TOWARDS THE TOTAL SYNTHESIS OF NOVENAMINE AND ITS ANALOGUES WITH MODIFICATIONS AT C-4 AND C-5

BY

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Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

In the Department of Chemistry

UNIVERSITY OF CAPE TOWN

August 2004

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ABSTRACT

Novenamine, a known antibacterial agent, is composed of 3-O-carbamoyl noviose (novobiose) glycosidically linked to a 4-hydroxy-3-amino-coumarin unit. Its activity as a DNA-gyrase inhibitor and the absence of analogues containing 4-epi-noviose provided the rationale for developing new synthetic routes to these analogues. This thesis describes the total synthesis of the methyl glycosides of noviose and C-4-epi-noviose, methodology for the introduction of the C-3 carbamoyl group to both isomers, an alternative synthesis of the coumarin component, progress towards a C-5 analogue of 4-epi-noviose and a model study of methodology for the glycosidic coupling of the coumarin unit to suitably activated sugars.

Methyl C-4-epi-novioside, a novel compound, was synthesized from D-ribose in 11 steps in 36% overall yield. Key steps in the synthetic route included introduction of the geminal dimethyl group at C-5 via Grignard methodology, careful choice of protecting group strategies, achieving of a non-trivial methylation of a hindered O-4 and successful oxidative cyclization to the final pyranose form of the product. The synthesis of methyl novioside was achieved in 12 steps and 22% overall yield in a divergent synthesis from a precursor to 4-epi-noviose, employing a key oxidation-reduction sequence to stereoselectively invert the configuration at C-4.

The chemoselective protection of the C-2 hydroxyl group of both sugar isomers was explored as a route for selective introduction of the carbamate at C-3. Benzoylation of the stannylene acetal of methyl novioside or methyl novioside itself gave exclusively the 3-acylated regioisomer while the outcome of acetylation and benzoylation of the stannylene acetal of methyl C-4-epi-novioside was dependent on the anomeric configuration, with the more readily available α-isomer yielding the 3-acyl regioisomers. However, direct selective protection of the equatorial C-2 hydroxyl of methyl C-4-epi-novioside as either a *t*-butyldimethylsilyl ether or benzoate ester was achievable in high yield. Subsequent introduction of the C-3 carbamate group to the 2-O-silylated sugar under mildly basic conditions resulted in migration of the silyl group from O-2 to O-3 and formation of the carbamate group at C-2. In contrast, carbamoylation of the 2-O-benzoylated sugar proceeded to give the 3-carbamate in high yield. Conversion of the methyl glycoside to glycosyl acetate in preparation for anomeric activation was accompanied by N-acylation of the carbamoyl group, and subsequent indiscriminate loss of the N-acylcarbamoyl group upon attempted removal of the esters with catalytic sodium methoxide. These results and experiences have clarified the options for completing the synthesis of epi-novobiose and the related analogue of novenamine.

The synthesis of the selectively protected coumarin component of novenamine was achieved in 5 steps in 40% overall yield from 2,4-dihydroxy-3-methylacetophenone, with new methodology introduced for protection of the 4-hydroxy and 3-amino groups of the coumarin as an oxazole.

Progress was made towards the synthesis of a C-5 analogue of 4-epi-noviose with stereoselective introduction of a methyl and *t*-butyl group at C-5 of D-ribose to replace the gem-dimethyl group, in the hope of achieving a conformationally-biased derivative of epi-noviose for evaluation of binding properties with the target enzyme.

Lastly, two model glycosidic coupling reactions were investigated based on coupling methodology introduced in the literature in the context of total syntheses of novobiocin and an analogue thereof.

LIST OF ABBREVIATIONS

$[\alpha]_D$	optical rotation
Ac	acetate or acetyl
Ar	aryl
ax	axial
Bn	benyl
BOM	benzyloxymethyl
bp	boiling point
br. s.	broad singlet
Bu	butyl
Bz	benzoyl
cat.	catalytic
conc.	Concentrated
d	doublet
DIAD	diisopropyl azodicarboxylate
DIBALH	diisobutylaluminium hydride
DIPEA	N,N-diisopropylethylamine
DHP	3,4-dihydro-2H-pyran
DMAP	dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
eq	equivalents or equatorial
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
g	grams
hr	hour
HMPA	hexamethylphosphoramide
HOMO	highest occupied molecular orbital
HRMS	high resonance mass spectrometry
IR	infra red spectroscopy
J	coupling constants
LUMO	lowest unoccupied molecular orbital
m	meta
m	molar
m/z	mass-to-charge ratio
m-CPBA	*meta*-chloroperbenzoic acid
Me	methyl
MEM	2-methoxyethoxymethyl

MeOH	methanol
mg	milligrams
MHz	Megahertz
min	minute
ml	milliliter(s)
mmHg	millimeter mercury, torr
mmol	millimole(s)
mol	mole(s)
mp	melting point
Ms	methanesulphonyl
MS	mass spectrometry
NBS	N-bromosuccinimide
nmr	nuclear magnetic resonance
NMO	4-methylmorpholine N-oxide
NOE	nuclear Overhauser effect
o	ortho
p	para
Pet ether	petroleum ether (boiling point range: 60-70°C)
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
ppm	parts per million
PPTS	3-[5-(sulfophenyl)-2-pyridyl]-1,2,4-triazin-5-ylbenzenesulfonic acid
q	quartet
quin	quintet
Red Al	sodium bis(2-methoxyethoxy)aluminium hydride
s	singlet
selectride	tri-*sec*-butylborohydride
S_N1	unimolecular nucleophilic substitution
S_N2	bimolecular nucleophilic substitution
t	triplet
t-But	*t*-butyl
TBDMS	*t*-butyl dimethylsilyl
THF	tetrahydrofuran
THP	tetrahydropyran
tlc	thin layer chromatography
TMS	tetramethylsilane
TMSOTf	trimethylsilyl trifluoromethanesulphonate
TPAP	tetrapropylammonium perruthenate
p-TsOH	*p*-toluenesulphonic acid

ACKNOWLEDGEMENTS

I would like to thank the following people for their contributions to this thesis:

Professor Roger Hunter, for showing me the beauty and complexity of synthetic organic chemistry with hours and hours of useful and inspirational discussions, the encouragement, guidance and support throughout this project and for whom I have endless respect;

Associate Professor David Gammon, for introducing me to this project, gently guiding me through it and for always having an open door policy for offering advice and useful discussions;

All my friends and colleagues that have passed through the Hunter/Gammon organic synthetic group, for their support and task-sharing, answers to the thousands of questions, useful discussions, and friendship;

All my past and present colleagues in the Department of Chemistry, particularly those in the Organic Synthesis laboratories;

Piero Benincasa, Dr. Phillip Boschoff, Noel Hendricks, Pete Roberts and Tommie van der Merwe for their analytical services and Dr. John Bacsa for performing the X-ray crystal structure determination;

The National Research Foundation and the Department of Chemistry for financial assistance;

My family, for their understanding and support, particularly through the tough times;

My husband, for his complete commitment, unwavering support and endurance of both my joys and tears throughout this project.

Finally, to my two children, Thomas and Rebecca, to whom I dedicate this thesis and whom I love dearly. My hope and dreams for them is that they too will set their goals to heights that appear unreachable but that are definitely obtainable.

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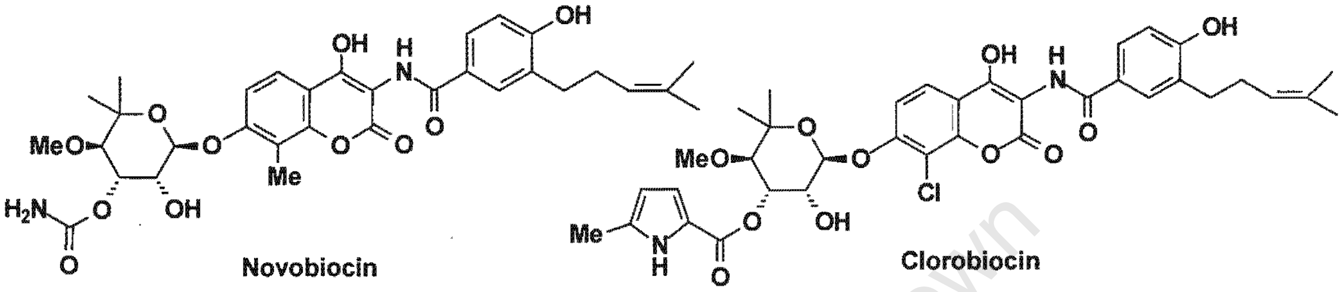
Chapter 1 : Introduction

1.1. Introduction to novobiocin

Novobiocin (Figure 1.1) is a naturally occurring antimicrobial agent that was first isolated in 1956 from several Streptomyces species including *S. spheroides* and *S. niveus*.[1]



Novobiocin **Clorobiocin**

Figure 1.1: Novobiocin and clorobiocin

Novobiocin, clorobiocin (Figure 1.1) and coumermycin A_1 (Figure 1.2) are closely related members of the coumarin-containing antibiotics that are active against gram-positive bacteria. These include methicillin-resistant strains of the staphylococci species, MRSA (methicillin-resistant *S. aureus*), as well as MRSE (methicillin-resistant S. *epidermidis*), which tend to spread in hospitals and are resistant to many antibiotics. They cause infections similar to those caused by sensitive strains of *S. aureus* such as lower respiratory and urinary tract infections, septicaemia, infection of sites involving invasive devices, pressure sores, burns, and ulcers and are currently a global concern in the treatment of bacterial infections.[2,3]

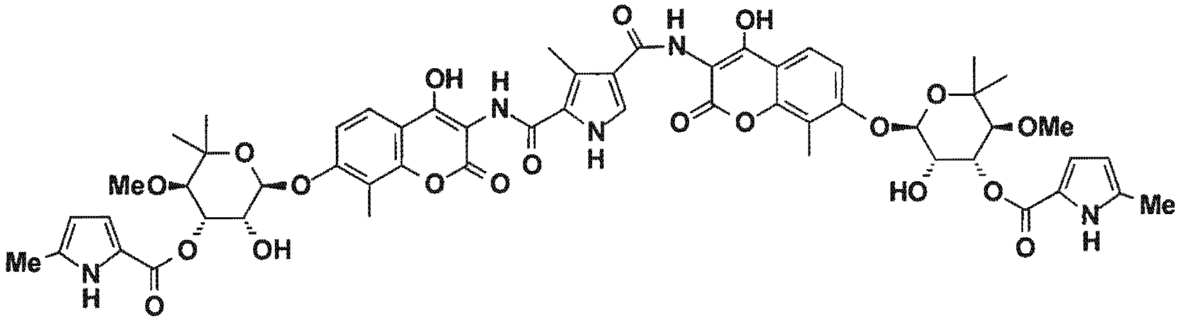
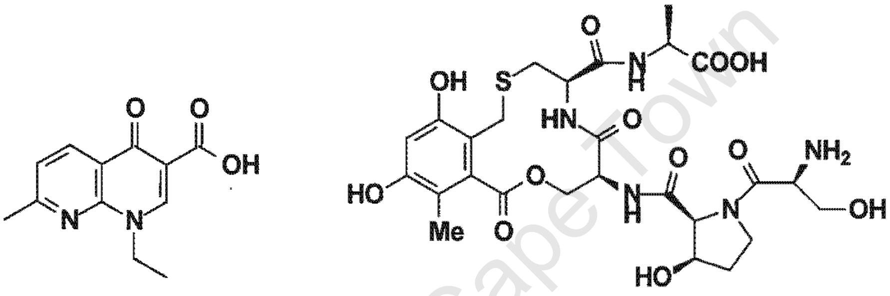


Figure 1.2: Coumermycin A_1

The coumarin-containing antibiotics are powerful DNA-gyrase inhibitors. DNA-gyrase is a type II topoisomerase and an essential prokaryotic enzyme. It utilizes the energy of ATP to induce supertwists in DNA and, along with the opposing action of DNA topoisomerase I, which relaxes supercoiled DNA, allows the bacteria to maintain the correct super-helical density of the chromosome required for replication, repair, recombination and the control of gene expression.[4] DNA-gyrase is composed of two proteins, A and B, which are encoded by the *gyr*A and *gyr*B genes respectively, and exist as an A_2B_2 heterotetramer.[5] The DNA-gyrase A (GyrA) subunits bind to DNA, while the DNA-gyrase B (GyrB) subunits carry the ATP binding site.[6] Since DNA-gyrase has no counterpart in eukaryotes, it is a very attractive drug-target for antibiotics.



Nalidixic acid **Cyclothialidine**

Figure 1.3: Nalidixic acid and Cyclothialidine

DNA-gyrase inhibitors include synthetic quinolones such as nalidixic acid (Figure 1.3) which act on subunit A, as well as cyclothialidines (Figure 1.3) and the coumarins, which act on subunit B. While the quinolones, which are broad-spectrum antibiotics, have been used successfully for the past 40 years as antibacterial agents, the coumarin drugs have failed to become clinically successful owing to poor water solubility and oral availability, low activity against gram-negative bacteria, toxicity, other undesirable side effects and the rapid materialization of coumarin-resistant organisms.[7]

The novobiocin molecule, broken down in Figure 1.4, comprises a 3-amino-4-hydroxy-7-noviosyl-8-methylcoumarin commonly referred to as novenamine coupled via an amide linkage to 4-hydroxy-3-[3'-methylbutenyl-2'-]benzoic acid. Novenamine can be further broken down into 3-amino-4,7-dihydroxycoumarin linked at its 7-position to novobiose, a 3-*O*-carbamoyl-4-*O*-methyl-5,5-dimethyl-L-lyxose sugar, via an α-glycosidic linkage.

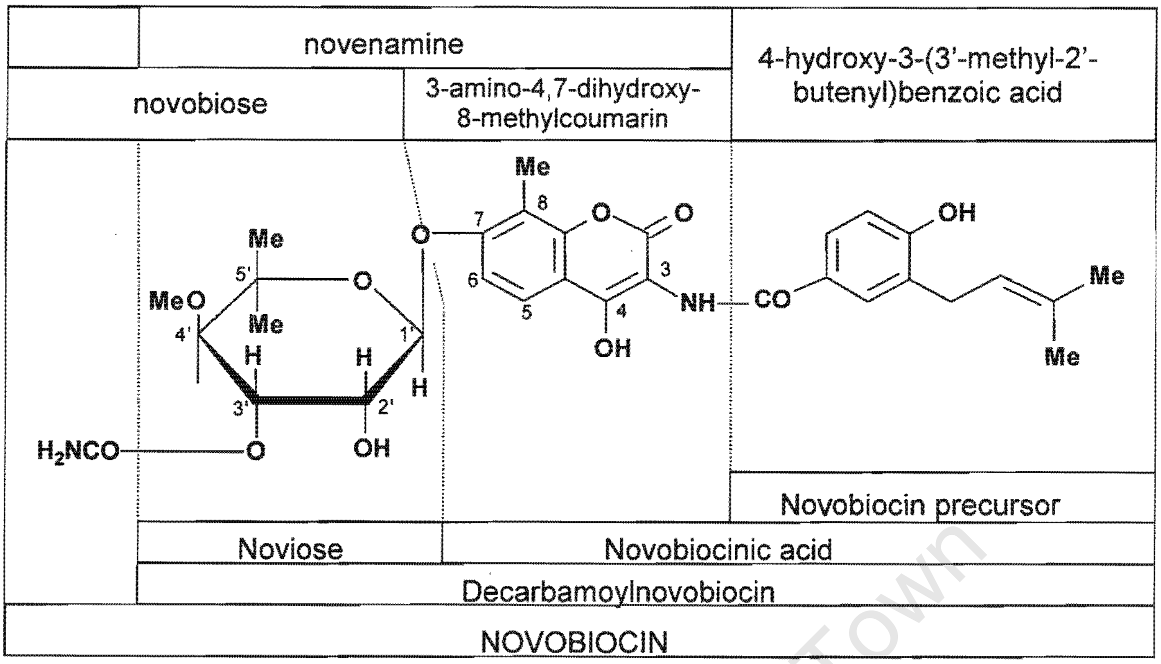


Figure 1.4: Structural breakdown of novobiocin

The decarbamoylated sugar is commonly referred to as L-noviose and is classified as an L-lyxose sugar. Lyxose is a trivial name given to one of the aldopentose sugars and exists in either the D- or L-form. According to the Rosanoff convention, all sugars represented as Fischer projections which have the hydroxyl group at the highest-numbered asymmetric centre (C-4 in the lyxose case) projecting to the right, belong to the D-series, while the others belong to the L-series. This convention is depicted in Figure 1.5 and results in two lyxose sugars that are enantiomers of each other. According to Freudenberg (1932), the glycosidic linkage is referred to as α- because "for D-glucose and all compounds of the D-series, α-anomers have the hydroxyl group at the anomeric centre projecting downwards in Haworth formulae; α-L-compounds have this group projecting upwards", as represented in Figure 1.5.[8]

In solution, α-L-novobiose exists preferentially in a thermodynamically-preferred $^{1}C_4$ ring conformation, with the anomeric substituent, the C-2' hydroxyl and one of the C-5' methyl groups as axial, and the C-3' carbamoyl, the C-4' methoxy and the second C-5' methyl group as equatorial substituents (Figure 1.7).

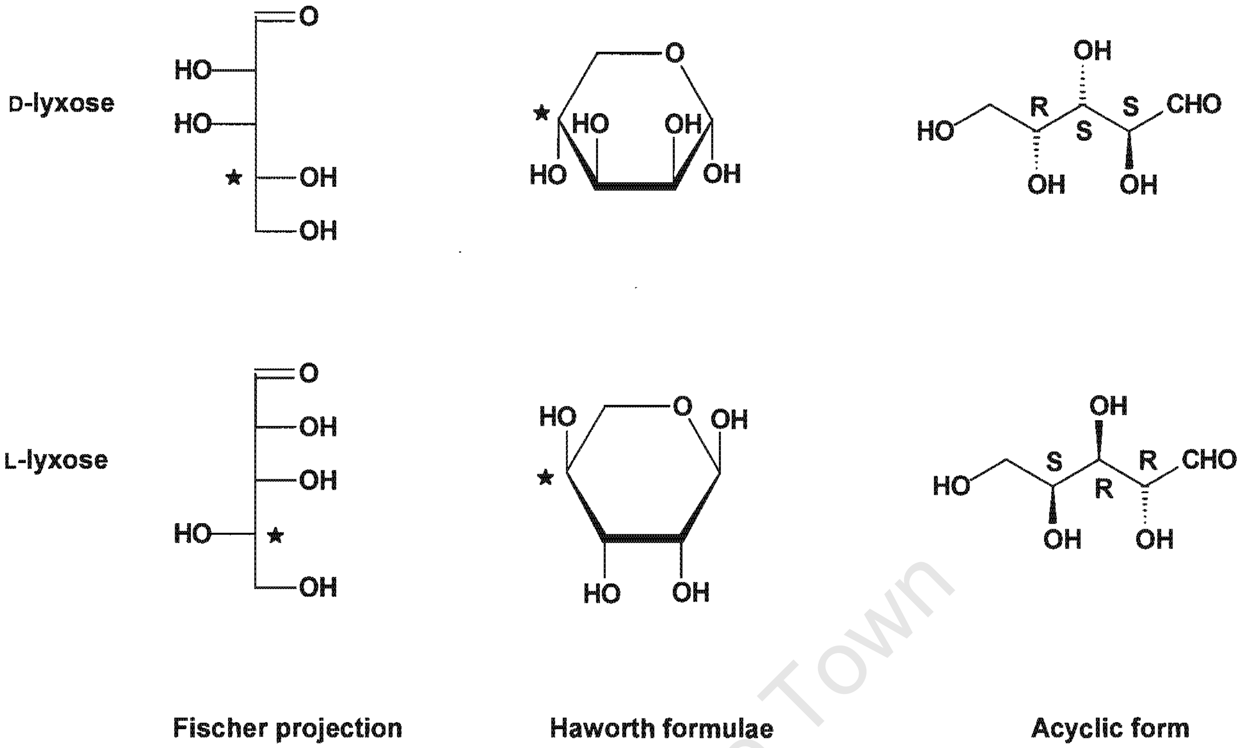


Figure 1.5: Representations of α-D- and α-L-lyxose. The asterisks denotes the centre whose configuration defines the D- or L- assignments.

Besides L-noviose, only one other sugar with gem-dimethyl groups at C-5 has been reported in the literature, namely a 4-*O*-isobutanoyl-5,5-dimethyl-β-D-rhamnose sugar found in lipiarmycin (Figure 1.6), making these C-5 branched-chain carbohydrates very rare natural compounds.

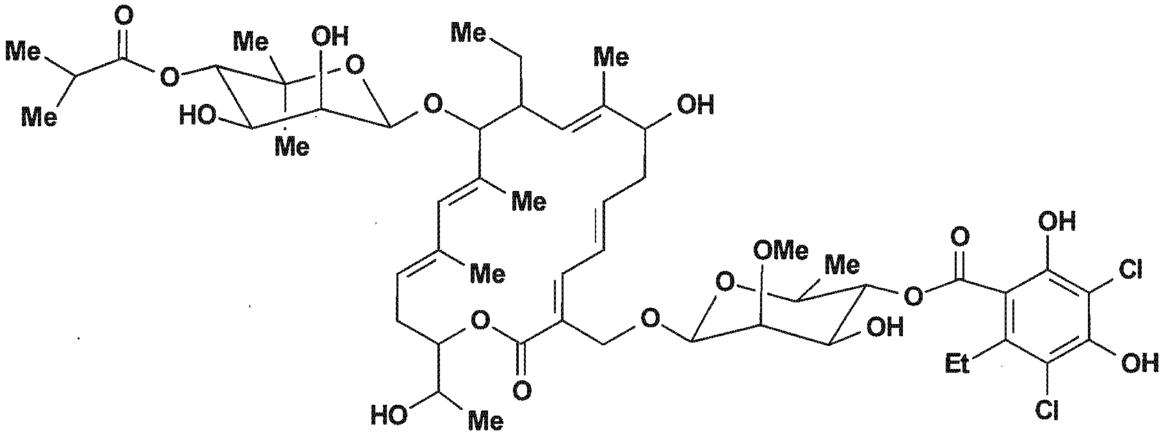


Figure 1.6: Lipiarmycin

1.2. Interaction between novobiocin and its target protein

Novobiocin binds reversibly and with high affinity to the amino-terminal 24K subdomain of (GyrB).[9] X-ray crystallographic studies of novobiocin bound to this fragment of GyrB from *Escherichia coli* have revealed several key interactions between the drug and protein (Figure 1.7).[10] The ester and carbonyl oxygens of the coumarin ring form hydrogen bonds with Arg136. Noviose alone has no anti-bacterial or anti-gyrase activity,[11] so it is believed that the interaction between Arg136 and the coumarin ring directs the sugar moiety to the appropriate site. Mutations of Arg136 have led to drug resistance, thus providing supporting evidence for the importance of this key interaction.

