arising from it) to be an essential structural motif for inhibition.
Various $O$-, $N$- and $S$- glycosides of acarbose have been prepared and their biological activity evaluated, yet none of these derivatives has displayed a significantly enhanced inhibitory action compared to acarbose.$^{41}$

Owing to the potent inhibitory action of acarbose against some enzymes that process $\alpha(1\rightarrow4)$ D-glucosidic linkages, such as $\alpha$-amylase, a number of compounds have been prepared that have structural features similar to acarbose, yet closely mimic the natural substrate of the enzyme under study.$^{42}$ For example, Brimacombe and co-workers prepared the carba-disaccharide (17) that proved to be a potent inhibitor of jack bean $\alpha$-mannosidase ($K_i$ 30 $\mu$M), yet was a poor inhibitor of other glycosidases.$^{43}$

\[
\begin{align*}
\text{(17)}
\end{align*}
\]

Ogawa and co-workers prepared the carba-disaccharides (18) and (19), which have a $\beta$-amino linkage yet an $\alpha$-aglycon. Both of these compounds displayed IC$_{50}$ values below 100 $\mu$M against yeast $\alpha$-D-glucosidase. However, they were very poor inhibitors of almond $\beta$-D-glucosidase.$^{44}$

\[
\begin{align*}
(18) \ R = H
(19) \ R = OH
\end{align*}
\]

Stick and co-workers prepared the all $\beta$-linked isomer (20), which in contrast to the above compounds, displayed strong inhibition of a $\beta$-glucosidase from $A$. $niger$. It should be noted that (20) gave a $K_i$ value an order of magnitude higher against the $\beta$-glucosidase isolated from $C$. $saccharolyticum$, highlighting the difficulty in comparing
inhibition results even within a class of enzymes. Stick and co-workers also prepared the 1-deoxy analogue (21) of (20), and the all-β analogues of methyl acarboside and methyl adiposin-2, (22) and (23) respectively. The inhibition results are shown below (Table 1).

![Chemical structures](image)

(20) 
(21) 
(22) R' = H, R'' = β-OMe  
(23) R = OH, R'' = OH

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<tr>
<td>β-glucosidase (C. saccharolyticum)</td>
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<tr>
<td>Cellulase - EGI (H. insolens)</td>
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Table 1 (n.d. - indicates no data)
In light of the inhibitory action displayed by methyl β-acarviosin (20) and other β-linked analogues of acarbose, it was thought worthy to prepare a suite of analogues of these compounds and to test their inhibitory activity against appropriate glucosidases. It should be noted that the target enzymes for study are glycan hydrolases - these enzymes contain multiple binding sites and are thus poorly inhibited by monosaccharide derivatives, hence the need for the synthesis of carba-saccharides similarly containing multiple residues. The carba-disaccharide (24) is aimed against β-D-xylosidases, with its S-linked analogue (25) useful for determining the importance of the nitrogen to binding to the enzyme. The ‘chain extended’ derivative (26) of methyl β-acarviosin should be of interest in showing the importance of binding in the -2 site of cellulases. The 1,3-linked carba-disaccharide (27) and 1,3;1,4 linked carba-trisaccharide (28) are aimed at β-1,3- and β-1,3;1,4- glucan hydrolases, respectively.

Additionally, both the construction of the amino cyclitol-carbohydrate linkage and the addition of sugar residues to the ‘left’ of the cyclitol unit are formidable synthetic
challenges and it was an aim of this work to extend the methodology to carry out such transformations.

Overview

Chapter 1 includes a review of the different approaches used to synthesise acarbose and its analogues, and describes a novel approach aimed at constructing the crucial cyclitol-carbohydrate linkage of this class of compounds. Chapter 2 describes the synthesis and biological testing of the cyclitols (29) and (30), and their carba-disaccharide analogues (24) and (25). Chapter 3 describes an attempted chemical synthesis, and ultimately successful chemo-enzymatic synthesis, of the 4'-glucosylated methyl β-acarviosin analogue (26). Chapter 4 describes the synthesis of the 1,3-linked isomer (27) of methyl β-acarviosin (20). Chapter 5 details the attempted synthesis of the 1,3;1,4 linked carba-disaccharide (28).

\[
\begin{align*}
(29) \ X &= \text{NH}_2 \\
(30) \ X &= \text{SH}
\end{align*}
\]
Chapter 1

Investigations into a Novel Approach for the Construction of the Amino Linkage of Acarbose Analogues
Introduction

The attachment of the carbocycle, by an amine linkage, to the carbohydrate chain presents itself as the most difficult feature of the synthesis of acarbose and its analogues. This task has largely been achieved in three ways: halide or sulfonate displacement by an amine, the ring opening of epoxides and azidirines and, lastly, the reductive condensation of a ketone with an amine. Following is a brief review of the major developments in the synthesis of acarbose and its analogues, with particular emphasis on β-linked analogues.

Halide or Sulfonate Displacement

In 1982, Sakari and Kuzuhara synthesised amylostatin XG (31), this being the first synthesis of an acarbose analogue.\(^4\)\(^5\) Their approach was the allylic substitution of the bromide (32) by the amine (33), to give the carba-trisaccharides (34) and (35) as an inseparable mixture, in 13% yield. A mixture of bromide diastereoisomers (32) was used as they were shown to be in equilibrium under the reaction conditions employed. Subsequent protecting group removal and acetolysis of the 1,6-anhydro group gave the per-acetate of the desired compound (31).
During studies directed towards the synthesis of β-adiposin (23), McAuliffe and Stick found that alkylation of the amine (36) with the triflate (37) gave only trace amounts of the desired carba-disaccharide (38), with the major products being those of elimination. When the trisaccharide triflate (39) was employed as the alkylating agent, none of the desired carba-tetrasaccharide could be detected.

Searching for a solution to the problem of elimination, Stick and co-workers found that by changing the protecting group at O3 of the triflate (37), from a benzyl to a benzoyl group, the yield of the alkylation improved markedly. Apparently, the benzoate at C3 suppresses the 3,4-elimination. Thus, alkylation of the amine (36) with the triflate (40) led to the formation of the carba-disaccharide (41), in 43% yield, and the alkene (42) in 24% yield.
With an alkylation strategy now available, Stick and co-workers were able to synthesise methyl β-acarboside (22).48 Thus, alkylation of the amine (36) with the triflate (43) furnished the carba-tetrasaccharide (44), albeit in very poor yield (4%).
The effect of triflate structure on alkylation reactions is highlighted in the synthesis of the carba-disaccharide (45), by Tagmose and Bols.\textsuperscript{49} The alkylation of the amine (46) with the primary triflate (47) afforded the 1,6-linked compound (45) in excellent yield (78%).
Epoxide Aminolysis

Much of the work on epoxide aminolysis belongs to Ogawa and co-workers. In 1983, this group reported the synthesis of adiposin-1 (48)\(^{50}\) - their strategy was the coupling of the racemic amine (49)* with the epoxide (50). Following acetylation of the reaction mixture, four compounds were identified - the two regeoisomers (51) and (52) each existing as two diastereoisomers. Diaxial and diequatorial opening of the epoxide occurred in roughly equal proportions. Ultimately the desired compound (48) was obtained, in 13% overall yield, the identity of which was verified by comparison with an authentic sample.

* Compounds depicted without emboldened bonds represent a racemic mixture.
Using the same approach, Ogawa synthesised amylostatin XG (31). Treatment of the amine (49) with the epoxide (53), followed by acetylation, gave the two regioisomers (54) and (55). Having a 6-deoxy functionality [on the epoxide (53)] had a dramatic effect on the ratio of isomers produced (when compared to the previously described coupling of the 6-hydroxy epoxide), with diaxial ring-opening predominating over the diequatorial by a factor of five. Ogawa speculates that the 6-hydroxy group, of (50), helps stabilise the conformation necessary for diequatorial opening.

This same methodology was extended to the synthesis of the saturated analogue (56) of adiposin-1 and to the carba-disaccharide (57).

Later, in work published in 1988, Ogawa reported the synthesis of acarbose and its 6'-hydroxylated derivative, adiposin-2. These syntheses were greatly simplified by using the amine (49) in optically pure form.
Ogawa extended this epoxide methodology to the synthesis of the β-linked carbo-
disaccharides (18) and (19).\textsuperscript{44} Thus, treatment of the amine (59) with the epoxide (58) gave the two regioisomers (60) and (61), in yields of 51% and 39%, respectively. Conversion of the primary hydroxyl group of (60) into an iodide, and subsequent reduction, led to the formation of 6-deoxy compound (18).

Additionally, the configuration at the anomeric centre seems to play an important role in the regioselectivity of such epoxide reactions; azide attacks the epoxide (62) nearly exclusively at C3 to give the 3-azido compound (63).\textsuperscript{55}
Whilst synthesising a related molecule, Paulsen made use of the epoxide (64) to couple to the amine (65).\textsuperscript{56} In contrast to the previously described examples of epoxide aminolysis, a single product (66) was produced, resulting from exclusive attack at C4. The coupled product (66) was transformed into the per-acetate (67) by acetolysis. Interestingly, deacetylation of (67) resulted in a spontaneous rearrangement to give the tricyclic compound (68). Ogawa later made use of similar 1,6:3,4-dianhydro sugars to synthesise several carba-disaccharides.\textsuperscript{57,58}
The effect of structure on the regioselectivity of epoxide opening is again highlighted in the synthesis of the trehalase inhibitor salbostatin. Coupling of the amine (49) and the epoxide (69), followed by acetylation, gave exclusively the unwanted product (70). A more favourable outcome was achieved when the unprotected epoxide (71) was used, with the product of diequatorial opening (72) (58%) predominating over that of diaxial opening.
Ogawa made use of a 1,6:3,4-dianhydro sugar similar to that used by Paulsen [(64)] to synthesise the carba-disaccharide (73), which was subsequently converted into the per-acetate (74) of methyl acarviosin (14a).\textsuperscript{60,61} This transformation was accomplished in a series of novel reactions. Firstly, (74) was converted into the dichloride (75). Treatment of the dichloride (75) with sodium acetate in DMF gave the aziridine (76). Opening of the aziridine ring with HCl afforded the chloride (77). Subsequent elimination, using DBU, gave the alkene (74) in 55% yield.
McAuliffe and Stick utilised the 1,6:3,4-dianhydo sugar (78) in successful syntheses of methyl 6-hydroxy-β-acarviosin (79) and β-adiposin-2 (22). Thus, the amine (36) was treated with the epoxide (78) to furnish the amino alcohol (80) in 67% yield. Subsequent transformations gave the carbamate (81), which was converted into the thioglycoside (82). Glycosylation of the alcohol (83) afforded the protected form of β-adiposin-2 (22). Additionally, the carbamate (81) was converted, via an anomeric bromide, into a protected form of methyl 6-hydroxy-β-acarviosin (79).
Employing a different approach, Ogawa used the racemic epoxides (84) and (85) to alkylate the amines (86a) and (86b). Thus, treatment of the epoxide (84) with the amines (86a) and (86b) gave, following saponification, acetylation and separation of the unwanted diastereoisomers, the carba-disaccharides (87a) and (87b), in yields of 46% and 10%, respectively. Similarly, treatment of the epoxide (85) with the amines (86a) and (86b) afforded the compounds (74) and (88) in 25% and 26% yields, respectively. In all cases, attack of the amine occurred exclusively at the allylic carbon. Subsequently, this methodology was used by Ogawa and Brimacombe to prepare various carba-oligosaccharides having the α-D-manno configuration.
Reductive Amination

In 1986, Horii and Fukase reported a reductive amination methodology to synthesise methyl acarviosin (14a) and its 6-hydroxy analogue (57).67,68 Thus, treatment of valienamine (13) with the ketone (89a), in the presence of NaCNBH₃, afforded the D-glucosamine (14a) in 34% yield. The D-galacto amine (90a) was a minor product, being produced in 10% yield. In a similar fashion, treatment of valienamine (13) with the ketone (89b) yielded the D-gluco amine (57) and the D-galacto amine (90b) in 15% and 5% yields, respectively.
Kuzuhara and co-workers synthesised the saturated analogue of acarbose, named ‘dihydroacarbose’ (15). Accordingly, condensation of the ketone (91) with the amine (92) gave the carba-tetrasaccharide (93) in 30% yield, as well as its isomer (94), isolated in only 4% yield.
This methodology could not be extended to the synthesis of acarbose itself; treatment of the amine (95) with the ketone (96) gave none of the desired carba-tetrasaccharide.

Whilst investigating possible syntheses of methyl β-acarviosin (20), Stick and co-workers treated the ketone (97) with the amine (98) in the presence of NaCNBH₃; however, no coupling product was observed.⁴⁷
Novel Methods

Knapp and co-workers utilised the thermal rearrangement of an $N$-substituted carboimidothioate to construct the imino linkage of the carba-disaccharide (99).\textsuperscript{70} Treatment of the sodium salt of the alcohol (100) with the isothiocyanate (101), followed by quenching with BnBr, gave the carboimidothioate (102). Thermolysis in refluxing toluene afforded the rearranged product (103) in 52% overall yield.

\begin{equation}
\text{(99)}
\end{equation}

\begin{equation}
\text{(100)}
\end{equation}

\begin{equation}
\text{(101)}
\end{equation}

\begin{equation}
\text{(102)}
\end{equation}

\begin{equation}
\text{(103)}
\end{equation}

An analogous sequence using a secondary isothiocyanate was also attempted. However, the thermolysis of (104) was unsuccessful, giving only the fragmentation products (105) and (106).
Discussion

None of the methods described above presents itself as an ideal way to construct the imino linkage of acarbose and its analogues. The success of triflate alkylations is very dependent on the structure of the triflate, thus lacking flexibility. Epoxide aminolysis can be highly effective, yet the structure of the epoxide must be such that regioselectivity problems are avoided. Additionally, the construction of the imino linkage would ideally coincide with the introduction of the amino functionality of the cyclitol, as per the work of Knapp described previously. This would reduce the number of steps required to synthesise these compounds. A reaction that could potentially satisfy this criterion is the Pd(0) catalysed allylic substitution of the tertiary acetate (107), to give the amine (108). This is an elegant method of introducing the amine functionality onto a cyclitol ring, which potentially could be used to construct additionally the imino linkage in β-linked analogues of acarbose. However, when cyclohexylamine was used, in place of benzylamine, only elimination products were observed, suggesting a carbohydrate amine would give similar results.
It was decided to reinvestigate this type of reaction, using an internal amine as the nucleophile, thus making the substitution an intramolecular process (Scheme 1).

Scheme 1

Such reactions have been used previously to construct nitrogen heterocycles. Godleski and co-workers used \( \pi \)-allyl palladium chemistry to construct 1-azaspirocycles, as shown by the conversion of (109) into (110) in > 95% yield.\(^73\) Presumably Et\(_3\)N is added so as to drive the reaction to completion; allylammonium salts are known substrates for Pd(0).\(^74\)
Trost and Cossey employed a similar methodology to construct the amino macrocycle (111). They found the best results were obtained when (112) was treated with 10 mol % Pd(PPh$_3$)$_4$ and 8 mol % 1,4-bis(diphenylphosphino)butane (dppb), giving (111) in 80-89% yield. Remarkably, only elimination products were formed when the ratio of dppb to Pd(PPh$_3$)$_4$ was >1.

Firstly, it was necessary to decide on an appropriate tether. In the field of intramolecular aglycon delivery, a number of methods have been developed for the temporary linking of donor and acceptor, prior to glycosylation. Intramolecular aglycon delivery is an elegant means of synthesising, otherwise difficult to form, glycosidic linkages, such as that of β-D-mannopyranosides. For example, intramolecular glycosylation of (113), connected by an isopropylidene acetal tether, gave only the β-linked disaccharide (114).

\[\text{AcO} \quad \text{H}_2\text{N} \quad \text{NHAc} \quad \text{O} \quad \text{AcO} \quad \text{H}_2\text{N} \quad \text{NHAc} \]

(112) (111)

Firstly, it was necessary to decide on an appropriate tether. In the field of intramolecular aglycon delivery, a number of methods have been developed for the temporary linking of donor and acceptor, prior to glycosylation. Intramolecular aglycon delivery is an elegant means of synthesising, otherwise difficult to form, glycosidic linkages, such as that of β-D-mannopyranosides. For example, intramolecular glycosylation of (113), connected by an isopropylidene acetal tether, gave only the β-linked disaccharide (114).

\[\text{BnO} \quad \text{BnO} \quad \text{BnO} \quad \text{O} \quad \text{Me} \quad \text{Me} \quad \text{OBn} \quad \text{SEt} \quad \text{O} \quad \text{OBn} \quad \text{BnO} \quad \text{BnO} \quad \text{BnO} \quad \text{OMe} \quad \text{BnO} \quad \text{BnO} \quad \text{BnO} \quad \text{OBn} \quad \text{BnO} \quad \text{BnO} \quad \text{OMe} \]

(113) (114)
a) NIS, 16 h, then H$_2$O
Some of the more common tethers and representative references are shown below (Table 1).

<table>
<thead>
<tr>
<th>Tether</th>
<th>References</th>
<th>Tether</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me Me</td>
<td>77,78</td>
<td>R R Si</td>
<td>79,80</td>
</tr>
<tr>
<td>RO OR</td>
<td></td>
<td>RO OR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R = Me, 'Bu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>81-84</td>
<td></td>
<td>80,85,86</td>
</tr>
<tr>
<td>OMe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RO OR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>86-88</td>
<td></td>
<td>89,90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RO (\text{C}_2\text{H}_4\text{O}_2\text{O}) _n OR</td>
<td>86-88</td>
<td>RO (\text{C}_6\text{H}_5\text{C}_2\text{H}_4\text{OR})</td>
<td>89,90</td>
</tr>
</tbody>
</table>

Table 1

Of these tethers, an ester linkage was thought to be ideal, owing to both the ease of installation and relative stability. From the esters available, phthalic, succinic or malonic, the more stable phthalic ester was chosen.
Thus, the initial target was the ester (115), which could be synthesised by condensation of the alcohol (116) and the acid (117) (Scheme 2).

\[
\begin{align*}
\text{AcO} & \quad \text{AcO} \\
\text{BnO} & \quad \text{BnO} \\
\text{BnO} & \quad \text{BnO} \\
\end{align*}
\]

(115)

The synthesis of the acid (117) required the blocking of the hydroxyl groups at C2 and C3 of methyl \(\beta\)-D-galactopyranoside (118), and this was achieved in a series of standard transformations. Firstly, (118) was treated with benzaldehyde dimethyl acetal and CSA in DMF, at 70° under reduced pressure. The reaction mixture was neutralised with Et\(_3\)N and then benzylated under normal conditions (NaH, BnBr) to give (119). Subsequently, the benzylidene functionality was removed by acid-catalysed hydrolysis (80% aq. AcOH, 100°) to furnish the diol (120).
The next task was to introduce an azide functionality at C4, firstly requiring the temporary blocking of the hydroxyl group at C6. Thus, (120) was regioselectively protected (TBDMSCl, imidazole, DMF), giving the silyl ether (121). A triflic acid ester was subsequently formed at C4, furnishing (122), which was transformed (NaN₃, DMF, 80°) into the azide (123) in 93% yield over the two steps.

Initially, the stereochemistry at C4 of the azide (123) could not be determined by ¹H n.m.r spectroscopy - the signals of H3, 4, and 5 showed second-order effects at 300 MHz. However, a decoupling experiment, which eliminated spin-spin coupling between H5 and H6, resolved the signal for H5 into a doublet; the magnitude of $J_{4,5}$, 9.4 Hz, is consistent with a D-gluco configuration (Figure 1).

![Figure 1. Decoupling experiment ($¹H$ n.m.r.) on H5 of the azide (123)](image-url)
To complete the synthesis of the acid (117), the silyl ether of (123) was removed by acid hydrolysis (80% aq. AcOH, 100°) to afford (124), and the liberated alcohol was esterified (phthalic anhydride, pyridine, DMAP).

\[
\text{OMe} \quad \text{OMe}
\]

(124)

(117)

The enone (125) was favoured as a potential precursor to the alcohol (116), requiring a hydroxymethylene extension and functionalisation at the newly formed tertiary centre. The enone (125) is easily prepared from methyl α-D-glucopyranoside.\(^9\)

\[
\text{O} \quad \text{BnO} \quad \text{BnO} \quad \text{BnO}
\]

(125)

\[
\text{AcO} \quad \text{OH} \quad \text{BnO} \quad \text{BnO} \quad \text{BnO}
\]

(116)

The first approach explored for the synthesis of the alcohol (116) was the acetylation of a suitable epoxide. Danishefsky and Park have previously prepared the epoxide (126), as a minor product (28%) from the epoxidation of the alkene (127).\(^9\) Thus, an alternative method to form an epoxide with the same stereochemistry as (126) was sought.

\[
\begin{align*}
\text{PMBO} & \quad \text{PMBO} \\
\text{O} & \quad \text{NHBn} \\
\text{O} & \quad \text{NH} \\
\text{K} & \quad \text{O} \\
\text{A} & \quad \text{BnO} \quad \text{BnO} \quad \text{BnO} \\
\text{AcO} & \quad \text{OH} \\
\end{align*}
\]

(127)

\[
\begin{align*}
\text{PMBO} & \quad \text{PMBO} \\
\text{O} & \quad \text{NHBn} \\
\text{O} & \quad \text{NH} \\
\text{K} & \quad \text{O} \\
\text{A} & \quad \text{BnO} \quad \text{BnO} \quad \text{BnO} \\
\text{AcO} & \quad \text{OH} \\
\end{align*}
\]

(126)

\[\text{PMBO, NaHCO}_3\]

a) \(m\)-CPBA, NaHCO\(_3\)
Two reagents that directly convert carbonyl compounds into epoxides are dimethylsulfonium methyliide and dimethylsulfoxonium methyliide, developed by Corey and Chaykovsky. As dimethylsulfoxonium methyliide has been known to cyclopropanate enones, only the reaction between dimethylsulfonium methyliide and the enone (125) was considered. Disappointingly, treatment of the enone (125) with dimethylsulfonium methyliide, followed by quenching the reaction with glacial AcOH, gave complex mixtures from which none of the desired product could be isolated.

![Complex mixtures](image)

Treatment of the enone (125) with the Grignard reagent derived from benzyl chloromethyl ether followed by quenching with acetic anhydride gives the acetate (107).

![Grignard reagent](image)

It was considered that an analogous Grignard reagent of the type ROCH2MgCl, where the R group is orthogonal to the benzyl group, could be a potential route to the alcohol (116). The first two reagents investigated were t-butyl chloromethyl ether and (t-butyldimethylsilyloxy)methyl chloride. Under typical Grignard conditions, no reaction of either of these reagents with the enone (125) was observed. However, success was encountered using chloromethyl 4-methoxybenzyl ether. This reagent was prepared by a modified procedure of Benneche and co-workers - only half an equivalent of sulfuryl chloride (instead of the reported one equivalent) was required to react fully with methylthiomethyl 4-methoxybenzyl ether. Addition of the enone (125) to a solution of 4-methoxybenzylloxymethylmagnesium chloride, followed by
quenching the reaction with Ac₂O, gave the acetate (128). The 4-methoxybenzyl group of (128) was then oxidatively cleaved with DDQ.

![Chemical structure of 128](image1)

![Chemical structure of 115](image2)

An initial reaction of this new product and the acid (115), in the presence of DCC and DMAP, proved very sluggish, with a new compound slowly forming over three days. Purification of the reaction mixture gave an inseparable mixture (30% mass return) of what appeared to be an esterified product, containing small amounts (10%) of an unidentified compound (¹H n.m.r.). This result was surprising, considering that such esterifications normally proceed in good yield, as illustrated by the following example.⁸⁰

![Chemical reaction](image3)

a) DCC, DMAP, CH₂Cl₂, 80%
A closer inspection of the $^1$H n.m.r. spectrum of the product obtained from the treatment of the acetate (128) with DDQ revealed why the esterification was so sluggish - cleavage of the 4-methoxybenzyl group coincided with the migration of the acetyl group to the primary hydroxyl, giving the primary acetate (129). A comparison of the $^1$H n.m.r. spectrum of the acetate (129) with that of similar compounds in the literature supports this claim.97

\[
\text{AcO} \quad \text{OPMB} \\
\text{BnO} \quad \text{OBn}
\]

(128) \quad (129)

\[
\begin{align*}
a) \text{DDQ}, \text{CH}_2\text{Cl}_2/\text{H}_2\text{O} \\
\text{AcO} \quad \text{OPMB} \\
\text{BnO} \quad \text{OBn}
\end{align*}
\]

This result led to a change in strategy for the formation of the tethered compound (115). The primary acetate of (129) was removed, using NaOMe in MeOH, to give the diol (130). This diol (130) has been synthesised previously, in a somewhat tedious manner, from the acid (131).98,99

\[
\begin{align*}
\text{HO} \\
\text{BnO} \\
\text{BnO} \\
\text{OBn}
\end{align*}
\]

(130)

\[
\begin{align*}
\text{CO}_2\text{H}
\end{align*}
\]

(131)

Exploiting the marked difference in reactivity between a tertiary and a primary alcohol, a one-pot synthesis of the tethered compound (115) was attempted. Thus, the diol (130) was treated with 1.25 equivalents of the acid (116), together with DCC and DMAP. T.l.c. analysis after one hour showed complete consumption of the diol (130), so five equivalents of AcOH, together with more DCC and DMAP, were added. T.l.c. analysis showed the slow formation of a new compound. Purification of the reaction mixture led to the ester (115) being isolated in good yield (74%). The $^1$H- and $^{13}$C- n.m.r. spectra of the ester (115) were able to be analysed fully with the aid of two-dimensional experiments.
Before a coupling reaction could be attempted, the azide functionality of (115) had to be reduced to an amine. With a range of functional groups in the molecule, a chemo-selective reducing agent was necessary. Propane-1,3-dithiol\textsuperscript{100} served this role well, reducing the azide of (115) to the amine (132) in excellent yield (82%).