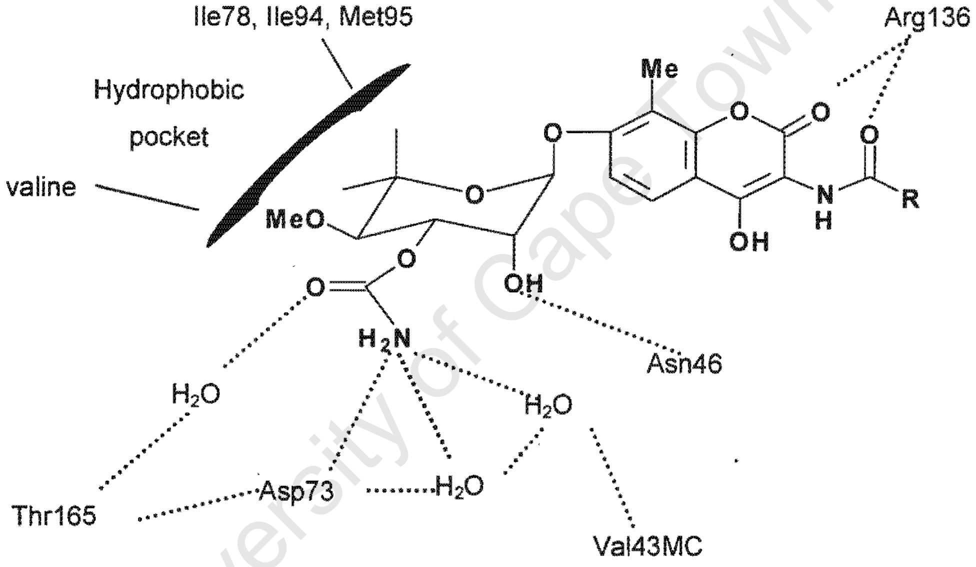
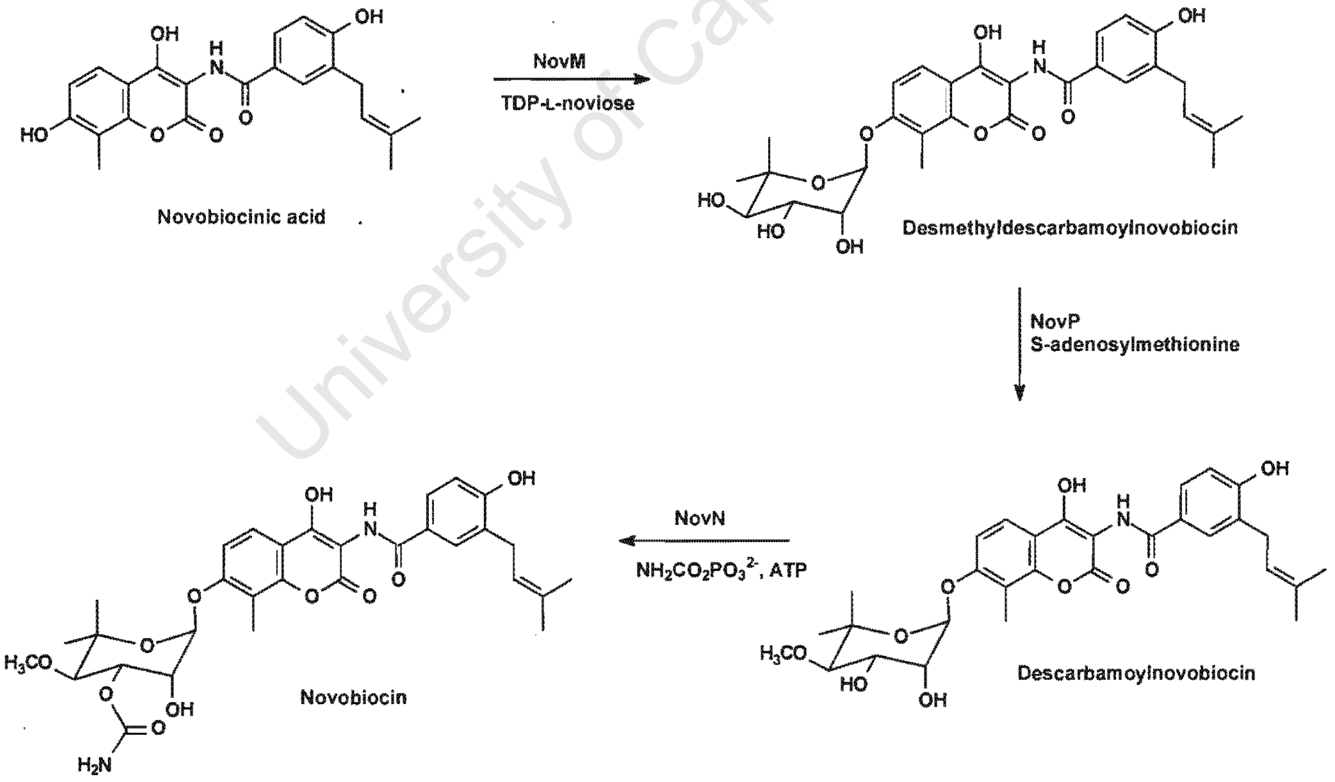


Figure 1.7: Novobiocin bound to DNA-gyrase B

Two other interactions are essential for drug-binding, these being the hydrogen bonding between the 2'-hydroxyl of noviose and the carbonyl group of Asn46, and the interaction of Asp73 and the 3'-O-carbamoyl group of noviose. Transfer of the carbamoyl group from C-3' to C-2' (isonovobiocin) results in a dramatically reduced affinity for DNA-gyrase and has been implicated in a mechanism for novobiocin resistance.[12] There is also a hydrogen-bonding interaction between the 3'-O-carbamoyl component and an ordered water molecule. The larger 5-methylpyrrolyl-2-acyl unit of clorobiocin displaces this water molecule and makes hydrophobic contacts with the enzyme's side chain, resulting in a 10-fold increase in antibacterial activity.[13] A number of hydrophobic contacts are made between the drug and protein. The 5',5'-dimethyl group interacts with the hydrophobic amino acids surrounding it, including Ile78, Ile94 and Met95, and the 4'-methoxy group sits in a hydrophobic pocket comprising mainly of valine (residues 43, 69, 71 and 120). The alkyl part of Arg76 lies above the plane of the coumarin ring, with Pro79 lying underneath, while the 4'-hydroxy-3'-isopentenylbenzoate aromatic ring wraps around Pro79. This ring appears to have little influence on the binding of the drug and is not crucial for gyrase B inhibition, but it does contribute to the intracellular uptake of the drug.

1.3. Biosynthesis of novobiocin

Early investigations of the biosynthesis of coumarin antibiotics revealed that the noviose unit is derived from D-glucose, and that the coumarin and prenylated 4-hydroxybenzoic acid components are derived from tyrosine.[14] As depicted in Scheme 1.1, recent work has revealed the enzymes involved in the final stages of novobiocin synthesis.[15]



Scheme 1.1: The final steps in novobiocin biosynthesis.

The glycosyltransferase NovM catalyzes the conversion of novobiocinic acid into desmethyldescarbamoylnovobiocin, the noviose being transferred from thymidine-5'-diphosph-β-L-noviose (TDP-β-L-noviose). The methyltransferase NovP then catalyzes the 4'-*O*-methylation of desmethyldescarbamoylnovobiocin to form descarbamoylnovobiocin via transfer from S-adenosylmethionine. The final step in the biosynthesis is the introduction of the 3'-*O*-carbamoyl group and this is achieved by the carbamoyltransferase NovN in the presence of carbamoyl phosphate and ATP. The availability of these three enzymes (NovM, NovP, and NovN) has paved the way for generating aminocoumarin libraries, thereby providing analogues for antibiotic-activity evaluation.

1.4. Structural elucidation studies of novobiocin

Extensive chemical modifications of novobiocin have been carried out, involving both semi-synthetic and synthetic approaches, in order to elucidate the biological mechanism. As mentioned previously, the N-acyl side chain of the coumarin is not essential for gyrase B inhibition but does contribute towards the intracellular uptake of the drug. According to Laurin *et al.*,[16] the high lipophilicity of these side chains may be responsible for the poor aqueous solubility of novobiocin, so they replaced the 3-acylamino group with more polar groups such as C(=O)R, C(=N-OR)R', CONHR and CONHOR resulting in greatly improved *in vitro* inhibition of DNA-gyrase. The C-3' carbamate of the sugar noviose has been replaced with many groups with little improvement. However, once it became established that the 5-methylpyrrolyl-2-carboxylate moiety of clorobiocin accounted for its increased antibacterial activity by contributing to higher hydrophobicity and higher membrane permeability, Periers *et al.* replaced the carbamate of novobiocin with 5-methylpyrrole-2-carboxylate and N-propargyloxycarbamate, with both showing improved antibacterial activity.[17]

A concerted effort has also been directed at replacing the 5',5'-dimethyl group of noviose with 5',5'-dialkyl groups. The presence of both alkyl groups as being essential for biological activity has been demonstrated by replacing noviose with L-rhamnose, an analogue bearing a 5'-hydrogen atom instead of the 5'-methyl axial group. This resulted in a five-fold reduction of inhibition of supercoiling of DNA-gyrase.[18] Recently, it was shown that the hydrophobic pockets of the enterococcal and streptococcal DNA-gyrase were slightly larger compared to that of gyrase B of *E. coli*. With this in mind, a 5',5'-spirocyclopentyl noviose derivative was synthesized which showed excellent biological activity, notably against the enterococcal family, and is currently undergoing further pharmacological evaluation.[2] Zeneca Pharmaceuticals recently (1997) published an analogue of novobiocin in which the noviose sugar was replaced with a 5',5'-dimethylcyclohexane ring, and the coumarin moiety was replaced with a 6',6'-spiroketal linked to a biphenyl ring (Figure 1. 8).[19]

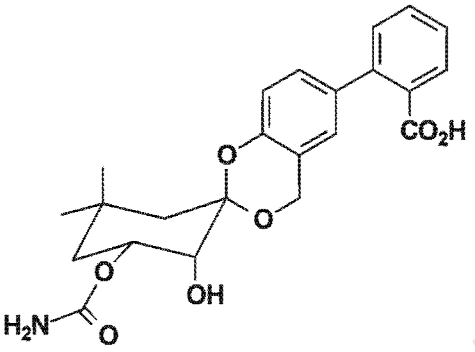
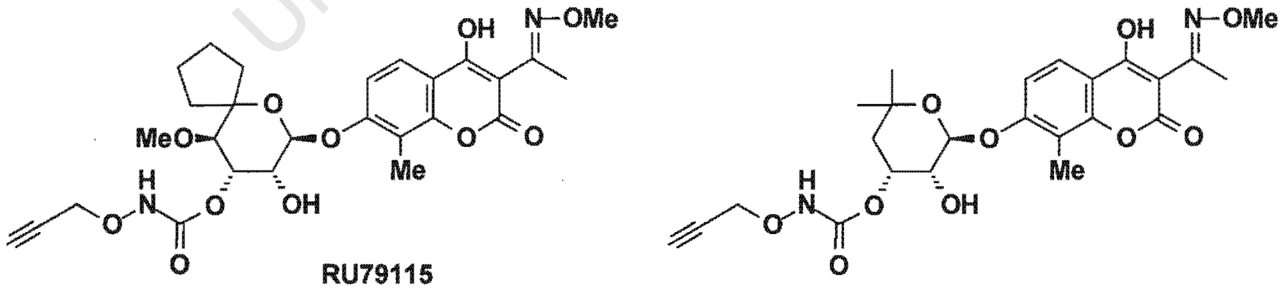


Figure 1.8: 6',6'-spiroketal derivative

This analogue, however, showed only weak biological activity, the explanation put forward being the absence of the 4'-OMe substituent that is involved in hydrophobic interactions with the surrounding amino acid residues of the gyrase B protein as well as in hydrogen bonding to the side chain of Asn46. This was corroborated recently when noviose was replaced with a 5',5'-dimethylcyclohexane ring, as well as with a 4'-desmethoxy analogue of RU79115 (Figure 1.9), with both showing weak biological activity.[19,20]



RU79115

Figure 1.9: RU79115 and a 4'-desmethoxy analogue thereof

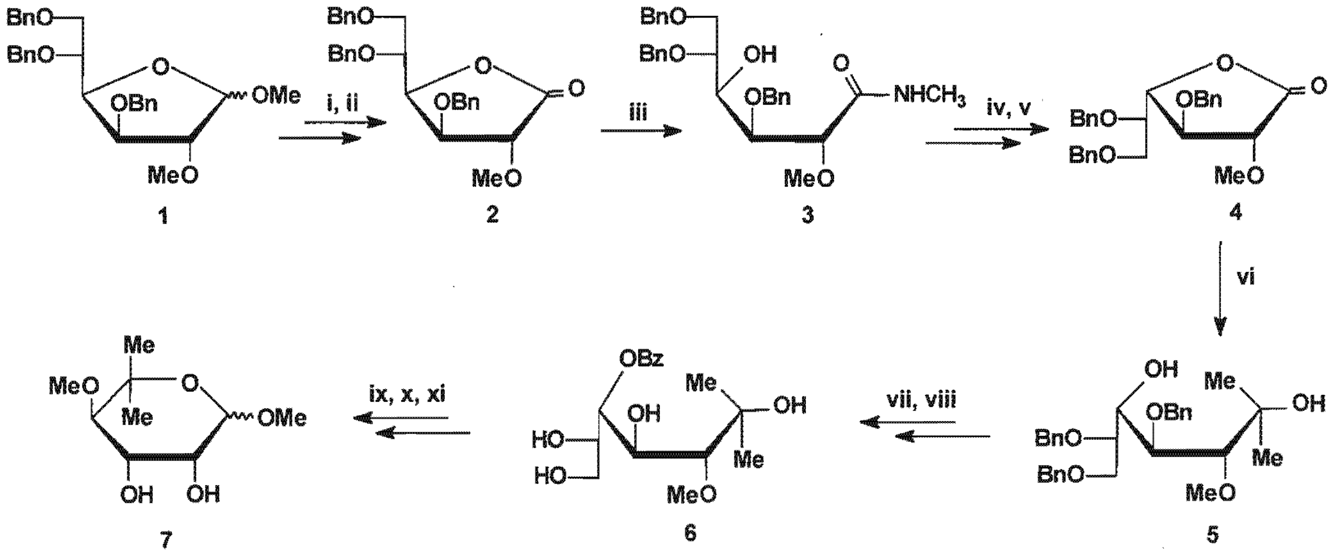
In summary, it has been shown that both noviose and the coumarin moiety are essential for biological activity. The C-3 N-acyl group of the coumarin can be replaced with isosteres, while both methyl groups on C-5' of noviose, while essential, may be replaced with longer-chain alkyl groups, both changes showing improved activity. The C-4' methoxy group of noviose is essential for activity, while the C-3' carbamate can be replaced with isosteres. However, the transfer of the carbamoyl group from C-3' to C-2' results in a dramatic reduction in the biological activity of novobiocin.

Despite all the research carried out on novobiocin over the last 40 years, a comprehensive structure-activity profile has yet to be established, thus providing opportunity for further research.

1.5. Syntheses of novobiocin, noviose and analogues

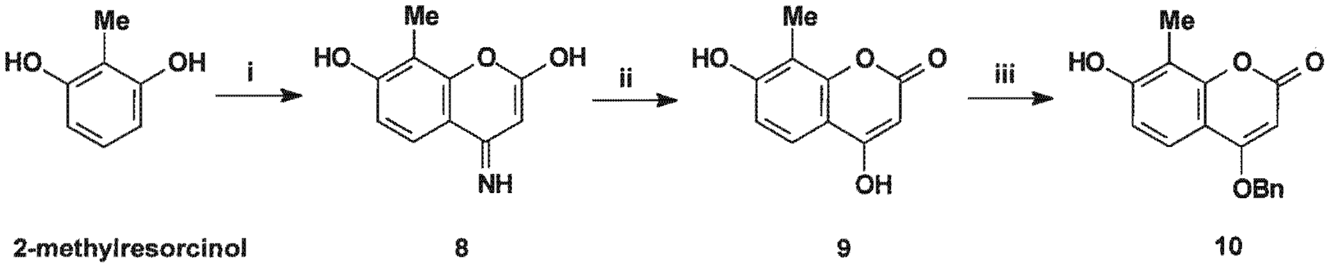
The first and only total synthesis of novobiocin was reported by Vaterlaus *et al.* in 1956.[21, 22, 23] The strategy involved the syntheses of (i) noviose from readily available methyl 3,5,6-tri-*O*-benzyl-2-*O*-methyl-D-glucofuranoside **1** (Scheme 1.2), and (ii) C-4 protected coumarin **10**. These two components were then coupled prior to the introduction of the sugar carbamoyl group and the coumarin N-acyl moiety.

The synthesis of noviose began with the conversion of methyl glucofuranoside **1** in two high-yielding steps into the corresponding *gluco*-γ-lactone **2** (Scheme 1.2). Nucleophilic opening by methylamine on **2** in methanol to generate the acyclic amide **3**, was followed by mesylation of the free secondary hydroxyl. Vigorous acid-hydrolysis (refluxing aqueous AcOH) of the amide followed by an S_N2 intramolecular nucleophilic displacement at C-4, with inversion of configuration, gave the *galacto*-γ-lactone **4**. The lactone was then subjected to a double Grignard reaction to yield acyclic diol **5**, which was regioselectively benzoylated at the secondary alcohol. Deprotection of the three benzyl ethers with Palladium-black furnished the tetra-ol **6**. Oxidative cleavage of the 5,6-diol with lead tetra-acetate, followed by cyclization, furnished a hemiacetal which was converted to the corresponding methyl glycoside. Finally, the benzoate was base-hydrolysed yielding methyl novioside **7** in 11 steps in a 2% overall yield from methyl 3,5,6-tri-*O*-benzyl-2-*O*-methyl-D-glucofuranoside **1**.



Scheme 1.2: *Reagents and conditions*: (i) 66% AcOH, reflux, 92%. (ii) $BaCO_3$, AcNHBr, 90% MeOH, room temperature, 82%. (iii) $MeNH_2$, MeOH, room temperature, 48%. (iv) MsCl, pyridine, CH_2Cl_2, 0°C – room temperature, 60%. (v) 66% AcOH, reflux, 73%. (vi) MgMeI (2 equiv.), ether, benzene, room temperature, 66%. (vii) BzCl, pyridine, 0°C – room temperature, 38%. (viii) Pd black, Norit-Supra-C, MeOH, 63%. (ix) $Pb(OAc)_4$, CH_2Cl_2, room temperature, 98%. (x) AcCl, MeOH, room temperature, 90%. (xi) 2 N NaOH, MeOH, 0°C, 70%.

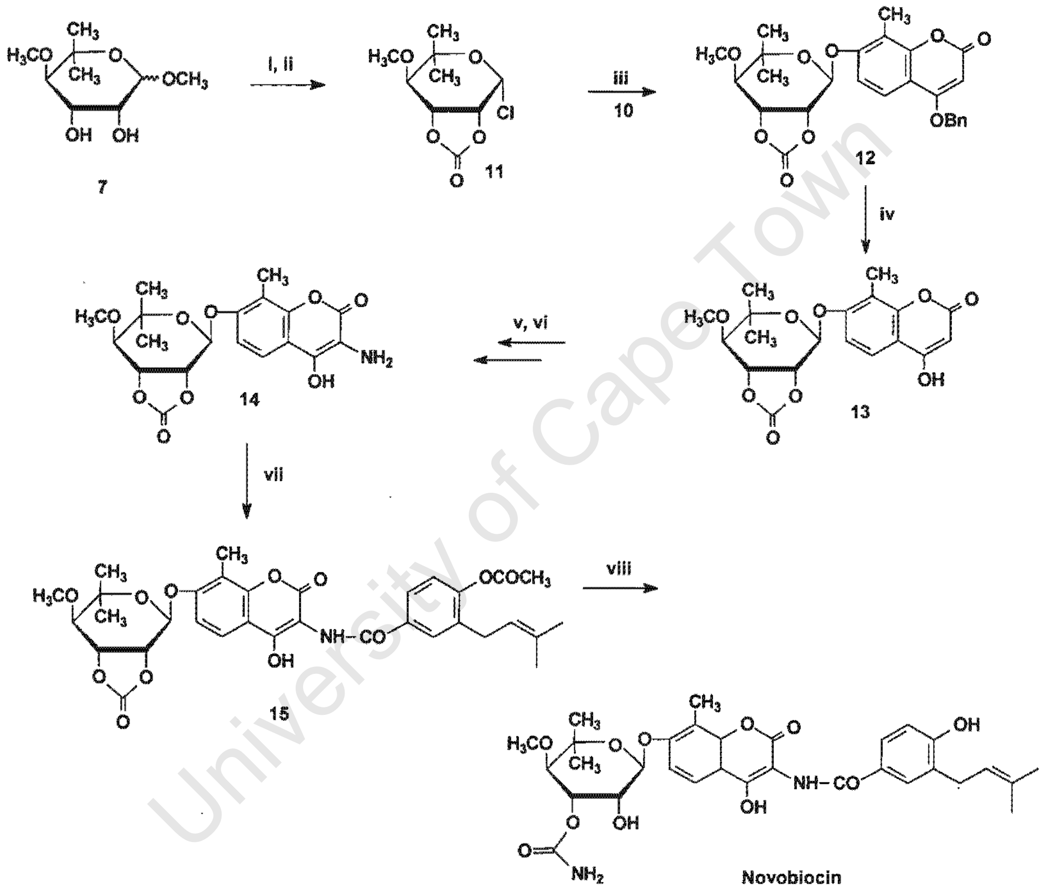
The synthesis of the coumarin moiety was achieved from 2-methylresorcinol in 3 steps in 13% overall yield and was based on the synthesis of novobiocinic acid introduced by Stammer *et al.* in 1958.[24] Thus, following a procedure introduced by Sonn in 1917,[25] condensation of 2-methylresorcinol with ethyl cyanoacetate in acid gave rise to the iminobenzopyran **8** (Scheme 1.3), the mechanism presumably being via C-4 nucleophilic addition onto the cyano group of ethyl cyanoacetate, followed by intramolecular cyclization onto the ester.



Scheme 1.3: *Reagents and conditions*: (i) Ethyl cyanoacetate, $ZnCl_2$, HCl gas, ether, 66%. (ii) 50% H_2SO_4, 50%. (iii) BnOH, conc. H_2SO_4, 39%.

Acid-hydrolysis of **8** yielded 4,7-dihydroxy-8-methylcoumarin **9**, which was regioselectively *O*-benzylated at its C-4 hydroxyl group employing acidic Michael substitution conditions to obtain coumarin aglycon **10**.

The synthesis of novobiocin was completed by the conversion of methyl novioside **7**, to its 2,3-*O*-carbonate (Scheme 1.4), followed by the direct conversion of the methyl glycoside to the anomerically stable β-L-glycosyl chloride **11** using the novel and unusual conditions of acetyl chloride and hydrogen chloride in nitromethane.



Scheme 1.4: *Reagents and conditions*: (i) Phosgene, benzene, pyridine, 67%. (ii) Nitromethane, AcCl. (iii) $CaSO_4$, Ag_2O, quinoline, 48%. (iv) 10% Pd/C, H_2, EtOAc, 50%. (v) Aniline, 1N HCl, $NaNO_2$, sodium acetate, 70%. (vi) 10% Pd/C, H_2, EtOAc, 51%. (vii) Pyridine, 4-acetoxy-3-isopenten-(2')-yl-benzoyl chloride, 25%. (viii) NH_3, 10%.

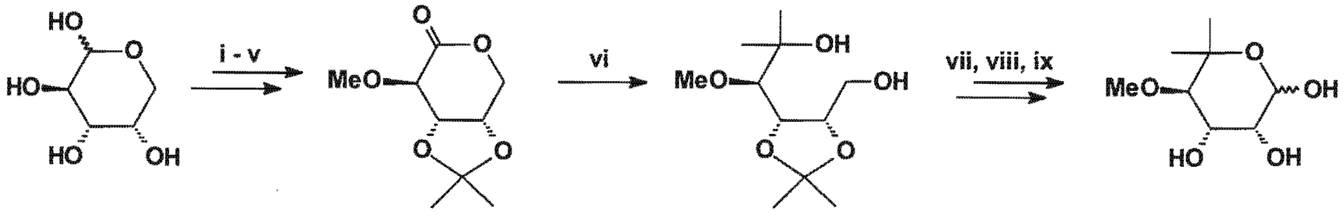
A known acylating reagent,[26] acetyl chloride in nitromethane presumably acylates the anomeric *O*-methyl, which ionises to form an intermediate oxocarbenium ion. This is then nucleophilically intercepted by chloride ion. Unfortunately, no yield was quoted for this conversion. The glycosyl chloride **11** was coupled with 4-benzyloxy-7-hydroxy-8-

methylcoumarin **10** using the well-known classical Koenigs-Knorr method employing silver oxide and quinoline to obtain the α-glycoside in 48% yield with inversion of configuration. Following debenzylation, the C-3 amino group was introduced via a two-step procedure involving: (i) electrophilic substitution with phenyldiazonium ion to form the azo compound, (ii) reductive cleavage of the azo bond with Pd/C and H_2 to give the amine **14**. The amine was then acylated, using standard conditions, with 4-acetoxy-3-(isopent-2'-enyl)-benzoyl chloride to yield the novobiocin precursor **15**, which underwent ammonolysis to yield a 4:1 mixture of novobiocin and isonovobiocin, the latter containing the carbamoyl group on the sugar C-2 axial hydroxyl group. Novobiocin was isolated by fractional crystallization in 10% yield from precursor **15**.

Numerous low-yielding steps were encountered in this total synthesis, including the formation of the amide **3** from lactone **2** (48%), the regioselective monobenzoylation of the galacto-diol (38% yield), the regioselective monobenzylation of 4,7-dihydroxy-8-methylcoumarin (39% yield), and the final ammonolysis step, which yielded a mixture of novobiocin and isonovobiocin that were difficult to separate and gave novobiocin in only 10% yield from **15**. Despite these low yielding steps, the synthesis was straightforward and the first of its kind, and with the development of improved methods and reagents, could be higher yielding.

In the total synthesis of an analogue of novobiocin in 1999, Laurin *et al.* also found the products of the final ammonolysis step difficult to separate.[16] Thus, in 2000, they published an improved total synthesis of an analogue of novobiocin, a precursor to RU79115,[17] in which noviose was synthesized from the sugar, L-arabinose, while the coumarin precursor was obtained from 2,4-dihydroxy-3-methylacetophenone, both starting materials being commercially available.

L-Arabinose, chosen to allow selective modifications at most positions, was converted in four protection-deprotection steps to a protected lactol which was oxidised with PCC to the lactone **16** (Scheme 1.5). The lactone was then reacted with two equivalents of methylmagnesium bromide to provide the acyclic diol **17**. Since it proved difficult to oxidise **17** directly to the lactol, diol **17** was first oxidatively cyclized to the lactone and then reduced to the lactol. Finally, acid-hydrolysis of the acetonide yielded noviose in 9 steps and in 20% overall yield from L-arabinose.

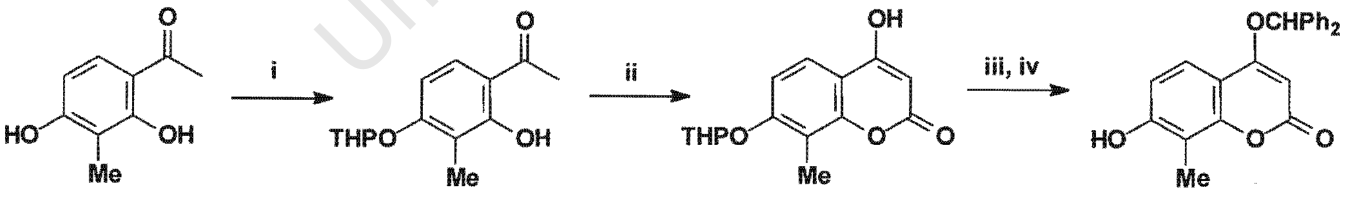


L-arabinose **16** **17** **noviose**

Scheme 1.5: *Reagents and conditions*: (i) BnOH, HCl gas, room temperature, 69%. (ii) 2,2-Dimethoxypropane, acetone, *p*-TsOH (cat.), room temperature, quant. (iii) *t*-BuOK, Me_2SO_4, THF, room temperature, quant. (iv) H_2, 10% Pd-C, EtOAc, quant. (v) PCC, CH_2Cl_2, room temperature, 87%. (vi) MeMgBr (2 eq), THF, 0°C, 86%. (vii) PCC, CH_2Cl_2, room temperature, 66%. (viii) DIBALH, THF, 0°C, 63%. (ix) H_2SO_4, H_2O, 65°C, 95%.

Although the synthesis does allow for selective modifications, there were three moderately-yielding steps, these being the initial benzylation of the anomeric hydroxyl group (69%), the oxidative cyclization of the diol to the lactone (66%) and its subsequent reduction to the lactol (63%). These steps reduced the overall yield considerably which is disadvantageous on a large scale.

In the coumarin part of Laurin's synthesis, in order to overcome chemoselectivity problems associated with selective protection of 4,7-dihydroxy-8-methylcoumarin, the 4-hydroxyl group, as the least hydrogen-bonded and sterically more accessible of the two hydroxyl groups of 2,4-dihydroxy-3-methylacetophenone, was first selectively protected as its THP derivative **18** (Scheme 1.6).

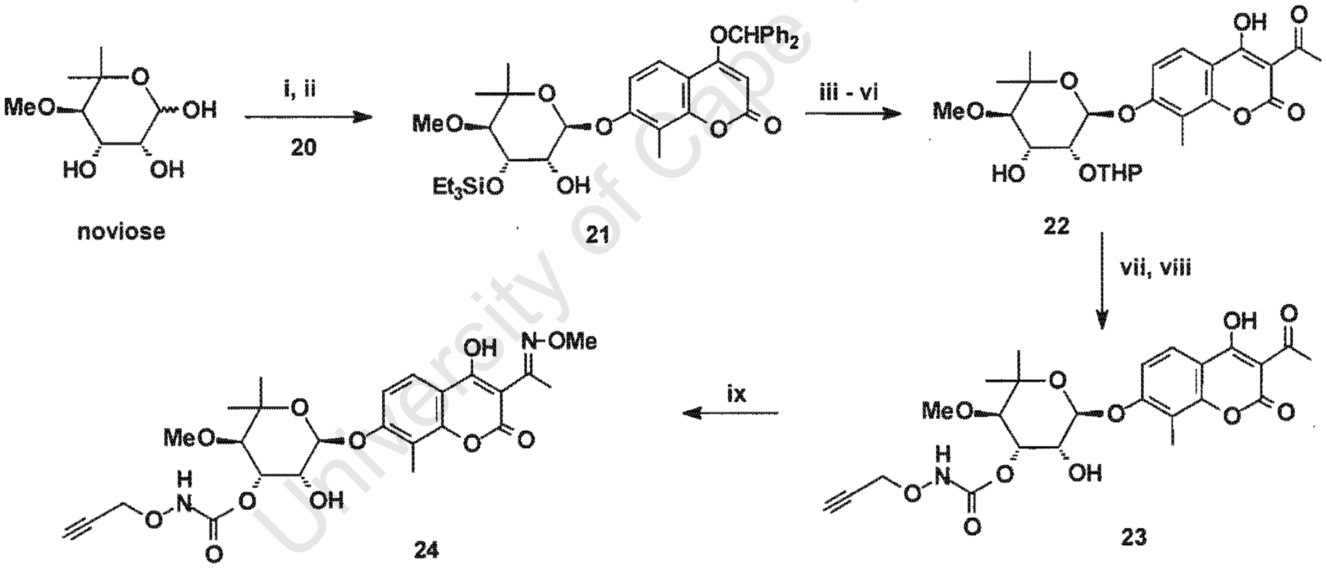


2,4-dihydroxy-3-methylacetophenone **18** **19** **20**

Scheme 1.6: *Reagents and conditions*: (i) DHP, *p*-TsOH (cat.), Et_2O, room temperature, 83%. (ii) NaH, $CO(OEt)_2$, toluene, reflux, 91%. (iii) Ph_2CN_2, DMF, 65°C, 55%. (iv) HCl, MeOH, CH_2Cl_2, room temperature, 90%.

Acetophenone **18** was then converted directly to the 4-hydroxycoumarin **19**, by a method first described by Barker *et al.* in 1971 involving base-mediated *C*-acylation with diethyl carbonate.[27] Subsequent protection of the coumarin 4-OH as a benzhydryl ether, followed by deprotection of the THP acetal yielded the mono-protected coumarin aglycon **20**, in 37% overall yield from 2,4-dihydroxy-3-methylacetophenone. Although the synthesis was an improvement on that described previously, protection of the coumarin 4-OH remained problematic, with the benzhydryl ether being the highest yielding (55%) of the protecting groups tried.

Under Mitsunobu conditions, noviose, as a mixture of anomers (the authors failed to mention the anomeric ratio), was coupled directly to the protected coumarin to yield the α-glycoside[*] as the major product (α:β = 4:1) in 67% yield after chromatographic separation (Scheme 1.7). This was an improvement on the yield obtained from the Ag_2O-quinoline coupling (48%) used by Vaterlaus (Scheme 1.4).



Scheme 1.7: *Reagents and conditions*: (i) PPh_3, $i PrO_2CN{=}NCO_2Et$, CH_2Cl_2, room temperature, 67%. (ii) Et_3SiCl, DIPEA, imidazole, CH_2Cl_2, room temperature, 70%. (iii) DHP, *p*-TsOH (cat.), CH_2Cl_2, room temperature. (iv) H_2, 10% Pd-C, THF, room temperature. (v) Ac_2O, DMAP, CH_2Cl_2, 0°C. (vi) Bu_4NF, THF, room temperature, 70% over 4 steps. (vii) (a) p-$NO_2C_6H_4OCOCl$, DMAP, CH_2Cl_2, 0°C; (b) $HC{\equiv}CCH_2ONH_2.HCl$, DMAP, DMF, room temperature. (viii) *p*-TsOH cat, MeOH, room temperature, 67% over 2 steps. (ix) $MeONH_2HCl$, KOAc, EtOH, room temperature, 80%.

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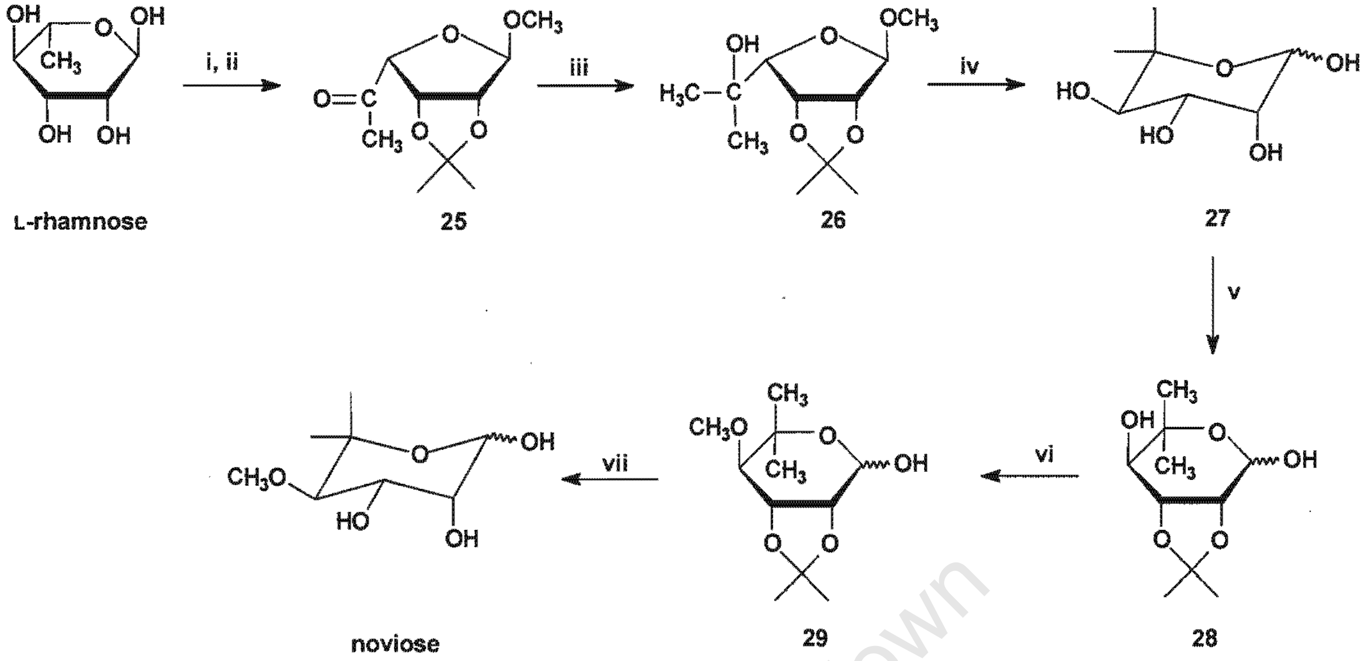
[*] Noviose is an L-sugar so when the anomeric centre projects upwards it is referred to as the α-anomer.

Furthermore, no glycosyl halide intermediate was required and regioselective displacement of the anomeric hydroxyl group over the C-2' and C-3' hydroxyl groups was achieved. The reaction proceeds via an S_N2 mechanism resulting in inversion of configuration, so the stereochemistry of the product depends on the configuration of the starting sugar.

Since the authors found the regioselective introduction of the carbamate onto C-3' via the 2',3'-*O*-carbonate difficult in terms of separation, the strategy developed by them involved sequential protection of the equatorial C-3' and C-2' hydroxyl groups, followed by deprotection of the C-3' hydroxyl group, thereby allowing the introduction of the carbamate directly onto C-3'. Thus the α-glycoside was regioselectively protected as a triethylsilyl ether at the equatorial C-3' hydroxyl to give **21** in good yield (70%). The axial C-2' hydroxyl was then protected as a THP acetal, the benhydryl ether hydrolysed and the coumarin C-3 position acetylated, the latter achieved directly by modifying both the base and solvent commonly used for O-acetylation, namely Et_3N and THF, to DMAP and CH_2Cl_2. Subsequent deprotection of the silyl ether yielded **22**, with the C-3' hydroxyl free. The N-propargyloxycarbamoyl moiety was then introduced via nucleophilic substution of the C-3 *p*-nitrophenyl carbonate derivative and the tetrahydropyran acetal acid-hydrolysed to give **23**. The final transformation involved conversion of the C-3 acetyl group into its *O*-methyl-oxime **24**.

Aside from the above two total syntheses, enantioselective syntheses of L-noviose have also been accomplished from L-rhamnose[28], D-gulonolactone[29], and a sugar building block derived from furfural[30]. The synthesis of D-noviose was achieved from (*1S,4R*)-4-acetoxy-5,5-dimethyl-2-cyclopenten-1-ol.[31]

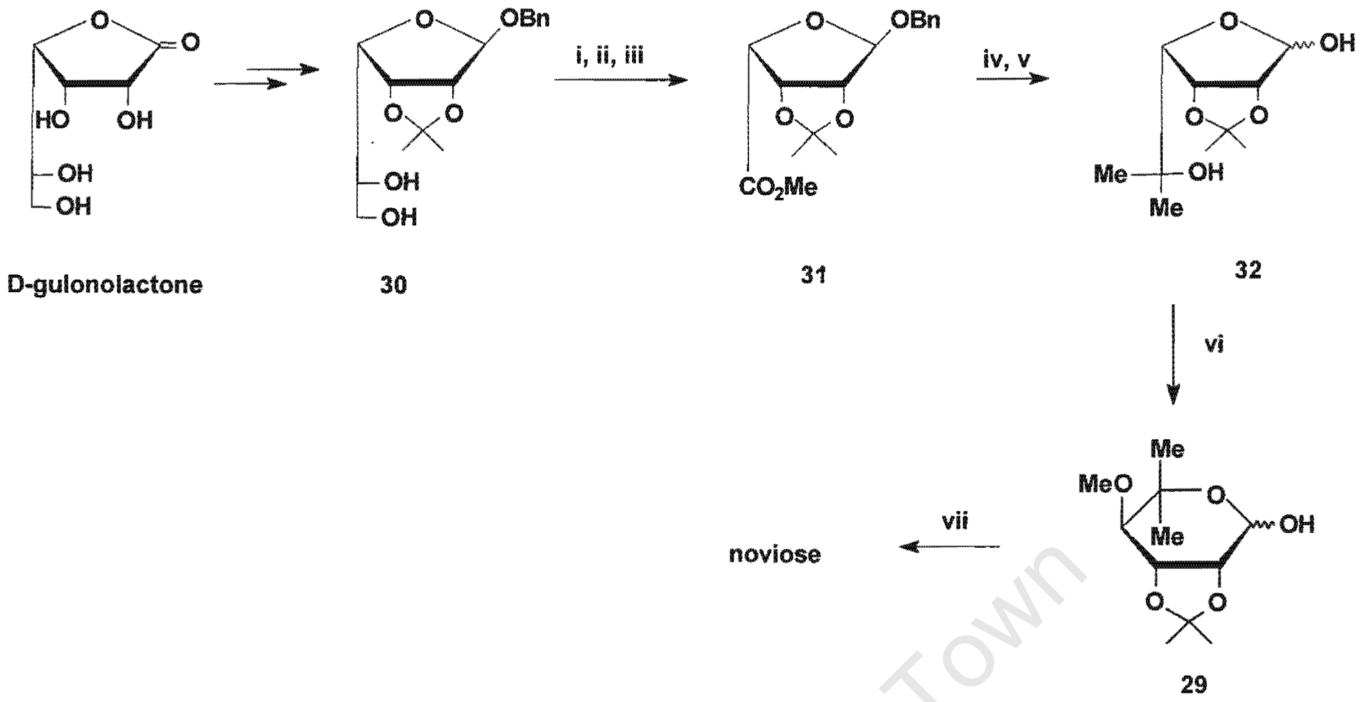
In 1986, Klemer *et al.* published the synthesis of noviose in 7 steps and in 33% overall yield from L-rhamnose.[28] Thus, L-rhamnose was converted in 2 steps to ketone **25** (Scheme 1.8), which was reacted in a single Grignard reaction to form methyl 2,3-*O*-isopropylidene-5,5-di-*C*-methyl-α-L-lyxofuranoside **26**. Hydrolysis of both the methyl glycoside and the acetonide group under strongly acidic conditions resulted in simultaneous transformation of the furanoid ring to its pyranoid form, yielding tetra-ol **27** in high yield. After reprotecting the 2,3-diol as the acetonide, the use of Me_2SO_4 and *tert-n*-butylammonium bromide in base resulted in novel phase-transfer catalyzed mono-methylation to give **29**.



Scheme 1.8: *Reagents and conditions*: (i) $(CH_3)_2C(OCH_3)_2$, *p*-TsOH (cat.), MeOH, 89%. (ii) CrO_3, pyridine, CH_2Cl_2, H_2SO_4, 94%. (iii) MeMgI, Et_2O, 95%. (iv) 1M H_2SO_4, 80°C, 95%. (v) Acetone, MeOH, H_2SO_4, 71%. (vi) 50% NaOH, toluene, $^nBu_4NBr$, Me_2SO_4, 81%. (vii) $EtOH/CF_3COOH/H_2O$ (90:9:1), 80°C, 75%.

Deprotection of the acetonide yielded noviose as a mixture of anomers. In the phase-transfer catalyzed mono-methylation step, both C-1 and C-4 hydroxyl groups were methylated, but owing to the hydrolytic susceptibility of 6-deoxysugars as a result of the electronic effect of replacing a hydroxyl group with hydrogen, rapid hydrolysis of the anomeric methoxy group took place. The synthesis required just 7 high-yielding steps, and lends itself to functional modifications at most positions.

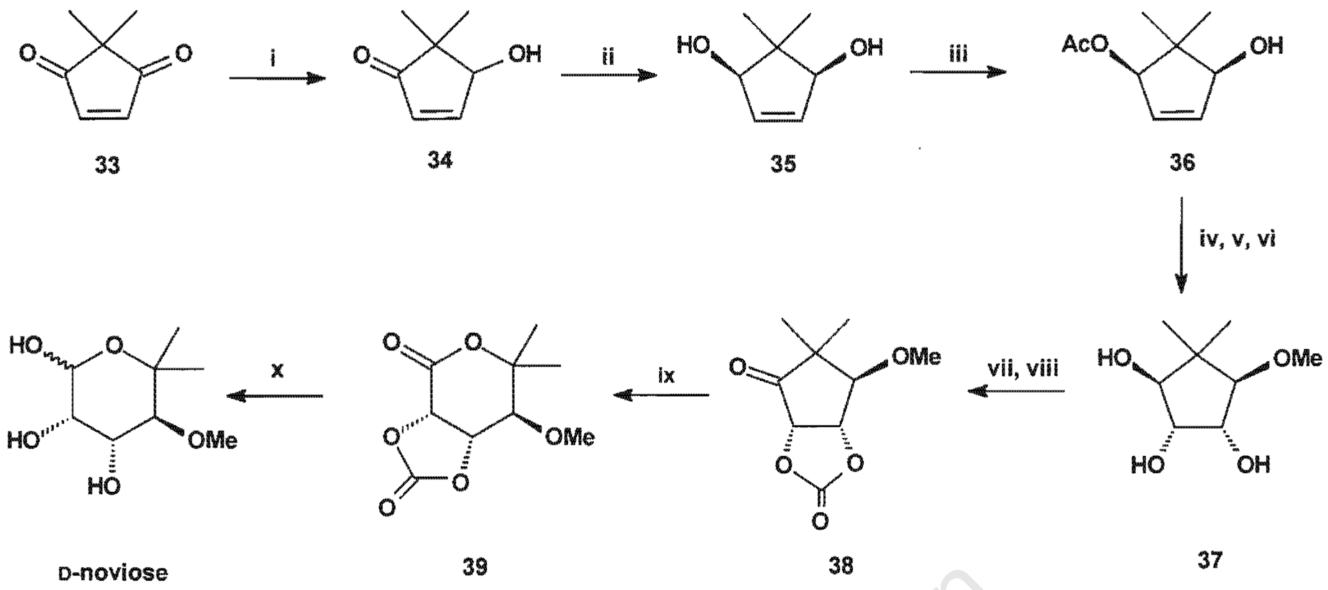
Ješelnik et al.[29] introduced a synthesis of noviose in 2003 based on Klemer and Waldmann's synthesis[28] but starting with D-gulonolactone. The sugar was first transformed by a known reaction sequence to its isopropylidene derivative **30** (Scheme 1.9). Periodate cleavage of the 5,6-diol yielded the aldehyde which was oxidized to the acid with Ag_2O, and esterified with diazomethane to form the methyl ester **31**. A double Grignard reaction gave the tertiary alcohol which was followed by debenzylation to yield the L-noviofuranose derivative **32**, which had physical data distinctly different from the data for the pyranose derivative **29** as described in the previous synthesis.



Scheme 1.9: *Reagents and conditions*: (i) $NaIO_4$, MeOH, H_2O, 73%. (ii) $AgNO_3$, KOH, EtOH, H_2O, 92%. (iii) CH_2N_2 in ether, 99%. (iv) MeMgCl, Et_2O, 95%. (v) 10% Pd/C, H_2, Et_2O, 99%. (vi) Me_2SO_4, 50% NaOH, H_2O-toluene/CH_2Cl_2, Bu_4NBr, 61%. (vii) EtOH/CF_3CO_2H/H_2O or DOWEX 50-W/H_2O.

Using the same phase-transfer conditions as in Klemer and Waldmann's synthesis,[28] the 4-OH group was regioselectively methylated with ring expansion to give **29**. Acid hydrolysis of the acetonide furnished noviose in 11 steps in an overall yield of 12% from D-gulonolactone.

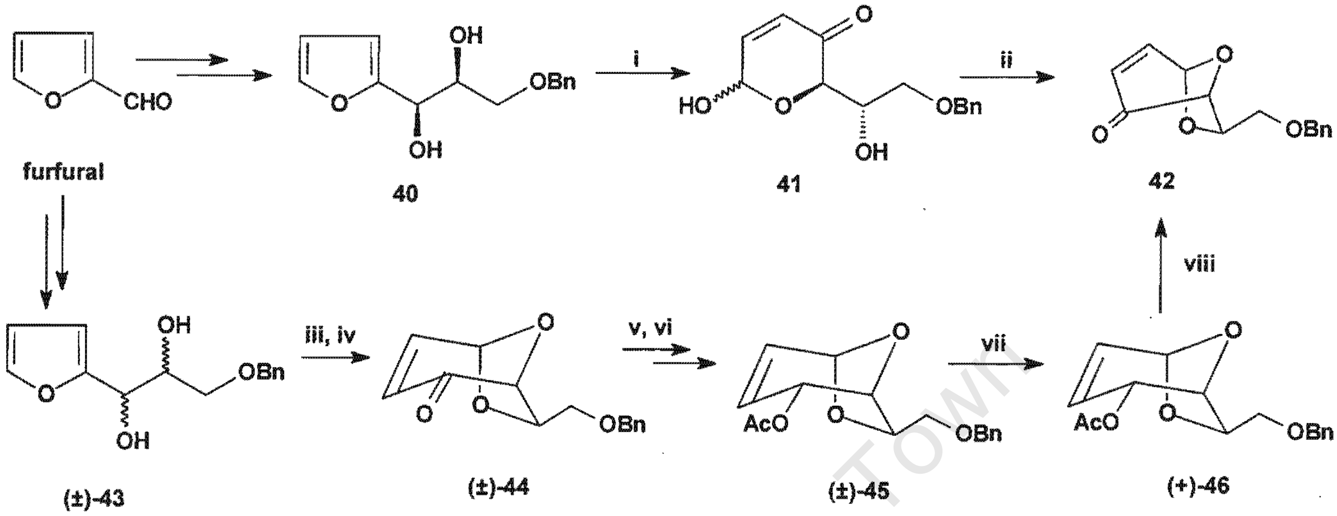
The first enantioselective synthesis of D-noviose from non-carbohydrate precursors was introduced by Pankau and Kreiser[31,32] in 1998 from the readily available enedione **33** (Scheme 1.10). Mono-reduction of **33** using Luche's reagent of sodium borohydride and $CeCl_3$ ensured 1,2-hydride addition and furnished **34**. A further molar equivalent of Luche's reagent resulted in the second hydride being delivered to the face opposite to the C-1 hydroxyl group giving the *cis*-diol **35**. Desymmetrization was achieved enzymatically by mono-acetylation with vinyl acetate in the presence of a lipase to give an almost quantitative yield of building block (1*S*, 4*R*)-4-acetoxy-5,5-dimethyl-2-cyclopentene-1-ol, **36**. Using diazomethane and catalytic amounts of BF_3-etherate, **36** was transformed to its methyl ether, via carbene insertion into the OH-bond.