With quantities of the amine (132) in hand, some alkylation reactions were attempted. However, under all reaction conditions tested, including those shown previously,\textsuperscript{73,75} none of the desired material (133) could be isolated. Under the reaction conditions employed, (132) proved quite unreactive, with extended reaction times required for its full consumption, leading to the formation of complex mixtures. Partial identification
of products formed, using $^1$H n.m.r. spectroscopy, suggested them to be the result of fragmentation of the phthalic acid tether.

\[
\text{O} \quad \text{AcO} \\
\text{BnO} \quad \text{BnO} \\
\text{BnO} \\
\text{H}_2\text{N} \\
\text{O} \quad \text{OMe} \\
\text{OBn} \quad \text{OBn}
\]

(132)

\[
\text{AcO} \quad \text{O} \\
\text{BnO} \quad \text{BnO} \\
\text{BnO} \\
\text{H}_2\text{N} \\
\text{O} \quad \text{OMe} \\
\text{OBn} \quad \text{OBn}
\]

(133)

dppp = bis(diphenylphosphino)pentane

Future Prospects

Clearly, a more stable linkage is required before a coupling reaction could possibly be successful. A $m$-xyylene moiety would be ideal, however the synthetic challenge towards such a compound seems formidable, with any potential benefit of the tether being outweighed by the difficulty in its synthesis.
Experimental

General

Melting points were determined on a Reichert hot stage (<230°) or Electrothermal melting point apparatus (>230°). Optical rotations were performed with a Perkin-Elmer 141 Polarimeter in a microcell (1 ml, 10 cm path length) in CHCl₃ at room temperature, unless stated otherwise.

¹H- and ¹³C-Nuclear magnetic resonance (n.m.r.) spectra were obtained on a Varian Gemini-200 (200 MHz for ¹H), a Bruker AM300 (300 MHz for ¹H and 75.5 MHz for ¹³C) or a Bruker ARX500 spectrometer (500 MHz for ¹H and 125.8 MHz for ¹³C). Unless stated otherwise, deuterated chloroform (CDCl₃) was used as the solvent with CHCl₃ (δ 7.24) or CDCl₃ (¹³C, δ 77.0) being employed as internal standards. N.m.r. spectra run in D₂O used internal HOD (¹H, δ 4.63), MeOH (¹³C, δ 49.00) or external 2,2-dimethyl-2-silapentane-5-sulfonate (δ 0.00) as standards.

Infrared spectra were recorded using a Biorad FTS-45 FTIR spectrometer or Mattson Galaxy FTIR spectrometer. Mass spectra were recorded with a VG-Autospec spectrometer using the fast atom bombardment technique (f.a.b.) and using 3-nitrobenzyl alcohol as a matrix, unless otherwise stated. Microanalyses were performed by M-H-W laboratories, Phoenix, Arizona; C.M.A.S., Melbourne, Victoria or the Chemistry Centre, Perth, Western Australia. Flash chromatography was performed on BDH silica gel or Geduran silica gel 60 with the specified solvents. Thin layer chromatography (t.l.c.) was effected on Merck silica gel 60 F₂₅₄ aluminium-backed plates, which were stained by heating (>200°) with either 5% sulfuric acid in EtOH, 1% PdCl₂ in 1 M HCl or aqueous 0.5% potassium permanganate solution.

All solvents except butanol, DMF, MeCN, PrOH and Pr²O were distilled prior to use and dried according to the methods of Burfield.¹⁰¹-¹⁰⁵ ‘Normal work up’ refers to dilution with water, extraction into an organic solvent, sequential washing with aqueous hydrochloric acid solution (1 M, where appropriate), saturated aqueous sodium bicarbonate and brine solutions, followed by drying over anhydrous magnesium sulfate (unless stated otherwise), filtration, evaporation of the solvent by means of a rotary evaporator at reduced pressure and drying of the residue at 1 mmHg.
Methyl 2,3-Di-O-benzyl-4,6-O-benzylidene-β-D-galactoside (119)

Methyl β-D-galactopyranoside (118) (7.2 g, 37 mmol) in DMF (50 mL) was treated with benzaldehyde dimethyl acetal (7.6 g, 50 mmol) and CSA (500 mg), under reduced pressure (60°, 2 h). Et₃N (1 mL) was added to the solution, which was then concentrated to half its volume. This solution was dilute with more DMF (50 mL), and NaH (4.4 g, 110 mmol, 60% dispersion in mineral oil) followed by BnBr (11.4 mL, 96 mmol) were added. The mixture was stirred (1 h) and then MeOH (2 mL) was cautiously added. Concentration, followed by a normal workup (CH₂Cl₂), gave an orange oil. This oil was purified by RSF (EtOAc/petrol, 1:10 to 1:3) to give the dibenzyl ether (119) as colourless crystals (11.6 g, 68%), m.p. 112° (Et₂O/petrol; lit.¹⁰⁶ 116.8°).

Methyl 2,3-Di-O-benzyl-6-O-(tert-butyldimethylsilyl)-β-D-galactopyranoside (121)

The dibenzyl ether of (119) (5.6 g, 12.1 mmol) was treated with aqueous AcOH (70 mL of 80%; 100°, 30 min). Concentration, followed by RSF (EtOAc/petrol, 1:1 to EtOAc), gave what was presumed to be methyl 2,3-di-O-benzyl-β-D-galactopyranoside (120), as a colourless oil. This oil was treated with tert-butyldimethylsilyl chloride (2.08 g, 13.3 mmol) and imidazole (1.03 g, 15.1 mmol) in DMF (20 mL; 1 d). MeOH (1 mL) was added and the mixture was stirred (20 min). Concentration, followed by normal workup (CH₂Cl₂) and flash chromatography (EtOAc/petrol, 1:9 to 2:8), gave the silyl ether
Methyl 4-Azido-2,3-di-O-benzyl-6-O-(tert-butyldimethylsilyl)-4-deoxy-\(\alpha\)-D-glucoside (123)

\(\text{Tf}_2\text{O} (0.82 \text{ mL}, 5.8 \text{ mmol})\) was added to the silyl ether (121) (1.9 g, 3.9 mmol) and pyridine (1.0 mL) in CH\(_2\text{Cl}_2\) (20 mL) and the mixture stirred (2 h, 0°). The addition of saturated NaHCO\(_3\) solution, followed by a normal workup (CH\(_2\text{Cl}_2\)), gave, presumably, methyl 2,3-di-O-benzyl-6-O-(tert-butyldimethylsilyl)-4-O-(trifluoromethylsulfonyl)-\(\alpha\)-D-galactoside (122) as a yellow oil. This oil was treated with NaN\(_3\) (0.65 g, 11.7 mmol) in DMF (1 h, 80°). Concentration and normal workup (CH\(_2\text{Cl}_2\)) gave an oil that was subjected to flash chromatography (EtOAc/petrol, 1:9) to give the azide (123), as an oil (1.93 g, 93%), [\(\alpha\)]\(_D\) +87° (Found: C, 64.2; H, 7.6; N, 7.9 C\(_{28}\)H\(_{39}\)N\(_3\)O\(_5\) requires C, 64.0; H, 7.5; N, 8.0%). \(^1\text{H}\) n.m.r. (300 MHz) \(\delta\) 0.10, s, 6H, SiMe; 0.92, s, 9H, CMe; 3.12-3.16, m, H5; 3.40, dd, \(J_{1,2} = J_{2,3}\) 7.9 Hz, H2; 3.46-3.62, m, H3,4; 3.54, s, OMe; 3.84, dd, \(J_{5,6} = J_{6,6}\) 11.5 Hz, H6; 3.98, dd, \(J_{5,6} = 2.0\) Hz, H6; 4.27, d, H1; 4.70-5.05, m, 4H, CH\(_2\)Ph; 7.28-7.43, m, 10H, Ph. \(^{13}\text{C}\) n.m.r. (75.5 MHz) \(\delta\) -5.23, -5.46, SiMe\(_2\); 18.35, CMe\(_3\); 25.87, CMe\(_3\); 56.48, OMe; 61.62, C4; 62.66, C6; 74.77, 75.71, 2C, CH\(_2\)Ph; 74.88, 77.42, 82.24, C2,3,4,5; 104.52, C1; 104.52-138.40, Ph.
The silyl ether (122) (1.93 g) was treated with aqueous AcOH (80%, 20 mL; 100°, 30 min). Concentration gave an oil that was subjected to flash chromatography (EtOAc/petrol, 2:3) to give the alcohol (124) as needles (1.38 g, 91%), m.p. 92-93° (Pr$_2$O), [α]$_D$ +128° (Found: C, 63.6; H, 6.2; N, 10.3. C$_{21}$H$_{25}$N$_3$O$_5$ requires C, 63.2; H, 6.3; N, 10.5%). $^1$H n.m.r. (300 MHz) δ 3.11-3.18, m, H5; 3.32-3.50, m, H2,3,4; 3.52, s, Me; 3.70, dd, $J_{5,6}$ 4.5, $J_{6,6}$ 12.1 Hz, H6; 3.84, dd, $J_{5,6}$ 2.6 Hz, H6; 4.28, d, $J_{1,2}$ 7.2 Hz, H1; 4.61-4.90, m, 4H, CH$_2$Ph; 7.14-7.48, m, 10H, Ph. $^{13}$C n.m.r. (75.5 MHz) δ 57.30, Me; 61.31, 74.19, 82.09, 82.68, C2,3,4,5; 62.12, C6; 74.80, 75.57, 2C, CH$_2$Ph; 104.74, C1; 127.76-138.16, Ph.

The alcohol (124) (0.94 g, 2.4 mmol) was treated with phthalic anhydride (1.73 g, 11.7 mmol) in the presence of DMAP (100 mg) in pyridine (10 mL; rt, 18 h). Water (1 mL) was added, so as to hydrolyse excess phthalic anhydride (t.l.c.). Concentration, followed by normal workup (CH$_2$Cl$_2$) and flash chromatography (MeOH/CHCl$_3$, 1:9), gave the acid (117) as a colourless oil (1.52 g, 86%), [α]$_D$ +93°. $^1$H n.m.r. (300 MHz) δ 3.40-3.59, m, H2,3,4,5; 3.56, s, Me; 4.32, d, $J_{1,2}$ 7.5 Hz, H1; 4.49, dd, $J_{5,6}$ 4.5, $J_{6,6}$ 12.0 Hz, H6; 4.63, dd, $J_{5,6}$ 2.2 Hz, H6; 4.67-4.96, m, 4H, CH$_2$Ph; 7.21-7.88, m, 14H, Ar. $^{13}$C n.m.r. (75.5 MHz) δ 57.34, Me; 61.44, 71.94, 81.90, 82.67, C2,3,4,5; 64.47,
(1R,4S,5R,6S)-4,5,6-Tribenzyloxy-1-C-(acetyloxymethyl)cyclohex-2-enol (129)

(i) Sulfuryl chloride (920 μL, 11.4 mmol) was added dropwise to a solution of 4-methoxybenzyl methylthiomethyl ether (4.15 g, 22.9 mmol) in CH₂Cl₂ (20 mL) at −78°. After 30 min., t.l.c analysis showed complete consumption of the 4-methoxybenzyl methylthiomethyl ether. The solution was concentrated to give a colourless oil, presumably chloromethyl 4-methoxybenzyl ether. A solution of this oil in THF (20 mL) was treated with magnesium metal (680 mg, 28 mmol) and HgCl₂ (50 mg; 0°, 1 h). The enone (125) (990 mg, 2.4 mmol) was added, and stirring was continued (30 min, 0°). Ac₂O (3.0 mL, 34 mmol) was then added and the mixture (10 min). The reaction mixture was poured into a solution of saturated NaHCO₃ solution, and the mixture was stirred (15 min) and filtered (Celite). Normal workup (CH₂Cl₂) on the filtrate, followed by flash chromatography (EtOAc/petrol, 3:17), gave an inseparable mixture of, presumably, the acetate (128) and aromatic impurities (¹H n.m.r.), as a colourless oil.

(ii) The above mixture was treated with DDQ (900 mg, 5.6 mmol) in CH₂Cl₂/water (20 mL, 95:5; 2 h). Saturated NaHCO₃ solution (100 mL) was added and the organic layer was separated. The aqueous layer was extracted with more CH₂Cl₂ (50 mL). The combined organic extracts were dried, filtered and concentrated to give a dark oil. This oil was purified by flash chromatography (EtOAc/petrol, 1:3) to give the acetate (129) as a colourless oil (720 mg, 62%), [α]D +24° (Found: C, 74.0; H, 6.6. C₃₀H₃₂O₆ requires C, 73.8; H, 6.6%). ¹H n.m.r. (300 MHz) δ 2.04, s, 3H, Ac; 3.72, d, J₂,6 9.8 Hz, H₆; 3.92, dd, J₄,₅ 6.6 Hz, H₅; 4.11, d, J₇,₇ 11.4 Hz, H₇; 4.17, m, H₄; 4.47, d, J₇,₇ 2.5 Hz, H₇; 4.62-4.93, m, 6H, CH₂Ph; 5.61, dd, J₂,₃ 10.4, J₂,₄ 2.0 Hz, H₂; 5.78, dd, J₃,₄ 2.5 Hz, H₃; 7.21-7.47, m, 15H, Ar. ¹³C n.m.r. (75.5 MHz) δ 20.94, Me; 67.65, 72.16, 74.96, 75.49, 4C, C₇, CH₂Ph; 74.60, C₁; 79.15, 81.34, 83.65, C₄,₅,₆; 127.74-138.35, C₂,₃, Ar;
A small piece of sodium metal was added to the acetate (129) (540 mg) in MeOH (5 mL; 0°C) and the solution was stirred (rt, 1 h), before being neutralised with resin [Dowex 50W-X8 (H+ form)] and filtered. The filtrate was concentrated to give a yellow oil. This oil was filtered through a plug of silica gel (EtOAc/petrol, 1:1) to give the diol (130) as a colourless oil (450 mg), [α]D +48°. The 1H n.m.r. (200 MHz) spectrum was consistent with that reported previously.99

\[
\text{(130)}
\]

\[
\text{(115)}
\]

*Methyl 4-Azido-2,3-di-O-benzyl-4-deoxy-6-(hydrogen 1,2-benzenedicarboxylate)-β-D-glucoside, ester with C-[(IR,4S,5R,6S)-1-acetoxy-4,5,6-tribenzylxycyclohex-2-enyl]methanol (115)*

The diol (130) (230 mg, 0.48 mmol) and the acid (117) (330 mg, 0.60 mmol) were treated with DCC (125 mg, 0.6 mmol) and DMAP (60 mg, 0.48 mmol) in CH2Cl2 (3 mL). After 2 h, t.l.c. analysis indicated the complete consumption of the diol. AcOH (140 µL, 2.4 mmol) and more DCC (500 mg, 2.5 mmol) and DMAP (300 mg, 2.5 mmol) were then added, and the solution was left for a further 5 h. Pre-adsorption onto silica gel, followed by purification on a short column of silica gel (EtOAc/toluene, 1:9), gave an oil that was further purified by flash chromatography (EtOAc/petrol, 1:4), to
give the ester (115) as a colourless oil (370, mg, 74%), [α]D +77°. 1H n.m.r. (600 MHz) δ 1.84, s, 3H, Ac; 3.38-3.42, m, H5; 3.44, dd, J1,2 7.9 Hz, J2,3 8.6 Hz, H2; 3.50-3.57, m, H3,4; 3.53, s, OMe; 3.97, dd, d, J4',5' 10.4, J5',6' 7.7 Hz, H5'; 4.28, d, H1; 4.33, ddd, J1',6' = J2',6' 2.3 Hz, H6'; 4.51, dd, J5,6 5.0, J6,6 12.0, H6; 4.55, d, J7,7' 11.3, H7'; 4.57, d, H4'; 4.58, dd, J5,6 2.2 Hz, H6; 4.64-4.93, m, 10H, CH2Ph; 4.87, d, H7; 5.74, dd, J1,2 10.5 Hz, H2; 5.89, dd, H1, 7.25-7.78, m, 29H, Ar. 13C n.m.r. (150 MHz) δ 21.86, COMe; 57.15, OMe; 61.69, C4; 64.48, C6; 66.60, C7'; 71.95, C5; 72.17, 74.76, 75.36, 75.61, 75.62, 5C, CH2Ph; 79.79, C6'; 80.26, C4'; 81.97, C2, 82.76; C3; 82.88, C5'; 83.66, C3'; 104.63, C1; 127.52-138.34, C1',2',Ar; 166.50, 167.25, 169.77, 3C, CO.

High-resolution mass spectrum (f.a.b.) m/z 1018.4028 [C59H60N3O13 (M+H)+ requires 1018.4126].

\[
\begin{align*}
\text{Methyl 4-Amino-2,3-di-O-benzyl-4-deoxy-6-(hydrogen 1,2-benzenedicarboxylate)-}\beta\text{-D-glycoside, ester with C-[(1R,4S,5R,6S)-1-acetyloxy-4,5,6-tribenzyloxyhex-2-enyl]methanol (131)}
\end{align*}
\]

The ester (115) (100 mg, 0.1 mmol) was treated with propane-1,3-dithiol (100 μL, 1.0 mmol) and Et3N (140 μL, 1.0 mmol) in MeOH (5 mL; 8 h). The solution was concentrated to give an orange oil, which was purified by flash chromatography (EtOAc/petrol/Et3N, 20:79:1 then EtOAc/petrol/EtOH/Et3N, 40:54:5:1) to give the amine (131) as a colourless oil (80 mg, 82%), [α]D +23°. 1H n.m.r. (300 MHz) δ 1.84, s, Ac; 2.85, dd, J3,4 = J4,5 9.6 Hz, H4; 3.30, dd, J2,3 9.6 Hz, H3; 3.36-3.42, m, H5; 3.43, dd, J1,2 7.7 Hz, H2; 3.55, s, OMe; 3.99, dd, J4,5 10.4, J5,6 7.7 Hz, H5'; 4.32, d, H1; 4.27-4.34, m, H6'; 4.48-4.99, m, 15H, H6,4',7',CH2Ph; 5.73, dd, J1,2 10.4, J2,6 2.2 Hz, H2; 5.89, dd, J1,6' 2.1 Hz, H1; 7.25-7.78, m, 29H, Ar. 13C n.m.r. (75.5 MHz) δ 21.84, COMe; 52.74, C4; 57.05, OMe; 64.93, 66.57, C6,7'; 72.25, 74.55, 75.22, 75.36, 75.57, 5C, CH2Ph; 74.77, 79.76, 80.16, 82.43, 82.89, 89.83, C2,3,5,4',5',6'; 83.58, C3'; 105.21,
C1; 127.13-138.23, C1',2',Ar; 166.55, 167.67, 169.76, 3C, CO. High-resolution mass spectrum (f.a.b.) $m/z$ 992.4227 [$C_{59}H_{62}NO_{13} (M+H)^{+}$ requires 992.4221].
Chapter 2

The Synthesis and Biological Evaluation of an $N$- and an $S$-linked Carba-disaccharide as $\beta$-D-Xylosidase Inhibitors
Introduction

The initial target, the amine (29), belongs to a purely synthetic class of compound known as the conduramines. The thiol (30) represents a new class of compounds, coined the ‘condurithiols’.

The nomenclature of the conduramines is derived from the conduritols. The conduramines differ from the conduritols by having an amino group in place of one of the conduritol’s hydroxyl groups. The history of the conduritols dates back to 1908 when Kübler isolated a polyol from the bark of the vine, *Marsdenia condurango*, which he named conduritol. In an elegant series of experiments, Dangschat and Fischer converted conduritol into mucic acid (134), establishing its relative stereochemistry as that of conduritol A (135). The five other possible diastereoisomers have been labelled conduritols B, C, D, E and F. Following this naming system, the amine (29) is known as conduramine B.
The first synthesis of the conduramines, albeit in racemic form, was by Nakajima and co-workers.\textsuperscript{112} Epoxidation of the \textit{trans}-diol (136) gave the epoxy-diol (137) [and its diastereoisomer (138)]. Allylic attack by ammonia on the epoxide (137) then gave conduramine B (30). This methodology was used to produce racemates of conduramines A, B, C and F.

In 1981, Paulsen and co-workers synthesised an optically active conduramine [conduramine F (139)], from quebrachitol (140).\textsuperscript{113} Since then, there have been many syntheses of optically active conduramines, starting with both optically active \textsuperscript{70,114-116} and inactive precursors.\textsuperscript{117-124}

Knapp and co-workers synthesised conduramine F (139) whilst investigating intramolecular amino delivery reactions for the synthesis of allylic \textit{N}-substituted derivatives of valienamine (13).\textsuperscript{70} Their approach involved a [3,3]-sigmatropic rearrangement of an \textit{N}-substituted carbonimidothioate to form the cyclitol carbon-nitrogen bond. Thus (as reported by Knapp), treatment of the sodium salt of the alcohol (141) with 4-methoxybenzyl isothiocyanate, followed by quenching with BnBr, gave the carbonimidothioate (142). A subsequent rearrangement in refluxing toluene gave the thiocarbamate (143) [62\% from (141)]. Deprotection steps then gave conduramine F, isolated as its \textit{per}-acetyl derivative (144).
Discussion

The rearrangement of an allylic carbonimidothioate presented itself as a route to conduramine B (29), using the diastereoisomer (145) of the alcohol (141). Thus, methyl α-D-glucopyranoside was converted, in seven steps, into the enone (125) and thence the alcohol (145). Treatment of the alcohol (145) with benzyl isothiocyanate and NaH, followed by quenching with BnBr, gave the carbonimidothioate (146).

Heating the carbonimidothioate (146) in refluxing toluene or xylene or DMF at 140° gave complex mixtures, from which the desired thiocarbamate (147) could not be isolated.
This result contrasts greatly with that of Knapp and co-workers, where the thermal rearrangement proceeded in good yield. Noticeably different between the two compounds is the location of the migrating group. In (146), the migrating group is *pseudo*-equatorial and remote from the double bond - the cyclohexene ring must undergo a conformational change for the migration to occur. In (142), the migrating group, being *pseudo*-axial, is already in position for migration to occur.

In order to reduce the steric bulk on the nitrogen of the migrating group, the rearrangement of a trichloroacetimidate was investigated.\textsuperscript{127} The [3,3]-sigmatropic rearrangement of an allylic trichloroacetimidate, named the Overman rearrangement, is extensively used in natural product synthesis. For example, while synthesising the cyclic guanidinium moiety of tetrodotoxin (148), Yamanto and Isobe employed a rearrangement of the trichloroacetimidate (149), to give the trichloroacetamide (150).\textsuperscript{128}

Initially, the sodium salt of the alcohol (145) was treated with trichloroacetonitrile, to furnish the trichloroacetimidate (151). Heating the trichloroacetimidate (151), in refluxing xylene, gave the trichloroacetamide (152) in an excellent yield (93%), with only one diastereoisomer with the desired stereochemistry ($J_{1,6}$ 7.0 Hz), apparent by $^1$H n.m.r. spectroscopy.
Hydrolysis of the trichloroacetamide (152), with aqueous NaOH in THF, gave the amine (153). Further deprotection, to remove the benzyl groups, was carried out using sodium in liquid ammonia, a chemo-selective method that does not reduce the double bond. This reduction proceeded in good yield (75%) to give, after ion-exchange and gel chromatography, conduramine B (29) isolated as the hydrochloride. A portion of the mixture was acetylated to give the amide (154), to satisfy characterisation of (29).

\[
\begin{align*}
\text{BnO} & \quad \text{NH}_2 \\
\text{BnO} & \quad \text{OBn} \\
\end{align*}
\]

(153)

\[
\begin{align*}
\text{HO} & \quad \text{OH} \\
\text{NH}_2 & \quad \text{OH} \\
\end{align*}
\]

(29)

\[
\begin{align*}
\text{AcO} & \quad \text{NHAc} \\
\text{OAc} & \quad \text{OAc} \\
\end{align*}
\]

(154)

The approach to the formation of the carbon-sulfur bond of the thiol (30) also involved a [3,3] sigmatropic rearrangement, in this case, that of an allylic xanthate.\(^{129}\) The ease with which allylic xanthates rearrange is shown in the example below, where simple distillation caused rearrangement of the xanthate (155) to the dithiocarbonate (156).\(^{130}\)

\[
\begin{align*}
\text{EtO}_2\text{C} & \quad \text{O} \\
\text{O} & \quad \text{SMe} \\
\text{S} & \quad \text{S} \\
\end{align*}
\]

(155)

\[
\begin{align*}
\text{MeS} & \quad \text{O} \\
\text{S} & \quad \text{S} \\
\text{EtO}_2\text{C} & \quad \text{EtO}_2\text{C} \\
\end{align*}
\]

(156)
For the synthesis of the thiol (30), the sodium salt of the alcohol (145) was treated with carbon disulfide, the reaction being greatly accelerated by the addition of a quarternary ammonium salt (TBAI). Once the alcohol was completely consumed (t.l.c.), methyl iodide was added to quench the reaction, presumably giving the xanthate (157). However, purification of the reaction mixture gave two compounds, the major one being the expected xanthate (157) and, somewhat surprisingly, the other being the rearranged dithiocarbonate (158). The xanthate (157) could be completely converted into the rearranged product (158) by heating in refluxing toluene for 30 minutes. Again, only one diastereoisomer was produced, with the desired stereochemistry ($J_{1,6}$ 7.2 Hz). This observation supports the ease of allylic xanthate rearrangement.