Scheme 1.10: *Reagents and conditions*: (i) $NaBH_4$ (1 equiv.), $CeCl_3.7H_2O$ (1 equiv.), MeOH, -78°C, 75%. (ii) $NaBH_4$ (1 equiv.), $CeCl_3.7H_2O$ (1 equiv.), MeOH, 0°C, 94%. (iii) Lipase, vinyl-OAc, THF, room temperature, quant. (iv) CH_2N_2, $BF_3.Et_2O$, Et_2O, 0°C, 78%. (v) OsO_4, NMO, acetone/water (8/1), 0°C, 86%. (vi) aq. KOH, MeOH, quant. (vii) $CO(OCCl_3)_2$, pyridine, CH_2Cl_2, -70°C, 84%. (viii) PDC, CH_2Cl_2, 20°C, 92%. (ix) *m*-CPBA, CH_2Cl_2, room temperature, 76%. (x) DIBALH, CH_2Cl_2, -78°C, 78%.

Cis-dihydroxylation from the α-face was achieved with NMO and catalytic OsO_4 in high yield and as a result of the two substituents on the β-face. Deacetylation to give triol **37** was followed by protection of the *syn*-diol as its carbonate.The remaining hydroxyl group was oxidized with PDC to give ketone **38**, which was transformed by a Baeyer-Villiger rearrangement, in high regioselectivity at the more substituted carbon, to afford the corresponding noviolactone **39**. Reduction, with concomitant deprotection of the cyclic carbonate, gave D-noviose in 31% yield over 7 steps from (1*S*, 4*R*)-4-acetoxy-5,5-dimethyl-2-cyclopentene-1-ol **36**. This synthesis is multifunctional as it starts from a very simple prochiral enedione that is converted in only a few steps to a chiral building block, namely **36**, that may be used as a precursor to many natural products. Most of the reagents employed in the synthesis are relatively cheap and readily available, and the lipase, which was immobilized on solid support, could be reused many times without loss of activity. Although this synthesis could be adapted for the preparation of L-noviose, the possibilities for functional modifications related to noviose are limited, so analogues of noviose would be difficult to synthesize, and the use of OsO_4 in the *cis*-dihydroxylation step makes large-scale synthesis unfeasible.

The most recent enantioselective synthesis was published by Ogasawara's group in 2000[30] in which a benzylated sugar building block **42**, was prepared from furfural in enantiomerically pure forms by either a chemical[33] or enzymatic procedure[34] (Scheme 1.11).

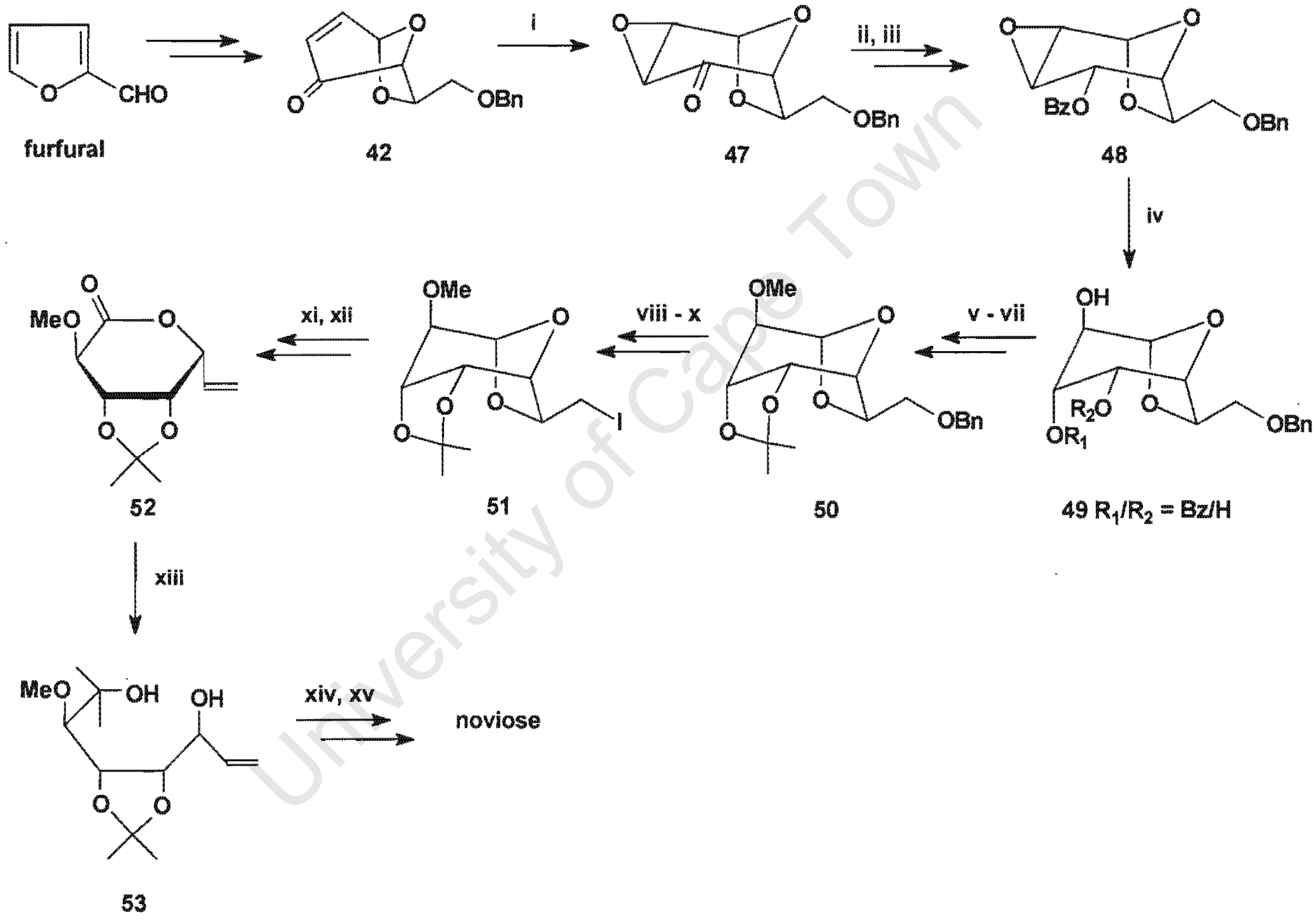


Scheme 1.11: *Reagents and conditions*: (i) NBS, NaOAc, aq. THF, 0°C, 51%. (ii) *p*-TsOH (cat.), benzene, reflux, 53%. (iii) *m*-CPBA, CH_2Cl_2, 0°C – room temperature. (iv) *p*-TsOH (cat.), benzene, reflux, 68% over 2 steps. (v) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, 0°C, 93%. (vi) Ac_2O, pyridine, room temperature, 94%. (vii) Lipase PS, phosphate buffer:acetone (9:1), 46%. (viii) PCC, NaOAc, molecular sieves, CH_2Cl_2, 93%.

The chemical method involved a Wittig reaction between furfural and methyl(triphenylphosphoranylidene) acetate to generate (*E*)-methyl-3-(2-furyl)propenoate, which was reduced to a primary alcohol and protected as a benzyl ether. A Sharpless asymmetric dihydroxylation (AD reaction) on the *E*-alkene furnished the cis-diol **40**. In the presence of NBS and sodium acetate in aqueous THF, the furan ring underwent ring expansion initiated by C-5 addition of a bromonium ion to give **41** as a mixture of anomers. Acid-catalyzed cyclization furnished enantiomerically pure **42**, via nucleophilic interception of an oxocarbenium ion intermediate.

The enzymatic procedure was developed as an alternative that could be carried out on a large scale without using the expensive Sharpless AD-mix reagents. The synthesis was similar to that of the chemical method except that *cis*-dihydroxylation with OsO_4 and NMO gave (±)-**43** as a racemic mixture, which underwent a peracid-mediated Achmatowicz rearrangement followed by acidic cyclization[35] to furnish (±)-**44**. The enone was reduced diastereoselectively to the allylic alcohol using Luche conditions, and the hydroxyl group was acetylated to afford racemic (±)-**45**. Enzymatic separation of the enantiomers was achieved by lipase-mediated hydrolysis of the acetate to furnish a single enantiomer in 46% yield. Oxidation back to the enone using PCC gave enantiomerically pure **42**.

Owing to its rigid framework, diastereoselective epoxidation of **42** with alkaline hydrogen peroxide gave the *exo*-epoxide **47**, as a single product (Scheme 1.12). Diastereoselective reduction under Luche conditions using sodium borohydride and $CeCl_3$ gave the *endo*-alcohol, which was protected as its benzoate **48**.



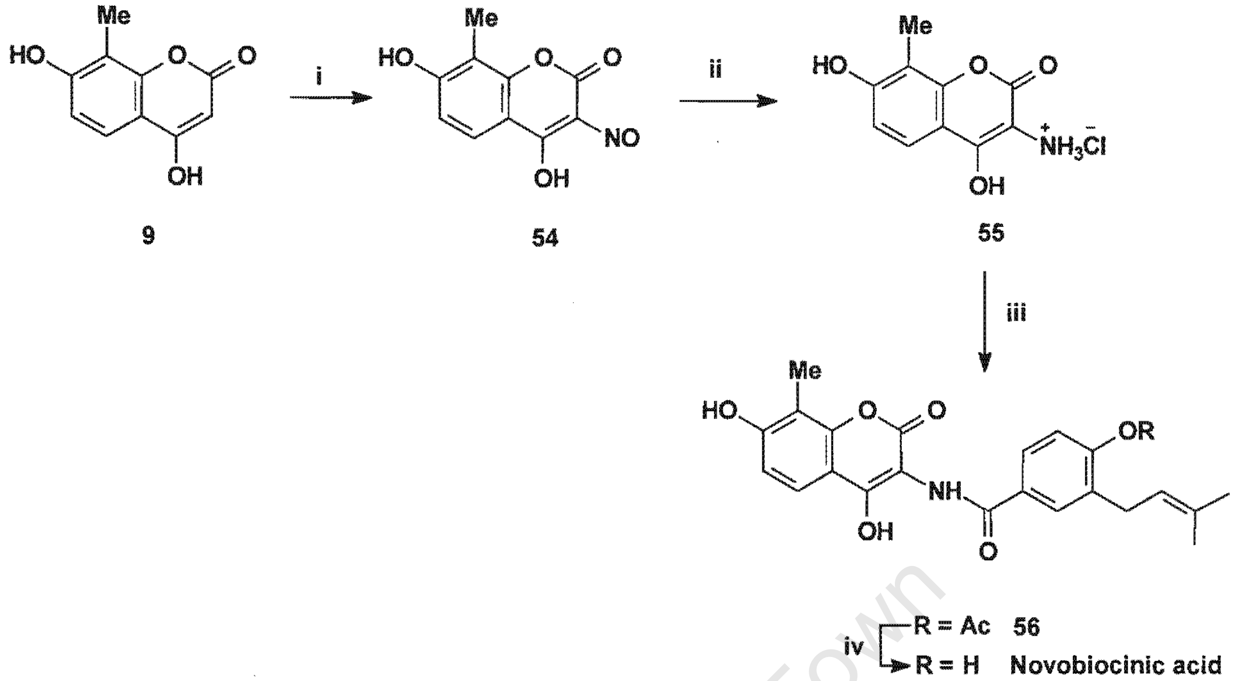
Scheme 1.12: *Reagents and conditions*: (i) 30% H_2O_2, 0.5N NaOH, THF, 0°C. (ii) $NaBH_4$-$CeCl_3.7H_2O$, MeOH, 0°C, 82% for 2 steps. (iii) BzCl, pyridine, CH_2Cl_2, 96%. (iv) $BF_3.OEt_2$, toluene. (v) NaOMe, MeOH. (vi) $Me_2C(OMe)_2$, PPTS (cat.), toluene, reflux, 84% overr 3 steps. (vii) MeI, NaH, THF, 99%. (viii) H_2, 10% Pd/C, MeOH, 91%. (ix) MsCl, Et_3N, CH_2Cl_2. (x) LiI, THF, reflux, 90% over 2 steps. (xi) Zn, AcOH:MeOH (1:10), 97%. (xii) TPAP-NMO, 4 Å molecular sieves, CH_2Cl_2, 90%. (xiii) MeLi, THF, 0°C, 83%. (xiv) OsO_4 (cat.), $NaIO_4$, 50% aq. THF. (xv) Dowex 50-W, H_2O, 70°C, 59% over 2 steps.

Regio- and diastereoselective Lewis-acid mediated rearrangement of the epoxide involving neighbouring group participation by the benzoate group produced the ring-opened alcohol as a mixture of benzoates **49**, which were hydrolysed without separation. Ketal protection of the *cis*-hydroxyl groups of the resulting triol, followed by methylation with methyl iodide under standard conditions gave the ketal methyl ether **50**. Removal of the benzyl ether by hydrogenolysis, standard elaboration of the resultant alcohol to iodide **52** via its mesylate, followed by reductive elimination and lactol oxidation furnished lactone **52**. The gem-dimethyl groups were introduced by treatment with methyllithium to give the acyclic diol **53**, and the alkene was oxidatively cleaved using Lemieux-Johnson conditions of catalytic OsO_4 and $NaIO_4$ to give the hemiacetal. Finally, acid-hydrolysis of the acetonide group gave noviose in 23% yield over 15 steps from **42**.

Although this is an elegant example of a completely diastereoselective total synthesis, it is too long and not suited to large scale as some of the reagents used are very expensive.

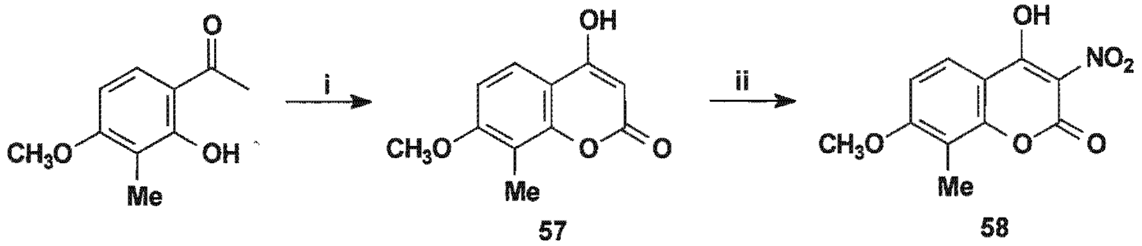
1.6. Synthesis of the coumarin moiety

The only total synthesis of novobiocinic acid was reported by Stammer *et al.* in 1958, and was developed to elucidate the structure of the coumarin moiety.[24] 2,7-Dihydroxy-8-methylcoumarin **9**, synthesized from 2-methylresorcinol and used by Vaterlaus *et al.* in the synthesis of novobiocin (see Scheme 1.3, page 10),[21] underwent electrophilic substitution with NO^+, formed from sodium nitrite and acetic acid, at the C-3 position to give the unstable nitroso compound **54**, which was reduced to the ammonium salt **55** (Scheme 1.13). N-acylation with 4-acetoxy-3-(3-methyl-2-butenyl)benzoyl chloride then gave mono-acetylated novobiocinic acid **56**, which was hydrolysed to novobiocinic acid. The synthesis was extremely low yielding (< 7%) but was successful in producing novobiocinic acid based on data from the degradation product of novobiocin.



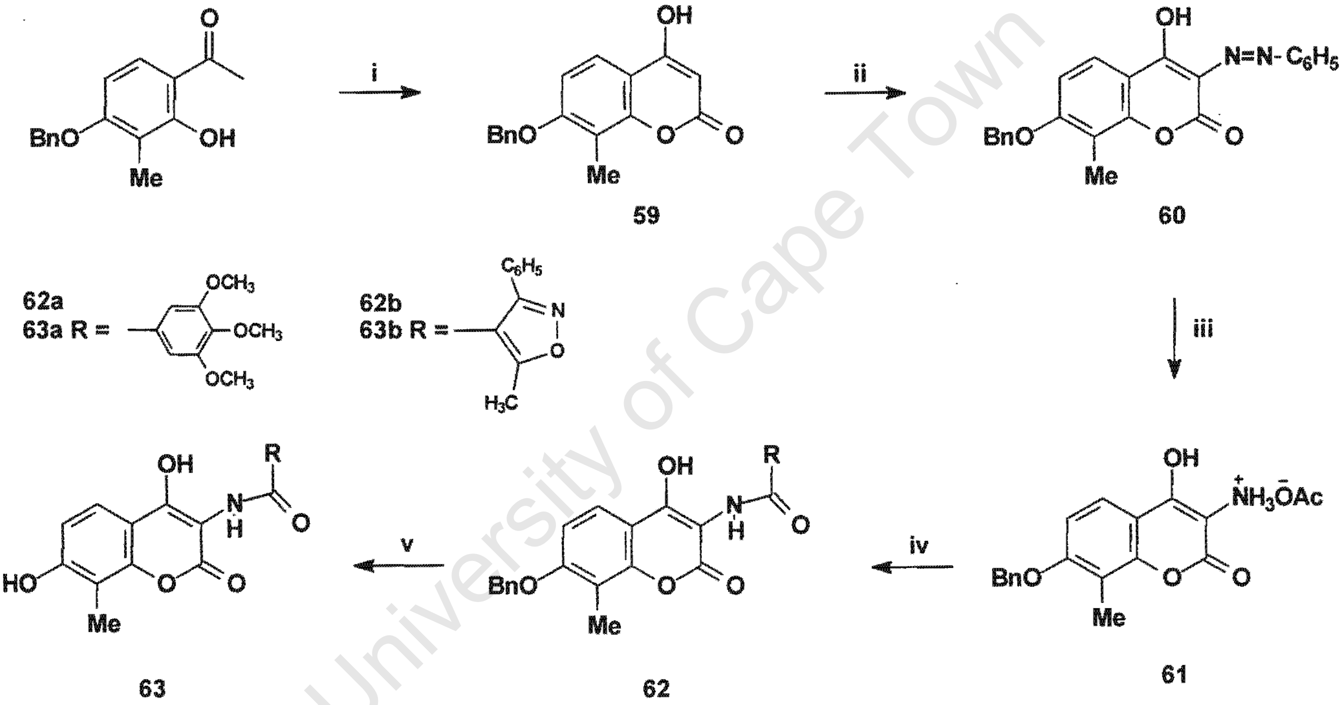
Scheme 1.13: *Reagents and conditions*: (i) Sodium nitrite, H_2O, AcOH, 47%. (ii) 10% Pd/C, 2.5N HCl, H_2, EtOH. (iii) 4-Acetoxy-3-(3-methyl-2-butenyl)benzoyl chloride, pyridine, 47%. (iv) 2.5N NaOH.

The synthesis of 4-hydroxy-7-methoxy-8-methyl-3-nitrocoumarin and report of its antiallergic activity was published by Cantello *et al.* in 1975.[36] Using the method described by Barker *et al.*,[27] commercially available 2-hydroxy-4-methoxy-3-methylacetophenone was converted to the corresponding coumarin **57** with sodium hydride and diethyl carbonate in refluxing benzene (Scheme 1.14). Based on a method described by Klosa,[37] nitration of **57** was effected in very high yield with fuming nitric acid in chloroform at room temperature to give **58**.



Scheme 1.14: *Reagents and conditions*: (i) $CO(OEt)_2$, NaH, benzene, reflux, 89%. (ii) Fuming HNO_3, $CHCl_3$, room temperature, 96%.

Bognar *et al.*[38] have published the synthesis and biological activity of numerous 3-acylamino-4-hydroxycoumarin derivatives, two of which were derived from 2,4-dihydroxy-3-methylacetophenone. In this publication, 4-benzyloxy-2-hydroxy-3-methylacetophenone was converted to the corresponding coumarin **59** in 72% yield using sodium metal and diethyl carbonate as described by Boyd *et al.* in 1948 (Scheme 1.15).[39] Using the well-known method patented by Okumura,[40] the 3-phenylazo moiety was introduced at C-3 of **59** via electrophilic substitution with phenyldiazonium ion to give **60** which was reduced with sodium dithionite to give **61** as a salt in 91% yield. The reduction step was developed by Bognar to avoid deprotection of the benzyl group.[38]



Scheme 1.15: *Reagents and conditions*: (i) Na (pulverized), $CO(OEt)_2$, heat, 72%. (ii) $PhNH_2$, H_2O, 35% HCl, $NaNO_2$, NaOAc, EtOH, 0°C, 80%. (iii) $Na_2S_2O_4$, CH_3COONa, EtOH, H_2O, reflux, 91%. (iv) RCOCl, THF, 4% NaOH. (v) 10% Pd/C, EtOH, 0.2M NaOH, 38% and 51% over 2 steps.

The N-acylation of ammonium salt **61** with two different acid chlorides in THF under Schotten-Baumann conditions (aqueous NaOH) afforded amides **62a** and **62b**, which were exposed to catalytic hydrogenolysis to give **63a** and **63b**, in 38% and 51% overall yield respectively from **61**. No reasons were given for these poor yields. Both **63a** and **63b** showed poor antibacterial and antifungal activity when compared with novobiocin, establishing the important role played by the noviosyl-sugar on the biological activity.

Interestingly, the synthesis of 4-hydroxycoumarins has been extensively documented in the literature, but A-ring substituted coumarin syntheses have not, with the above mentioned coumarin syntheses being the only publications related to the noviosyl-coumarin unit.

1.7. Overview of Thesis objective

A structure-activity aspect that has yet to be investigated in the novobiocin field based on analogues that have been synthesized, are the roles played by the configuration of the C-4 methoxy group and the conformation of the noviose ring on the biological activity of novobiocin. The C-4 methoxy group (positioned equatorially) of noviose has been shown to be essential for enzyme receptor interaction, as it is involved in hydrophobic interactions with the surrounding amino acid residues, and replacement of it with a hydrogen has resulted in greatly reduced antibacterial activity. The $^1C_4$ chair conformation preferentially adopted by noviose is also assumed to be vital for enzyme binding. A conformational analysis for both chair conformations of noviose reveals 3 axial substituents for each chair. In each set of interactions there is an unfavourable 1,3-diaxial interaction as shown in Figure 1.10 involving two large groups (> hydrogen group).