Treatment of the dithiocarbonate (158) with aqueous sodium hydroxide in MeOH and THF gave the thiol (159). Debenzylation of the thiol (159), again using sodium in liquid ammonia, followed by acetylation, gave the thioacetate (160), a direct precursor of the thiol (30). Conventional deacetylation of the thioacetate (160), using NaOMe in MeOH, gave appreciable amounts of the disulfide (161), a problem that could be averted by the addition of ethanethiol to the reaction mixture.\textsuperscript{131}
Attention now turned to the synthesis of the carba-disaccharide (24), with the key reaction being the formation of the new nitrogen-carbon bond. Of the several methods utilised to construct nitrogen-carbon bonds in similar systems, an alkylation with a triflate [(162)] was thought most suitable (Scheme 1).
The initial approach undertaken for the synthesis of the triflate (162) involved a selective benzylation of methyl α-L-arabinopyranoside (163)\textsuperscript{132} to give, hopefully, the dibenzoate (164), relying on the lower reactivity of axial versus equatorial hydroxyl groups. However, when methyl α-L-arabinopyranoside (163) was treated with BzCl at low temperature (-20°), both the desired dibenzoate (164) and the regioisomer (165) were the main products, in roughly a 1:1 ratio and proved to be inseparable by flash chromatography. This result was surprising, considering that methyl β-D-galactopyranoside can be selectively benzyolated to give the tribenzoate (166) in reasonable yield (55%).\textsuperscript{133}

Knowing the reactivity of O3 to be far greater than O4 towards acylation, an alternative route to the triflate (162) presented itself. Thus, benzylation of methyl 3,4-\textit{O}-isopropylidene-α-L-arabinoside gave the benzoate (167).\textsuperscript{134} Hydrolysis of the isopropylidene acetal, using aqueous AcOH at 100°, followed by regioselective benzyolation of O3 over O4 (-20°), gave the dibenzoate (164) in good yield [71% from (167)]. Finally, treatment of (164) with Tf\textsubscript{2}O and pyridine gave the triflate (162).

With quantities of the triflate (162) in hand, some alkylations were now attempted. The amine (153) and the triflate (162), in a ratio of 5:2, were heated in 1,3-dimethyl-2-imidazolidinone (DMI) at 60° for 20 hours, to give the disaccharide (168) in 56% yield.
(based on the triflate). Unreacted amine (153) was recovered and recycled for further reactions. It was necessary to ensure complete consumption of the triflate (162), as separation of the triflate from the disaccharide (168) proved difficult.

Several cases of nucleophilic substitution of carbohydrate triflates by thiolates have appeared in the literature, normally proceeding in good to excellent yield. For example, the sulfur-linked analogues of maltotriose (169),\textsuperscript{135} cellotriose (170)\textsuperscript{136} and chitobiose (171)\textsuperscript{137} have been synthesised using this methodology.
Common in the construction of the sulfur linkage is the initial formation of a thiolate ion, by a strong base such as NaOMe, followed by nucleophilic substitution of a leaving group. While these reactions normally proceed in good yield, a drawback is that the required strong base may attack other functional groups. A simpler, yet just as effective, method is the reaction of the thiol and triflate with the non-nucleophilic base, DBU (172). Thus, treatment of the thiol (159) and the triflate (162) with DBU (172) in toluene for six hours gave the sulfide (173) in 72% yield. The higher yield of the alkylation involving the thiol (159) [than that involving the amine (152)] with the triflate (162) is not surprising - while possessing similar basicity, thiolates make better nucleophiles than amines.

The secondary amine (168) and the sulfide (173) were both deprotected according to the following sequence of reactions. Firstly, the benzoyl groups were removed, using methanolic NaOMe, followed by removal of the benzyl groups, using sodium in liquid ammonia. Subsequent acetylation afforded the per-acetylated pseudo-disaccharides (174) and (175), being direct precursors of the target compounds (24) and (25).

The secondary amine (24), the sulfide (25) and the primary amine (29) were tested as
inhibitors of a retaining xylosidase from the bacterium, *Thermoanaerobacterium saccharolyticum*, using phenyl β-D-xylopyranoside as substrate. The thiol (30) was not tested owing to its instability (disulfide formation) in aqueous solution. The secondary amine (24) showed the greatest inhibition ($K_i$ 40 µM). The primary amine (29) and sulfide (25) had $K_i$ values of 2.2 and 10.2 mM, respectively. These $K_i$ values show the importance of the positively charged nitrogen for enzyme-inhibitor binding, the charged nitrogen presumably interacting with a carboxylate residue located in the active site. Additionally, the marked difference in $K_i$ values between the secondary amine (39) and primary amine (29) highlights the importance of the aglycon site to inhibitor binding.

Interestingly, the secondary amine (24) exhibited time dependant inhibition. That is, the inhibition of the xylosidase increases over a period of a few minutes, to then become steady state. Titled 'slow binding inhibition', this phenomenon occurs in a number of different enzymes, including glycosidases.

* Kinetic measurements carried out at pH 5.5.
Experimental

O-[(1S,4S,5R,6R)-4,5,6-Tribenzyloxyhex-2-en-1-yl] Trichloroacetimidate (151)

Trichloroacetonitrile (0.77 mL, 7.7 mmol) was added to a mixture of the allylic alcohol (145)\(^{125}\) (2.0 g, 4.8 mmol) and pre-washed (petrol) NaH (180 mg, 8 mmol) in CH\(_2\)Cl\(_2\) (50 mL) under nitrogen. After one hour, the mixture was filtered (Celite) and the filtrate was concentrated to give a dark syrup. Flash chromatography (EtOAc/petrol, 1:9) gave the trichloroacetimidate (151) as colourless, chunky crystals (2.35 g, 87%), a portion of which was recrystallised, m.p. 62-64° (EtOAc/petrol), \([\alpha]_D +103^\circ\) (Found: C, 62.3; H, 5.1. C\(_{29}\)H\(_{28}\)C\(_3\)N\(_4\) requires C, 62.1; H, 5.0%). \(^1\)H n.m.r. (300 MHz) \(\delta\) 3.83, dd, \(J_{1,6}\) 7.7, \(J_{5,6}\) 10.5 Hz, H\(_6\); 3.94, dd, \(J_{4,5}\) 7.8 Hz, H\(_5\); 4.25-4.31, m, H\(_4\); 4.68-4.95, m, 6H, CH\(_2\); 5.71-5.87, m, H\(_{1,2,3}\); 7.23-7.36, m, 15H, Ph; 8.44, s, NH. \(^{13}\)C n.m.r. (75.5 MHz) \(\delta\) 72.55, 75.45, 75.64, 3C, CH\(_2\); 79.65, 79.86, 81.75, 83.23, C\(_{1,4,5,6}\); 91.36, CCl\(_3\); 124.59-138.52, C\(_{2,3}\),Ph; 162.06, CN.

\[
\begin{align*}
\text{HO} & \quad \text{OBn} \\
BnO & \quad \text{BnO}
\end{align*}
\]

(145)

\[
\begin{align*}
\text{HN} & \quad \text{CCl}_3 \\
O & \quad \text{CCl}_3 \\
\text{BnO} & \quad \text{BnO} \\
\text{OBn} & \quad \text{OBn}
\end{align*}
\]

(151)

\[
\begin{align*}
\text{HN} & \quad \text{CCl}_3 \\
O & \quad \text{O} \\
\text{BnO} & \quad \text{BnO} \\
\text{OBn} & \quad \text{OBn}
\end{align*}
\]

(152)

N-[(1R,4R,5S,6S)-4,5,6-Tribenzyloxyhex-2-en-1-yl] Trichloroacetamide (152)

The trichloroacetimidate (151) (900 mg) was dissolved in xylene (30 mL) and heated at reflux for nine hours under nitrogen. Concentration of the solution afforded a yellow solid which was purified by flash chromatography (EtOAc/petrol, 1:9) to give the trichloroacetamide (152) as white plates (840 mg, 93%), a portion of which was recrystallised, m.p. 107-110° (EtOAc/petrol), \([\alpha]_D -147^\circ\) (Found: C, 62.2; H, 5.2%). C\(_{29}\)H\(_{28}\)C\(_3\)N\(_4\) requires C, 62.1; H, 5.0%). \(^1\)H n.m.r. (500 MHz) \(\delta\) 3.71, dd, \(J_{1,6}\) 7.0, \(J_{5,6}\) 8.0 Hz, H\(_6\); 3.90, dd, \(J_{4,5}\) 5.9 Hz, H\(_5\); 4.14-4.17, m, H\(_1\); 4.60-4.64, m, H\(_4\); 4.64-4.82, m, 6H, CH\(_2\); 5.78-5.85, m, H\(_{2,3}\); 6.87, d, \(J_{1,NH}\) 8.5 Hz, NH; 7.23-7.32, m, 15H, Ph. \(^{13}\)C n.m.r. (125.8 MHz) \(\delta\) 52.05, C\(_1\); 72.07, 74.10, 74.56, 3C, CH\(_2\); 76.75, 78.20, 81.00, C\(_{4,5,6}\); 92.41, CCl\(_3\); 126.06-138.04, C\(_{2,3}\),Ph; 161.42, CO.
N-[(1R,4R,5S,6S)-4,5,6-Tribenzyloxy-cyclohex-2-en-1-yl]amine (153)

The trichloroacetamide (152) (2.0 g) in ethanol (40 mL) was treated with aqueous NaOH (20 mL of 6 M) and left to stir overnight, during which the cloudy mixture became clear. Removal of volatile solvents, followed by normal workup (CH₂Cl₂), gave a pale yellow solid which was recrystallized to afford the amine (153) as white flakes (1.3 g, 86%), m.p. 56-58° (Pr’₂O/petrol), [α]D -158°. ¹H n.m.r. (300 MHz) δ 3.27, dd, J=6.8 Hz, H₆; 3.43-3.49, m, H₁; 3.77, dd, J₄,5 7.7 Hz, H₅; 4.20-4.26, m, H₄; 4.65-5.05, m, 6H, CH₂; 5.56, 5.64, 2 ddd, J₁,₂ ≈ J₁,₃ ≈ J₂,₄ ≈ J₃,₄ 1.8, J₂,₃ 10.2 Hz, H₂,₃; 7.25-7.41, m, 15H, Ph. ¹³C n.m.r. (75.5 MHz) δ 54.14, Cl; 72.10, 75.26, 74.45, 3C, CH₂; 80.98, 84.10, 85.60, C₄,5,6; 126.35-138.63, C₂,3,PH. High-resolution mass spectrum (f.a.b.) m/z 416.2221 (C₂₇H₃₀NO₃ [M+H]+) requires 416.2226.

(1S,2S,3R,6R)-6-Aminocyclohex-4-ene-1,2,3-triol, (-)-Conduramine B (29)

Small pieces of sodium metal were added to a solution of the amine (153) (370 mg) in THF (10 mL) and ammonia (30 mL) at -78° under nitrogen until a blue colour persisted for one hour. NH₄Cl was added so as to dissipate the blue colour and the solution was left to stand at room temperature until the ammonia had evaporated. The colourless mixture was concentrated to leave an orange residue, that was dissolved in water. This aqueous solution was washed with ether, acidified with HCl (1 M) and freeze-dried. The residue was dissolved in water and applied to a column of cation-exchange resin (Dowex 50W-X2, H⁺). The column was washed with water, then eluted with aqueous ammonia (4 M). Freeze-drying of the ammoniacal eluant gave a tan solid which was
dissolved in water and filtered through a short column of Sephadex A-25 (H₂O). Acidification of the filtrate with HCl (1 M), followed by freeze-drying, gave the hydrochloride of (-)-conduramine B (29) as pale brown needles (120 mg, 75%), m.p. 202° (d.), [α]D −22° (MeOH). ¹H n.m.r. (300 MHz, D₂O) δ 3.39, dd, J₁,₂ 10.4, J₁,₆ 8.0 Hz, H₁; 3.52, dd, J₂,₃ 9.0 Hz, H₂; 3.72-3.78, m, H₆; 4.02-4.08, m, H₃; 5.50, 5.72, 2ddd, J₃,₄ = J₃,₅ = J₄,₆ = J₅,₆ 2.3, J₄,₅ 10.4 Hz, H₄,₅. ¹³C n.m.r. (75.5 MHz) δ 54.30, C₆; 71.32, 71.70, 75.56, C1,2,3; 121.96, 133.71, C4,5. High-resolution mass spectrum (f.a.b.) m/z 146.0812 (C₆H₁₂NO₃ [(M–Cl)+] requires 146.0817). A portion of the hydrochloride was acetylated under normal conditions (Ac₂O/pyridine/DMAP) to afford, after normal workup (CH₂Cl₂) and flash chromatography (EtOAc), the acetamide (154) as white needles, m.p. 139-40° (Pr₂O), [α]D −169° (Found: C, 53.5; H, 6.1. C₂₉H₃₃N₀₄ requires C, 53.7; H, 6.1%). ¹H n.m.r. (300 MHz) δ 1.95, 2.03, 2.05, 3s, 12H, Me; 4.79-4.88, m, H₁; 5.06, dd, J₁,₆ 9.1, J₅,₆ 10.7 Hz, H₆; 5.33, dd, J₄,₅ 7.8 Hz, H₅; 5.49-5.57, m, H₄; 5.57-5.68, m, H₂,₃; 5.80, d, J₁,NH 8.8 Hz, NH. ¹³C n.m.r. (75.5 MHz) δ 20.63, 20.67, 20.88, 23.20, 4C, Me; 50.92, C₁; 71.38, 71.72, 71.84, C₄,5,6; 125.99, 129.67, C₂,3; 169.69, 169.98, 170.41, 171.23, 4C, CO.

O-[1S,4S,5R,6S)-4,5,6-Tribenzyloxyxycyclohex-2-en-1-yl] S-Methyl Dithiocarbonate (157)

The allylic alcohol (145)¹²⁵ (200 mg, 0.48 mmol), TBAI (18 mg, 0.05 mmol) and CS₂ (75 µL, 1.2 mmol) were added to a stirred suspension of pre-washed (petrol) NaH (18 mg, 0.7 mmol) in THF (10 mL) under nitrogen. After three hours, t.l.c. analysis showed complete consumption of the allylic alcohol. MeI (37 µL, 0.60 mmol) was then added to the yellow suspension with stirring (10 min) and the reaction was quenched with water (5 mL). Concentration of the mixture, followed by normal workup (CH₂Cl₂), afforded a yellow oil which was used without further purification for the next step. A portion of the oil was purified by flash chromatography (EtOAc/petrol, 1:9) to afford
the xanthate (157) as a colourless oil, [α]D +69°. 1H n.m.r. (300 MHz) δ 2.58, s, Me; 3.89, 4.00, 2 dd, J1,6 ≈ J4,5 7.6, J5,6 10.4 Hz, H5,6; 4.28-4.32, m, H4; 4.72-5.01, m, 6H, CH2; 5.78, 5.88, 2 ddd, J1,2 ≈ J1,3 ≈ J2,4 ≈ J3,4 2.1, J2,3 10.4 Hz, H2,3; 6.47-6.52, m, H1; 7.25-7.48, m, 15H, Ph. 13C n.m.r. (75.5 MHz) δ 19.11, Me; 72.42, 75.10, 75.56, 3C, CH2; 79.42, 81.20, 83.20, 83.49, C1,4,5,6; 124.65-138.38, C2,3,Ph; 215.36, C=S.

S-[(lR,4R,5S,6R)-4,5,6-Tribenzyloxycyclohex-2-en-1-yl] S-Methyl Dithiocarbonate (158)

The crude xanthate (157) was dissolved in toluene (30 mL) and heated under reflux for 30 min. The solution was concentrated and the residual oil was subjected to flash chromatography (EtOAc/petrol, 1:9) to afford the dithiocarbonate (158) as a colourless oil [206 mg, 85% from (145)], [α]D -112° (Found C, 68.5; H, 6.2. C28H30O4S2 requires C, 68.8; H, 6.0%). 1H n.m.r. (300 MHz) δ 2.57, s, Me; 3.75, 3.82, 2dd, J1,6, J4,5 7.2, 8.3, J5,6 9.7 Hz, H5,6; 4.22-4.28, m, H4; 4.39-4.46, m, H1; 4.71-4.93, m, 6H, CH2; 5.66, 5.76, 2ddd, J1,2 ≈ J1,3 ≈ J2,4 ≈ J3,4 2.2, J2,3 10.2 Hz, H2,3; 7.22-7.45, m, 15H, Ph. 13C n.m.r. (75.5 MHz) 13.09, Me; 48.43, C1; 72.38, 75.26, 75.82, 3C, CH2; 79.39, 81.10, 84.12, C4,5,6; 127.38-138.54, C2,3,Ph; 188.75, CO.

![Thiol structure](159)

(1R,4R,5S,6R)-4,5,6-Tribenzyloxycyclohex-2-ene-1-thiol (159)

A solution of the dithiocarbonate (158) (510 mg) in THF (20 mL) and MeOH (10 mL) was treated with aqueous NaOH (5 mL of 5 M) at room temperature for 25 min. The solution was neutralized with AcOH (70%) and the volatile solvents were removed. Normal workup (CH2Cl2) and flash chromatography (EtOAc/petrol, 1:9) gave the thiol (159) as white flakes (370 mg, 86%), m.p. 65-66° (Pr2O/petrol), [α]D -145°. 1H n.m.r. (300 MHz) δ 1.92, d, J1,SH 7.1 Hz, SH; 3.56, dd, J4,5 9.8, J5,6 9.6 Hz, H5; 3.58-3.62, m, H1; 3.69, dd, J1,6 7.5 Hz, H6; 4.21-4.37, m, H4; 4.65-4.98, m, 6H, CH2; 5.58-5.68, m, H2,3; 7.20-7.48, m, 15H, Ph. 13C n.m.r. (75.5 MHz) δ 42.80, C1; 72.43, 75.34, 76.21,
The thiol (36) (150 mg) was debenzylated and acetylated as for the amine (153) to afford, after normal workup (EtOAc) and flash chromatography (EtOAc/petrol, 1:3), the thioacetate (160) (98 mg, 84%) as white needles, m.p. 96-98° (EtOH), [α]D -146° (Found C, 51.2; H, 5.0. C_{14}H_{18}O_{7}S requires C, 51.2; H, 4.9%). ¹H n.m.r. (300 MHz) δ 2.00, 2.03, 2s, 9H, OAc; 2.32, s, SAc; 4.32-4.41, m, H1; 5.22-5.36, m, H5,6; 5.50-5.56, m, H4; 5.57-5.69, m, H2,3. ¹³C n.m.r. (75.5 MHz) 20.67, 20.89, 3C, OCOCH₃; 30.44, SCOCH₃; 43.97, C1; 70.24, 70.97, 71.90, C4,5,6; 125.97, 129.08, C2,3; 169.78, 170.22, 3C, CO; 193.73, SCO. High-resolution mass spectrum (f.a.b.) m/z 331.0855 (C_{14}H_{19}O_{7}S [(M+H)^+]) requires 331.0852).

Bis[(1R,4R,5S,6R)-4,5,6-Trihydroxycyclohex-2-en-1-yl] Disulfide (161)

A solution of the thioacetate (160) (45 mg, 0.13 mmol) in MeOH (5 mL) was treated with NaOMe (260 µL of 1 M in MeOH, 0.26 mmol) at room temperature for 30 min. Cation-exchange resin (Dowex 50W-X8, H⁺) was added and the subsequent mixture was stirred for 30 min. Filtration and concentration, followed by freeze-drying of the residue, gave the disulfide (161) (23 mg) as a pale yellow powder, m.p. 162-168°, [α]D -383° (MeOH). ¹H n.m.r. (300 MHz, CD₃OD) δ 3.29-3.36, m, H1; 3.30, dd, J_{4,5} 7.9, J_{5,6} 10.0 Hz, H5; 3.48, dd, J_{1,6} 8.7 Hz, H6; 3.96-4.00, m, H4; 5.48-5.62, m, H2,3. ¹³C n.m.r.(75.5 MHz) δ 56.68, C1; 73.30, 74.50, 78.63, C4,5,6; 128.83, 132.54, C2,3.
(1S,2S,3R,6R)-6-Sulfanylcyclohex-4-ene-1,2,3-triol (30)

A solution of the thioacetate (160) (57 mg, 0.17 mmol) and EtSH (11 μL, 0.17 mmol) in MeOH (5 mL) was treated with NaOMe (1.0 mL of 0.5 M in MeOH, 0.50 mmol) at room temperature for one hour. Cation-exchange resin (Dowex 50W-X8, H⁺) was added and the subsequent mixture was stirred for 30 min. Filtration and concentration gave the thiol (30) (30 mg) as a pale yellow solid, m.p. 100-105°, [α]D -220° (MeOH).

\[ \text{Mass spectrum (f.a.b.) } m/z \ 322 \ (M^+) \]

**Methyl 2-O-Benzoyl-3,4-O-isopropylidene-α-L-arabinoside (167)**

BzCl (1.2 mL, 10 mmol) was added to a stirred solution of methyl 3,4-O-isopropylidene-α-L-arabinoside (166)\(^{134}\) (1.8 g, 9.3 mmol) in CH₂Cl₂ (20 mL) and pyridine (9 mL) at 0° under nitrogen. After 30 min, water (2 mL) was added and stirring was continued for five min. The reaction mixture was concentrated and the residue was subjected to a normal workup (CH₂Cl₂) to afford a white residue which was recrystallised to afford the **benzoate (167)** as colourless needles (2.15 g, 78%), m.p. 123-127° (Pr\(\text{O} /\text{petrol})\), [α]D +31° (Found; C, 62.7; H, 6.5. C\(_{16}\)H\(_{20}\)O\(_6\) requires C, 62.3; H, 6.5%). \[ \text{1H n.m.r. (300 MHz, CD₃OD) } \delta 1.36, 1.59, 2s, CMe₂; 3.43, s, OMe; 4.12, dd, J\(_{4,5}\) 4.4, J\(_{5,5}\) 12.9 Hz, H5; 4.12, dd, J\(_{4,5}\) 4.1 Hz, H5; 4.29-4.38, m, H3,4; 4.47, d, J\(_{1,2}\) 6.0 Hz, H1; 5.29, dd, J\(_{2,3}\) 6.0 Hz, H2; 7.40-8.09, m, Ph. \] \[ \text{13C n.m.r. (75.5 MHz) } \delta 26.13, 27.77, CMe₂; 56.1, OMe; 61.77, C5; 72.05, 72.62, 75.72, C2,3,4; 100.66, C1; 110.50, CMe₂; 128.06-133.14, Ph; 167.27, CO. \]
Methyl 2,3-Di-O-benzoyl-α-L-arabinopyranoside (164)

A mixture of the benzoate (167) (1.17 g) and aqueous AcOH (20 mL of 80%) was heated at 100° for 15 min. The mixture was concentrated, followed by co-evaporation with dry pyridine (2 x 20 mL). The resultant residue was treated with BzCl (510 μL, 4.4 mmol), pyridine (5 mL) and CH₂Cl₂ (15 mL) at -40° under nitrogen. The mixture was allowed to warm to -30° over 25 min. MeOH (2 mL) was added and stirring was continued for five min. Normal workup (CH₂Cl₂) and flash chromatography (EtOAc/petrol, 2:3) gave the dibenzoate (164) as a colourless foam (1.0 g, 71% over two steps), a portion of which was crystallised to give white needles, m.p. 99° (PrO), [α]D +116° (Found: C, 64.3; H, 5.2. C₂₀H₂₀O₇ requires C, 64.5; H, 5.4%). ¹H n.m.r. (300 MHz) δ 3.52, s, OMe; 3.77, dd, J₄,₅ 2.3, J₅,₅ 12.4 Hz, H₅; 4.12, dd, J₄,₅ 4.4 Hz, H₅; 4.27-4.32, m, H₄; 4.59, d, J₁₂,₅ 6.0 Hz, H₁; 5.38, dd, J₂,₃ 8.4, J₃,₄ 3.2 Hz, H₃; 5.61, dd, H₂; 7.36-8.04, m, 10H, Ph. ¹³C n.m.r. (75.5 MHz) δ 56.50, OMe; 64.31, C₅; 66.46, 69.72, 72.96, C₂,3,₄; 101.36, C₁; 128.35-133.43, Ph; 165.27, 166.00, 2C, CO.

Methyl 2,3-Di-O-benzoyl-4-O-trifluoromethylsulfonyl-α-L-arabinoside (162)

Tf₂O (160 μL, 1.2 mmol) in CH₂Cl₂ (5 mL) was added dropwise to a solution of the dibenzoate (164) (270 mg, 0.73 mmol) and pyridine (130 μL, 1.6 mmol) in CH₂Cl₂ (10 mL) at -78° under nitrogen. The stirred solution was allowed to warm to -50° over one hour and was then poured into ice-cold, saturated NaHCO₃ solution (30 mL). The organic layer was separated and the aqueous layer was extracted twice with CH₂Cl₂. The combined organic extracts were dried and concentrated. Flash chromatography
(EtOAc/petrol, 1:3) of the residue gave the triflate (162) as lustrous, plates (320 mg, 84%), m.p. 117-118° (Pr<sub>2</sub>O/petrol), [α]_D +99° (Found; C, 50.1; H, 3.8. C<sub>21</sub>H<sub>19</sub>F<sub>3</sub>O<sub>9</sub>S requires C, 50.0; H, 3.8%). ¹H n.m.r. (300 MHz) δ 3.53, s, OMe; 3.93, dd, J<sub>4,5</sub> 1.5, J<sub>5,5</sub> 13.6 Hz, H5; 4.36, dd, J<sub>4,5</sub> 3.3 Hz, H5; 4.62, d, J<sub>1,2</sub> 6.7 Hz, H1; 5.33-5.42, m, H4; 5.53, dd, J<sub>2,3</sub> 9.4, J<sub>3,4</sub> 3.2 Hz, H3; 5.65, dd, H2; 7.37-8.03, m, m, 10H, Ph. ¹³C n.m.r. (75.5 MHz) δ 56.91, OMe; 62.91, C5; 68.96, 69.86, 81.47, C2,3,4; 101.84, C1; 118.36, q, J<sub>C,F</sub> 319 Hz, CF<sub>3</sub>; 128.24-133.82, Ph; 164.92, 165.53, 2C, CO.

(Methyl 2,3-Di-O-benzoyl-4-deoxy-4-[(1R,4R,5S,6S)-4,5,6-tribenzyloxycyclohex-2-en-1-yl]amino-ß-D-xyloside (168)

A solution of the amine (153) (450 mg, 1.1 mmol) and the triflate (162) (220 mg, 0.44 mmol) in DMI (5 mL) was heated at 60° for 20 hours. The deep orange solution was cooled to room temperature and Et₃N (200 μL, 1.4 mmol) was added. After five minutes, the solution was subjected to normal workup (Et₂O) to afford an orange oil. This oil was subjected to flash chromatography (EtOAc/petrol/Et₃N, 20:79:1 to EtOH/EtOAc/petrol/Et₃N, 4:20:75:1) to give, firstly, the amine (168) as needles (190 mg, 56%), m.p. 110-111° (Pr<sub>2</sub>O/petrol), [α]_D -64° (Found; C, 73.2; H, 6.1%. C<sub>47</sub>H<sub>47</sub>N<sub>9</sub>O<sub>9</sub> requires C, 73.3; H, 6.1%). ¹H n.m.r. (500 MHz) δ 3.17-3.23, m, H4; 3.32, dd, J<sub>4,5</sub> 9.2, J<sub>5,5</sub> 11.9 Hz, H5; 3.36-3.43, m, H1',6'; 3.50, s, OMe; 3.71, dd, J<sub>4,5</sub> = J<sub>5,6</sub> 4.5 Hz, H5'; 4.11-4.14, m, H4'; 4.18, dd, J<sub>4,5</sub> 4.5 Hz, H5; 4.52-4.56, m, H1; 4.59-4.95, m, 6H, CH<sub>2</sub>Ph; 5.28-5.36, m, H2,3; 5.35-5.49, m, H2',3'; 7.30-7.97, m, Ph. ¹³C n.m.r. (125.8 MHz) δ 56.11, 59.47, C1',4; 56.65, OMe; 64.95, C5; 71.69, 74.42, 80.09, 83.17, 83.98, C2,3,4',5',6'; 72.16, 75.24, 75.58, 3C, CH<sub>2</sub>Ph; 101.99, C1; 126.08-138.62, C2',3',Ph; 165.32, 166.57, 2C, CO. Next to elute from the column was the amine (153) (220 mg).
Methyl 2,3-Di-O-acetyl-4-deoxy-4-[[1R,4R,5S,6S]-4,5,6-triacetoxycyclohex-2-en-1-yl]amino-$\beta$-D-xyloside (174)

Sodium metal (5 mg) was added to a solution of the amine (168) (120 mg) in dry MeOH (10 mL) and THF (5 mL). After 40 min, the solution was concentrated. The residue was debenzylated and subsequently acetylated as described for the amine (153). Normal workup (CH$_2$Cl$_2$) and flash chromatography (EtOAc/petrol/Et$_3$N, 30:69:1 to 50:49:1) afforded the amine (174) as a foam (70 mg, 88%), [α]$_D$ -180° (Found; C, 54.2; H, 6.4%. C$_{22}$H$_{31}$NO$_2$ requires C, 54.4; H, 6.4%). $^1$H n.m.r (300 MHz) δ 1.98, 2.01, 2.02, 2.03, 4s, 15H, Me; 2.82-2.91, m, H4; 3.11, dd, J$_{4,5}$ 9.7, J$_{5,5}$ 11.9 Hz, H5; 3.30-3.42, m, H1'; 3.43, s, OMe; 3.89, dd, J$_{4,5}$ 4.7 Hz, H5; 4.25-4.30, m, H1; 4.78-4.83, m, H2,3; 4.95, dd, J$_{1',6'}$ 8.6, J$_{5',6'}$ 10.7 Hz, H6', 5.22, dd, J$_{4',5}$ 8.0 Hz, H5'; 5.46-5.49, m, H4'; 5.58-5.76, m, H2',3'. $^{13}$C n.m.r. (75.5 MHz) δ 20.63, 20.67, 20.71, 20.82, 5C, Me; 56.25, 57.77, C1',4; 56.67, OMe; 64.91, C5; 71.40, 71.72, 71.87, 73.18, 74.19, C2,3,4',5',6'; 101.80, C1; 125.06, 130.50, C2',3'; 169.65, 170.05, 170.243, 170.82, 5C, CO.

Methyl 2,3-Di-O-benzoyl-4-deoxy-4-[[1R,4R,5S,6S]-4,5,6-tribenzoyloxy cyclohex-2-en-1-yl]thio-$\beta$-D-xyloside (173)

A solution of the triflate (162) (170 mg, 0.34 mmol), the thiol (159) (160 mg, 0.38 mmol) and DBU (56 µL, 0.38 mmol) in toluene (3 mL) was stirred (6 h). The solution was then diluted with more toluene and washed with water and brine and dried. Concentration of the solution afforded a colourless oil which was purified by flash chromatography (EtOAc/petrol, 1:9 to 1:3) to give the D-xyloside (173) as a colourless oil (193 mg, 72%), a portion of which was crystallised to give needles, m.p. 108-
109°(Pr12O), [α]D –5.7° (Found; C, 71.4; H, 6.0. C47H46O9S requires C, 71.6; H, 6.0%).

1H n.m.r. (300 MHz) δ 3.12-3.23, m, H4; 3.45-3.71, m, 5H, H5,1',5',6'; 3.51, s, Me; 4.18-4.27, m, H4'; 4.56, d, J1,2 7.1 Hz, H1; 4.59-4.89, m, 6H, CH2Ph; 5.32, dd, J2,3 8.9 Hz, H2; 5.48, dd, J3,4 10.2 Hz, H3; 5.50-5.68, m, H2',3'; 7.11-8.98, Ph. 13C n.m.r. (75.5 MHz) δ 44.53, 50.23, C4,1'; 56.81, Me; 68.33, C5; 72.53, 72.96, 76.62, 83.06, 84.40, C2,3,3',4',5'; 72.63, 75.40, 76.19, 3C, CH2Ph; 102.14, C1; 127.49-138.52, C2,3',Ph; 165.34, 165.62, 2C, CO.

AcO
AcO
S

(175)

Methyl 2,3-Di-O-acetyl-4-deoxy-4-[(IR,4R,5S,6S)-4,5,6-triacetyloxycyclohex-2-en-1-yl]thio-D-xyloside (175)

The xyloside (173) (250 mg) was debenzoylated, debenzylated and acetylated as described for the amine (168) to afford, after normal workup (EtOAc) and flash chromatography (EtOAc/petrol, 3:7), the sulfide (175) as a white foam (92 mg, 55%), [α]D –190° (Found; C, 51.0; H, 6.0. C22H30O12S requires C, 51.0; H, 6.2 %). 1H n.m.r. (300 MHz) δ 2.02, 2.04, 2.06, 2.08, 15H, Me; 2.90-3.00, m, H4; 3.32, dd, J4,5 = J5,5 11.3 Hz, H5; 3.48, s, OMe; 3.55-3.61, m, H1'; 4.06, dd, J4,5 5.0 Hz, H5; 4.31, d, J1,2 7.4 Hz, H1; 4.80-4.99, m, H5',6'; 5.15-5.26, m, H2,3; 5.52-5.58, m, H4'; 5.61-5.78, m, H2',3'. 13C n.m.r. (75.5 MHz) δ 20.65, 20.73, 20.85, 5C, Me; 46.04, 46.95, C1',4; 56.84, OMe; 65.98, C5; 71.01, 72.35, 72.55, 72.77, C2,3,4',5',6'; 102.05, C1; 126.55, 129.23, C2,3'; 169.66-170.09, 5C, CO.
Kinetic Analysis

Inhibition constants were determined at 37° using a 0.05 M sodium citrate buffer (pH 5.5) and phenyl β-D-xylopyranoside as the substrate. Measurements were started at the addition of the enzyme. Measurements of the increase in absorption (at 277 nm) with time in a continuous assay yielded reaction rates. Michaelis parameters ($V_{\text{max}}$ and $K_m$) were extracted from these data by best fit to the Michaelis-Menten equation ($V_{\text{max}} = 0.808 \text{ A/min}, K_m = 2.05 \text{ mM}$). $K_i$ values were obtained by measuring rates in a series of cells at a fixed substrate concentration, in the presence of a range of inhibitor concentrations. The observed rates were plotted in the form of a Dixon plot, and the $K_i$ value was determined by an intersection of this line with a horizontal line drawn through $1/V_{\text{max}}$. The per-acetates were deacetylated (NaOMe, MeOH) until pure (by t.l.c.). The methanolic solutions were neutralised, filtered and the filtrate concentrated. The residues were purified by gel chromatography and then dried under high vacuum prior to the inhibition assay.

\[ V_{\text{max}} \text{ Determination} \]

\[
\begin{align*}
\text{1 / Velocity (V)} & = \text{R}^2 = 0.9982 \\
\text{V} = \Delta\text{abs. / min.} & \\
\text{1 / Substrate Concentration (mM)} & \\
\end{align*}
\]
\( K_i \) Determination of the Secondary Amine (24)

\[
\frac{1}{V} = \frac{1}{V_{\text{max}}} + \frac{K_i}{V_{\text{max}}} \cdot C
\]

\( V = \frac{\Delta \text{abs.}}{\text{min.}} \)

\[
R^2 = 0.9989
\]

\( 1/V_{\text{max}} \)

\[ \text{Inhibitor Concentration (μM)} \]

\( K_i \) Determination of the Sulfide (25)

\[
\frac{1}{V} = \frac{1}{V_{\text{max}}} + \frac{K_i}{V_{\text{max}}} \cdot C
\]

\( V = \frac{\Delta \text{abs.}}{\text{min.}} \)

\[
R^2 = 0.9925
\]

\[ \text{Inhibitor Concentration (mM)} \]
Determining the Amine (29)

\[ K_i \]

\[ V = \Delta \text{abs.} / \text{min.} \]

\[ \frac{1}{V} = \frac{1}{V_{\text{max}}} \]

\[ R^2 = 0.9953 \]

Inhibitor Concentration (mM)
Chapter 3

The Synthesis of a 4'-Glycosylated Derivative of Methyl β-Acarviosin
Discussion

The major challenge in a synthesis of the carba-trisaccharide (26) is the attachment of the two sugar residues to the core epivalienamine moiety (176), by glycosylation with a glycosyl donor (177) and by alkylation with a triflate (178) (Scheme 1). With respect to the glycosylation, extensive protecting group manipulation would be required to furnish a free hydroxyl group on the epivalienamine moiety (176), thus greatly complicating the synthesis of the carba-trisaccharide (26).

A synthesis starting from cellobiose, with chemical manipulation to produce the epivalienamine unit, would overcome this requirement for extensive protecting group manipulations by eliminating the need for a glycosylation. An excellent synthesis of the epivalienamine derivative (36), from methyl α-D-glucopyranoside, exists in the literature91 and it was decided to adapt this sequence, starting from cellobiose.
Below is the proposed retrosynthetic analysis for the synthesis of the carba-trisaccharide (26) (Scheme 2). The synthesis begins with the conversion of cellobiose (179) into a methyl glycoside. In order to manipulate the hydroxyl group at C6, the other primary hydroxy group must be blocked. A benzylidene acetal was thought ideal for this purpose. The acetal (180) could then be transformed into the amine (181), in a fashion analogous to that reported for the conversion of methyl α-D-glucopyranoside into the amine (36). Alkylation with the triflate (178), followed by deprotection steps, would then furnish the target compound (26).

Scheme 2
The transformation from a hemiacetal to a methyl glycoside may be carried out in one step (HCl, MeOH) but, based on previous experience, such reactions normally give an anomeric mixture, which can be difficult to purify. Instead, methyl β-cellobioside (182) was prepared from cellobiose (179) in a series of standard reactions. Firstly, cellobiose was acetylated (NaOAc, Ac₂O) to give the octa-acetate (183). Treatment of (183) with HBr in AcOH gave the cellobiosyl bromide (184), which was subsequently converted (Ag₂CO₃, MeOH) into the β-cellobioside (185). Finally, transesterification (Na, MeOH) of the acetyl groups afforded the polyol (182). This four step sequence conveniently furnished methyl β-cellobioside (182) in 67% overall yield from cellobiose.

The next task was to block the hydroxyl groups at C4' and C6' as a benzylidene acetal. Under the conditions as described by Takeo and co-workers (benzaldehyde dimethyl acetal in DMF under reduced pressure), varying yields of the benzylidene acetal (180) were obtained (48% to 73%), with several unidentified side-products also being produced (t.l.c.). Alternatively, the general benzylideneation procedure of Stütz (benzaldehyde dimethyl acetal and anhydrous HBF₄) was investigated, yet offered no advantage over the previously described method. This methodology appeared to furnish the benzylidene acetal (180) exclusively (t.l.c.), yet subsequent isolation of the product (180), from the one equivalent of HBF₄ used proved troublesome.
A regioselective iodination was now performed on the free primary hydroxyl group of the benzylidene acetal (180). This transformation has been carried out in two steps by Takeo and co-workers: first the installation of a tosylate at C6, followed by displacement of the tosylate with iodide, to give (186). More convenient was the general method of Garegg (I₂, imidazole, Ph₃P), which allowed the direct formation of the iodide (186) from the alcohol (180). Subsequent acetylation (Ac₂O, pyridine, DMAP) afforded (187). The installation of acetyl protecting groups warrants a special mention, as they are replaced with benzyl groups after only one subsequent step. Benzyl groups cannot be introduced at this stage as the conditions necessary for their introduction (NaH, BnBr) lead to the formation of 3,6-anhydro sugars when there is a good leaving group at C6.49

![Chemical Structures](image)

Dehydroiodination of (187) was performed with DBU, in refluxing THF, to give the alkene (188). Interestingly, this transformation was complete in thirty minutes, as opposed to the analogous dehydroiodination of the iodide (189), which takes eight hours. Perhaps this is owing to the axial methyl aglycon of (189) inhibiting the approach of DBU to H5.

![Chemical Structures](image)

Deacetylation (NaOMe, MeOH) of the alkene (188), followed by benzylation (BnBr, NaH), gave (190). Ferrier rearrangement [(Hg(CF₃COO))₂] of (190) gave an inseparable mixture of compounds, presumably the β-hydroxy ketones (191a) and (191b). Subsequent dehydration (MsCl, Et₃N) furnished the enone (192).
The addition the Grignard reagent derived from benzyl chloromethyl ether to the enone (125), as described by Nicotra and co-workers, proceeds with >95 % stereoselectivity, giving the pseudo-equatorial alcohol (193).

A similarly stereoselective addition was expected, when using the carba-disaccharide enone (192).
Surprisingly, treatment of the enone (192) with the Grignard reagent derived from benzyl chloromethyl ether proved to be non-stereoselective. The two diastereoisomers (194) and (195) were produced in a 1:1 ratio (t.l.c.), with the isolated yields of pseudo-equatorial (194) and pseudo-axial alcohols (195) being 34 and 40% yields, respectively. Complicating things further, the purification of the diasteroisomers (194) and (195) by flash chromatography was extremely difficult, owing to their similar polarities.

The stereochemistry, at C1, of each of the alcohols was established by NOE experiments (Figure 1). A noticeable correlation exists between H5 and 7 of the pseudo-equatorial alcohol (194), which is lacking for the pseudo-axial alcohol (195).

Figure 1. Partial NOE spectra of the pseudo-axial alcohol (195) (left) and pseudo-equatorial alcohol (194) (right).
It is interesting to speculate why this Grignard addition fails to proceed stereoselectively, compared to the analogous addition to the enone (125). Presumably, the oxygen at C6 [of the enone (125)] plays a central role in directing addition from below the carbocyclic ring. However, the oxygen at C6 of the carba-disaccharide enone (192) exhibits no such chelation control. Still and McDonald found a similar outcome when examining the effect of a range of oxygen protecting groups on the stereoselectivity of addition of butylmagnesium bromide to chiral α-alkoxyketones.\textsuperscript{147} As shown below, a benzyl protecting group gave excellent chelation control whilst a THP acetal exhibited only moderate control. Based on these findings, and the similarities between a sugar residue and a THP ether, it is not surprising, in hindsight, that the addition of the Grignard reagent to the enone (192) proceeded non-stereoselectively.

\[
\begin{align*}
\text{C}_7\text{H}_{15} \quad \text{H} \quad \text{OR} & \quad \overset{\text{C}_4\text{H}_9\text{MgBr}}{\xrightarrow{\text{THF}}} & \quad \text{C}_7\text{H}_{15} \quad \text{H} \quad \text{OR} \quad \text{C}_4\text{H}_9 \quad \text{OH} \\
\text{R} = \text{Bn} & & \text{Threo} & & \text{R} = \text{THP} & & \text{Threo/Erythro} \\
\text{Threo/Erythro} & & \text{200} & & \text{3} 
\end{align*}
\]

Efforts were made to increase the stereoselectivity of the Grignard addition to the enone (192). These included a low temperature reaction (-78°), the changing of the solvent from THF to toluene,\textsuperscript{148} and the addition of cerium(III) chloride\textsuperscript{149} to the reaction mixture. However, under all of these conditions, no addition of the Grignard reagent was observed. The scope of the reaction seemed to be limited by the relative unreactivity of the Grignard reagent.
The introduction of the hydroxymethyl functionality by other means was considered, yet none seemed to offer an advantage to the method described above. For example, Tagmose and Bols found that the addition of benzyloxymethylolithium to the cyclohexenone (196) proceeded non-stereoselectively at $-78^\circ$, giving a 1:1 ratio of the diastereoisomers (197) and (198). Some selectivity was observed when conducting the reaction at $-110^\circ$; the pseudo-axial alcohol (198) was produced in a 6-fold excess over the pseudo-equatorial alcohol.

![Chemical structure of 196, 197, 198](image1)

Continuing with the planned synthesis of the amine (181), the pseudo-equatorial alcohol (194) was acetylated, under forcing condition ($60^\circ$, 5 h), to give the acetate (199) in good yield. The next step of the sequence was to introduce the azide functionality by means of Pd(0)-catalysed allylic substitution. Disappointingly, prolonged exposure of the acetate (199) to sodium azide and Pd(PPh$_3$)$_4$, in a solution of refluxing THF/water, gave none of the desired azide (200).

![Chemical structure of 199, 200](image2)

Panza and co-workers, during a synthesis of the valienamine derivative (201), treated the alcohol (193) with thionyl chloride, to give the rearranged chloride (202). Substitution with azide and subsequent reduction gave the amine (201) (Scheme 3).
It was hoped that an analogous sequence, using the pseudo-axial alcohol (195), would procure the amine (181). However, treatment of the alcohol (195) with thionyl chloride, under the conditions described by Panza,71 afforded none of the desired chloride (203).

With these disappointing results, the viability of the sequence was questioned. While undoubtedly there are other methods that could be attempted to convert either of the alcohols (194) and (195) into the amine (181), the difficulty in procuring significant quantities of these alcohols curtailed the attractiveness of any further investigations.
The glycosylation of the known carba-disaccharide (20) was now viewed as an alternative means of procuring the carba-trisaccharide (26). Conceivably, protecting group manipulations on (20) could provide an appropriate acceptor for glycosylation by conventional chemical techniques. To avoid any such manipulations, it was decided to pursue an enzyme-assisted, transglycosylation approach - a glycosynthase seemed ideal for this purpose (Scheme 4).

Following is a very brief review on glycosynthases and some of their applications in synthetic carbohydrate chemistry.
**Glycosynthases**

Glycosidases are normally thought of as enzymes that catalyse the hydrolysis of glycosidic linkages. However, glycosidases that operate by a double displacement mechanism, involving a covalent glycosyl-enzyme intermediate, can also be used to form glycosidic linkages. This type of transglycosylation takes place by the interception of the normal glycosyl-enzyme complex with a sugar acceptor - thus forming a new glycosidic bond. The practical use of glycosidases to form glycosidic linkages is limited by the reversibility of the transglycosylation; the product can be a substrate for the enzyme. Withers and co-workers came up with a simple yet ingenious solution to this problem, by mutating a glycosidase so that the glycosidic linkage can be formed but not hydrolysed.\(^{150}\) The mechanism of action of such a mutant glycosidase, named a glycosynthase, is shown below (Figure 2). In this case, the active site carboxyl residue, essential for hydrolysis of a glycosidic linkage, was replaced with a neutral group by site directed mutagenesis of the active site glutamate.

A α-D-glycosyl fluoride was selected as the donor for two reasons; firstly, the good leaving ability of the fluoride atom (\(k_{cat} 5.5 \text{ min}^{-1}\) and \(K_m 53 \text{ mM}\) for Abg wild type),\(^{151}\) which ensures sufficient reactivity necessary for the transglycosylation reaction to occur and, secondly, the unnatural α-D-anomer reduces the incidence of
binding in the +1 subsite, where it could itself act as an acceptor. The scope of the reaction extends to using both α-D-glucopyranosyl fluoride and α-D-galactopyranosyl fluoride as donors and a wide suite of acceptors, including the D-gluco (205), D-manno (206), D-xylo (207), and 2-deoxy-2-fluoro D-gluco (208) sugars. Generally, the transglycosylation proceeds with both excellent regio- and stereoselectivity - the D-gluco and D-manno acceptors, (205), (206) and (208), give β-1,4- linked oligosaccharides, whilst the D-xylo acceptor (207) gives β-1,3-linkages. Additionally, the presence of an aryl glycoside, which binds well to the +1 (aglycon) site, greatly accelerates the transglycosylation, particularly with monosaccharides.