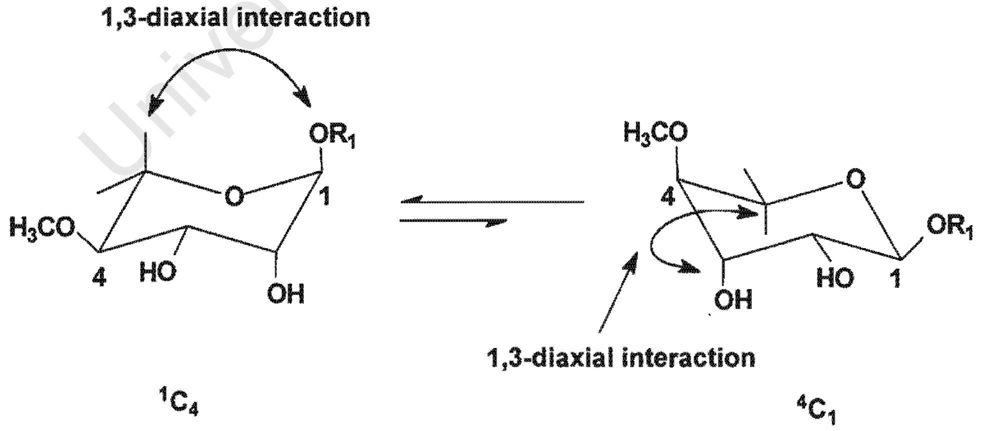


Figure 1.10: $^1C_4$ versus $^4C_1$ conformation of noviose

The $^1C_4$ conformer is favoured however, because (i) the large methoxy group (A value 3.1 kJ/mol) is equatorial whereas in the $^4C_1$ conformer it is axial, and (ii) the axial α-anomeric group imparts a greater thermodynamic stability compared to the equatorial β-anomeric group in the $^4C_1$ conformer, the stability being ascribed to the well known "anomeric effect". An interpretation of the anomeric effect, first described by Edward,[41] suggests that this stabilization is due to orbital overlap between the axial lone pair of electrons on the ring oxygen atom and the anti-periplanar antibonding σ^*-orbital of the C-O bond. This leads to a shortening of the bond connecting the ring oxygen atom to the anomeric centre and a lengthening of the axial α-anomeric C-O bond relative to the respective bond lengths in the equatorial β-anomer. Resonance depiction of this concept is illustrated in Figure 1.11.

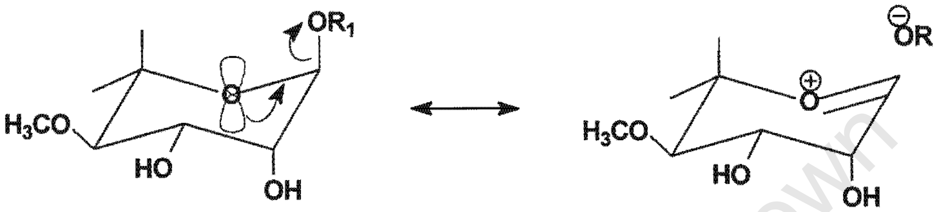


Figure 1.11: Resonance stabilisation of the anomeric centre

A consequence of this bond-lengthening is that the axial OR group is further away from the anomeric centre and hence the 1,3-diaxial interaction with the C-5 methyl is reduced.

Whereas noviose preferentially adopts the $^1C_4$ conformation, no interest has been shown in the C-4 epimer of noviose, presumably because of the assumption that the ring will flip to a biologically inactive $^4C_1$ conformation. Thus it seemed appropriate to explore the C-4 substituent with the aim of:

(i) appraising the configuration and biological activity of 4-epi-noviose[†] and

(ii) substituting 4-epi-noviose stereoselectively at the 5- position to bias the chair conformation back into a $^1C_4$ form, while retaining the 4-OMe epimeric configuration (Figure 1.12).

[†] A term adopted in the thesis for the C-4 epimer of noviose

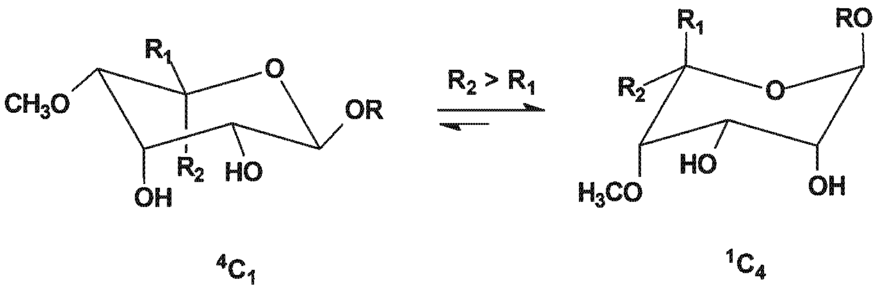


Figure 1.12: The chair would adopt the $^1C_4$ conformation provided R_2 is much larger than R_1.

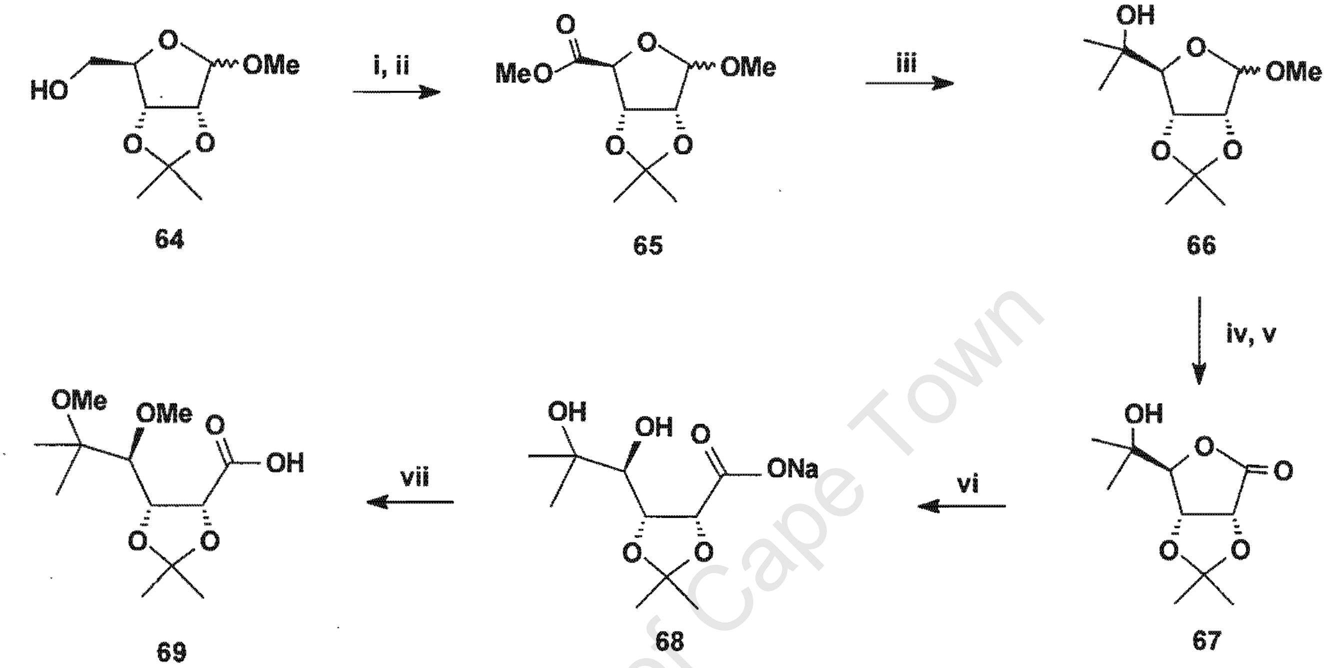
Hence, the aims of the project were:

1. To develop a versatile divergent synthesis to both noviose and 4-epi-noviose.
2. To convert 4-epi-noviose to 4-epi-novenamine and evaluate the biological activity.
3. To stereoselectively substitute one of the 5,5-dimethyl groups with a bulky alkyl group, thus providing a means of evaluating the biological activity of the $^1C_4$ chair form of 4-epi-noviose.

1.8. Syntheses of 4-epi-noviose intermediates

A literature survey revealed that, to date, 4-epi-noviose has not been synthesized. Although several syntheses of noviose have been described, none of them allow for the direct synthesis of 4-epi-noviose. Methyl 3,5,6-tri-*O*-benzyl-2-*O*-methyl-D-glucofuranoside, L-arabinose, L-rhamnose, and D-gulonolactone have a predetermined chirality at C-4, while the enantioselective syntheses induce the chirality at C-4 through a diastereoselective process. Two syntheses for an intermediate to 4-epi-noviose have been described, the first in 1958 from D-ribose, and the second from furan in 1991.

In 1958, in an endeavour to confirm the configuration of noviose as a lyxose and not, as earlier work had proposed, a ribose sugar, a team from Merck & Co. published a synthesis of 2,3-isopropylidene-5,5-dimethyl-4,5-di-*O*-methyl-D-ribonic acid **69**, from D-ribose.[42] Thus, methyl 2,3-isopropylidene-D-ribofuranoside **64** (Scheme 1.16), easily prepared from D-ribose, was oxidized with alkaline permanganate to the carboxylic acid and esterified with ethereal diazomethane to give the ester **65**. A Grignard reaction using an excess of methylmagnesium iodide gave the tertiary alcohol **66**, in good yield. Acidic hydrolysis of both the ketal and the glycosidic methyl groups gave a triol, which was followed by oxidation of the anomeric hydroxyl with bromine. The 2,3-diol was again protected as its ketal to furnish the lactone **67**, which was hydrolysed with aqueous sodium hydroxide to give the acyclic sodium salt **68**. Finally, on treatment with NaH and methyl iodide, both the tertiary and secondary hydroxyl groups were methylated to give **69**.



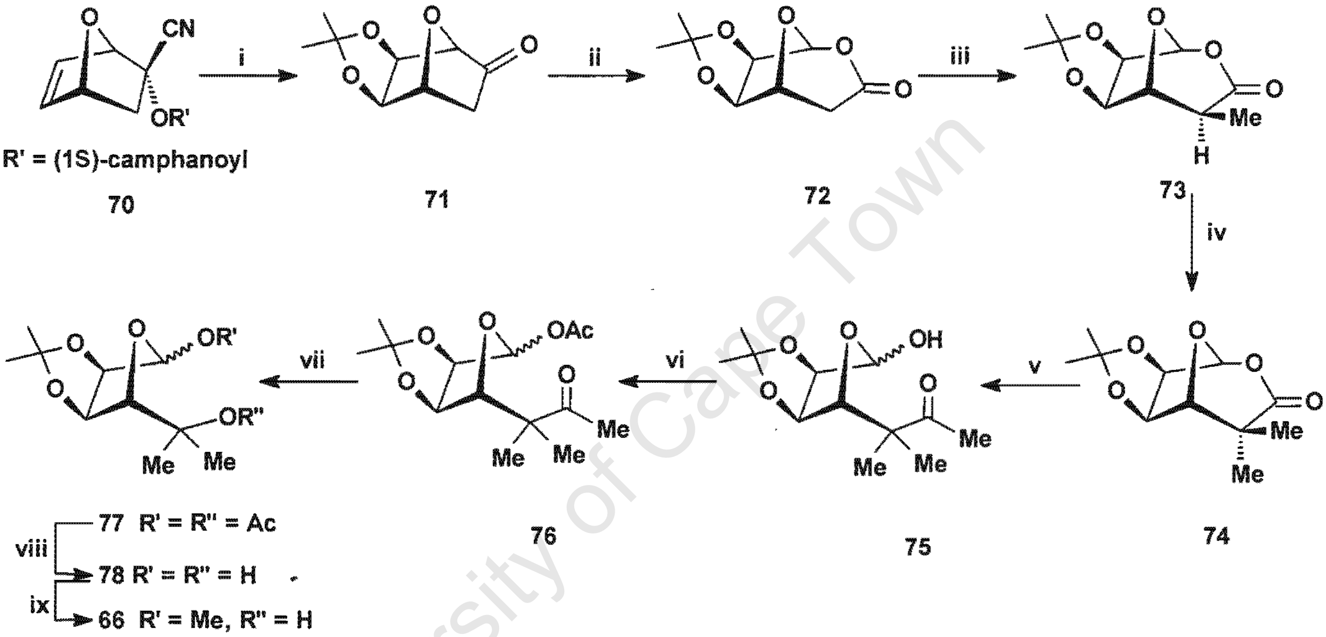
Scheme 1.16: *Reagents and conditions*: (i) KMnO$_4$, H$_2$O, KOH, room temperature, 31%. (ii) CH$_2$N$_2$, Et$_2$O, room temperature, 81%. (iii) MeMgI, Et$_2$O, reflux, 73%. (iv) (a) 0.1N HCl, reflux; (b) Br$_2$, NaNO$_2$, room temperature; 60% over 2 steps. (v) Acetone, CaCl$_2$, HCl, room temperature. (vi) 0.1N NaOH, room temperature. (vii) NH$_3$, Na, MeI, reflux.

The infrared spectrum of **69** was compared to that of 2,3-isopropylidene-5-O-methylnovionic acid derived from the degradation of novobiocin, and showed a marked difference in the absorption in the 800 to 1000 cm^{-1} region, providing proof that noviose was not a ribose sugar. The disadvantages of this synthesis include:

(i) the first oxidation step was very low yielding (31%) and would need to be improved for a viable synthesis,

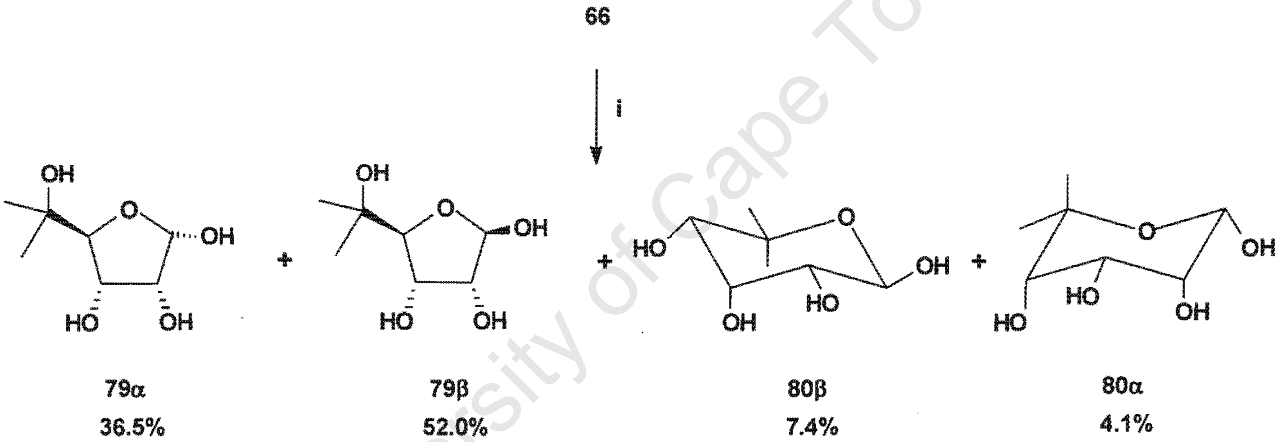
(ii) methylation of **68** gave both the secondary and tertiary methyl ethers, so to achieve chemoselectivity, the tertiary alcohol would need to be protected prior to ring opening.

Similiarly, in 1991 Wagner and Vogel reported a total synthesis of methyl 2,3-*O*-isopropylidene-5,5-di-*C*-methyl-D-ribofuranoside **66**, from the optically pure Diels-Alder product **70**, derived from the cycloaddition of furan and 1-cyanovinyl(1*S*)-camphanate (Scheme 1.17).[43] *Cis*-dihydroxylation of **70** from the *exo*-face, followed by diol ketal protection and hydrolysis yielded ketone **71**. Baeyer-Villiger oxidation of **71**, inserting oxygen at the more substituted carbon, affording the corresponding ketone **72** in high yield, which was α-methylated with LHMDS (enolate formation) and MeI, to give the dimethylated ketone **74**.



Scheme 1.17: *Reagents and conditions*: (i) (ii) 3-chloroperoxybenzoic acid, $NaHCO_3$, CH_2Cl_2, 20°C, 98%. (iii) $(Me_3Si)_2NLi$, THF, -65°C, MeI, 98%. (iv) $(Me_3Si)_2NLi$, THF, -65°C, MeI, 96%. (v) Me_3SiCH_2Li, THF, -65°C, MeOH. (vi) Ac_2O, pyridine, THF, room temperature, 97%. (vii) CF_3CO_3H, CH_2Cl_2, room temperature, 95%. (viii) K_2CO_3, MeOH, room temperature, 98%. (ix) 2,2-dimethoxypropane, *p*-TsOH, MeOH, room temperature, 90%.

The authors found that higher yields were obtained if the methylation was performed in two steps rather than in a single step (94% vs 74%). The generation of the methylketone **75** was achieved via nucleophilic opening of lactone **74** with Me_3SiCH_2Li to form a β-ketosilane intermediate which desilylated to the methyl ketone following an acidic work-up. Acetylation of the secondary hydroxyl group to furnish **76**, was followed by a regioselective Baeyer-Villiger oxidation to afford the diacetate **77**. Hydrolysis with K_2CO_3 in MeOH gave **78** and finally, the anomeric hydroxyl was selectively protected as its methyl ether to give **66** in 59% overall yield from furan. Although product **66** was achieved with high regioselectivity as a result of the biased nature of the Diels-Alder adduct, the synthesis was long, with nine steps required compared to three in the previous synthesis (Scheme 1.16). Furthermore, the use of expensive reagents makes large scale work impractical. A crucial aspect of this publication is that anomeric and ketal hydrolysis (aq. H_2SO_4, 80°C) of methyl 2,3-*O*-isopropylidene-5,5-di-*C*-methyl-D-ribofuranoside **66**, did not furnish 4-epi-noviose; instead, both the unprotected furanose and pyranose sugars **79** and **80** respectively, were formed in a ratio of 89:11 (Scheme 1.18). This is in total contrast to the corresponding L-lyxose derivative, where, as shown by Klemer *et al.*, acid hydrolysis of methyl 2,3-*O*-isopropylidene-5,5-di-*C*-methyl-L-lyxofuranoside **26** (Scheme 1.8, page 16), exclusively furnished the pyranose form in 95% yield.[28] Since the pyranose form was required from methyl 2,3-*O*-isopropylidene-5,5-di-*C*-methyl-D-ribofuranoside **66**, a different strategy would need to be developed.



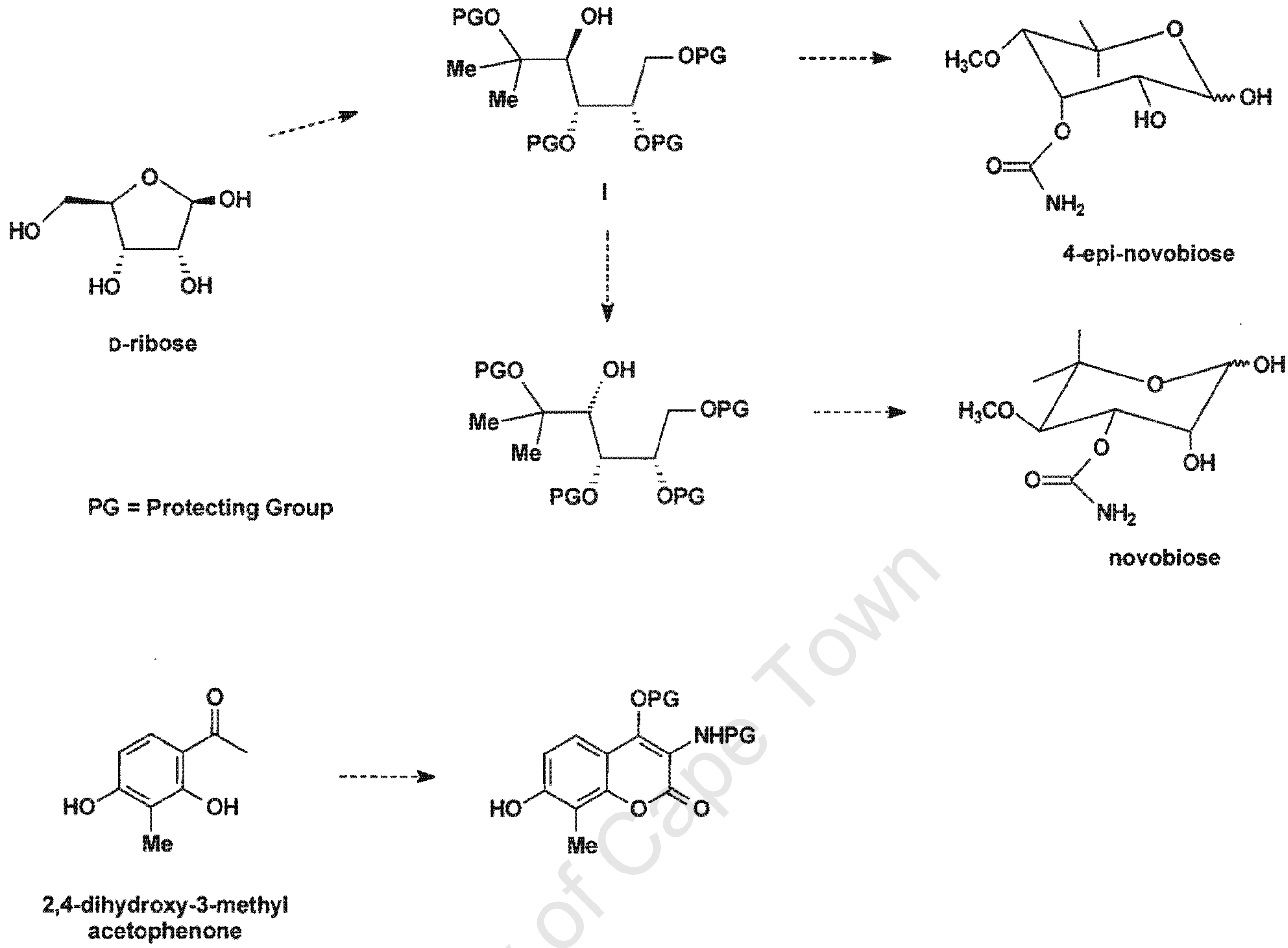
Scheme 1.18: *Reagents and conditions*: (i) 0.5M H_2SO_4, 80°C, 94%.

1.9. Strategy for the synthesis of novenamine and 4-epi-novenamine

1.9.1. Novobiose and 4-epi-novobiose

An outline of the target molecules is shown schematically in Scheme 1.19. The highlights of this strategy envisaged, were:

- The choice of D-ribose as a cheap and readily available chiral starting material, on the basis of the inherent chirality at C-4 and the likelihood, according to the Felkin-Ahn stereoselective induction model, of the stereoselective introduction of different alkyl groups on C-5.



Scheme 1.19: Strategy for the synthesis of novobiose, 4-epi-novobiose and the coumarin substituent.

- The synthesis of the target molecules via the acyclic precursor **I** as shown in Scheme 1.19, owing to the difficulty in converting a furanose D-ribose derivative directly into its corresponding pyranose form.
- The use of a common synthetic precursor **I** for both sugar isomers, as an attractive feature of this divergent synthesis.
- The novel introduction of the C-3 carbamoyl group before coupling of the sugar unit to the coumarin, thus minimizing the number of steps required after glycosylation.
- The challenge of regioselectively protecting the C-2 hydroxyl group of both sugar isomers, thus allowing the introduction of the carbamate onto the C-3 hydroxyl group. These regioselective protections loomed as significant but not insurmountable challenges, with the possible exploitation of the different reactivities of the axial and

equatorial hydroxyl groups, as well as the use of stannylene acetals suggesting themselves as potentially useful methodologies.

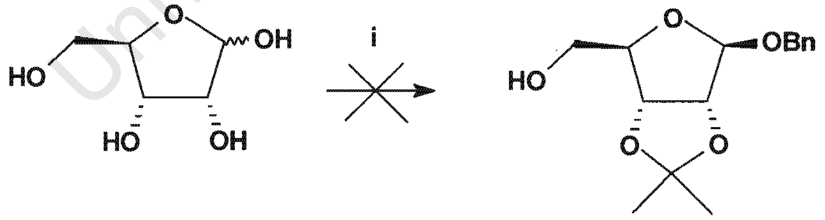
- The synthesis of the coumarin unit from commercially available 2,4-dihydroxy-3-methylacetophenone with the challenge being the novel introduction of the amino group at an earlier stage, possibly in a masked form such as an oxazole, in order to minimize post-glycosylation manipulations.

Chapter 2 : Synthesis of noviose and 4-epi-noviose

One of the aims of this project was to develop a versatile divergent synthesis to both noviose and 4-epi-noviose. In order to achieve this aim, D-ribose was selected as the synthetic starting material on the basis of its relatively low cost, ready availability and the capacity to convert it into an acyclic precursor common to both sugars, as outlined in Scheme 1.19.