Additionally, some rather novel compounds have been shown to act as acceptors, including the isofagomine derivative (209) and the oxazine (210). Both of these acceptors gave β-1,4- linked oligosaccharides. Interestingly, modification of the hydroxyl groups of the acceptor can alter the regioselectivity of glycosylation. For example, the thiol (211) gives exclusively a β-1,3- linked disaccharide, whilst the benzoate (212) gives predominantly a β-1,2- linked disaccharide.
Since the original publication in 1998, additional glycosynthases have been cloned that give $\beta(1\rightarrow4),^{155,156} \beta(1\rightarrow3)^{157}$ and branching glycosylation [$\beta(1\rightarrow3), \beta(1\rightarrow4)$ and $\beta(1\rightarrow6)]^{158}$. Additionally, the utility of the original glycosynthase has been enhanced by mutating the nucleophilic amino acid from alanine to serine.\textsuperscript{155} This enzyme, coined a 'turbo-glycosynthase', gives up to a 24-fold improvement in synthetic rates over the original glycosynthase. This glycosynthase is used in the work described below.

**Discussion**

Before a glycosynthase reaction could be attempted, the donor, $\alpha$-D-glucopyranosyl fluoride (204), and acceptor, methyl $\beta$-acarviosin (20), had to be synthesised. The glycosyl fluoride (204) was synthesised in two steps from $\beta$-D-glucose penta-acetate (213). Firstly, treatment of (213) with HF-pyridine gave the protected glycosyl fluoride (214).\textsuperscript{159} Deacetylation (Na, MeOH) then furnished the fluoride (204). Careful attention had to be paid to the deacetylation of (214), as extended reaction times led to the decomposition (t.l.c.) of the product.

a) HF, pyridine; b) NaOMe, MeOH
The carba-disaccharide (20) has been synthesised previously in a lengthy sequence.\(^{47}\) However, quite conveniently, quantities of a precursor (215) to this compound (20) were available. The synthesis of (215) is described in Chapter 5. Thus, (215) was debenzoylated (Na, MeOH), debenzylated (Na, NH\(_3\)) and acetylated (Ac\(_2\)O, pyridine) to give (216). Subsequent deacetylation (Na, MeOH), followed by purification through a short column of Sephadex (A-25), gave the polyol (20).

![Chemical structures](image)

(215) \(R = \text{Bz}, R' = \text{Bn}, R'' = \text{H}\)
(216) \(R = R' = R'' = \text{Ac}\)
(20) \(R = R' = R'' = \text{H}\)

A solution of the carba-disaccharide (20) and \(\alpha\)-D-glucosyl fluoride (204) (1.5 equiv.) in aqueous NH\(_4\)HCO\(_3\) was treated with the glycosynthase AbgGlu358Ser. T.l.c. analysis of the reaction mixture showed the slow consumption of the donor (204) and the formation of two new compounds. On complete consumption of the donor (three days), the reaction mixture was lyophilised. Initial attempts at purifying this mixture by size exclusion chromatography (LH-20) failed. More satisfactory was the acetylation of the mixture, followed by flash chromatography. The major product isolated was the carba-trisaccharide (217) (42%); additionally, acetylated starting material (216) (24%) and the carba-tetrasaccharide (218) (6%) were also isolated.

The \(^1\)H n.m.r. spectrum of the carba-trisaccharide (217) revealed both the stereoselectivity and regioselectivity of the transglycosylation reaction. Firstly, the magnitude of the coupling constant, \(J_{1',2''}\) of 8.0 Hz showed that a \(\beta\)-linkage had been formed. The chemical shift of the signal for H4' of the carba-trisaccharide (217), at 4.20 ppm, is consistent with a glycosyl residue attached to O4'. It should be noted that the chemical shift of the signal for H4' of the carba-disaccharide (216) is at 5.67 ppm, consistent with an acetyl group at O4'.
Figure 3. Partial H,H-Cosy of the carba-trisaccharide (217)

a) AbgGlu358Ser, aq. NH₄HCO₃; b) Ac₂O, pyridine
The carba-trisaccharide (26) and carba-tetrasaccharide (219) have been assayed (performed by Dr Jon Fairweather in the laboratory of Prof. Marc Claeyssens) against a range of cellulases. At the same time, methyl β-acarviosin (20), methyl β-acarboside (22) and β-adiposin-2 (23), were also assayed. The results appear below (Table 1).

<table>
<thead>
<tr>
<th>Kᵢ (µM)</th>
<th>EGII (T. reesei)</th>
<th>CelC (C. thermocellum)</th>
<th>CBHII (T. reesei)</th>
<th>CBHI (T. reesei)</th>
<th>EGI (T. reesei)</th>
<th>EGI (H. insolens)</th>
<th>CelD (C. thermocellum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(22)</td>
<td>&gt;1000</td>
<td>nt</td>
<td>&gt;1000</td>
<td>180*</td>
<td>&gt;1000</td>
<td>110*</td>
<td>224*</td>
</tr>
<tr>
<td>(23)</td>
<td>210</td>
<td>nt</td>
<td>&gt;1000</td>
<td>190*</td>
<td>nt</td>
<td>&gt;1000</td>
<td>nt</td>
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<tr>
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<td>nt</td>
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<td>nt</td>
</tr>
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<td>nt</td>
<td>nt</td>
<td>410</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>(219)</td>
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<td>90</td>
<td>100</td>
<td>15</td>
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</table>

Table 1 (*indicates that the putative inhibitor was in fact a substrate for the enzyme. nt - not tested).

Particularly interesting is the comparison of the activities of the three carba-tetrasaccharides (219), (22) and (23), with (219) showing the greatest inhibition against the enzymes tested. This is perhaps owing to the other carba-tetrasaccharides being hydrolysed (in many cases) by the enzymes under study, whereas (219) was unaffected.
Experimental

\[
\begin{align*}
\text{Methyl } \beta\text{-Cellobioside (182)}
\end{align*}
\]

A mixture of cellobiose (20 g, 58 mmol) and NaOAc (10 g) was heated in Ac₂O (180 mL) under reflux, until the reaction mixture became clear (30 min). The reaction mixture was cooled (rt), and then poured into ice-cold water, resulting in the formation of a milky precipitate. This precipitate was collected and subjected to a normal workup (CH₂Cl₂) to give a colourless solid. This solid in CH₃COOH (100 mL) was treated with HBr in CH₃COOH (30% w/v, 19 mL; overnight). The solution was diluted with ice-cold water and subjected to a normal workup (CH₂Cl₂) to give an off-white solid. This solid was added to a mixture of Ag₂CO₃ (12 g, 44 mmol) and powdered 3 Å molecular sieves (10 g) in MeOH (100 mL) and CH₂Cl₂ (100 mL). The resultant mixture was stirred (3 h) in the absence of light. The mixture was filtered through a plug of silica (EtOAc) and the filtrate was concentrated to give a colourless solid. A small piece of sodium metal was added to a solution of this solid in MeOH (100 mL). Shortly afterwards, a solid began to crystallise from solution. After 1 h, the mixture was cooled and the solid collected. The filtrate was neutralised with resin [Dowex 50W-X8 (H⁺ form)], filtered and the filtrate concentrated to give a yellow residue. This residue and the previously collected solid were combined and recrystallised to give the β-cellobioside (182) as a microcrystalline solid (13.9 g, 67%), m.p. 192-194° (EtOH/MeOH; lit.¹⁶⁰ 198°).
Methyl 2,3-Di-O-acetyl-6-deoxy-6-iodo-4-O-(2,3-di-O-acetyl-4,6-O-benzylidene-β-D-glucosyl)-β-D-glucoside (187)

I$_2$ (800 mg, 3.4 mmol) was added to a solution of the benzylidene (180)\textsuperscript{144} (1.0 g, 2.2 mmol), Ph$_3$P (890 mg, 3.4 mmol) and imidazole (460 mg, 6.8 mmol) in dioxane (5 mL). The resultant white mixture was then heated (65°, 30 min). After the mixture was cooled (rt), H$_2$O (2 mL) was added to quench the reaction. The solution was concentrated, and the residue was treated with Ac$_2$O/pyridine (15 mL, 1:2) and DMAP (50 mg; 5 h). MeOH (3 mL) was added to the mixture, which was then concentrated. Normal workup (CH$_2$Cl$_2$) gave a crystalline mass, which was recrystallised to give the iodide (187) as needles, (1.2 g, 72%), m.p. 275° (d.) [EtOH/CHCl$_3$; lit.\textsuperscript{144} 268-70° (d.)], [α]$_D$ −48.5° (lit.\textsuperscript{144} −47.8°). $^1$H n.m.r. (500 MHz) δ 2.00, 2.03, 2.03, 2.12, 4s, 12H, Ac; 3.23-3.30, m, H5,6; 3.48-3.52, m, H5'; 3.51, s, OMe; 3.56-3.73, m, H4,6,4',6'; 4.33-4.37, m, H6', 4.44, d, J$_{1,2}$ 7.9 Hz, H1; 4.71, d, J$_{1',2'}$ 7.8 Hz, H1'; 4.88, dd, J$_{2,3}$ 9.5 Hz, H2; 4.94, dd, J$_{2',3'}$ 9.2 Hz, H2'; 5.17, dd, J$_{3,4}$ 8.9 Hz, H3; 5.28, dd, J$_{3',4'}$ 9.3 Hz, H3'; 5.47, s, CHPH; 7.34-7.42, m, Ph. $^{13}$C n.m.r. (125 MHz) δ 4.25, C6; 20.77, 20.90, 20.92, 4C, COMe; 57.01, OMe; 66.32, C5'; 68.36, C6'; 71.55, 71.81, 72.57, 72.59, C2,3,2',3'; 73.47, C5; 77.86, 80.40, C4,4'; 100.92, Cl; 101.34, C1'; 101.50, CHPH; 126.05-136.52, Ph; 169.25, 169.48, 169.61, 170.15, 4C, CO.
Methyl 2,3-Di-O-acetyl-6-deoxy-4-O-(2,3-di-O-acetyl-4,6-O-benzylidene-β-D-glucosyl)-β-D-xylo-hex-5-enoside (188)

The iodide (187) (1.0 g, 1.4 mmol) was treated with DBU (690 μL, 4.60 mmol) in THF (10 mL) at reflux (30 min). The solution was concentrated to give an orange oil. This oil was diluted in EtOAc (40 mL), and the resultant solution was washed with cold aqueous HCl (1 M), followed by a normal workup to give an off-white solid. Recrystallisation of this solid gave the alkene (188) as needles (0.71 g, 86%), m.p. 175° (EtOH), [α]D -112° (Found: C, 56.6; H, 5.9. C28H34O14 requires C, 56.5; H, 5.9%).

1H n.m.r. (300 MHz) δ 2.04, 2.06, 2.07, 2.08, 4s, 12H, Ac; 3.45-3.82, m, H4,,5',6'; 3.53, s, OMe; 4.32-4.42, m, H1,6'; 4.62-4.68, m, H2,6; 4.73, d, J1',2' 7.8 Hz, H1'; 4.80, dd, J4,6 ≈ J6,6 1.2 Hz, H6; 4.92, dd, J2,3 ≈ J3,4 5.1 Hz, H3; 4.98-5.07, m, H4,2'; 5.30, dd, J2',3' ≈ J3',4' 9.3 Hz, H3', 5.50, s, CHPh; 7.32-7.48, Ph. 13C n.m.r. (75.5 MHz) δ 14.54, 20.71, 20.81, 21.00, 4C, COMe; 56.99, OMe; 65.30, 66.40, 68.47, 71.87, 72.28, 77.41, 78.05, C2,3,4,2',3',4',5'; 68.37, C6'; 96.61, 100.86, 101.48, C1,1',CHPh; 126.08-144.34, C5,6,Ph; 169.39, 169.44, 169.94, 170.03, 4C, CO.

Methyl 2,3-Di-O-benzyl-6-deoxy-4-O-(2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucosyl)-β-D-xylo-hex-5-enoside (190)

A small piece of sodium metal was added to the alkene (188) (590 mg, 1.0 mmol) in MeOH (10 mL) at 0°. The solution was left to stand (rt, 1 h) and was then concentrated,
co-evaporating with toluene (2 x 10 mL). NaH (220 mg, 5.6 mmol, 60% dispersion in mineral oil) was added to a solution of the residue in DMF (10 mL). BnBr (520 μL, 4.4 mmol) was then added dropwise, and the resultant mixture was stirred (30 min.). EtOH (1 mL) was added to decompose the excess sodium hydride. Concentration and normal workup (CH₂Cl₂) gave a yellow oil that was purified by RSF (toluene to EtOAc/toluene, 1:3) to give the alkene (190) as a microcrystalline powder (760 mg, 97%), m.p. 93-95° (Pr°), [α]D -47° (Found: C, 73.2; H, 6.6. C₄₈H₅₀O₁₀ requires C, 73.3: H, 6.4%). ¹H n.m.r. (300 MHz) δ 3.36-3.83, m, H₂,3,2',3,,4',5',6'; 3.48, s, OMe; 4.32, dd, J₅,₆ 6.0, J₆,₆' 11.6 Hz, H₆'; 4.43, d, J₁,₂ 6.9 Hz, H₁; 4.60-4.96, m, 12H, H₄,6,6',₁',CH₂Ph; 5.58, s, CHPh; 7.16-7.52, m, 25H, Ph. ¹³C n.m.r. (75.5 MHz) δ 56.90, OMe; 66.00, 75.67, 80.81, 81.04, 81.20, 81.80, 82.05, C₂,3,4,2',3',4',5'; 68.85, 73.77, 73.92, 75.21, 75.71, 5C, C₆',CH₂Ph; 95.98, 100.80, 101.16; C₁,₁',CHPh; 126.01-152.30, C₅,₆,Ph.

The alkene (190) (460 mg, 0.60 mmol) was treated with Hg(CF₃COO)₂ (50 mg) in acetone/H₂O (9 mL, 2:1; overnight). Volatile solvents were removed and the resultant aqueous solution was subjected to a normal workup (EtOAc), followed by RSF (EtOAc/petrol, 7:14), to give a colourless oil. MsCl (95 μL, 1.2 mmol) was added to this oil in Et₃N (330 μL, 2.4 mmol) and CH₂Cl₂ (10 mL) at −10°. The solution was then left to stand (rt, 1 h). Normal workup (CH₂Cl₂), followed by flash chromatography (EtOAc/petrol, 1:9), gave the enone (192) as a colourless oil (590 mg, 80%), [α]D 5.7° (Found: C, 74.6; H, 6.1. C₄₇H₄₆O₉ requires C, 74.8; H, 6.1%). ¹H n.m.r. (500 MHz) 3.38-3.44, m, H₅'; 3.57, dd, J₁',₂' 7.3, J₂',₃' 8.2 Hz, H₂'; 3.68, dd, J₅',₆' = J₆',₆' 10.3 Hz, H₆'; 3.73, dd, J₃',₄' = J₄',₅' 9.4 Hz, H₄'; 3.83, dd, H₃'; 3.94, dd, J₄,₅ 8.1, J₅,₆ 10.6 Hz,
H5; 4.29, dd, J5',6' 5.0 Hz, H6'; 4.42, ddd, J2,4 = J3,4 2.2 Hz, H4; 4.54, d, H6; 4.73-5.13, m, 9H, H1',CH2Ph; 5.50, s, CHPh; 6.06, dd, J2,3 10.4 Hz, H3; 6.85, dd, H2; 7.25-7.50, m, 25H, Ph. 13C n.m.r. (125 MHz) δ 65.72, 80.72, 81.30, 82.30, 83.75, 78.47, 80.08, C4,5,6,2',3',4',5'; 69.92, 73.81, 74.77, 75.13, 75.39, 5C, C6',CH2Ph; 101.02, 102.48, Cl',CHPh; 125.98-148.24, C2,3,Ph; 196.01, Cl.

A mixture of magnesium (370 mg, 15 mmol), benzyl chloromethyl ether (1.9 mL, 14 mmol) and HgCl2 (50 mg) in THF (15 mL) was stirred (90 min, 0°). The enone (192) (2.0 g, 2.6 mmol) was added and the mixture was stirred (1 h). The reaction mixture was poured into saturated NaHCO3 solution and the suspension was stirred (10 min). Normal workup (CH2Cl2), followed by flash chromatography (EtOAc/petrol, 15:85) gave firstly the alcohol (195) (900 mg, 40%) as needles, m.p. 124° (Pr2iO), [α]D 2.7° (Found: C, 75.0; H, 6.5, C56H56O10 requires C, 75.2: H, 6.5%). 1H n.m.r. (500 MHz) δ 3.16-3.24, m, H5'; 3.24, d, J7,7' 9.1 Hz, H7; 3.45-3.52, m, H2',6'; 3.60-3.67, m H7,3',4'; 3.94, dd, J4,5 7.2, J5,6 9.6 Hz, H5; 4.09, d, H6; 4.13-4.17, m, H4; 4.23-4.32, m, H6'; 4.33-4.98, m, 10H, CH2Ph; 4.39, d, J1',2' 7.7 Hz, H1'; 5.52, s, CHPh; 5.63, dd, J2,3 10.1, J3,4 2.0 Hz, H2; 5.90, dd, J3,4 2.2 Hz, H3; 7.21-7.52, m, 30H, Ph. 13C n.m.r. (125 MHz) δ 65.72, 80.72, 81.30, 82.30, 83.75, 78.47, 80.08, C4,5,6,2',3',4',5'; 69.92, 73.81, 74.77, 75.13, 75.39, 5C, C6',CH2Ph; 101.02, 102.48, Cl',CHPh; 125.98-148.24, C2,3,Ph; 196.01, Cl.
Next to elute was the alcohol (194) as needles (760 mg, 34%), m.p. 141° (Pr2O), [α]D −13° (Found: C, 75.2; H, 6.6%). 1H n.m.r. (500 MHz) δ 3.37-3.43, m, H5'; 3.50-3.54, m, H7,2'; 3.59, d, J7,7 9.2 Hz, H7; 3.66, dd, J5',6' = J6',6' 10.3 Hz, H6'; 3.72, dd, J3',4' = J4',5' 9.3 Hz, H4'; 3.87, dd, J2',3' 9.0 Hz, H3'; 4.03, dd, J4,5 6.6, J5,6 10.2 Hz, H5; 4.10, d, H6; 4.20-4.26, m, H4,6'; 4.24-5.00, m, 11H, CH2Ph; 5.56, s, CHPh; 5.65, dd, J2,3 10.4, J2,4 2.0 Hz, H2; 5.75, dd, J3,4 2.5 Hz, H3; 7.23-7.50, m, 30H, Ph. 13C n.m.r. (125 MHz) δ 65.81, 78.29, 78.77, 79.36, 81.46, 81.84, 81.87, C4,5,6,2',3',4',5'; 68.75, 72.04, 72.73, 73.20, 74.57, 74.87, 75.45, 7C, C7,6',CH2Ph; 125.98-137.88, Ph.

The alcohol (194) (540 mg, 0.62 mmol) was treated with Ac2O (1 mL) and pyridine (10 mL) in the presence of DMAP (120 mg, 1.0 mmol; 60°, 4 h). MeOH (1 mL) was added to the now dark solution. Concentration of the mixture followed by a normal workup (EtOAc), gave a brown oil. This oil was purified by flash chromatography (EtOAc/petrol, 1:4) to give the acetate (199) as a colourless oil (440 mg, 79%), [α]D −2.2°. 1H n.m.r. (600 MHz) δ 1.98, s, Ac; 3.30-3.35, m, H5'; 3.43, dd, J1,2' 7.6, J2,3' 8.5 Hz, H2'; 3.64, dd, J5',6' = J6',6' 10.4 Hz, H6'; 3.68, dd, J3',4' = J4',5' 9.3 Hz, H4'; 3.78, dd, H3'; 3.82, d, J7,7 10.0 Hz, H7; 3.87, d, H7; 4.15, dd, J3,4 9.6, J5,6 7.4 Hz, H5; 4.26-4.32, m, H6,6'; 4.28-4.96, m, 10H, CH2Ph; 4.80, d, H1'; 4.93, d, H4; 5.53, s, CHPh;
5.82, dd, $J_{1,2} 10.4$, $J_{1,6} 2.4$ Hz, H1; 5.89, dd, $J_{2,6} 2.1$ Hz, H2; 7.25-7.50, m, 30H, Ph.
$^{13}$C n.m.r. (125 MHz) $\delta$ 22.08, Ac; 66.20, 77.19, 79.11, 81.28, 81.43, 81.66, 82.26, C4,5,6,2',3',4',5'; 68.83, 71.75, 72.24, 73.43, 74.68, 75.03, 75.07, 7C, C7,6',CH2Ph; 85.06, C3; 101.06, CHPh; 102.44, C1'; 125.96-138.31, C1,2,Ph; 169.70, CO. High-resolution mass spectrum (f.a.b.) $m/z$ 919.4050 [C$_{57}$H$_{59}$O$_{16}$ (M+H)$^+$ requires 919.4057].

**Methyl 2,3-Di-O-acetyl-4,6-dideoxy-4-[(1'R,4'R,5'S,6'S)-4',5',6'-triacetyloxy-3'- (acetyloxymethyl)cyclohex-2'-enyl]amino-$\beta$-D-glucoside (216)**

A small piece of sodium metal was added to the amine (215) (280 mg) in MeOH (10 mL). The solution was left to stand overnight, then concentrated. Small pieces of sodium metal were added to the residue in THF (10 mL) and NH$_3$ (30 mL) at -78° until a blue colour persisted for 1 h. NH$_4$Cl was added so as to dissipate the blue colour. Evaporation of the solvents gave an off-white residue that was then treated with Ac$_2$O/pyridine (6 mL, 1:2) for 5 h. Normal workup (CH$_2$Cl$_2$), followed by flash chromatography (EtOAc/petrol, 4:6), gave the hexa-acetate (216) as a colourless oil (138 mg, 68%). The $^1$H n.m.r. (300 MHz) spectrum was consistent with that recorded previously.$^{47}$
Transglycosylation of Methyl 4,6-Dideoxy-4-\([1'R,4'R,5'S,6'S]-4',5',6'-\)trihydroxy-3'-(hydroxymethyl)cyclohex-2'-enyl]amino-\(\beta\)-D-glucoside (20)

A solution of \(\alpha\)-D-glucopyranosyl fluoride (204) (64 mg, 0.32 mmol) and the \(\beta\)-D-glucoside (20) (80 mg, 0.23 mmol) in NH\(_4\)HCO\(_3\) (3 mL of 0.15 M) was treated with AbgGlu358Ser (1 mg; rt, 3 d). The solution was lyophilised and the resultant residue was treated with Ac\(_2\)O (1 mL) and pyridine (3 mL). Concentration of the mixture, followed by flash chromatography (EtOAc/petrol, 4:6 to 6:4), gave, firstly, the carba-disaccharide (216) (32 mg, 24%), followed by the carba-trisaccharide (217) as a colourless oil (88 mg, 42%), [\(\alpha\)]\(_D\) \(-82^\circ\) (Found: C, 50.2; H 6.0. C\(_{38}\)H\(_{54}\)N\(_2\)O\(_{24}\) requires C, 50.2; H, 6.0%). \(^1\)H n.m.r. (500 MHz) \(\delta\) 1.33, d, 3H, J\(_{5,6}\) 6.2 Hz, H6; 1.98, 2.00, 2.03, 2.04, 2.05, 2.08, 2.10, 8 s, 27H, Ac; 2.47, dd, J\(_{3,4}\) = J\(_{4,5}\) 9.3 Hz, H4; 3.18-3.28, m, H5,1'; 3.46, s, OMe; 3.67-3.41, m, H5''; 4.05, dd, J\(_{5'',6''}\) = 2.3, J\(_{6'',6''}\) 12.4 Hz, H6''; 4.20, br d, J\(_{4,5'}\) 5.7 Hz, H4'; 4.28, d, J\(_{1,2}\) 7.7 Hz, H1; 4.33, dd, J\(_{5'',6''}\) 6.6 Hz, H6''; 4.49, d, J\(_{7,7'}\) 13.0 Hz, H7'; 4.57, d, H7'; 4.68, d, J\(_{1''},2''\) 8.0 Hz, H1''; 4.81-4.95, m, H2,3,6',2''; 5.05, dd, J\(_{3'',4''}\) = J\(_{4'',5''}\) 9.4 Hz, H4''; 5.15, dd, J\(_{2'',3''}\) 9.4 Hz, H3''; 5.36, dd, J\(_{5',6'}\) 8.5 Hz, H5'; 5.84, br s, H2'. \(^{13}\)C n.m.r. (125 MHz) \(\delta\) 17.78, C6; 20.54, 20.58, 20.66, 20.69, 20.75, 20.81, 20.88, 9C, COMe; 56.75, OMe; 56.88, C1'; 61.64, C6''; 62.49, C4; 63.76, C7''; 67.91, C4''; 70.86, C5'; 71.51, 71.78, 71.92, 72.09, C2,6',2'',5''; 73.03, C3''; 73.89,
C5; 74.82, C3; 76.84, C4'; 101.16, C1; 101.43, C1''; 128.60, C2'; 131.16, C3'; 169.30, 169.36, 169.54, 169.68, 170.02, 170.06, 170.26, 170.62, 171.37, 9C, CO.