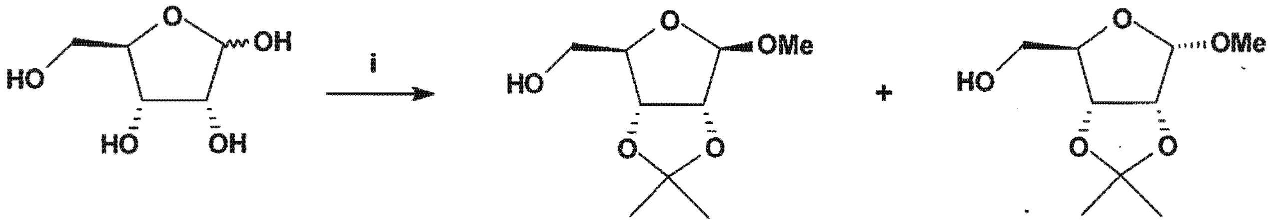
2.1. Synthesis of 4-epi-noviose

As with most syntheses involving carbohydrates, the first task involved selective orthogonal protection of a poly-hydroxylated molecule to ensure chemoselectivity in subsequent reactions. Numerous methods to achieve the selective protection of D-ribose have been published. Since our intention was to selectively manipulate both the primary hydroxyl group at C-5 and the anomeric position, the synthesis of benzyl 2,3-*O*-isopropylidene-β-D-ribofuranoside from D-ribose was an attractive proposition because, although it was a low-yielding reaction (50%),[44] the anomeric hydroxyl group could be selectively regenerated by catalytic hydrogenolysis leaving the 2,3-isopropylidene group intact. Thus, following the published method, D-ribose was refluxed in acetone, excess benzyl alcohol and concentrated H_2SO_4 (Scheme 2.1). Frustratingly, however, a complex mixture of products resulted as shown by tlc, and no major product could be isolated from the reaction.



Scheme 2.1: *Reagents and conditions*: (i) BnOH, H_2SO_4, acetone, reflux.

An alternative was to form the methyl glycoside and protect the 2,3-*cis* diol as an acetal. The synthesis of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside is well documented in the literature, with the original preparation having been reported by Levene and Stiller in 1934 using acetone, methanol and concentrated H_2SO_4 as well as $CuSO_4$ to act as a dehydrating agent.[45] Modifications of this synthesis have included the use of 2,2-dimethoxypropane, methanol and HCl gas,[46] and acetone, methanol and HCl gas.[47] In a variation to these methods, D-ribose was dissolved in methanol, cooled to 0°C and treated slowly with concentrated H_2SO_4 (1 equiv.) (Scheme 2.2).



64β **(95:5)** **64α**

Scheme 2.2: *Reagents and conditions*: (i) MeOH, acetone, conc. H_2SO_4, 85%.

Following disappearance of the starting material (monitored by tlc), excess acetone was added and the reaction allowed to stir at room temperature until complete, which was typically about 2 hours after acetone addition. On a relatively large scale (10 g), an aqueous work-up was avoided by quenching the reaction with KOH in methanol, filtering through Celite® to remove the finely granulated inorganic salts and evaporating the filtrate *in vacuo*. Two products were observed by tlc and these were separated by column chromatography. The major product, β-anomer **64β**, was isolated in 81% yield and the ^1H and ^{13}C NMR spectra, as well as the $[\alpha]_D$ corresponded to that reported in the literature.[46] The ^1H NMR spectrum of **64β** revealed a very distinctive coupling pattern which was observed for the subsequent β-ribofuranoside series and so will be discussed in full at this point. Vicinal coupling was observed between H-2 and H-3 (*J* 6.0 Hz) whereas none was seen between H-2 and H-1 and between H-3 and H-4. H-4 appeared as a triplet (*J* 2.8 Hz) with coupling to the two H-5 protons. Examination of models as depicted schematically in Figure 2.1 (β-anomer), reveals that the dihedral angles between H-1 and H-2 and between H-3 and H-4 are each about 90°, giving zero couplings according to the Karplus equation as observed in the ^1H NMR spectrum.

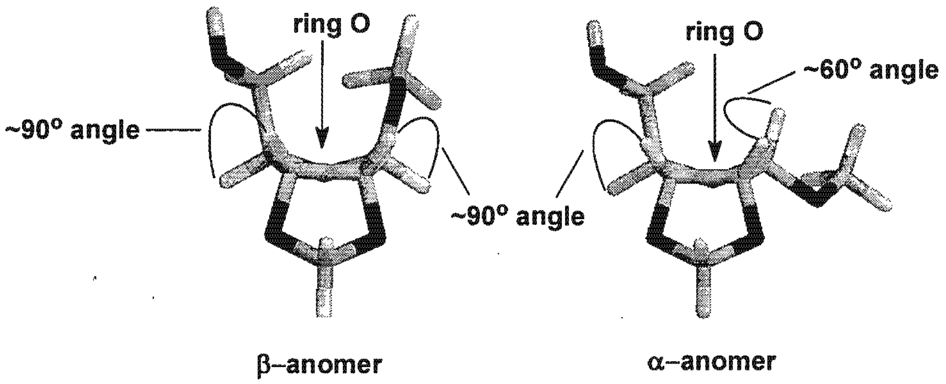
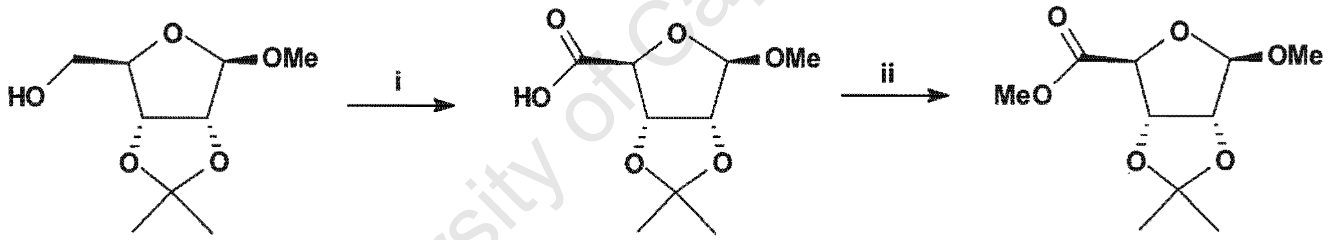


Figure 2.1: Illustration of dihedral angles for methyl 2,3-O-isopropylidene-D-ribofuranoside (β- and α- anomers).

Surprisingly, a full characterization for the α-anomer, isolated in 4% yield, had yet to be reported in the literature despite the fact that it was first isolated in 1966, so a full characterization was carried out. The anomeric carbon appeared at δ_C 102.8 in the ^{13}C NMR spectrum of **64α**, significantly further upfield than that for the β-anomer at δ_C 109.9. This difference in chemical shifts is commonly observed in carbohydrate chemistry and is indeed a method for distinguishing between α- and β-anomers using ^{13}C NMR.[48] Similarly, upfield shifts for the C-2, C-3 and C-4 carbon signals, compared with those for the β-anomer, were also observed. The C-4 carbon signal appeared significantly upfield at δ_C 81.6, a characteristic of the furanose ring form when compared to the pyranose ring form, where the C-4 carbon signal would typically appear at about δ_C 68.8.[49] The ^1H NMR was poorly resolved at 400 MHz and did not allow for determination of any coupling constants. A D_2O wash only partially simplified the spectrum, with the H-5 protons resolved as a doublet of doublets (J_{AB} 12.0 Hz, $J_{1,2}$ 3.8 Hz). H-2 and H-3 had similar chemical shifts and H-4 was observed as a multiplet. In theory, H-1 should have appeared as a doublet, coupling to H-2; instead it appeared as a multiplet with variable small coupling constants (< 2 Hz). These small couplings are presumably a result of coupling to H-2, predicted to be approximately 2 – 3 Hz consistent with a dihedral angle of 60° (Figure 2.1 [α-anomer]), as well as possibly through-bond coupling with H-3. High-resolution mass spectometry gave a molecular mass consistent with the assigned structure.

With protected D-ribose **64β** in hand, attention was turned to oxidation of the primary alcohol. In the synthesis of methyl 2,3-O-isopropylidene-4-methoxycarbonyl-β-D-ribofuranoside, **65**, the primary alcohol of protected D-ribose **64** was oxidized to the corresponding carboxylic acid with alkaline permanganate (Scheme 1.16, page 27).[42] As the yield for this reaction was very low (31%), it was decided to seek an alternative and the use of catalytic RuO_4 was selected. This method dates from the introduction of RuO_4 as an organic oxidant by Djerassi and Engle in 1953.[50] A catalytic procedure was subsequently developed whereby stoichiometric aqueous sodium periodate was used as a co-oxidant in CCl_4.[51] Sharpless then modified the method even further with the addition of acetonitrile as a co-solvent, as it was observed that insoluble lower-valent ruthenium carboxylate complexes might be responsible for loss of catalyst activity resulting in sluggish or failed reactions,[52] and acetonitrile disrupted these insoluble complexes, returning the ruthenium to the catalytic cycle.

Applying this latter method, methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside **64β** was successfully oxidized to the corresponding carboxylic acid and, following work-up, immediately esterified by dissolving in DMF and treating with anhydrous K_2CO_3 and MeI (Scheme 2.3).



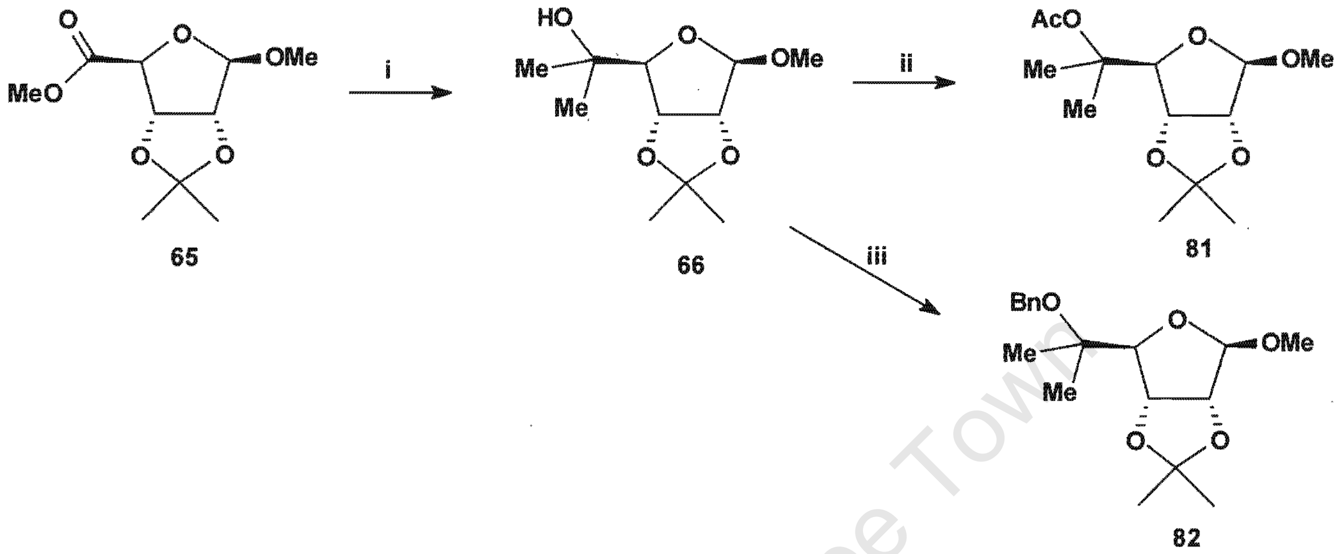
64β

65

Scheme 2.3: *Reagents and conditions*: (i) $RuO_4.H_2O$ (cat.), 10% $NaIO_4$, CCl_4/CH_3CN (1:1), room temperature. (ii) K_2CO_3, MeI, DMF, room temperature, 93% over 2 steps.

Column chromatography was used to purify the crude material, furnishing pure ester **65** in 93% yield over the two steps and as the β-anomer, identical in every respect to the reported compound.[53]

The next step in the reaction sequence was the introduction of the C-5 *gem*-dimethyl group. The use of the well-established Grignard methodology suggested itself, and methyl ester **65** was duly refluxed with two molar equivalents of methylmagnesium iodide in ether, and then subjected to an acidic work-up, to give tertiary alcohol **66** as an oil in 92% yield (Scheme 2.4). This was confirmed by the observation of a six-proton singlet at δ_H 1.24 in the ^1H NMR spectrum and two signals at δ_C 24.7 and 27.0 in the ^{13}C NMR spectrum of **66** for the newly introduced diastereotopic *gem*-methyl groups, in accordance to those reported in the literature.[43]



Scheme 2.4: *Reagents and conditions*: (i) MeMgI (2 equiv.), ether, reflux, 92%. (ii) Et_3N, Ac_2O, DMAP, CH_2Cl_2, room temperature, 54%. (iii) NaH, THF, reflux 2 hours, BnBr, $^nBu_4NI$, reflux, 98%.

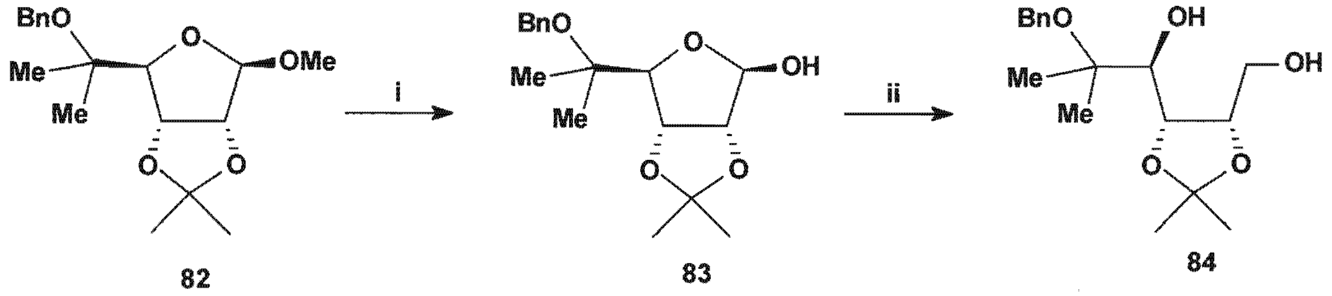
The aim was then to convert **66** to a hemiacetal and reduce it to an acyclic triol, protect the primary alcohol and then regioselectively methylate the secondary alcohol. This required that the tertiary alcohol be protected prior to ring opening (for an analogy see Scheme 1.16, page 27).

Initially, the choice of protecting group for the tertiary hydroxyl group was an acetic acid ester. However, when tertiary alcohol **66** was mixed with triethylamine, acetic anhydride and DMAP in CH_2Cl_2 and stirred overnight, acetylated **81** could only be isolated in 54% yield (Scheme 2.4).[‡] NMR revealed the presence of the acetate ester with the acetate methyl group resonating characteristically as a singlet at δ_H 1.98 in the ^{1}H NMR spectrum, and its carbonyl carbon being detected at δ_C 170.2 in the ^{13}C NMR spectrum.

[‡] Since **81** was not fully characterised and hence, not included in the experimental section, the NMR data are included here: δ_H (400 MHz, $CDCl_3$): 1.34, 1.35, 1.50, 1.53 (12H, 4 x s, 4 x CH_3), 1.98 (3H, s, $-OCOC\underline{H}_3$), 3.36 (3H, s, -OMe), 4.06 (1H, d, *J* 2.0 Hz, H-4), 4.52 (1H, d, *J* 6.4 Hz, H-2), 4.77 (1H, dd, *J* 2.0 and 6.4 Hz, H-3), 5.00 (1H, s, H-1); δ_C (100 MHz, $CDCl_3$): 22.0 ($-OCO\underline{C}H_3$), 22.2, 22.4, 25.3, 26.9 (4 x CH_3), 55.1 (-OMe), 80.6 (C-5), 81.3 (C-3), 85.9 (C-2), 93.4 (C-4), 110.0 (C-1), 112.6 (C-6), 170.2 ($-O\underline{C}OCH_3$).

In order to improve the yield of this reaction, alternative acylating conditions that could have been investigated include the use of a stronger base such as sodium hydride. However, the fear that the acetyl group might possibly migrate to the less-hindered secondary hydroxyl group under basic conditions following the reduction of the hemiacetal, prompted an alternative protecting group to be sought. Thus, protection as a benzyl ether was considered, since the latter is resistant to both base and aqueous acid anticipated for subsequent steps, and selectively removeable in the presence of a methyl ether. The fact that benzylation is moderately sterically demanding, and that protection of a hindered tertiary alcohol was required, promoted the use of BnBr and $^nBu_4NI$ following alkoxide formation with NaH in THF.[54] In order to achieve acceptable yields, it was necessary to reflux **66** with NaH in THF for 2 hours to allow complete formation of the anion before adding the BnBr and $^nBu_4NI$ (Scheme 2.4). Using these conditions resulted in complete conversion to the benzyl ether **82** after a further two hours. The product was isolated by chromatography in 98% yield as an oil with the presence of the characteristic benzyl methylene protons resonating as a singlet at δ_H 4.50 in the ^1H NMR spectrum, and the absence of the hydroxyl proton, which was observed at δ_H 3.33 in the starting material **66**, evidence that the tertiary hydroxyl group had been benzylated. The IR spectrum of **82** confirmed the absence of the broad -OH stretch seen at 3442 cm^{-1} in the spectrum of **66.** Similarly, the ^{13}C NMR spectrum was assigned with the aid of an HSQC experiment and revealed the correct number of resonances with C-5 exhibiting a downfield shift from δ_C 70.1 in **66** to δ_C 75.6, consistent with hydroxyl substitution.

Treatment of **82** with aqueous HCl in dioxane at 60°C resulted in hydrolysis of both the methyl glycoside and the 2,3-*O*-acetal, so the latter was re-introduced by suspending the crude product in acetone, cooling to 0°C and slowly adding concentrated H_2SO_4 (Scheme 2.5). A mixture of both the 1,2- and 2,3-acetonides was obtained as observed by Hughes et al.,[55] but careful separation of the products by column chromatography gave the 2,3-*O*-isopropylidene acetal **83** in 81% overall yield from **82.**



Scheme 2.5: *Reagents and conditions*: (i) (a) 1M HCl, dioxane, 60°C, (b) Acetone, H_2SO_4, 0°C, 81% over 2 steps. (ii) $LiAlH_4$, THF, 0°C, 94%.

The ^{1}H NMR spectrum of **83** revealed the absence of the *O*-Me protons observed as a singlet at δ_H 3.39 in the spectrum of **82**, as well as the presence of a hydroxyl proton at δ_H 4.56. The coupling constant for the hydroxyl proton at δ_H 4.56 was 12.3 Hz with H-1, signifying that the acetal had formed between the C-2 and C-3 hydroxyl groups, leaving a free –OH at the anomeric centre. A D_2O wash reduced the H-1 doublet to a singlet, with the disappearance of the hydroxyl proton observed at δ_H 4.56. H-2 resonated as a doublet (*J* 6.0 Hz) at δ_H 4.35, coupling to H-3 (d, *J* 6.0 Hz) and with no coupling observed to H-1, confirming the presence of the β-anomer, formed presumably to minimize C_1–O/C_2– O torsional strain.

Reduction of hemiacetal **83** was realised in high yield (95%) with lithium aluminium hydride in THF at 0°C to give the acyclic diol **84** (Scheme 2.5). The ^{1}H NMR spectrum of **84** revealed the diastereotopic nature of the H-1 protons, with one resonating at δ 3.68 (dd, J_{AB} 11.6 Hz, $J_{1,2}$ 4.8 Hz), and the other at δ 3.89 (dd, J_{AB} 11.6 Hz, $J_{1,2}$ 7.6 Hz), indicative of geminal protons adjacent to a chiral centre. A large coupling was observed between H-3 and H-4 ($^3J_{3,4}$ 9.3 Hz), indicating an anti-periplanar relationship in the conformationally more flexible acyclic structure, and the dominant conformer consistent with these data is illustrated in Figure 2.2. In addition, the spectrum revealed the appearance of a broad singlet at δ 3.10 integrating for two protons for the two hydroxyl groups. The ^{13}C NMR spectrum showed a large upfield shift for C-1, from δ 103.7 in **83**, to δ 61.1, providing further evidence for the successful reduction of the hemiacetal.

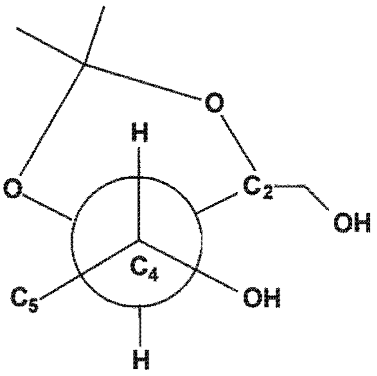
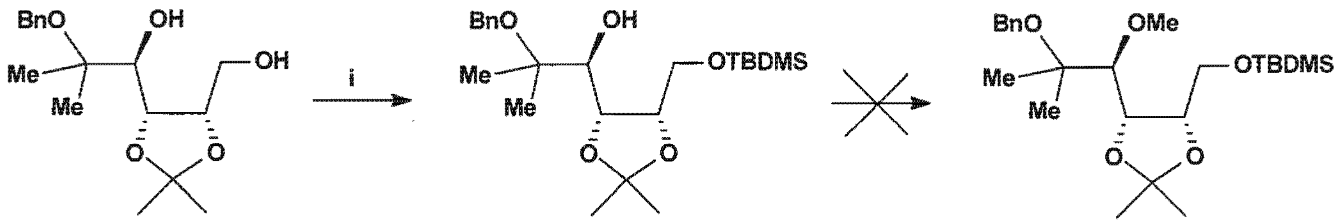


Figure 2.2: Newman projection for the C-4 and C-3 bond of the dominant conformer of **84**.

Following the successful reduction, the next step required the regioselective protection of the primary alcohol. The relative ease with which a TBDMS protecting group can be introduced onto a primary hydroxyl group in the presence of a secondary hydroxyl group, as well as the ease of its removal under mild conditions, made it the candidate of choice for regioselectively protecting **84**.

Application of the standard conditions of TBDMSCl and imidazole in CH_2Cl_2 furnished **85** in 88% yield (Scheme 2.6).[§] The appearance of the characteristic six- and nine-proton singlets for the *tert*-butyldimethylsilyl group at δ_H 0.12 and 0.92 in the ^{1}H NMR spectrum respectively, and the absence of one of the hydroxyl proton signals observed in the spectrum of **84** provided evidence of mono-silylation. Furthermore, coupling observed between the –OH and H-4 (*J* 3.6 Hz) indicated the presence of a hydroxyl group on C-4, thus verifying that regioselective protection of the primary hydroxyl group had taken place.

[§] Since **85** was not fully characterised and hence, not included in the experimental section, the NMR data are included here: δ_H (300 MHz, $CDCl_3$): 0.12 (6H, s, $-OSi(C\underline{H}_3)_2C(CH_3)_3$), 0.92 (9H, s, $-OSi(CH_3)_2C(C\underline{H}_3)_3$), 1.34, 1.35, 1.39, 1.41 (12H, 4 x s, 4 x CH_3), 3.64 (1H, dd, *J* 4.5 and 10.5 Hz, H-1), 3.74 (1H, d, *J* 3.6 Hz, -OH), 3.79 (1H, dd, *J* 3.6 and 9.3 Hz, H-4), 3.89 (1H, dd, *J* 7.5 and 10.5 Hz, H-1), 4.26 (1H, m, H-2), 4.33 (1H, dd, J 5.7 and 9.3 Hz, H-3), 4.56 (2H, s, $-OC\underline{H}_2C_6H_5$), 7.24 – 7.3 (5H, m, $-OCH_2C_6\underline{H}_5$); δ_C (75.5 MHz, $CDCl_3$): -5.4, -5.5 ($-OSi(\underline{C}H_3)_2C(CH_3)_3$), 18.3 ($-OSi(CH_3)_2\underline{C}(CH_3)_3$), 21.7, 22.8, 25.5, 28.2 (4 x CH_3), 25.9 ($-OSi(CH_3)_2C(\underline{C}H_3)_3$), 62.4 (C-1), 63.9 ($-O\underline{C}H_2C_6H_5$), 73.6 (C-4), 76.6 (C-3), 77.5 (C-2), 78.5 (C-5), 108.6 (C-6), 127.1, 127.3, 128.2, 139.9 ($-OCH_2\underline{C}_6H_5$).



84

85

Scheme 2.6: *Reagents and conditions*: (i) TBDMSCl, imidazole, DMF, room temperature, 88%.