Next to elute was the carba-tetrasaccharide (218) as a colourless oil (16 mg, 6%), [α]D –28°. 1H n.m.r. (600 MHz) δ 1.33, d, 3H, J5,6 6.1 Hz, H6; 1.97, 1.98, 2.00, 2.00, 2.07, 2.11, 2.14, 7 s, 21H, Ac; 2.03-2.04, m, 15H, Ac; 2.51, br s, H4; 3.07-3.14, m, H5; 3.34 br s, H1'; 3.47, s, OMe; 3.60-3.75, m, H4'',5'',5''''; 4.04, dd, J5'',6'' 2.2, J6'',6' 12.5 Hz, H6''; 4.08, dd, J5'',6'' 5.5, J6'',6' 12.1 Hz, H6''; 4.19, br s, H4'; 4.31, br s, H7'; 4.37, dd, J5'',6'' 4.3 Hz, H6''; 4.39, br d, J7',7' 10.8 Hz, H7'; 4.46, d, J1,2 7.9 Hz, H1; 4.58, br d, H6''''; 4.64, br d, J1',2' 7.9 Hz, H1''; 4.83-4.94, m, H2,3,6',2'',1''',2'''; 5.06, dd, J3''',4''' 9.8 Hz, H4''''; 5.13, m, H3'',3''''; 5.33, dd, J4'',5' 5.7, J5'',6' 8.4 Hz, H5'; 5.83, br s, H2'. 13C n.m.r. (125 MHz) δ 17.77, C6; 20.47, 20.53, 20.59, 20.66, 20.70, 20.77, 20.81, 20.91, 12C, COMe; 56.77, OMe; 57.00, C1'; 61.46, 62.15, 63.74, C7',6'',6''''; 62.57, C4; 67.69, 71.06, 71.52, 71.69, 71.87, 71.96, 72.10, 72.70, 72.81, 72.90, 73.92, 74.80, 76.34, 76.94, C2,3,5,4',5',6',2'',3'',4'',5'',6'',2''',3''',4''',5''''; 100.88, 101.18, 101.34, C1,1'',1''''; 128.61, C2'; 131.34, C3'; 169.14, 169.31, 169.48, 169.57, 169.69, 169.80, 169.95, 170.05, 170.21, 170.35, 170.52, 171.39, 12C, CO. High-resolution mass spectrum (f.a.b.) m/z 1164.3886 [C50H70N030 (M+H)+ requires 1164.3983].
Chapter 4

The Synthesis of an Isomer of

Methyl β-Acarviosin
Discussion

The strategy for the synthesis of the carba-disaccharide (27) involved the alkylation of the amine (36) by the triflate (220) (Scheme 1). This approach was undertaken based upon the success of similar alkylations, both described previously and published elsewhere.47

Firstly, attention focused on the synthesis of the triflate (220). The obvious choice of starting material for the synthesis of the triflate (220) is D-allose; however, this sugar is prohibitively expensive. Alternatively, a route to the triflate (220), via an inversion of stereochemistry at C3 of a D-glucoside, was sought. Perhaps the easiest such route to a D-alloside is the oxidation of ‘diacetone glucose’ (221) to the ketone (222), and subsequent reduction to give the alcohol (223).161
However, the necessary conversion from furanose to pyranose forms is often problematic, as illustrated by the following example (Scheme 2).\textsuperscript{162}

![Chemical structures showing the conversion](image)

Scheme 2. a) TFA, H\textsubscript{2}O then BzCl, pyr.

A novel conversion of a D-glucoside into a D-alloside involves the regioselective nucleophilic substitution of the dimesylate (224), to furnish the benzoate (225).\textsuperscript{144} Mono-substitution is ensured by the difficulty of substitution at C2. Subsequent reduction, with lithium aluminium hydride, affords the diol (226).

![Chemical structures showing the novel conversion](image)

While this methodology presented itself as an attractive route to a D-alloside, it was thought that a Mitsunobu inversion of the alcohol (227) would be more expedient, as the necessary benzoyl group at O2 is already installed. It should be noted that, unlike diacetone glucose (221), oxidation and subsequent reduction of the alcohol (227) returns only starting material.\textsuperscript{162}

![Chemical structure of the 2-O-benzoyl compound](image)

The 2-O-benzoyl compound (227) was prepared from the diol (228), using the benzoylating reagent, 1-(benzoyloxy)benzotriazole (Scheme 3).\textsuperscript{163} In this case the benzoylation is not regioselective, giving a 1:1 mixture of the two regioisomers, (227)
and (229). However, dibenzoylation was avoided (a problem encountered when using \text{BzCl} in pyridine). Additionally, the unwanted 3-\text{O}-benzoyl compound (229) could be deacylated, to recover the diol (228), then used in any subsequent benzoylations. Pretreatment of (228) with \text{Bu}_2\text{SnO}, followed by treatment with BzCl, similarly gave a 1:1 mixture of (227) and (229).

\begin{center}
\begin{tikzpicture}
\node (228) at (0,0) {\text{Ph} \text{O} \text{O} \text{O} \text{O} \text{HO} \text{HO} \text{OMe}};
\node (227) at (2,0) {\text{Ph} \text{O} \text{O} \text{O} \text{O} \text{BzO} \text{HO} \text{OMe} + \text{BzO} \text{OMe}};
\node (229) at (4,0) {\text{Ph} \text{O} \text{O} \text{O} \text{O} \text{OMe}};
\node (a) at (1,0) {\text{a}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 3.} a) 1-(benzoyloxy)benzotriazole, Et$_3$N, THF

The next task was the inversion of stereochemistry at C3, utilising the Mitsunobu reaction, via an esterification/de-esterification procedure. Chloroacetic acid was chosen as the carboxylic acid for two reasons. Firstly, alongside 4-nitrobenzoic acid, chloroacetic acid is the acid of choice for Mitsunobu esterifications.\textsuperscript{164,165} Secondly, chloroacetates can be selectively cleaved in the presence of other esters using a number of reagents. The suggested mechanism of cleavage of chloroacetates with thiourea is shown below.\textsuperscript{166}

\begin{center}
\begin{tikzpicture}
\node (1) at (0,0) {\text{Cl} \text{H}_2\text{N} \text{S} \text{H}_2\text{N}};
\node (2) at (1,0) {\text{OR}};
\node (3) at (2,0) {\text{OR}};
\node (4) at (3,0) {\text{OR}};
\node (5) at (4,0) {\text{S}};
\node (6) at (5,0) {\text{NH}_2};
\node (7) at (6,0) {\text{HN}};
\node (8) at (7,0) {\text{HN}};
\node (9) at (8,0) {\text{S}};
\node (10) at (9,0) {\text{NH}};
\node (11) at (10,0) {\text{HN}};
\node (12) at (11,0) {\text{HN}};
\node (13) at (12,0) {\text{R}};
\node (14) at (13,0) {\text{HCl}};
\node (15) at (14,0) {\text{ROH}};
\end{tikzpicture}
\end{center}
Thus, treatment of the alcohol (227) with chloroacetic acid, triphenylphosphine and diethyl azodicarboxylate (DEAD) gave the chloroacetate (230). Removal of the chloroacetyl group, using thiourea and 2,6-lutidine, gave the D-allo alcohol (231) in good yield [83% from the alcohol (227)]. The magnitude of the coupling constants, $J_{2,3}$ 2.8 Hz and $J_{3,4}$ 2.7 Hz, confirmed the D-allo configuration.

The next step was deoxygenation at C6, and this seemed best approached by installation of a halogen at C6. A useful transformation of benzylidene acetals is their ring-opening with $N$-bromosuccinimide (independently discovered by Hanessian\textsuperscript{167} and Hullar\textsuperscript{168}). In the case of 4,6-O-benzylidene acetals, 4-O-benzoyl-6-bromo derivatives are produced. Thus, (231) was treated with calcium carbonate\textsuperscript{169} and freshly recrystallised $N$-bromosuccinimide, in refluxing carbon tetrachloride, to afford the bromide (232) in excellent yield.

Several methods to convert the bromide (232) into the 6-deoxy derivative (233) were attempted. Hydrogenolysis, in the presence of palladium-on-charcoal, proved ineffective with only starting material being isolated after extended reaction times. Reaction of the bromide (232) with sodium borohydride in DMSO\textsuperscript{170} gave only degradation products. However, treatment of the bromide (232) with tributyltin hydride in refluxing toluene proved more successful, with the alkane (233) being obtained in excellent yield. Alternatively, \textit{in situ} generation of tributyltin hydride, from bis-tributyltin oxide and polymethylhydrosiloxane, proved less successful.\textsuperscript{171}
Finally, treatment of the alcohol (233) with Tf$_2$O, in the presence of pyridine, afforded the triflate (220).

The amine (36) was prepared from methyl α-D-glucopyranoside following the procedure of McAuliffe and Stick,\textsuperscript{91} itself based on the procedure of Panza and co-workers.\textsuperscript{71} One small alteration to the published synthesis was made: sodium sulfide was used instead of hydrogen sulfide for the reduction of the azide (234). While hydrogen sulfide effectively reduces the azide (234), sodium sulfide proved much safer and convenient to use. The synthetic scheme is shown below (Scheme 4).
Treatment of the amine (36) with the triflate (220), in 1,3-dimethylimidazolidin-2-one, afforded the carba-disaccharide (235) in excellent yield (55%) (Scheme 5). This result was very pleasing, as the yields of similar alkylations are usually much lower. A possible explanation for the good yield obtained is that the triflate group of (220) is flanked by two benzoyl groups, which have been shown to suppress competing elimination reactions.

Interestingly, the $^1$H n.m.r. (500 MHz) spectrum of the carba-disaccharide (235) contained a number of broadened signals, notably for the (non-aromatic) ring hydrogens. These effects have been seen before on similar compounds and can arise when nitrogen is substituted with bulky or electron-withdrawing groups, resulting in slow inversion about the nitrogen atom. This inversion is usually a rapid process on the n.m.r. time scale and, indeed, was less obvious in the $^1$H n.m.r. (300 MHz) spectrum (Figure 1).

Scheme 5. a) DMI, r.t., 14 h
Next, the carba-disaccharide (235) was subjected to, firstly, methanolic NaOMe, followed by sodium in liquid ammonia and, finally, acetic anhydride and pyridine, to afford the per-acetate (236) in fair yield (59%).

\[
\text{(236)}
\]
It was thought that the glycosylation of the 1,3-linked carba-disaccharide (27) at the 4'-position would provide interesting compounds, such as (237), as potential inhibitors of 1,3:1,4-β-glucan hydrolases. However, treatment of the carba-disaccharide (27) with α-D-glucopyranosyl fluoride (204) in the presence of the glycosynthase, AbgGlu358Ala, resulted in no reaction, even with extended reaction times (Scheme 6). It appeared as though the carba-disaccharide (27) was not an appropriate acceptor for the mutant hydrolase.

Scheme 6. a) AbgGlu358Ala, NH₄HCO₃ (150 mM)
Experimental

\begin{align*}
\text{Methyl 2-O-Benzoyl-4,6-O-benzylidene-\(\beta\)-D-alloside (231)}
\end{align*}

(i) DEAD (630 \(\mu\)L, 4.00 mmol) was added to the alcohol (227) \(^{163}\) (780 mg, 2.0 mmol), Ph\(_3\)P (1.0 g, 4.0 mmol) and chloroacetic acid (280 mg, 3.0 mmol) in \(\text{C}_6\text{H}_6\) (50 mL) and THF (10 mL) at 0\(^\circ\). After seven hours, the solution was concentrated. The residue was subjected to flash chromatography (EtOAc/petrol, 3:20 to 2:4) to give the chloroacetate (230) as an oil that was carried through to the next step. \(^1\)H n.m.r. (300 MHz) \(\delta\) 3.56, s, Me; 3.80-3.90, m, H4,6; 4.02-4.08, m, H5; 4.11, 4.17, ABq, \(J\) 14.4 Hz, \(\text{CH}_2\text{Cl}\); 4.45, dd, \(J_{5,6}\) 5.1, \(J_{6,6}\) 10.5 Hz, H6; 4.92, d, \(J_{1,2}\) 8.2 Hz, H1; 5.18, dd, \(J_{3,4}\) 3.1 Hz, H2; 5.59, s, \(\text{CHPh}\); 5.98, b t, H3; 7.38-8.03, 10H, Ph.

(ii) The chloroacetate (230) was treated with thiourea (1.5 g, 20 mmol) and 2,6-lutidine (230 \(\mu\)L, 2.0 mmol) in \(\text{CH}_2\text{Cl}_2/\text{MeOH}\) (40 mL, 1:1) at reflux (12 h). Concentration of the mixture, followed by flash chromatography (EtOAc/petrol, 3:20 to 1:3) of the residue, gave the alcohol (231) \([0.64 \text{ g, 83% from (227)}]\) as fine needles, m.p. 177-178\(^\circ\) (Pr\(^2\)O), \([\alpha]_D\) -58\(^\circ\) (Found: C, 65.4; H, 5.9. \(\text{C}_{22}\text{H}_{22}\text{O}_7\) requires C, 65.3: H, 5.7%). \(^1\)H n.m.r. (300 MHz) \(\delta\) 3.54, s, Me; 3.73, dd, \(J_{3,4}\) 2.4, \(J_{4,5}\) 9.4 Hz, H4; 3.82, dd, \(J_{5,6} = J_{6,6}\) 10.3 Hz, H6; 4.05-4.18, m, H5; 4.44, dd, \(J_{5,6}\) 5.0 Hz, H6; 5.57-5.63, m, H3; 5.01, m, H1,2; 5.61, s, \(\text{CHPh}\); 7.37-8.15, m, 10H, Ph. \(^{13}\)C n.m.r. (75.5 MHz) \(\delta\) 57.38, Me; 63.19, 67.96, 72.23, 78.41, C2,3,4,5; 69.06, C6; 100.03, 101.79, C1,CHPh; 126.14-136.90, Ph; 165.39, CO.
Methyl 2,4-Di-O-benzoyl-6-bromo-6-deoxy-\(\beta\)-D-alloside (232)

A mixture of the acetal (231) (290 mg, 0.75 mmol), NBS (150 mg, 0.82 mmol) and CaCO\(_3\) (80 mg, 0.82 mmol) in CCl\(_4\) (10 mL) was heated under reflux (3 h), over which time the mixture changed colour from red to yellow. The mixture was concentrated and subjected to a normal workup (CH\(_2\)Cl\(_2\)) to give a residue that was purified by flash chromatography (EtOAc/petrol, 1:4) to yield the bromide (232) (350 mg, 77\%) as cubes, m.p. 140-141° (Pr\(_2^i\)O/petrol), \([\alpha]_D\) -14° (Found: C, 54.3; H, 4.4. C\(_{22}\)H\(_{21}\)BrO\(_7\) requires C, 54.2; H, 4.6\%). \(^1\)H n.m.r. (300 MHz) \(\delta\) 3.52, dd, \(J_{5,6}\) 7.0, \(J_{6,6}\) 11.2 Hz, H\(_6\); 3.57, s, Me; 3.61, dd, \(J_{5,6}\) 2.8, H\(_6\); 4.57-4.64, m, H\(_5\); 4.67-4.68, m, H\(_3\); 5.03, d, \(J_{1,2}\) 7.8 Hz, H\(_1\); 5.07, dd, \(J_{2,3}\) 2.8 Hz, H\(_2\); 5.10, dd, \(J_{3,4}\) 2.7, \(J_{4,5}\) 9.1 Hz, H\(_4\); 7.38-8.09, 10H, Ph. \(^{13}\)C n.m.r. (75.5 MHz) \(\delta\) 32.03, C\(_6\); 56.94, Me; 67.68, 70.90, 71.58, 71.85, C\(_{2,3,4,5}\); 99.29, C\(_1\); 128.38-133.64, Ph; 165.04, 165.14, 2C, CO.

\[
\text{CH}_3
\]
\[
\begin{array}{c}
\text{Br} \\
\text{O} \\
\text{OMe} \\
\text{HO} \\
\text{BzO}
\end{array}
\]

(232)

Methyl 2,4-Di-O-benzoyl-6-deoxy-\(\beta\)-D-alloside (233)

The bromide (232) (590 mg, 1.3 mmol) was treated with Bu\(_3\)SnH (0.42 mL, 2.1 mmol) in toluene (7 mL) (70°, 3 h). The solution was concentrated and the residue was partitioned between MeCN and petrol. Concentration of the MeCN extract gave an oil that was purified by flash chromatography (EtOAc/petrol, 3:20 to 1:3) to give the 6-deoxy-\(\beta\)-D-alloside (233) as a glass (420 mg, 81\%), \([\alpha]_D\) -11° (Found: C, 65.3; H, 5.9. C\(_{22}\)H\(_{22}\)O\(_7\) requires C, 65.3; H, 5.7\%). \(^1\)H n.m.r. (300 MHz) \(\delta\) 1.33, d, \(J_{5,6}\) 6.3 Hz, 3H, H\(_6\); 3.56, s, OMe; 4.23-4.34, m, H\(_5\); 4.65, m, H\(_3\); 4.94, dd, \(J_{3,4}\) 2.7 \(J_{4,5}\) 9.7 Hz, H\(_4\); 4.99, d, \(J_{1,2}\) 8.1 Hz, H\(_1\); 5.09, dd, \(J_{2,3}\) 2.9 Hz, H\(_2\); 7.71-8.13, m, 10H, Ph. \(^{13}\)C n.m.r. (75.5 MHz) \(\delta\) 17.60, C\(_6\); 56.88, OMe; 67.52, 68.02, 72.16, 74.16, C\(_{2,3,4,5}\); 99.16, C\(_1\);
Methyl 2,4-Di-O-benzoyl-6-deoxy-3-O-trifluoromethylsulfonyl-β-D-alloside (220)

Tf₂O (190 μL, 1.6 mmol) was added to the alcohol (233) (390 mg, 0.98 mmol) and pyridine (150 μL, 2.2 mmol) in CH₂Cl₂ (5 mL) at -20°. The mixture was allowed to warm to room temperature (2 h), and was then quenched with saturated NaHCO₃ solution (5 mL). Normal workup (CH₂Cl₂) and flash chromatography (EtOAc/petrol, 3:20 to 1:4) gave the triflate (220) as a glass (440 mg, 86%), [α]D -2.5°. ¹H n.m.r. (300 MHz) δ 1.38, d, J₅,₆ 6.2 Hz, H₆; 3.57, s, OMe; 4.18-4.30, m, H₅, H₇; 4.92, d, J₁,₂ 8.2 Hz, H₁; 5.10, dd, J₄,₅ 9.9, J₃,₄ 2.3 Hz, H₄; 5.28, dd, J₂,₃ 2.3 Hz, H₂; 5.64, m, H₃; 7.38-8.12, m, 10H, Ph. ¹³C n.m.r. (75.5 MHz) δ 17.38, Me; 57.1, OMe; 67.92, 69.18, 71.02, C₂,4,5; 84.52, C₃; 98.90, Cl; 118.19, q, J₃,F 319 Hz, CF₃; 128.20-138.83, Ph; 164.87, 164.99, 2C, CO. High-resolution mass spectrum (f.a.b.) m/z 519.0951 [C₂₂H₂₂F₃O₉S (M+H)⁺ requires 519.0937].
A mixture of the azide (234) (3.3 g, 6.0 mmol),91 Na2S.9H2O (2.75 g, 12.0 mmol) and Et3N (200 μL) in MeOH (50 mL) was heated under reflux for 3 h. Concentration of the mixture, followed by a normal workup (CH2Cl2) and flash chromatography (toluene/EtOAc/EtOH/Et3N, 60:35:4:1) gave the amine (36) as a waxy solid (2.4 g, 76%). The 1H n.m.r. (300 MHz) spectrum was consistent with that recorded previously.71

Methyl 2,4-Di-O-benzoyl-4,6-dideoxy-3-[(1R,4R,5S,6S)-4',5',6'-tribenzyloxy-3'-(benzyloxymethyl)cyclohex-2'-enyl]amino]-β-D-glucoside (235)

A solution of the triflate (220) (280 mg, 0.50 mmol) and the amine (36)91 (650 mg, 1.25 mmol) in DMI (1.5 mL) was left to stand overnight. Normal workup (Et2O), followed by flash chromatography (EtOAc/petrol/Et3N, 15:84:1 to 25:74:1), gave the carba-disaccharide (235) as a glass (250 mg, 55%), [α]D -67° (Found: C, 74.3; H, 6.2. C56H57NO10 requires C, 74.4: H, 6.4%). 1H n.m.r. (500 MHz) δ 1.32, d, J5,6 6.2 Hz, 3H, H6; 3.17-3.28, m, H1'; 3.32-3.37, m, H3; 3.42-3.56, m, H6',7'; 3.47, s, OMe; 3.72-3.89, m, H5; 3.92-4.03, m, H5'; 4.07, d, J7,7' 11.7 Hz, H7'; 4.16, br d, J4',5' 11.6 Hz, H4'; 4.36-4.65, m, 9H, H1,CH2Ph; 4.86-4.97, m, H4; 5.10-5.25, m, H2; 5.57, br s, H2'; 6.95-8.02, m, Ph. 13C n.m.r. (125.8 MHz) δ 17.89, C6; 56.61, OMe; 58.46, 59.87, 71.16, 74.93, 76.46, 78.90, 81.88, 83.74, C2,3,4,5,1',4',5',6'; 70.51, 71.72, 74.18, 74.38,
C7',CH2Ph; 102.29, C1; 127.44-138.34, C2',3',Ph; 165.27, 165.63, 2C, CO (the signal for either C7' or a CH2Ph could not be located).

![Chemical Structure](image)

*Methyl 2,4-Di-O-acetyl-4,6-dideoxy-3-[[(1'R,4'R,5'S,6'S)-4',5',6'-triacetyloxy-3'-acetyloxymethyl]cyclohex-2'eny]amino]-β-D-glucoside (236)*

A small piece of sodium metal was added to a solution of the carba-disaccaride (235) (95 mg, 0.10 mmol) in MeOH (10 mL). The solution was left to stand overnight, after which time it was concentrated. Small pieces of sodium metal were added to a solution of the residue in THF (10 mL) and NH3 (30 mL) at -78° until a blue colour persisted for 1 h. NH4Cl was added so as to dissipate the blue colour. Evaporation of the solvents gave an off-white residue that was then treated with Ac2O/pyridine (6 mL, 1:2) over 5 h. Normal workup (CH2Cl2), followed by flash chromatography (EtOAc/petrol, 4:6) gave the per-acetate (236) as needles (37 mg, 59 %), m.p. 158° (PrO/EtOH), [α]D -67°, (Found: C, 52.9; H, 6.6. C26H37O14N requires C, 53.1: H, 6.4%). ^1H n.m.r. (300 MHz) δ 1.20, d, 3H, J5,6 6.2 Hz, H6; 1.98, 2.02, 2.04, 2.08, 2.10, 6s, 18H, Ac; 2.88, dd, J2,3 ≈ J3,4 10.0 Hz, H3; 3.43, s, OMe; 3.43-3.58, m, H5,1'; 4.26, d, J1,2 7.9 Hz, H1; 4.36, d, J7,7' 12.8 Hz, H7', 4.56, dd, J4,5 9.7 Hz, H4; 4.62, br s, H7'; 4.74, dd, H2; 4.91, dd, J1',6' 8.9, J5',6' 10.7 Hz, H6'; 5.19, dd, J4',5' 7.7 Hz, H5'; 5.68, br s, H4', 5.72, br s, H2'. ^13C n.m.r. (75.5 MHz) δ 17.55, C6; 20.63, 20.67, 20.71, 20.89, 20.97, 6C, COMe; 56.42, 57.45, 59.98, 70.63, 71.21, 72.85, 74.06, 75.80, C2,3,4,5,1',4',5',6'; 62.93, C7'; 101.83, C1; 120.24, C2', 130.66, C3'; 169.98, 170.21, 170.32, 170.37, 6C, CO.
**The Attempted Synthesis of the Trisaccharide (237)**

A small piece of sodium metal was added to a solution of the *per*-acetate (236) (47 mg, 0.08 mmol) in MeOH (5 mL). After 2 h the solution was neutralised with resin [Dowex 50W-X8 (H+ form)], filtered and the filtrate concentrated. To a solution of the residue in aqueous NH$_4$HCO$_3$ (400 µL, 150 mM) was added α-D-glucosyl fluoride (204) (18 mg, 1.2 eq.) and Abg E358a (10 µL from a stock solution of 12.9 mg/mL). The solution was left for a week at room temperature. T.l.c. analysis showed that no reaction had occurred.
Chapter 5

The Attempted Synthesis of a 3'-Glycosylated Derivative of Methyl β-Acarviosin
Introduction

Without a suitable enzyme available to effect the construction of ϒ-1,3-linkages, the synthesis of the carba-trisaccharide (28) was approached on a purely chemical basis. Thus it was hoped that the crucial glycosidic linkage at the non-reducing end of the carba-trisaccharide (28) could be constructed by glycosylation with a suitable donor (238) and the carba-disaccharide (215) (Scheme 1).

An additional impetus for undertaking this approach was that, by using a cellobiosyl donor, it could offer a means of procuring the carba-tetrasaccharide (239). Conceivably (239) could be a better inhibitor than (28) as it more closely mimics the natural substrate of (1,3; 1,4) ϒ-glucan hydrolases.
The glycosylation of the carba-disaccharide (215) is potentially problematic owing to the presence of the secondary amine. However, Ogawa and Miyamoto have successfully conducted glycosylations on a similar system to synthesise some components of the antibiotic validamycin complex, which exhibit inhibitory action against *Rhizoctonia solani* (sheath blight diseases of rice plant).\textsuperscript{175} Outlined below is a series of glycosylations used to synthesise one of these components, Validamycin F (Scheme 2).

\begin{center}
\textbf{Scheme 2.} a) AgOTf, TMU, CH\textsubscript{2}Cl\textsubscript{2}, 39%; b) several steps; c) AgOTf, TMU, CH\textsubscript{2}Cl\textsubscript{2}, 55%.
\end{center}
Ogawa and Miyamoto used an AgOTf-promoted glycosylation of the alcohol (240) with the bromide (241), to afford the carba-trisaccharide (242). Protecting group manipulations on (242) then gave the alcohol (243), which, upon glycosylation with the glycosyl bromide (244), again promoted with AgOTf, gave the carba-tetrasaccharide (245). The success, albeit in modest yields, of both glycosylations instilled confidence in the strategy adopted for the synthesis of the carba-trisaccharide (28). Particularly relevant to the glycosylation of the carba-disaccharide (215) is the successful glycosylation with the relatively unreactive (disarmed) donor (241).

Prior to glycosylation, the carba-disaccharide acceptor (215) had to be synthesised. Ogawa's strategy for the synthesis of validamycin F relied upon a straightforward protecting group protocol to furnish free hydroxyl groups at C4 and then subsequently C4' (see below for numbering) of validoxyamine A (246). However, a sole free hydroxyl group at C5' of the carba-disaccharide (20) is not so easily attained.
As it is much easier to differentiate between hydroxyl groups on a monosaccharide rather than on a disaccharide, it was thought that an alkylation of the protected epivalienamine derivative (247) with the triflate (178) would provide a direct access to the carba-disaccharide (215) (Scheme 3).

Several reactions in the synthesis of the epivalienamine derivative (247) require that the hydroxyl group at C3 of the precursor D-glucoside must be blocked with a suitable protecting group. Obviously, this protecting group must be orthogonal to the benzyl groups on the molecule. A 4-methoxybenzyl ether was thought to be ideal for this purpose as, in many respects, its chemistry is similar to that of the benzyl group (thus avoiding complications during the synthesis), yet it can be easily removed oxidatively in the presence of benzyl groups. Thus, the first task was to synthesise a suitably protected methyl D-glucoside, such as (248). This compound could then be transformed into the azide (249) according to literature precedents.91
Several methods, each relying on the different reactivities of the hydroxy groups of D-glucose, exist to gain access to the hydroxyl group at C3. Of these methods, the conversion of D-glucose to give the commercially available 'diacetone glucose' (DAG) (221) is surely the most straightforward. Additionally, there are some rather novel methods to gain access to these compounds.

One such method is the regioselective introduction of alkyl or acyl groups at O2 of the benzylidene acetal (250). The regioselectivity of these reactions is enhanced by the use of stannylene acetals, which are prepared in situ by heating the diol (250) with Bu₂SnO, driven to completion by the azeotropic removal of water. For instance, benzylation of the dibutylstannylene acetal of (250) gave the 2-O-benzoyl derivative (251) in 93% yield.¹⁷⁶
Additionally, there are some other, less common, yet potentially useful, methods to gain access to these sugars. The benzylation of methyl α-D-glucopyranoside, gives, under the correct conditions, the alcohol (252) in 50% yield. This degree of selectivity is quite remarkable, considering the normal lack of selectivity in such alkylations.

The benzyl groups of methyl 2,3,4,6-tetra-O-benzyl-α-D-glucoside (253) show some degree of selectivity to acetolysis. After a brief exposure to Ac₂O/H₂SO₄, the 1,3,6-tri-O-acetyl derivative (254) is the major product formed, which could, conceivably, be converted into the 3,6-diol (255) by treatment with HCl/MeOH.

Discussion

As stated above, DAG (221) is a readily available derivative of D-glucose exhibiting a free hydroxyl group at C3 and thus a simple synthetic approach from DAG was envisaged. Towards this end, DAG (221) was treated with NaH and 4-methoxybenzyl chloride (PMBCl) to give the ether (256). It was hoped that methanolysis of (256) would give the methyl D-glucopyranoside (257). However, treatment of the ether (256) with HCl in MeOH gave a compound that was not UV active (t.l.c.), indicating that the 4-methoxybenzyl group had been cleaved. Furthermore, this compound seemed similar
(t.l.c.) to an authentic sample of methyl α-D-glucopyranoside.

With this approach thwarted, the previously mentioned stannylene acetal chemistry was explored (Scheme 4). Treatment of the stannylene acetal of the diol (250) with BnBr gave predominately the 2-<9-benzyl compound (258). The free hydroxy group at C3 was then protected with a 4-methoxybenzyl group under normal conditions to furnish (259). Subsequently, it was hoped that the benzylidene ring could be transformed, using LiAlH4 and AlCl3, to give the alcohol (248). However, under these conditions the 4-methoxybenzyl group was again cleaved.

Scheme 4. a) Bu2SnO, BnBr, Bu4NI, C6H6; b) PMBCl, NaH, DMF; c) LiAlH4, AlCl3, Et2O/DCM
Alternatively, it was thought that the benzylidene acetal of (259) could be removed and the hydroxyl group at C4 blocked as a benzyl ether later in the synthesis. Wary of the sensitivity of 4-methoxybenzyl ethers to acid, it was nevertheless hoped that the benzylidene acetal of (259) could be removed with aqueous acid without affecting the 4-methoxybenzyl ether. However, heating (259) in 80% aqueous AcOH did not give clean conversion to the 4,6-diol (260); the benzylidene acetal and 4-methoxybenzyl ether seemed to have similar rates of hydrolysis under these conditions. T.l.c. analysis of the reaction mixture showed the presence of three new compounds, in roughly equal proportions, presumably (260), (258) and (261). Treatment of the benzylidene acetal (259) with aqueous HBF$_4$ also gave similar results.

A 4-methoxybenzyldene acetal reportedly undergoes acid hydrolysis ten times faster than a benzylidene acetal, so, based on the previous results it was hoped that a 4-methoxybenzyldene acetal could be removed selectively in the presence of a 4-methoxybenzyl ether. Towards this end, methyl α-D-glucopyranoside was transformed into the 4-methoxybenzyldene acetal (262). Benzylation of the stannylene acetal of (262) gave two compounds (t.l.c.), presumably (mainly) the 2-O-benzyl ether (263a) and the 3-O-benzyl ether (264b). However, unlike for the analogous reaction with the benzylidene acetal (250), the two ethers had extremely similar polarities, making
purification by flash chromatography extremely tedious, and thus rendering any thoughts of a large scale reaction redundant. Purification by crystallisation also proved unsatisfactory.

Following these rather frustrating results, an alternative means of cleaving a benzylidene acetal in the presence of a 4-methoxybenzyl ether was investigated. A particularly mild method of benzylidene acetal cleavage utilizes I₂ in refluxing MeOH. Gratifyingly, this methodology afforded the 4,6-diol (260) in excellent yield, with the 4-methoxybenzyl ether unaffected by these reaction conditions.

With a suitable route to the diol (260) in hand, multi-gram quantities of this compound were required for the extended synthesis of the carba-disaccharide (215). However, the conversion of the diol (250) into the 2-O-benzyl ether (258) proved to be a stumbling block to this objective, as significant amounts of the 3-O-benzyl ether (264) were also produced.
The method followed for the conversion of (250) into (258), as described by Boons and co-workers, involved the treatment of the stannylene acetal of (250) with BnBr and tetrabutylammonium iodide (TBAI) in benzene.\textsuperscript{181,182} The reported yield of the 2-O-benzyl compound (258) is 83\%, but here much lower yields (from 50\% to 60\%) were obtained. An investigation of the literature found that others had also noticed such a discrepancy in yields when following this procedure.\textsuperscript{186,187} Some different methods for the benzylation of the dibutylstannylene acetal of (250) are shown below (Table 1).

<table>
<thead>
<tr>
<th>solvent/nucleophile</th>
<th>temp (°)</th>
<th>time (h)</th>
<th>% yield</th>
<th>% yield</th>
<th>ratio (O2:O3)</th>
<th>Reference</th>
</tr>
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<tr>
<td>DMF</td>
<td>100</td>
<td>2</td>
<td>70</td>
<td>20</td>
<td>3.5:1</td>
<td>188</td>
</tr>
<tr>
<td>DMF</td>
<td>100</td>
<td>3</td>
<td>46</td>
<td>19</td>
<td>2.4:1</td>
<td>187</td>
</tr>
<tr>
<td>BnBr</td>
<td>85</td>
<td>30</td>
<td>74</td>
<td>8</td>
<td>9.2:1</td>
<td>186</td>
</tr>
<tr>
<td>DMF/CsF</td>
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<td>16</td>
<td>25</td>
<td>52</td>
<td>1:2</td>
<td>187</td>
</tr>
<tr>
<td>C\textsubscript{6}H\textsubscript{6}/TBAI</td>
<td>50</td>
<td>48</td>
<td>41</td>
<td>15</td>
<td>2.7:1</td>
<td>187</td>
</tr>
<tr>
<td>MeCN/TBAI</td>
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<td>16</td>
<td>49</td>
<td>13</td>
<td>3.8:1</td>
<td>187</td>
</tr>
</tbody>
</table>

Table 1

As shown above, the best regioselectivity was realised when BnBr was solvent (and reactant), giving the 2-O-benzyl ether (258) and the 3-O-benzyl ether (264) in a ratio of 74:8, as reported by Grindley and Qin.\textsuperscript{186} These authors rationalised the good regioselectivity attained, relative to that of other reaction conditions, by postulating that the regioselectivity of alkylation is greatest when the dialkylstannylene acetal exists primarily as a dimer. The authors have shown that in non-polar solvents, without the presence of nucleophiles (such as TBAI), dimers are the major constituent, thus the regioselectivity of alkylation is greatest when conducting the reaction under these conditions.

However, using BnBr as a solvent, on a large scale, seemed both rather hazardous and expensive. Extending the rationale of using a non-polar solvent, the alkylation was
conducted in heptane. Heptane was chosen because it is non-polar and has a boiling point of approximately 95°, a suitable temperature to conduct the experiment. Thus, treatment of the stannylene acetal of (250) with BnBr in refluxing heptane for three days gave, after purification, the 2-O-benzyl ether (258) in 76% yield, and a small amount of the 3-O-benzyl ether (264) (7%), representing a ratio of 10.6:1. This now represents the best method for the regioselective benzylation of the dibutylstannylene acetal of the diol (250).

Returning to the diol (260), the next step required was the installation of an iodine atom at C6. This transformation was effected by the general procedure of Garegg (I₂, Ph₃P, imidazole),¹⁴⁶ to furnish the iodide (265). Iodine at the 6-position of carbohydrates may be eliminated using a number of reagents, including AgF, DBU and NaH. As NaH is also commonly used in benzylations, it was thought that benzylation of O4 and a dehydroiodination could be carried out in one step. Thus, treatment of the iodide (265) with BnBr and NaH in DMF, for 18 h, gave the enol ether (266) in good yield (82%).

![Chemical Structures]

a) I₂, Ph₃P, imidazole, toluene; b) NaH, BnBr, DMF
The path from the alkene (266) to the azide (249) was straightforward (Scheme 5), being carried out in a fashion analogous to that for the published synthesis of the all-benzylated analogue. The 4-methoxybenzyl ether was unaffected by any of the reaction conditions in this sequence, and all yields obtained were similar to those obtained with the all-benzyl protected analogue.

![Scheme 5](image)

**Scheme 5.** a) Hg(CF₃COO)₂, acetone/H₂O; b) MsCl, Et₃N, CH₂Cl₂; c) i) BnOCH₂Cl, Mg, THF; ii) Ac₂O, pyridine, DMAP; d) NaN₃, Pd(Ph₃P)₄, THF/H₂O.

With the azide (249) in hand, it was thought to remove the 4-methoxybenzyl ether (oxidatively) first and then to reduce the azide functionality subsequently. A reversal of this order could result in oxidation of the amine functionality. Thus, treatment of (249) with DDQ, utilising the conditions of Oikawa and co-workers, smoothly gave the alcohol (267) in good yield (74%). The azide (267) was then reduced to give the amine (247).

![Scheme 5](image)

**Scheme 5.** a) DDQ, DCM/water; b) propane-1,3-dithiol, Et₃N, MeOH
With the amine (247) in hand, attention turned to the synthesis of a suitable alkylating agent. Stick and co-workers have used the triflate (40) previously to alkylate the amine (36).\(^{47}\)

While the preparation of the triflate (40) is quite straightforward, the synthesis of the 2,3-di-\(O\)-benzoyl triflate (178) proved to be, operationally, much simpler (Scheme 6). Thus, the iodide (268)\(^{189}\) was reduced by the procedure of Trumtel and co-workers, to furnish (269).\(^{189}\) Benzoylation of the crude reaction mixture then gave the benzoate (270) in 71\% overall yield from the iodide (268). Acid hydrolysis of the isopropylidene group of (270), followed by a low temperature regioselective benzoylation at O3, afforded the dibenzoate (271). Finally, a triflate was introduced at C4 under normal conditions. The dibenzoate (271)\(^{190,191}\) and the triflate (178)\(^{190}\) have been prepared previously, yet the published syntheses were either rather long\(^{191}\) or originating from D-fucose,\(^{190}\) which is prohibitively expensive. Furthermore, these publications were not accompanied by experimental details.

```
Scheme 6. a) Pd/C, H\(_2\), Et\(_3\)NH, EtOAc/petrol; b) BzCl, pyridine; c) AcOH/H\(_2\)O; d) BzCl, pyridine; e) Tf\(_2\)O, pyridine
```
The alkylation of the amine (247) with the triflate (178) proceeded moderately well, for this type of reaction, giving the carba-disaccharide (215) in 36% yield.

![Chemical structures](image-url)

a) DMI, 3 d
On the basis of the successful glycosylations performed by Miyamoto and Ogawa, as described previously in this Chapter, it was hoped that an AgOTf-promoted glycosylation of the carba-disaccharide (215) by the D-glucosyl bromide (273) would provide access to the carba-trisaccharide (274). Towards this end, the D-glucosyl bromide (273) and the carba-disaccharide (215) were treated with AgOTf, N,N-tetramethylurea (TMU) and powdered 4 Å molecular sieves in CH2Cl2. The addition of TMU to the reaction mixture serves two purposes, firstly to neutralise liberated HBr and, secondly, the conjugate acid of TMU isomerises ortho-acetates into glycosides. Disappointingly, after 24 h, t.l.c. analysis showed both donor (273) and acceptor (215) to be still present, with a trace of hydrolysis product from the D-glucosyl bromide (273). After the addition of more D-glucosyl bromide (273), AgOTf and TMU and extended reaction times (48 h), t.l.c. analysis showed that small amounts of a new, UV-active compound had been formed. Purification of the reaction mixture showed that what had thought to be a single compound was in fact a mixture (1H n.m.r.); additionally, the mixture represented a mass return of less than 5%. The experiment was subsequently repeated and a similar result attained.

\[
\begin{align*}
\text{(273)} & \quad + \quad \text{(215)} \\
\text{a)} & \quad \text{AgOTf, TMU, 4 Å molecular seives, CH2Cl2}
\end{align*}
\]
The disappointing result obtained with the D-glucosyl bromide (273) led to a consideration of other glycosyl donors. The next considered was the D-glucosyl trichloroacetimidate (275).\textsuperscript{193}

\begin{center}
\includegraphics[width=0.8\textwidth]{images.png}
\end{center}

Some different promoters for the glycosylation with the trichloroacetimidate (275) were investigated. Firstly, treatment of the trichloroacetimidate (275) and the carba-disaccharide (215) with \( \text{Et}_2\text{O} \cdot \text{BF}_3 \), resulted in no reaction, even after extended reaction times. Using \( \text{TMSOTf} \) as promoter, this quickly led to the formation of a new, less polar compound (t.l.c.). Isolation and \( ^1\text{H} \) n.m.r. analysis of this compound suggested it to be the trimethylsilyl ether (276), as shown by a singlet integrating for nine hydrogens close to 0 ppm.

\begin{center}
\includegraphics[width=0.8\textwidth]{images.png}
\end{center}

Treatment of the carba-disaccharide (215) with the trichloroacetimidate (275) over three hours, using \( \text{AgOTf} \) as promoter,\textsuperscript{194} led to the formation of a new, more polar compound (t.l.c.). The \( ^1\text{H} \) n.m.r. spectrum of this new compound showed a singlet at 2.35 ppm, characteristic of the methyl group of an ortho-ester (acetyl methyl groups normally resonate at approximately 2.0 ppm). The suspicion of ortho-ester formation was confirmed when the treatment of this compound (277) with aqueous acid returned the carba-disaccharide (215). Formation of an ortho-ester in this case is perhaps not surprising, considering that the standard method for the preparation of ortho-esters is
the treatment of a glycosyl bromide and an alcohol with AgOTf and a base.\textsuperscript{195} Attempts at the rearrangement of the ortho-ester (277) to the glycoside (274) with TMSOTf\textsuperscript{195} were unsuccessful.

A thioglycoside was now considered for the glycosylation of the alcohol (215). Yudovich and co-workers have successfully glycosylated the morphine derivative (278) using this approach.\textsuperscript{196} Importantly, regarding the glycosylation of the carbo-disaccharide (215), the morphine derivative also has an amine functionality. The method involved treating a mixture of the acceptor (278), donor (279), 4Å molecular sieves and NIS in CH\textsubscript{2}Cl\textsubscript{2} with 1.05 equivalents of triflic acid, which led to the exclusive formation of the β-linked compound (280) in good yield (87%). Presumably, the triflic acid protonates the amine of (278), after which the addition of a slight excess of triflic acid promotes the glycosylation. Additionally, the authors found that using a glycosyl donor with benzoyl protecting groups gave a much better yield than when acetyl groups were involved.

\[
\begin{align*}
\text{COOMe} & \quad \text{BzO} \\
\text{BzO} & \quad \text{BzO} \\
\text{BzO} & \quad \text{SEt} \\
\text{PhO} & \quad \text{H} \\
\text{HO} & \quad \text{N} \\
\text{Me} & \quad (279) \\
\end{align*}
\]

\[
\begin{align*}
\text{PhO} & \quad \text{H} \\
\text{HO} & \quad \text{N} \\
\text{Me} & \quad (278) \\
\end{align*}
\]

\[
\begin{align*}
\text{PhO} & \quad \text{H} \\
\text{HO} & \quad \text{N} \\
\text{Me} & \quad (280) \\
\end{align*}
\]

\text{a) NIS, TfOH, CH\textsubscript{2}Cl\textsubscript{2}.}
Disappointingly applying the same procedure as described above, to the glycosylation of the carba-disaccharide (215) with the thioglycoside (281) led to no glycosylated product being formed. After two hours, some more triflic acid was added, but this seemed to decompose the acceptor (215) (t.l.c.).

\[
\text{SPh}
\]

First developed by Kahne, glycosyl sulfoxides are formidable donors that have been successful where other donors have failed. A case in point is the glycosylation of the extremely hindered hydroxyl group of the deoxycholic acid derivative (282). A number of different glycosylation methods had been explored, yet all gave low yields (0% to 30%); additionally, when products were attained, extended reaction times were required. However, glycosylation with the sulfoxide (283) gave, exclusively, the β-linked glycoside (284) in 83% yield. Quite remarkably, the glycosylation is conducted at low temperature (−78° to −24°).

\[
\begin{align*}
\text{(283)} & \quad \text{(282)} \\
\text{a) Tf}_2\text{O, 2,6-di-tert-butyl-4-methylpyridine, CH}_2\text{Cl}_2
\end{align*}
\]
A significant drawback with sulfoxide donors is that if a participating group is required at O2 (to effect a $\beta$-linkage), an acetyl group may not be used, owing to ortho-ester formation. Pivaloyl groups may be used instead, but they are harder to install and, particularly, to remove than acetyl groups.

The general procedure as described by Kahne was followed when carrying out the sulfoxide glycosylation. Thus, the sulfoxide (283) was activated by the addition of half an equivalent of Tf$_2$O at low temperature ($-60^\circ$), followed by addition of the alcohol (215) after 15 minutes. Gratifyingly, t.l.c. analysis after a further 15 minutes showed complete conversion of the alcohol (215) to a less polar compound. Disappointingly, though, $^1$H n.m.r. (500 MHz) analysis of the product showed that a mixture of compounds, in a ratio of 1:2, had been formed. Although the identity of the two compounds could not be confirmed, both showed signals characteristic for each residue of the expected carba-trisaccharide, indicating that in all likelihood an $\alpha/\beta$ mixture [(285) and (286)] had been formed. All attempts at resolving this mixture with flash chromatography, using a variety of solvent systems, failed.
Quite obviously, the secondary amine of the carba-disaccharide (215) was having an adverse effect on these glycosylations. However, attempts to protect this sort of nitrogen atom (as an amide or carbamate) even in the fully O-protected amine (216) proved unsuccessful. The unreactiveness of the amine (216) towards trifluoroacetic anhydride is particularly noteworthy.
With little success to show from the above approach, the alternative of reversing the order of glycosylation and alkylation was investigated (Scheme 7).
Unlike the previous glycosylations attempted, the glycosylation of the azide (267) with the trichloroacetimidate (275), using catalytic amounts of TMSOTf as promoter, proceeded smoothly, giving the carba-disaccharide (287) in 74% yield. The β-linkage was confirmed from the magnitude of $J_{1',2'}$ (8.2 Hz).

![Chemical Structures](267.png) ![Chemical Structures](287.png)

Problems were encountered with the reduction of the azide group of (287). The previously successful method of propane-1,3-dithiol in refluxing MeOH led to the formation of a complex reaction mixture, which was difficult to purify by flash chromatography owing to the presence of the primary amine. With just small quantities of the azide (287) in hand, an attempt was made to change the acetyl groups to benzyl groups prior to reduction. However, normal deacetylation (NaOMe, MeOH) and benzylation conditions (NaH, BnBr, DMF) again led to the formation of a complex reaction mixture, perhaps owing to the reduction of the azide group by NaH.
Experimental

*Methyl 2-O-Benzyl-4,6-O-benzylidene-α-D-glucoside (258)*

Methyl 4,6-O-benzylidene-α-D-glucoside (250)\(^{180}\) (2.5 g, 8.7 mmol) and Bu₂SnO (2.4 g, 9.6 mmol) in C₆H₆ (100 mL) were heated under reflux (12 h) with the azeotropic removal of water. The now clear solution was concentrated. Heptane (50 mL) and BnBr (2.1 mL, 17.5 mmol) were added, and the solution was heated under reflux (3 d). Concentration of the solution, followed by flash chromatography (EtOAc/toluene, 1:9 to 3:17) gave, firstly, (258) as a colourless solid (2.48 g, 76%), m.p. 129.0-130.5° (EtOAc/petrol; lit.\(^{200}\) 129.5°), [α]D +35° (lit.\(^{200}\) +35°), followed by (264), also as a colourless solid (230 mg, 7%), m.p. 182-183° (EtOAc/petrol; lit.\(^{200}\) 185°), [α]D +71° (lit.\(^{200}\) +84°).

*Methyl 2-O-Benzyl-4,6-O-benzylidene-3-O-(4-methoxybenzyl)-α-D-glucoside (259)*

The alcohol (258) (24.1 g, 60 mmol) was treated with NaH (3.2 g, 84 mmol, 60% dispersion in mineral oil) and 4-methoxybenzyl chloride (10.0 mL, 72 mmol) in DMF (250 mL; 2 h). MeOH (2 mL) was added and the mixture was concentrated to give an orange oil. This oil was subjected to a normal workup (CH₂Cl₂) to give a crystalline mass, which was used without further purification in the next step. A small portion was recrystallised to give the ether (259) as needles, m.p. 93° (Pr₂IO), [α]D –33° (Found: C, 70.5; H, 6.6. C₂₉H₃₂O₇ requires C, 70.5; H, 6.6%). ¹H n.m.r. (300 MHz) δ 3.42, s,
OMe; 3.55, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 9.6 Hz, H2; 3.58, dd, $J_{3,4} = J_{4,5}$ 9.6 Hz, H4; 3.72, dd, $J_{5,6} = J_{6,6}$ 9.6 Hz, H6; 3.80, s, ArOMe; 3.79-3.85, m, H5; 4.03, dd, H3; 4.27, dd, $J_{5,6}$ 4.7 Hz, H6; 4.60, d, H1; 4.68-4.89, m, 4H, CH$_2$Ar; 5.56, s, CHPh; 6.88-7.51, m, 14H, Ar.