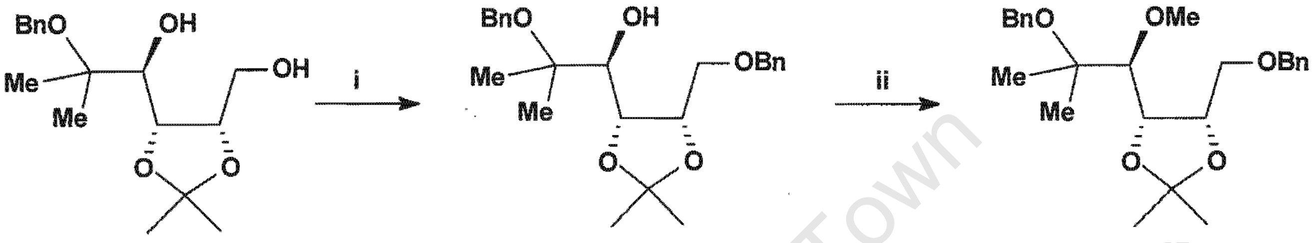
The next step in the synthesis was the methylation of the C-4 hydroxyl group. As shown in Table 2.1, numerous methods were attempted with little or no success. Methylation was eventually achieved, but only in modest yield, with *n*-BuLi in the presence of HMPA, presumably because HMPA as a polar aprotic solvent powerfully solvates the lithium cation, thus enhancing the nucleophilicity of the anion for nucleophilic attack. However, the secondary C-4 alkoxide was presumably severely hindered by the adjacent bulky silyl and benzyl ethers, thus restricting the accessibility of the electrophile.

Reagents	Conditions	Yield of methylation
Me_2SO_4, NaH, THF	Reflux	Decomposition of starting material
a) n-BuLi; b) MeI, THF	-78°C - rt	No reaction
NaH, MeI, DMF	0°C	Decomposition of starting material
MeI, Ag_2O, DMF[56]	0°C to 60°C	No reaction
KOH, Me_2SO_4, 1,4-dioxane	Reflux	No reaction
a) n-BuLi; b) Me_2SO_4, HMPA/THF	-78°C	35%

Table 2.1: Summary of the methods used for the methylation of **85**.

It was necessary, therefore, to find a less bulky protecting group for O-1. Once again, the benzyl ether was selected on the basis of the C-5 hydroxyl already being protected as a benzyl ether, allowing for simultaneous deprotection of O-1 and O-5. Furthermore, under appropriate conditions, a primary alcohol can be selectively benzylated in the presence of a more hindered alcohol.[54] Thus, treatment of **84** with one equivalent of NaH in THF followed by one equivalent of benzyl bromide resulted in clean conversion of the starting material to the mono-benzylated product **86**, which was isolated after column chromatography in 94% yield (Scheme 2.7).

The ^{1}H NMR spectrum of **86** revealed the appearance of additional benzylic methylene proton signals at δ_H 4.60 compared to **84**, and the absence of the signal for one of the hydroxyl protons, observed at δ_H 3.33 in **84**. As with the silylation, a coupling was observed between the –OH and H-4 (*J* 3.3 Hz), providing evidence that the primary hydroxyl group had been regioselectively benzylated.

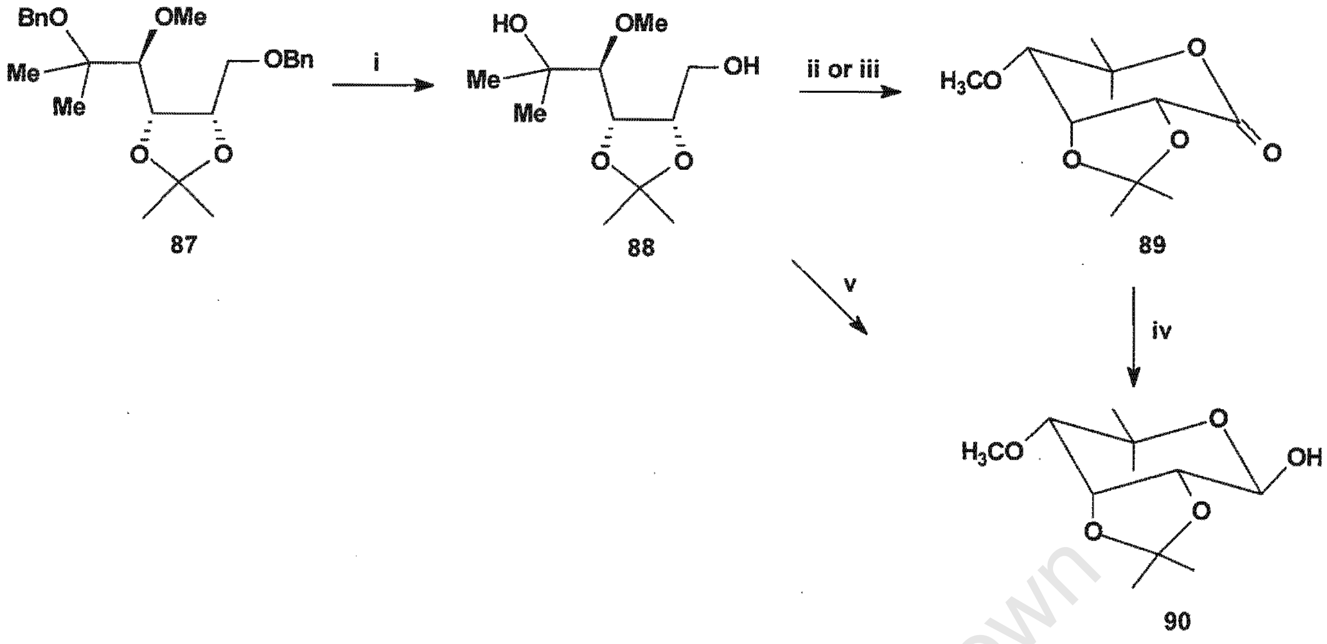


84 **86** **87**

Scheme 2.7: *Reagents and conditions*: (i) NaH, BnBr, THF, room temperature, 94%. (ii) NaH, MeI, THF, reflux, 98%.

Pleasingly, the methylation of **86** was then achieved using methodology analogous to that used for the benzylation of the tertiary alcohol **66** to give **82**. This gave methyl ether **87** in 98% yield after chromatography. The appearance of the characteristic methoxy methyl protons at δ_H 3.47 in the ^{1}H NMR spectrum and the absence of the signal for the hydroxyl proton observed at δ_H 3.32 in **86** provided evidence for methylation at O-4.

Exhaustive debenzylation of **87** to afford **88** was achieved in near quantitative yield by hydrogenolysis with catalytic amounts of 10% palladium on carbon in ethanol at room temperature (Scheme 2.8). Both the ^{13}C and ^{1}H NMR spectra of **88** revealed the disappearance of the characteristic benzyl peaks, while its IR spectrum exhibited a broad band in the hydroxyl region (3521 cm^{-1}) that was not present in **87**.



Scheme 2.8: *Reagents and conditions*: (i) 10% Pd/C, H_2, EtOH, 99%. (ii) DMSO (2 equiv.), $(COCl)_2$ (2 equiv.), Et_3N, CH_2Cl_2, -68°C – room temperature, 81%. (iii) TPAP, NMO, 4Å molecular sieves, CH_2Cl_2, room temperature, 72%. (iv) DIBALH, toluene, -78°C, 68%. (v) DMSO (1 equiv.), $(COCl)_2$ (1 equiv.), Et_3N, CH_2Cl_2, -68°C – 0°C, 81%.

With the relevant hydroxyl groups now exposed, the stage was set for the oxidative cyclization to the required lactone or lactol. Numerous methods are available for the selective oxidation of a primary alcohol to its corresponding aldehyde, all of which are based on either:

(i) reagents involving metals in a high oxidation state such as pyridinium chlorochromate (PCC), pyridinium dichromate (PDC) and tetra-*n*-propylammonium perruthenate (TPAP)[57]

(ii) halogens, sulfur or nitrogen in a high oxidation state, examples being Dess-Martin periodinane,[58] and Swern oxidation using DMSO, oxalyl chloride and triethylamine (Figure 2.3).

The relatively mild conditions, the ease of work-up and the availability of reagents made Swern and TPAP oxidations the methods of choice.

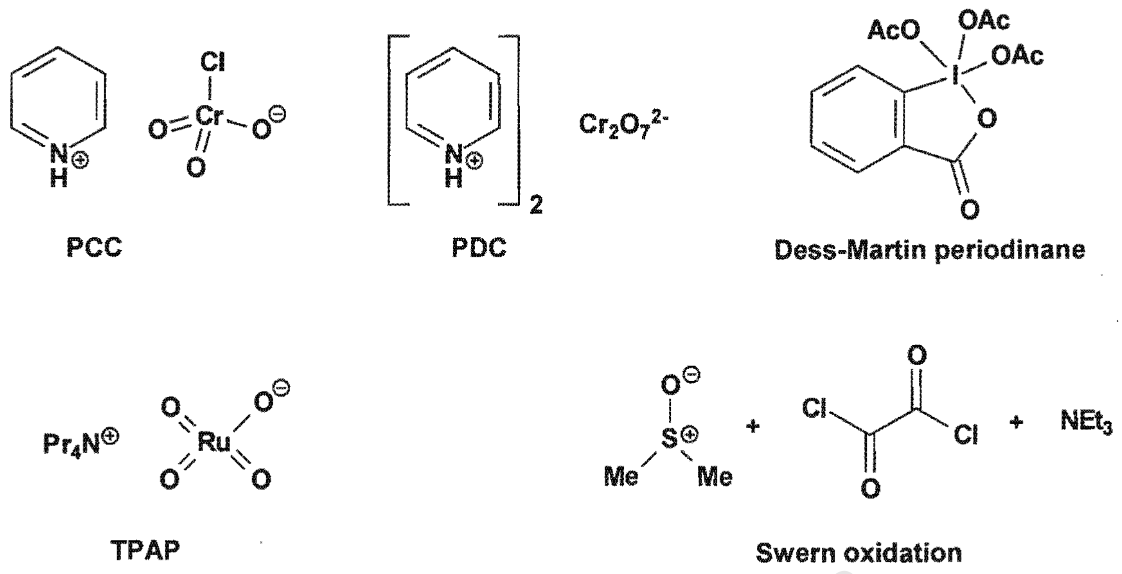


Figure 2.3: Reagents used for oxidizing primary alcohols to aldehydes.

Similar to RuO_4, TPAP, introduced by Steven Ley in 1987, is expensive, but may be used catalytically in conjunction with stoichiometric amounts of N-methylmorpholine-N-oxide (NMO). NMO is reduced to the amine during the course of the reaction, reoxidizing the ruthenium back to Ru(VII) (Figure 2.4).

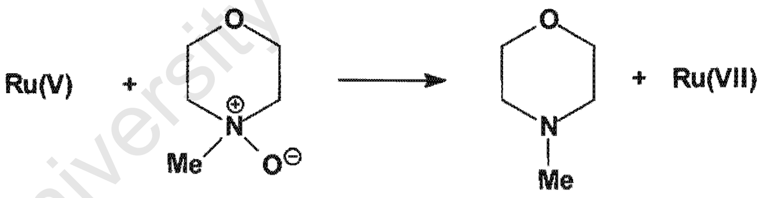


Figure 2.4: Regeneration of Ru(VII) by NMO.

The acyclic diol **88** was accordingly treated with catalytic TPAP (10 mol %) and a slight excess of NMO (1.2 mol equivalents) in CH_2Cl_2 containing 4Å molecular sieves at room temperature (Scheme 2.8). After two hours, tlc revealed the presence of two compounds, the more polar of which correlated to the starting material, so a further equivalent of NMO was added. After consumption of the starting material, a simple work-up involving the evaporation of excess solvent *in vacuo* followed by column chromatography gave lactone **89** in 72% yield. This was deduced from the ^1H NMR spectrum which revealed the absence of an anomeric proton signal, typically seen between δ_H 5.0 and 6.0, and the ^{13}C NMR and IR spectra providing evidence for a carbonyl group with signals at δ_C 169.0 and 1748 cm^{-1}, respectively.

The formation of the lactone **89** and not a lactol was confirmed by both elemental microanalysis and high-resolution mass spectrometry. An examination of the ^{1}H NMR spectrum revealed an interesting coupling pattern. The relatively large coupling constant of 8.7 Hz between H-2 and H-3 and the coupling constant of 3.9 Hz between H-3 and H-4 suggests that the rigid 2,3-*O*-isopropylidene group, together with the sp^2 centre at C-1, bring about near co-planarity of C-1, C-2, C-3 and the ring oxygen, resulting in a dihedral angle between H-2 and H-3 of close to 0° and between H-3 and H-4 of about 60° as depicted schematically in the model in Figure 2.5.

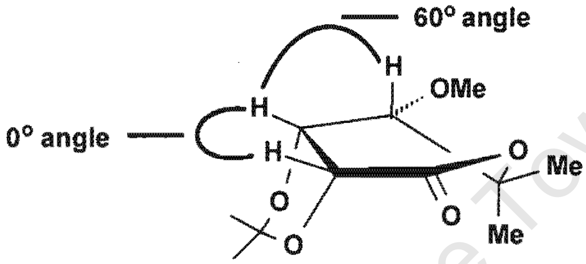


Figure 2.5: Conformational changes to lactone **89** caused by the rigid isopropylidene ring.

Since it was not possible to prevent the overoxidation of the lactol to the lactone under TPAP conditions, a Swern oxidation was attempted.

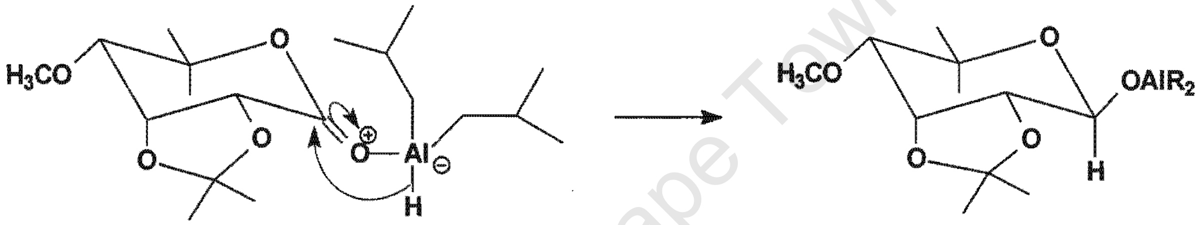
Swern oxidation, the mechanistic details of which are well established, uses a sulfoxide [S(IV)] as the oxidising agent to oxidise an alcohol to an aldehyde and in the process the sulfoxide is reduced to the sulfide. The results of the Swern oxidation are summarised in Table 2.2.

No. of equivalents DMSO and oxalyl chloride	Product
1	Inseparable mixture of lactone **89** and lactol **90**
2	Lactone **89**

Table 2.2: Results of the oxidation of **88** using different equivalents of DMSO and oxalyl chloride.

Oxidation of **88** under standard conditions (-68°C – room temperature) and using one equivalent of DMSO and oxalyl chloride disappointingly afforded an inseparable mixture of lactol and lactone as judged from the ^{1}H NMR spectrum of the product mixture. In contrast, when 2 equivalents of DMSO and oxalyl chloride were used, only the lactone **89** was isolated in 81% yield (Scheme 2.8).

Since it was difficult to obtain the lactol directly, it was decided to prepare it via back-reduction of the lactone. DIBALH (diisobutylaluminium hydride) is commonly used for the reduction of lactones to lactols since a stable, cyclic tetrahedral intermediate is formed (Scheme 2.9), which collapses to the lactol only on an acidic aqueous work-up, thus preventing further reduction to the acyclic diol.[59]



Scheme 2.9: Mechanism for the reduction of the lactone to the lactol using DIBALH.

To this effect, lactone **89** was treated with DIBALH in toluene at –78°C, providing lactol **90** in 15 minutes in 68% yield after chromatography (Scheme 2.8). A minor, more polar spot was observed on tlc during the reaction, but it was not isolated. DIBALH reduction of ketals to ethers is known[60] and may provide an explanation as to why a low yield of 68% was obtained. A 68% yield was considered acceptable at this stage, and so other reducing agents were not screened, in which over-reduction back to diol **88** might have been a complication. The ^{1}H NMR spectrum of **90** (Figure 2.6) revealed the presence of a single anomer, with the anomeric proton signal appearing as a triplet (J 6.0 Hz) at δ_H 5.02 with coupling to both H-2 and the anomeric hydroxyl as confirmed by a D_2O wash which collapsed the signal to a doublet.

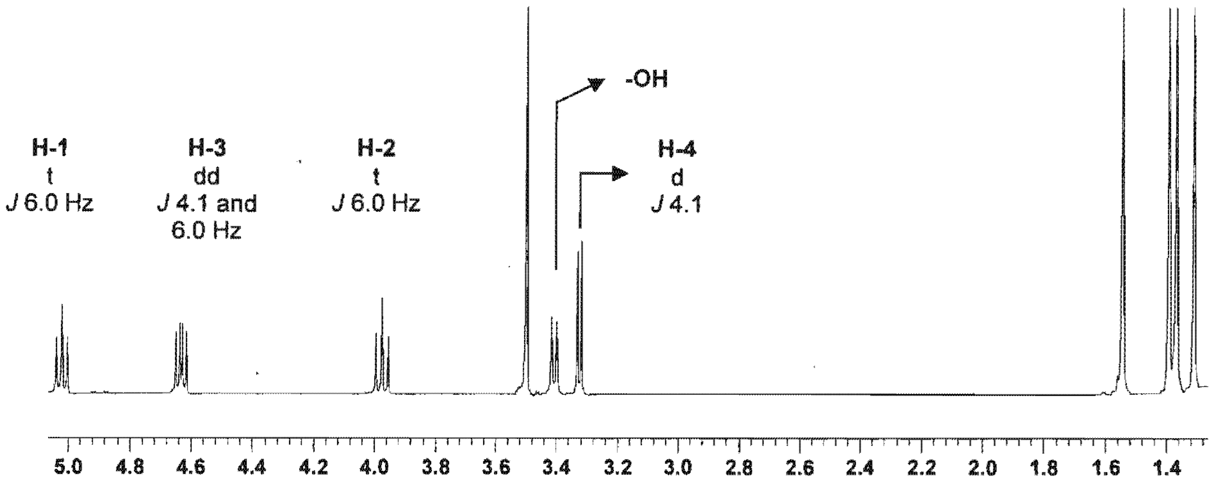


Figure 2.6: ^{1}H NMR spectrum of lactol **90**.

The anomeric configuration was assigned as β, with a reduced coupling constant between H-1 and H-2 (*J* 6.0 Hz) induced by the flattening effect of the fused ring system at C-2 and C-3 as seen schematically in the model in Figure 2.7. According to this model, based on the Karplus equation, the dihedral angle between H-1 and H-2 is reduced from 180° to about 150° and the angle between H-2 and H-3 from 60° to about 30°. This results in a reduced coupling constant of *J* 6.0 Hz between H-1 and H-2 and the same value between H-2 and H-3 as predicted by the Karplus equation.

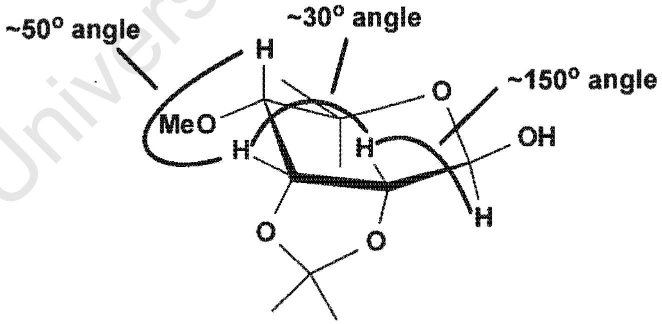


Figure 2.7: Conformational changes to lactol **90** caused by the rigid isopropylidene ring.

Unfortunately the yield for the reduction of lactone **89** to lactol **90** could not be improved above 68% so the conditions for the Swern oxidation of acyclic diol **88** were re-evaluated. In a crucial modification, the acyclic diol **88**, dissolved in CH_2Cl_2 was added to one equivalent of DMSO and oxalyl chloride in CH_2Cl_2 at –68°C. After 15 minutes, triethylamine was added. However, instead of allowing the reaction to reach room temperature, it was quenched at 0°C with saturated Na_2CO_3, and then subjected to a standard work-up. Gratifyingly, the ^1H NMR revealed the presence of only lactol **90**, which was subsequently isolated in 81% yield with no trace of lactone **89**. This yield was an improvement on that obtained for the two-step procedure of oxidising diol **88** to lactone **89** and then reducing back to lactol **90**, achieved in a combined yield of only 55% (Table 2.3).

Procedure	Yield of lactol **90**
Swern oxidation, quenched at 0°C	81%
Swern oxidation to lactone (quench at room temperature), then back-reduction to lactol	55% for the two steps

Table 2.3: Summary of the two procedures utilized in the synthesis of lactol **90**.

Fortunately, on recrystallization with ethyl acetate and hexane, lactol **90** gave diffraction-quality crystals and a crystal structure could be obtained (Figure 2.8). The $^4C_1$ conformation of the ring was confirmed, as well as the flattening effects described above. The predicted dihedral angles shown schematically in Figure 2.7 versus the dihedral angles measured from the crystal structure of lactol **90** are summarised in Table 2.4 and are in reasonable agreement with one another.

	Predicted dihedral angle	Measured dihedral angle
H1 – H2	150°	160.2°
H2 – H3	30°	36.0°
H3 – H4	50°	42.1°

Table 2.4: Predicted versus measured dihedral angles for lactol **90**.

As can be seen in Table 2.4, the X-ray-measured dihedral angles are slightly higher than those predicted from the ^1H NMR data for H1/H2 and H2/H3. However, the 400 MHz spectrometer did not make a distinction between the value of these angles and hence, H-2 resonates as a triplet in the ^1H NMR spectrum, coupling with the same *J* value to both H-1 and H-3.

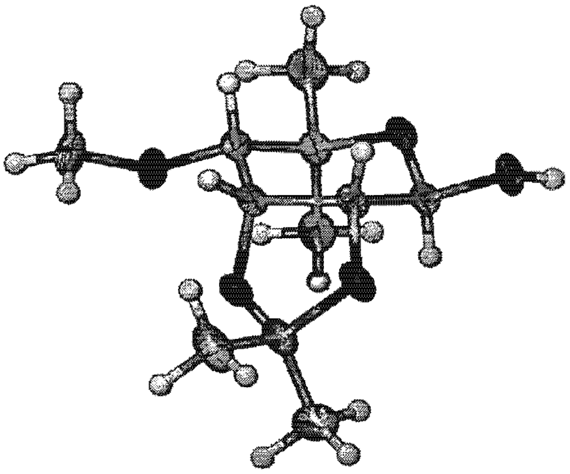
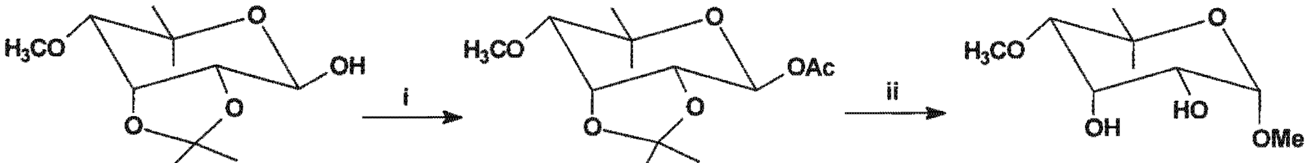


Figure 2.8: Crystal structure for lactol **90**.

This was the first synthesis and crystal structure of 2,3-*O*-isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranose and was accepted for publication in 2002.[61]

The next step in the synthetic strategy involved the regioselective introduction of the C-3 carbamoyl group (see Chapter 3). In order to allow for the selective manipulation of the C-2 and C-3 hydroxyl groups, the strategy entailed the protection of the anomeric hydroxyl group, followed by hydrolysis of the ketal group, thereby selectively liberating the C-2 and C-3 hydroxyl groups.

The anomeric hydroxyl group of lactol **90** was protected as an acetate under standard conditions to furnish **91** in 79% yield, and in the final step this was simultaneously deprotected and inadvertently converted to its methyl glycoside by refluxing with Dowex resin in 95% methanol (Scheme 2.10). This gave **92** in 95% yield (α:β = 14:1), inseparable from its β-anomer.