$^{13}$C n.m.r. (75.5 MHz) $\delta$ 55.25, 55.33, 2C, Me; 62.34, 78.28, 79.14, 82.12, C2,3,4,5; 69.06, 73.79, 75.04, 3C, C6,CH$_2$Ar; 99.26, C1; 101.24, CHPh; 113.72-159.18, Ar.

$\text{HO-\text{-PMB}} \text{-X-\text{-Ar}}$

$\text{BnO}$

(260) OMe

Methyl 2-O-Benzyl-3-O-(4-methoxybenzyl)-$\alpha$-D-glucopyranoside (260)

The crude benzylidene acetal (259) (from the previous step) was treated with I$_2$ (2 g) in MeOH (200 mL), at reflux (5 h). Powdered Na$_2$S$_2$O$_3$ was added, so as to decolourise the solution. The mixture was filtered and the filtrate was concentrated to give a colourless oil. This oil was purified by flash chromatography (EtOAc/petrol, 1:1) to give the diol (260) as a colourless oil (22.9 g, 88% over two steps), $[\alpha]_D +12^*$ (Found: C, 65.3; H, 7.0. C$_{22}$H$_{28}$O$_7$ requires C, 65.2; H, 6.9%). $^1$H n.m.r. (300 MHz) $\delta$ 3.36, s, OMe; 3.36-3.62, m, H2,4,5; 3.68-3.80, m, 3H, H3,6; 3.79, s, ArOMe; 4.59, d, $J_{1,2}$ 3.5 Hz, H1; 4.61-4.96, m, 4H, CH$_2$Ar; 6.88-7.36, m, 9H, Ar. $^{13}$C n.m.r. (75.5 MHz) $\delta$ 55.14, 55.20, 2C, OMe; 70.27, 70.63, 79.71, 80.86, C2,3,4,5; 62.34, 73.09, 74.96, 3C, C6,CH$_2$Ar; 98.13, C1; 113.98-159.31, Ar.

$\text{HO-\text{-PMB}} \text{-I-\text{-Ar}}$

$\text{BnO}$

(265) OMe

Methyl 2-O-Benzyl-6-deoxy-6-iodo-3-O-(4-methoxybenzyl)-$\alpha$-D-glucopyranoside (265)
I₂ (18.7 g, 74 mmol) was slowly added to a mixture of imidazole (10.9 g, 160 mmol), PPh₃ (20.7 g, 78 mmol) and the diol (260) (22.7 g, 52.5 mmol) in toluene (200 mL). The mixture was vigorously stirred (1 h, 70°). The reaction mixture was cooled (rt) and then diluted with saturated NaHCO₃ solution (100 mL). I₂ was then added, until the solution remained dark (5 min). Na₂S₂O₃ solution (1 M) was then added to decolourise the reaction mixture. Normal workup (toluene) followed by RSF (EtOAc/toluene, 1:4) gave the iodide (265) as a colourless oil (25.7 g, 87%), [α]D +15° (Found: C, 51.5; H, 5.4. C₂₂H₂₇I₂O₆ requires C, 51.4; H, 5.3%). ¹H n.m.r. (300 MHz) δ 3.11-3.22, m H₂,5; 3.32, dd, J₅,6 2.1, J₆,6 9.4 Hz, H₆; 3.34, s, OMe; 3.38-3.46, m, H₄,6; 3.65, dd, J₂,₃ = J₃,₄ 9.0 Hz, H₃; 3.72, s, ArOMe; 4.48-4.88, m, 4H, CH₂Ar; 4.54, d, J₁,₂ 3.5 Hz, H₁; 6.80-7.36, m, 9H, Ar. ¹³C n.m.r. (75.5 MHz) δ 6.98, C₆; 55.27, 55.53, 2C, OMe; 69.78, 73.60, 79.83, 80.30, C₂,3,4,5; 73.14, 74.96, 2C, CH₂Ar; 98.13, C₁; 114.09-159.45, Ar.

Methyl 2,4-Di-O-benzyl-6-deoxy-3-O-(4-methoxybenzyl)-α-D-xylo-hex-5-enoside (266)

The iodide (265) (25.7 g, 46 mmol) was treated with NaH (8.0 g, 200 mmol, 60% dispersion in mineral oil) and BnBr (7.0 mL, 65 mmol) in DMF (250 mL; 18 h). MeOH (10 mL) was added cautiously and the solution was concentrated. Normal workup (CH₂Cl₂) gave a dark oil that was purified by flash chromatography (EtOAc/petrol, 3:17) to give the alkene (266) as a colourless oil (16.3 g, 82%), [α]D −38°. ¹H n.m.r. (300 MHz) δ 3.42, s, OMe; 3.59, dd, J₁,₂ 3.4, J₂,₃ 9.0 Hz, H₂; 3.80, s, ArOMe; 3.90, ddd, J₃,₄ 9.0, J₄,₆ = J₄,₆ 1.8 Hz, H₄, 4.23, m, H₃; 4.61, d, H₁; 6.65-4.87, m, 8H, H₆,CH₂Ar; 6.83-7.38, 14H, Ar. ¹³C n.m.r. (75.5 MHz) δ 55.24, 55.43, 2C, OMe; 73.59, 75.46, 76.57, 3C, CH₂Ar; 79.24, 79.51, 80.92, C₂,3,4; 96.77, C₆; 99.04, C₁; 113.76-159.18, C₅,Ar.
The alkene (266) (16.1 g, 37 mmol) was treated with Hg(CF₃COO)₂ (500 mg) in acetone/water (300 mL, 2:1; 12 h). The reaction mixture was partially concentrated and the resultant aqueous solution was extracted with CH₂Cl₂ (3 x 70 mL). The extracts were dried (MgSO₄), filtered and concentrated. RSF (EtOAc/petrol, 1:2) of the residue gave a colourless oil. Mesyl chloride (11 mL) was added dropwise to this oil and Et₃N (35 mL) in CH₂Cl₂ (200 mL) at −10°. The solution was then stirred (1 h, rt). Normal workup (CH₂Cl₂) gave an oil that was purified by flash chromatography (EtOAc/petrol, 1:9 to 1:4) to give the enone (288) as a colourless oil (12.0 g, 80%), [α]D +62° (Found: C, 76.0; H, 6.1. C₂₈H₂₈O₅ requires C, 75.7; H, 6.4%). ¹H n.m.r. (300 MHz) δ 3.78, s, OMe; 3.91, dd, J₄,₅ 7.7, J₅,₆ 10.7 Hz, H₅; 3.99, d, H₆; 4.30, ddd, J₂,₄ 2.0, J₃,₄ 2.4 Hz, H₄; 4.68-5.08, m, 6H, CH₂Ar; 5.99, dd, J₂,₃ 10.4 Hz, H₃; 6.77, dd, H₂; 6.79-7.46, m, 14H, Ar. ¹³C n.m.r. (75.5 MHz) δ 55.22, OMe; 73.57, 74.48, 75.36, 3C, CH₂Ar; 78.96, 83.88, 84.31, C₄,₅,₆; 113.74-159.25, C₂,₃,Ar; 197.38, C1.

(1R,4S,5R,6S)-4,6-Dibenzyloxy-1-C-(benzyloxymethyl)-5-[(4-methoxybenzyl)oxy]cyclohex-2-enol (289)

A mixture of magnesium (4.5 g, 180 mmol), benzyl chloromethyl ether (11.0 mL) and HgCl₂ (200 mg) in THF (100 mL) was stirred (90 min, 0°). The enone (288) (11.8 g, 30 mmol) in THF (50 mL) was added to the mixture, and stirring was continued (90 min,
Saturated NaHCO₃ solution (100 mL) was added and the mixture was left to stand (10 min). The mixture was filtered (Celite) and the filtrate was extracted with CH₂Cl₂. The combined organic extracts were dried (MgSO₄) and filtered. Concentration of the filtrate gave an oil that was purified by flash chromatography (EtOAc/petrol, 3:17) to give the alcohol (289) as a colourless oil (9.5 g, 64%), [α]D −58° (Found: C, 76.2; H, 7.0. C₃₆H₃₈O₆ requires C, 76.3; H, 6.8%). ¹H n.m.r. (300 MHz) δ 3.63, d, J₇,₇ 9.2 Hz, H₇; 3.65-3.90, m, H₄,₅,₇; 3.81, s, OMe; 4.18, d, J₅,₆ 6.8 Hz, H₆; 4.58-4.88, m, 8H, CH₂Ar; 5.73, s, H₂,₃; 6.81-7.42, m, 19H, Ar. ¹³C n.m.r. (75.5 MHz) δ 55.25, OMe; 72.08, 73.08, 73.95, 74.77, 75.59, 5C, C7,CH₂Ar; 75.70, C1; 79.63, 81.27, 83.91, C4,5,6; 113.72-159.15, C2,3,Ar.

(3R,4S,5R,6S)-3-Acetoxy-4,6-dibenzyloxy-3-(benzyloxymethyl)-5-[(4-methoxybenzyl)oxy]cyclohexene (290)

The alcohol (289) (9.3 g, 16.4 mmol) was treated with Ac₂O (5.0 ml, 48 mmol) and DMAP (2.0 g, 24 mmol) in pyridine (40 mL; 3 h, 55°). MeOH (3 mL) was added and then the dark solution was concentrated. Normal workup (EtOAc), followed by flash chromatography (EtOAc/petrol, 1:9), gave the acetate (290) as a colourless oil (8.2 g, 82%), [α]D +63° (Found: C, 74.9; H, 6.6. C₃₈H₄₀O₇ requires C, 75.0; H, 6.6%). ¹H n.m.r. (300 MHz) δ 1.86, s, Ac; 3.80, s, OMe; 3.88, m, 2H, H₇; 4.07, dd, J₄,₅ 10.3, J₅,₆ 7.8 Hz, H₅; 4.32, ddd, J₁,₆ 2.0, J₂,₆ 1.7 Hz, H₆; 4.50, d, H₄; 4.56-4.89, m, 8H, CH₂Ar; 5.83, dd, J₁,₂ 10.5 Hz, H₁; 5.90, dd, H₂; 6.83-7.42, m, 19H, Ar. ¹³C n.m.r. (75.5 MHz) δ 22.02, Ac; 55.24, OMe; 71.95, 72.24, 73.62, 74.97, 75.55, 5C, C7,CH₂Ar; 79.93, 82.46, 85.07, C4,5,6; 80.95, C3; 113.68-159.10, C1,2,Ar; 170.01, CO.
(3R,4S,5S,6R)-3-Azido-4,6-Dibenzyloxy-1-(benzyloxymethyl)-5-[(4-methoxybenzyl)oxy]cyclohexene (249)

Pd(PPh₃)₄ (800 mg, 0.61 mmol) was added to the acetate (290) (8.0 g, 13.2 mmol) and NaN₃ (5.2 g, 80 mmol) in deoxygenated THF/water (240 mL, 2:1). The yellow solution was heated at reflux (2 h) under argon. Volatile solvents were removed and the resultant solution was extracted with CH₂Cl₂. The combined extracts were dried (MgSO₄) and filtered. The filtrate was concentrated to give an orange oil that was purified by flash chromatography (EtOAc/petrol, 1:9) to give the azide (249) as needles (4.7 g, 59%), m.p. 50-51° (Et₂O/petrol), [α]D-58°.

**1H n.m.r.** (300 MHz) δ 3.62, dd, J₃,₄ 8.5, J₄,₅ 10.5 Hz, H₄; 3.80, s, OMe; 3.83, dd, J₅,₆ 7.8 Hz, H₅; 3.70, d, J₇,₇ 12.2 Hz, H₇; 4.06-4.22, m, H₃,₇; 4.24, d, H₆; 4.42-4.89, m, 8H, CH₂Ar; 5.80, br s, H₂; 6.82-7.41, m, 19H, Ar.

**13C n.m.r.** (75.5 MHz) δ 55.26, OMe; 63.52, C₃; 69.62, 72.39, 74.91, 75.14, 75.39, 5C, C₇,CH₂Ar; 79.76, 82.61, 84.05, C₄,5,6; 113.85-138.46, C₁,2,Ar.

(1S,2R,5R,6S)-5-Azido-2,6-dibenzyloxy-3-(benzyloxymethyl)cyclohex-3-enol (267)

The azide (249) (4.5 g, 7.6 mmol) was treated with DDQ (2.6 g, 11.4 mmol) in CH₂Cl₂/water (100 mL, 95:5; 2 h). Normal workup (CH₂Cl₂) followed by flash chromatography (CH₂Cl₂/petrol, 2:3 to EtOAc/petrol, 1:4) gave the alcohol (267) as a
colourless oil (2.4 g, 74%), $[\alpha]_D^{25} -49^\circ$ (Found: C, 71.4; H, 6.0. C$_{28}$H$_{29}$N$_3$O$_4$ requires C, 71.3; H, 6.2%). $^1$H n.m.r. (300 MHz) $\delta$  3.48, dd, $J_{1,6}$ 10.4, $J_{5,6}$ 8.5 Hz, H$_6$; 3.90, dd, $J_{1,2}$ 7.8 Hz, H$_1$; 3.98, br d, $J_{7,7}$ 12.8 Hz, H$_7$; 4.08-4.22, m, H$_2$,H$_5$,H$_7$; 4.47-5.02, m, 6H, CH$_2$Ph; 5.65-5.69, br s, H$_2$; 7.22-7.45, 15H, Ph. $^{13}$C n.m.r. (75.5 MHz) $\delta$  63.05, C$_5$; 69.54, 72.53, 74.43, 75.20, 4C, C$_7$,CH$_2$Ph; 76.13, 79.34, 82.11, C$_{1,2}$,6; 127.73-138.38, C$_{3,4}$,Ph.

![Structural formula](image)

$(1S,2R,5R,6S)$-5-Amino-2,6-dibenzyloxy-3-(benzyloxymethyl)cyclohex-3-enol (247)

The azide (267) (1.25 g, 2.65 mmol) was treated with Et$_3$N (3.7 mL, 26.5 mmol) and propane-1,3-dithiol (2.9 mL, 26.5 mmol) in MeOH (20 mL) at reflux (5 h). The solution was concentrated to give an orange residue that was purified by flash chromatography (EtOAc/toluene/Et$_3$N, 20:79:1 to EtOAc/toluene/EtOH/Et$_3$N, 30:64:5:1) to give the amine (247) as a colourless oil (1.15 g, 97%), $[\alpha]_D^{25} -71^\circ$. $^1$H n.m.r. (300 MHz) $\delta$ 3.23, dd, $J_{1,6}$ 9.8, $J_{5,6}$ 7.2 Hz, H$_6$; 3.43-3.50, m, H$_5$; 3.92, br d, $J_{7,7}$ 11.7 Hz, H$_7$; 3.95, dd, $J_{1,2}$ 9.5 Hz, H$_1$; 4.20-4.28, m, H$_2$,H$_7$; 4.48-4.93, m, 6H, CH$_2$Ph; 5.62, br s, H$_4$; 7.21-7.42, m, 15H, Ph. $^{13}$C n.m.r. (75.5 MHz) $\delta$ 52.99, C$_5$; 70.22, 70.55, 73.99, 74.98, 4C, C$_6$,CH$_2$Ph; 76.20, 80.25, 85.23, C$_{1,2}$,6; 127.61-138.67, C$_{3,4}$,Ph. High-resolution mass spectrum (f.a.b.) $m/z$ 446.2346 [C$_{28}$H$_{32}$NO$_4$ (M+H)$^+$ requires 446.2331].
Methyl 2-O-Benzoyl-6-deoxy-3,4-O-isopropylidene-β-D-galactoside (270)

A mixture of the iodide (268) (2.4 g, 7.0 mmol), Et₂NH (1.5 mL, 14 mmol) and Pd-on-carbon (300 mg of 10%) in EtOAc/petrol (40 mL, 1:1) was stirred overnight under an atmosphere of hydrogen. The mixture was filtered (Celite) and the filtrate concentrated. The residue in CH₂Cl₂ (20 mL) was treated with BzCl (900 μL, 7.7 mmol) and Et₃N (2.0 mL, 14 mmol; overnight). MeOH (1 mL) was added and the solution was stirred for a further 5 min. Normal workup (CH₂Cl₂) gave a white solid that was taken up in Et₂O/petrol and filtered through a plug of silica. Concentration of the filtrate, followed by recrystallisation of the residue, gave the benzoate (270) as cubes (1.6 g, 71%), m.p. 102-103° (Et₂O/petrol), [α]D +59°. ¹H n.m.r. (300 MHz) δ 1.35, 1.63, 2s, CMe₂, 1.46, d, J₅,₆ 6.6 Hz, 3H, H₆; 3.46, s, OMe; 3.95, dq, J₄,₅ 2.1 Hz, H₅; 4.08, dd, J₃,₄ 5.4 Hz, H₄; 4.32, dd, J₂,₃ 7.45 Hz, H₃; 4.59, d, J₁,₂ 8.2 Hz, H₁; 5.23, dd, H₂; 7.31-7.80, m, 5H, Ph. ¹³C n.m.r. (75.5 MHz) δ 16.55, C₆; 26.38, 27.78, CMe₂; 56.50, OMe; 69.02, 73.60, 76.52, 77.27, C₂,3,4,5; 101.38, C₁; 110.28, CMe₂; 128.24-132.96, Ph; 165.45, CO.

Methyl 2,3-Di-O-benzoyl-6-deoxy-β-D-galactopyranoside (271)

The isopropylidene acetal (269) (580 mg, 1.8 mmol) was treated with aqueous CH₃COOH (20 mL, 80%; 20 min, 100°). The solution was concentrated, followed by repeated co-evaporation with toluene. The residue was taken up in CH₂Cl₂ (10 mL)
and treated with BzCl (230 µL, 2.0 mmol) and pyridine (500 µL; -50°). The solution was allowed to warm to room temperature overnight, after which MeOH (1 mL) was added. Normal workup (CH₂Cl₂) followed by flash chromatography (EtOAc/petrol, 1:3 to 1:1) gave the *dibenzoate* (271) as needles (600 mg, 86%), m.p. 131° (PrO/petrol), [α]D +121° (Found: C, 65.4; H, 5.7. C₂₁H₂₂O₇ requires C, 65.3; H, 5.7%). ¹H n.m.r. (300 MHz) δ 1.42, d, J₅,₆ 6.5 Hz, 3H, H₆; 3.53, s, OMe; 3.89, dq, J₄,₅ 0.9 Hz, H₅; 4.08-4.11, m, H₄; 4.59, d, J₂₂ 8.0 Hz, H₁; 5.30, dd, J₂,₃ 10.4, J₃,₄ 3.2 Hz, H₃; 5.69, dd, H₂; 7.31-7.80, m, 10H, Ph. ¹³C n.m.r. (75.5 MHz) δ 16.14, C₆; 56.82, OMe; 69.48, 70.16, 70.55, 74.85, C₂,₃,₄,₅; 102.11, C₁; 128.28-133.36, Ph; 165.44, 165.96, 2C, CO.

The dibenzoate (271) (600 mg, 1.55 mmol) was treated with Tf₂O (330 µL) and pyridine (1 mL) in CH₂Cl₂ (10 mL; 0°, 20 min). The addition of saturated NaHCO₃ solution, followed by normal workup (CH₂Cl₂) and flash chromatography (EtOAc/petrol, 1:4), gave the triflate (178) as a microcrystalline powder (700 mg, 87%), m.p. 128° (Et₂O/petrol), [α]D +64°. ¹H n.m.r. (300 MHz) δ 1.48, d, J₅,₆ 6.6 Hz, 3H, H₆; 3.55, s, OMe; 4.09, br q, H₅; 4.68, d, J₁,₂ 7.8 Hz, H₁; 5.24, br d, J₃,₄ 3.0 Hz, H₄; 5.57, dd, J₂,₃ 10.8 Hz, H₃; 5.68, dd, H₂; 7.32-8.03, m, 10H, Ph. ¹³C n.m.r. (75.5 MHz) δ 16.40, C₆; 56.98, OMe; 68.49, 68.86, 71.14, 84.90, C₂,₃,₄,₅; 101.97, C₁; 118.32, q, Jₐ,C 318.9 Hz, CF₃; 128.28-133.36, Ph; 165.01, 165.78, 2C, CO.
The amine (247) (1.92 g, 4.32 mmol) and the triflate (178) (900 mg, 1.74 mmol) were stirred in DMI (3 mL; 4 d, rt). The deep orange solution was concentrated (70°, 1 mm Hg) and the residue partitioned between CH$_2$Cl$_2$ and saturated NaHCO$_3$ solution. The organic layer was separated and the aqueous layer was extracted with more CH$_2$Cl$_2$. The combined organic extracts were dried (MgSO$_4$) and concentrated. Flash chromatography (EtOAc/petrol, 3:17 to 1:3) of the residue gave firstly the carba-disaccharide (215) as a pale yellow oil (520 mg, 36%), [α]$_D$ -12°. $^1$H n.m.r. (300 MHz) δ 1.51, d, J$_{5,6}$ 6.5 Hz, 3H, H$_6$; 2.69, br t, J$_{3,4}$ = J$_{4,5}$ 9.3 Hz, H$_4$; 3.17-3.28, m, H1',6',7'; 3.42-3.48, m, H5; 3.47, s, OMe; 3.80-3.84, m, 2H, H5',CH$_2$Ph; 3.98-4.03, m, H1,4',7'; 4.58-5.10, m, 5H, CH$_2$Ph; 5.34-5.46, m, H2,3; 5.57, br s, H2'; 6.95-8.02, m, 25H, Ph. $^{13}$C n.m.r. (100 MHz) δ 18.12, C6; 56.80, OMe; 60.11, 62.96, 72.37, 74.65, 75.15, 75.67, 78.87, 82.10, C2,3,4,5,1',4',5',6'; 70.53, 71.91, 73.92, 75.91, 4C, C7',CH$_2$Ph; 101.50, C1; 127.39-138.66, C2',3',Ph; 165.32, 16521, 2C, CO. Further elution gave the amine (247) (1.30 g).
(3R,4S,5S,6R)-3-Azido-4,6-dibenzyloxy-1-benzyloxymethyl-5-[(2,3,4,6-tetra-O-acetyl-β-D-glucosyl)oxy]cyclohexene (287)

The alcohol (267) (180 mg, 0.39 mmol), trichloroacetimidate (275) (380 mg, 0.78 mmol) and 4Å molecular sieves (500 mg) in CH₂Cl₂ (5 mL) were stirred (2 h). TMSOTf (2 drops) was added and the mixture was stirred (1 h). Solid NaHCO₃ (500 mg) was added and the mixture filtered through a plug of silica (EtOAc). Concentration of the filtrate, followed by flash chromatography (EtOAc/petrol, 3:7) of the residue, gave the D-glucoside (287) as a colourless oil (74%, 230 mg), [α]D -94°. ¹H n.m.r. (600 MHz) δ 1.93, 2.01, 2.03, 2.08, 4s, 12H, Ac; 3.57, dd, J₂₃,4 8.6, J₅₆,₆' 10.0 Hz, H₄; 3.56-3.62, m, H₅'; 3.91, br d, J₇,₇ 12.4 Hz, H₇; 4.02, dd, J₅',₆ 2.3, J₆',₆' 12.4, H₆'; 4.07-4.11, m, H₃,₇; 4.19, dd, J₅',₆' 4.5 Hz, H₆; 4.21, br d, J₅,₆ 7.4 Hz, H₆; 4.23, dd, H₅; 4.43-4.97, m, 6H, CH₂Ph; 5.08-5.13, m, H₂',₄'; 5.17, t, J₂',₃' = J₃',₄' 9.4 Hz, H₃'; 5.21, d, J₁',₂' 8.1 Hz; H₁'; 5.62, br s, H₂; 7.18-7.47, m, 15H, Ph. ¹³C n.m.r. (150 MHz) δ 20.57, 20.59, 20.60, 20.90, 4C, Ac; 61.83, 69.56, 72.64, 74.52, 75.50, 5C, C₇',CH₂Ph; 63.52, 68.27, 71.87, 71.98, 72.95, 78.00, 79.81, 82.74, C₃,₄,₅,₆,₂',₃',₄',₅'; 99.80, C₁'; 122.39-138.38, C₁,₂,Ph; 169.13, 169.40, 170.21, 170.61, 4C, CO. High-resolution mass spectrum (f.a.b.) m/z 802.3198 [C₄₂H₄₈N₃O₁₃ (M+H)+ requires 802.3187].
References

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