Major anomer

90 **91** **92**

Scheme 2.10: *Reagents and conditions*: (i) Ac_2O, Et_3N, DMAP, CH_2Cl_2, room temperature, 79%. (ii) Dowex 50WX2-200, 95% MeOH, reflux, 95%.

The mixture of anomers presumably arises from the formation of an oxocarbenium ion intermediate following the acid-catalysed loss of the acetyl group, with attack of the methanol possible from either face. However, the hydrolysis of the bulky ketal group, as well as the anomeric effect (discussed in Section 1.7) favours the formation of the α-anomer. The two anomers could easily be distinguished in the ^{1}H NMR spectrum of the mixture and the anomeric ratio was calculated from the H-1 signals seen at δ_H 4.7 (d, $J_{1,2}$ 4.8 Hz) for the α-anomer and at δ_H 4.5 (d, $J_{1,2}$ 7.5 Hz) for the β-anomer. The coupling constant between H-2 and H-3 was $J_{2,3}$ 3.3 Hz and between H-3 and H-4 was $J_{3,4}$ 3.3 Hz, both couplings characteristic of equatorial-axial relationships, indicating that the conformational strain asserted by the 2,3-*O*-isopropylidene ring had been released and that the sugar ring now adopted a normal chair conformation.

In summary D-ribose was converted to methyl 4-*O*-methyl-5,5-di-*C*-methyl-D-ribopyranoside **92**, the methyl glycoside of 4-epi-noviose, in 11 steps in 36% overall yield.

2.2. Synthesis of noviose

A syntheis of noviose from the key intermediate **86** (Scheme 2.7, page 40) required inversion of configuration at C-4. Various methods are available to achieve inversion of configuration at a hydroxyl-bearing carbon, including: (i) S_N2 attack of a nucleophile at a chiral centre bearing a good leaving group; (ii) Mitsunobu reaction using *p*-nitrobenzoic acid,[62] and (iii) oxidation to the ketone followed by stereoselective reduction.

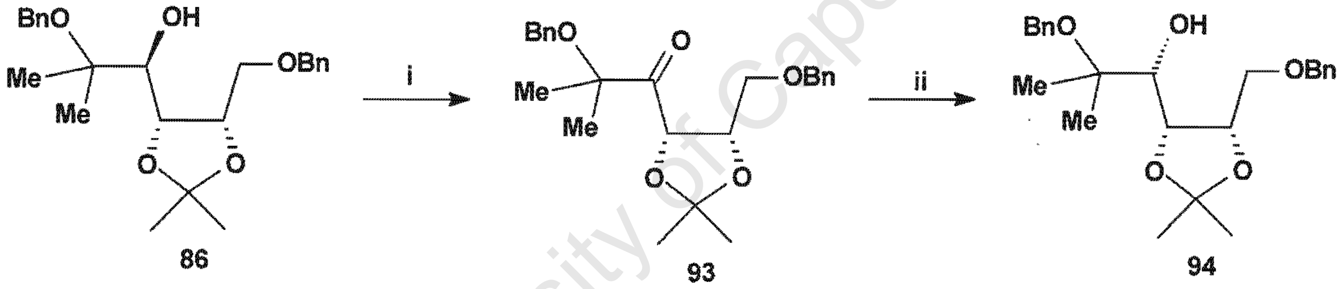
It was decided to pursue a Mitsunobu strategy first. The Mitsunobu reaction with *p*-nitrobenzoic acid involves S_N2 nucleophilic attack of a *p*-nitrobenzoate ion at the hydroxyl-bearing carbon to generate a *p*-nitrobenzoic acid ester, the mechanism of which is well established[63] and hence will not be discussed. Hydrolysis of the ester regenerates the alcohol but with inversion of configuration at the hydroxyl-bearing carbon centre.

Using this method, secondary alcohol **86** was treated with *p*-nitrobenzoic acid, DIAD (diisopropyl azodicarboxylate) and triphenylphosphine in CH_2Cl_2 at 0°C to room temperature. Unfortunately, no reaction occurred (monitored by tlc for consumption of starting material and generation of $Ph_3P{=}O$), and starting material was recovered.

The next step was to convert the secondary alcohol **86** into a good leaving group that could then undergo S_N2 nucleophilic substitution. In 1993, Moriarty *et al.* described a method for the inversion of configuration of α-trisubstituted secondary alcohols in which the hindered alcohol was first converted to a trifluoromethanesulfonic acid (triflate) ester and then treated with KNO_2 to form a nitrous acid ester which was hydrolysed to the alcohol.[64] To this end, **86** was mixed with pyridine and triflic anhydride in CH_2Cl_2 at –78°C. Conversion of the starting material to the triflate ester was extremely slow (monitored by tlc) so the reaction was warmed to 0°C, which resulted in decomposition of the starting material.

The failure of both the Mitsunobu and triflate ester reactions was attributed to the extremely hindered nature of the C-4 secondary alcohol. This point of view is supported by the difficulty encountered in methylating **86** (Schemes 2.6 and 2.7), in which vigorous conditions (refluxing in the presence of NaH) were required.

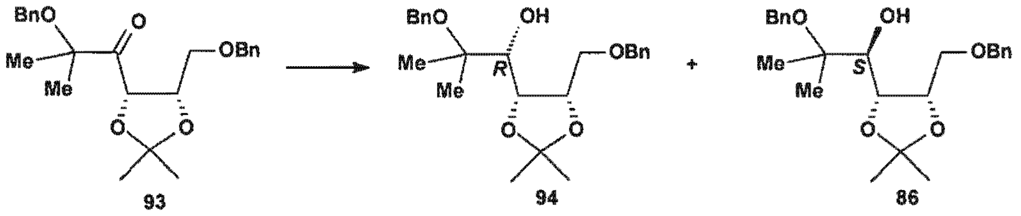
Consequently, it was decided to put these steric effects to good use in a diastereoselctive ketone reduction. Thus, the final method investigated was the diastereoselective reduction of a carbonyl group. Swern oxidation of the free hydroxyl group of **86** afforded ketone **93** in near quantitative yield (Scheme 2.11).



Scheme 2.11: *Reagents and conditions*: (i) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -68°C – room temperature, 99%. (ii) K-Selectride®, toluene, 0°C – room temperature, 72%.

The ^1H NMR spectrum of **93** revealed the absence of the C-4' hydroxyl proton signal resonating at δ_H 3.32 in **86**, and its ^{13}C NMR spectrum revealed a carbonyl carbon at δ_C 207.3. Furthermore, a carbonyl stretch was observed at 1732 cm^{-1} in its IR spectrum.

The diastereoselective reduction of **93** with various reducing agents was carried out and the results presented in Table 2.5. Reduction of **93** with $LiAlH_4$ resulted in a 2:1 ratio of **94** and **86** respectively.

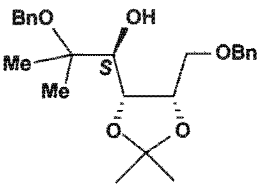


Reducing agent	Yield	94	86
$LiAlH_4$, THF, 0°C	>95%	2	1
DIBALH (1 M in toluene), toluene, -78°C	>95%	<1	>99
L-Selectride®, toluene, -78°C	>95%	1	4
K-Selectride®, toluene, 0°C to rt	72%	>99	<1

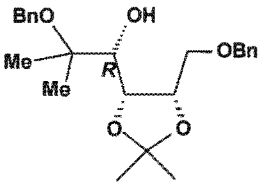
Table 2.5: Diastereoselective reduction of **93** with various reducing agents.

On tlc, the two products were very close in Rf and could not be separated cleanly so the ratio of the products was calculated from the C-4 hydroxyl proton signals resonating at δ_H 3.34 for **86** (d, $J_{OH,4}$ 3.0 Hz) and δ_H 2.74 for **94** (d, $J_{OH,4}$ 8.7 Hz) in the ^{1}H NMR spectrum.

DIBALH (diisobutylaluminium hydride) reduction of **93** in toluene at –78°C gave only **86** in high yield (>95%). Lithium and potassium tri(*sec*-butyl)borohydrides (L and K-Selectrides)[65,66] are very bulky reducing reagents that have been employed in highly diastereoselective reductions of aliphatic carbonyl compounds. Surprisingly, while reduction of **93** with L-Selectride® in toluene or THF at –78°C gave a virtually quantitative mixture of **86** and **94** in a ratio of 4:1, reduction of **93** with K-Selectride® in toluene (0°C - room temperature) yielded exclusively the desired diastereoisomer **94** in 74% yield. At lower temperatures (-78°C), the reaction was very sluggish, so K-Selectride® was added at 0°C and the reaction allowed to reach room temperature, with consumption of the starting material typically being complete within 1 hour. The presence of a single diastereoisomer **94** was established by the ^{1}H NMR spectrum, in which a C-4 hydroxyl proton was observed at δ_H 2.74 as well as a signal at δ_H 4.47 for H-4. No coupling was observed between H-4 and H-3 in contrast to diastereoisomer **86** where a strong coupling ($J_{3,4}$ 9.6 Hz) was observed. A comparison of the key resonances of **86** and **94** is summarised in Table 2.6.



86



94

Nucleus	**Signal (CDCl$_3$, ppm)** **86**	**94**
H-1	3.57 (1H, dd, *J* 6.3 and 9.6 Hz) 3.83 (1H, dd, *J* 5.3 and 9.6 Hz)	3.60 (2H, m)
H-2	4.44 (q, J 5.3, 5.7 and 6.3 Hz)	4.25 (m)
H-3	4.22 (dd, *J* 5.7 and 9.6 Hz)	4.25 (m)
H-4	3.61 (dd, *J* 3.3 and 9.6 Hz)	4.47 (d, *J* 8.8 Hz)
C-1	69.2	70.1
C-2	77.2	77.6
C-3	76.6	76.0
C-4	73.6	74.2
C-5	77.6	77.4

Table 2.6: A comparison of the NMR data for **86** and **94**.

The mode of action for K and L-Selectride® was anticipated to be similiar as the only difference between them is their counterions, namely K$^+$ and Li$^+$ respectively. However, reduction of **93** with K- and L-Selectride® resulted in the prevalence of opposite diastereomers. The observed diastereoselectivity of the sterically demanding and non-chelating K-Selectride® may be rationalized using the Felkin-Ahn model depicted in Figure 2.9a. In this model the electronegative C-O ketal bond is placed perpendicular to the carbonyl bond in the dominant transition-state conformer. This ensures that the π* orbital of the C=O and the σ* orbital of the C-O are stereoelectronically aligned to allow maximum overlap, resulting in the formation of a new, lower-energy LUMO. Subsequently, the hydride is delivered to the less hindered face at the Bürgi-Dunitz preferred angle of 107° from the C=O bond.

By comparison, reduction with DIBALH and L-Selectride® may be explained by Houk's or the Felkin-Ahn chelated model illustrated in Figure 2.9b in which the metal (Al or Li) chelates to the carbonyl prior to the delivery of the hydride. In this case the largest group, namely C-2, now assumes a perpendicular orientation in view of the carbonyl group's greater steric bulk.

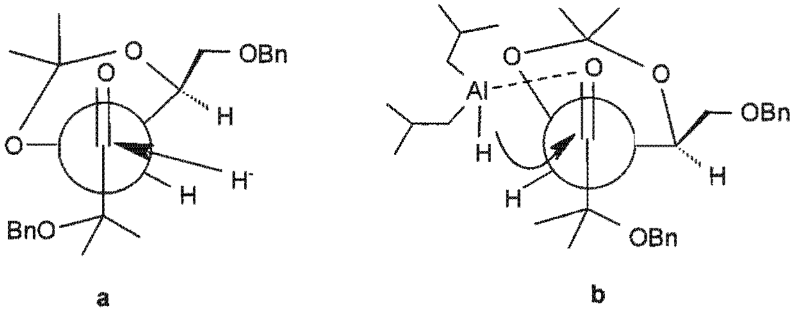
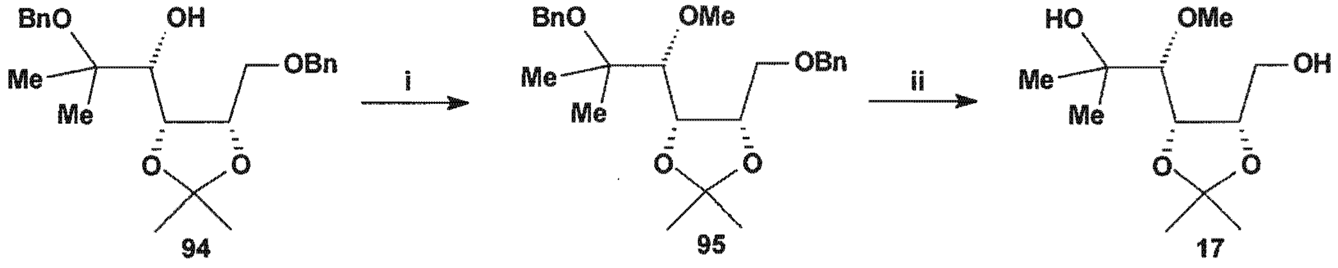


Figure 2.9: (a) Felkin-Ahn model; (b) Houk's model with DIBALH.

The aluminium of DIBALH is a much stronger Lewis acid than the lithium of L-Selectride®, and hence will form a stronger Lewis acid-base complex with the carbonyl oxygen. This would bring the isobutyl groups of DIBALH closer in proximity to the carbonyl centre and hence would cause more steric hindrance than L-Selectride® even though L-Selectride® is a bulkier reducing agent. This provides a plausible explanation for the observed diastereoselectivities between DIBALH and L-Selectride®.

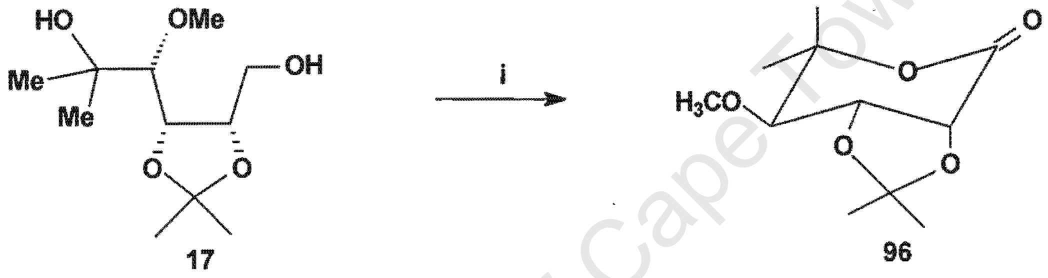
With the correct diastereomer in hand, alcohol **94** was then methylated using methodology identical to that used for the methylation of **86**, to afford **95** in 95% yield (Scheme 2.12). Unlike the methylation of alcohol **86**, it was observed in this case that it was unnecessary to reflux the mixture after the addition of iodomethane as methylation occurred at room temperature; however the reaction was much faster at reflux temperature. The ^{1}H NMR spectrum of **95** revealed the absence of the hydroxyl proton signal, observed at δ_H 2.74 for **94**, and the appearance of a new methoxy methyl signal resonating at δ_H 3.60.



Scheme 2.12: *Reagents and conditions*: (i) NaH, MeI, THF, reflux, 95%. (ii) 10% Pd/C, H_2, EtOH, 95%.

To complete the synthesis, catalytic hydrogenolysis of **95** was performed to furnish **17** (see Scheme 1.5, page 13) in high yield (95%) (Scheme 2.12). The presence of two hydroxyl groups led to poor resolution in the ^{1}H NMR spectrum of **17**, which was addressed by carrying out a D_2O wash. Thus, the appearance of the hydroxyl protons as broad singlets resonating at δ_H 2.65 and 2.99 before the D_2O wash, and the absence of the characteristic benzyl methylene protons at δ_H 4.48 and 4.52 for **95**, provided evidence that exhaustive debenzylation had taken place.

Oxidative cyclization of diol **17** to lactone **96** was achieved with TPAP and NMO in 91% yield (Scheme 2.13).



Scheme 2.13: *Reagents and conditions*: (i) TPAP, NMO, 4Å molecular sieves, CH_2Cl_2, 0°C – room temperature, 91%.

The ^{1}H NMR spectrum of **96** revealed the absence of the anomeric proton, typically between δ_H 4.0 and 5.0, while the ^{13}C NMR spectrum showed the presence of a carbonyl carbon, consistent with formation of a lactone. Further confirmation of lactone formation was provided by the IR spectrum which showed the presence of a carbonyl group at 1768 cm^{-1}. As with lactone **89**, the 2,3-*O*-isopropylidene group caused a change in the conformation of the sugar ring, with a flattening around carbons C-1, C-2 and C-3 as depicted schematically in Figure 2.10. Thus, the coupling between H-3 and H-4 ($J_{3,4}$ 9.3 Hz) was as expected for an axial-axial relationship, while the coupling between H-2 and H-3 ($J_{2,3}$ 10.8 Hz) was much larger than expected for an axial-equatorial relationship as the flattening effect caused the dihedral angle between H-2 and H-3 to change from 60° to about 0°.

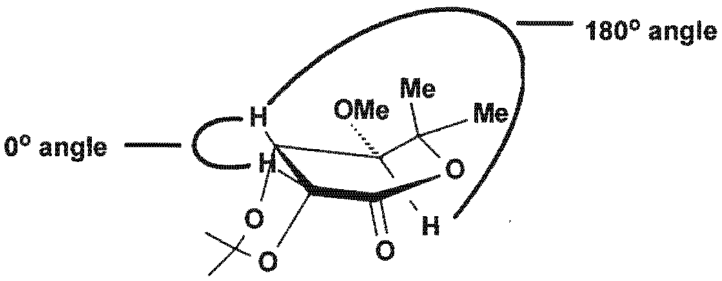
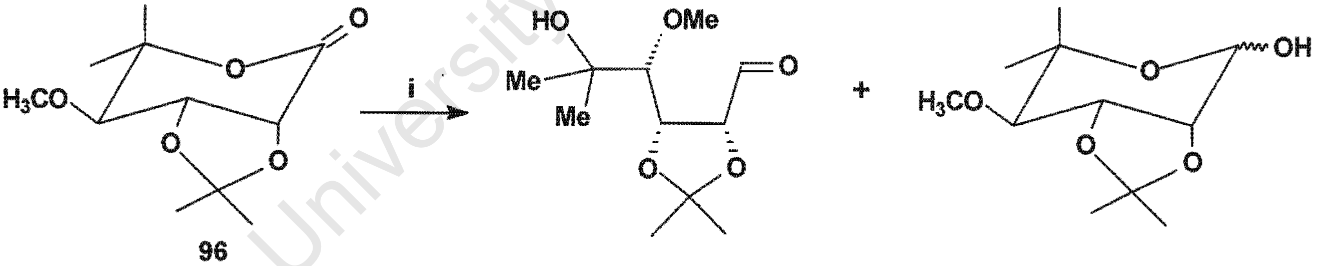


Figure 2.10: Conformational changes to lactone **96** caused by the rigid isopropylidene ring.

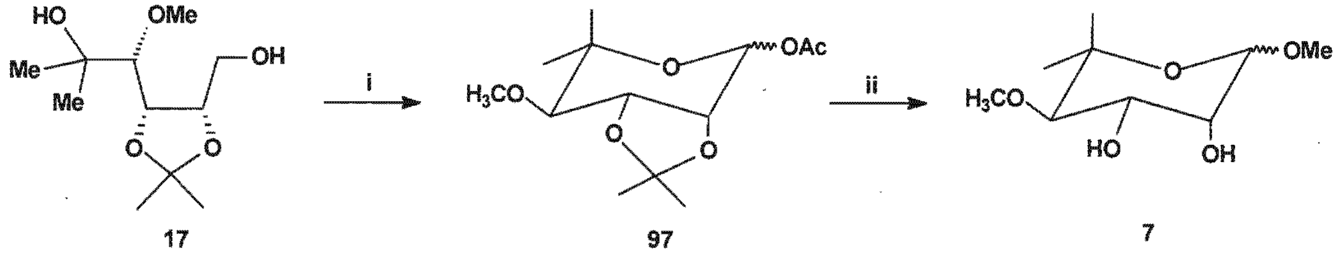
Interestingly, reduction of lactone **96** with DIBALH in toluene yielded a complex mixture of both α- and β-anomeric lactols as well as the open chain aldehyde, the ratio of which was difficult to determine from the ^{1}H NMR and ^{13}C NMR spectra (Scheme 2.14). The aldehyde proton was clearly seen at δ_H 9.80 as well as both α- and β- anomeric protons at ~ δ_H 5, although these signals were unresolved so coupling constants could not be determined. The aldehyde carbon was also clearly seen in the ^{13}C NMR spectrum at δ_C 202.1. This phenomenon was not reported by Laurin *et al.* in the synthesis of noviose from L-arabinose (Scheme 1.5, page 13),[16] and most likely is a result of the rigid 2,3-*O*-isopropylidene group preventing ring-closure. This result was also in contrast to the reduction of lactone **89** in the synthesis of 4-epi-noviose which gave lactol **90** only.



96

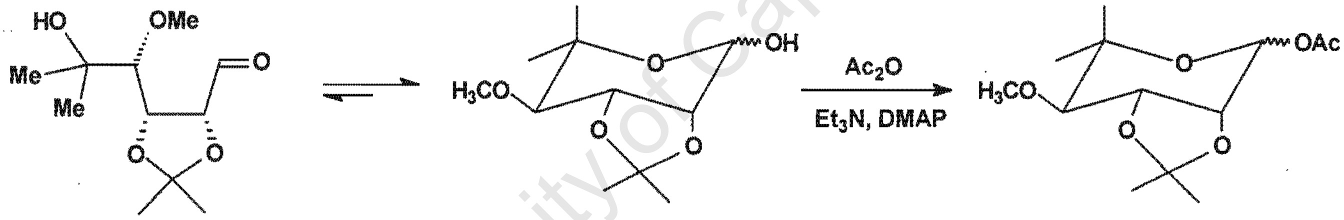
Scheme 2.14: *Reagents and conditions:* (i) DIBALH, toluene, -78°C, 89%.

In order to avoid separation of this complex mixture of lactol and acyclic aldehyde, acyclic diol **17** was oxidized under the same modified Swern conditions employed for the conversion of diol **88** to lactol **90**. The crude material was acetylated without further purification to furnish acetylated lactol **97** as an inseparable 2:1 mixture of β- and α-anomers in 69% yield from **17** (Scheme 2.15).



Scheme 2.15: *Reagents and conditions*: (i) (a) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -68°C – 0°C; (b) Et_3N, Ac_2O, DMAP, CH_2Cl_2, room temperature, 69% over 2 steps. (ii) Dowex 50WX2-200, 95% MeOH, reflux, 97%.

The proposed mechanism for this reaction is illustrated in Scheme 2.16. Acetylation of the tertiary hydroxyl group is seemingly slow under these conditions, as was demonstrated in Scheme 2.4 (Page 36). So, presumably the key issue in the acetylation is trapping the cyclic lactol as an anomeric acetate, resulting in further cyclic hemi-acetal formation from the aldehyde.

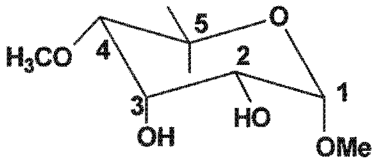


Scheme 2.16: Proposed mechanism for ring closure and acetylation of the resultant anomeric hydroxyl group.

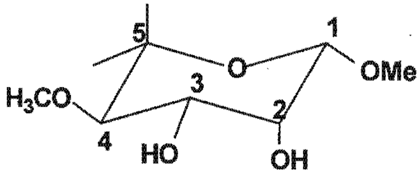
The two anomeric carbon signals were observed at δ_C 101.2 and δ_C 96.5 in the ^{13}C NMR spectrum of **97**. Based on the earlier observation that the α-anomeric carbon resonates further upfield than the β-anomeric carbon, the signal at δ_C 101.2 in the ^{13}C NMR spectrum was assigned to the β-anomer and the signal at δ_C 96.5 to the α-anomer. A COSY 2D-NMR spectrum was then used to distinguish the α- and β-anomeric proton signals in the ^1H NMR spectrum in view of their identical J values. The ratio of anomers was calculated from integration of the H-1 signals in the ^1H NMR spectrum, the α-anomeric signal appearing at δ_H 6.08 ($J_{1,2}$ 3.2 Hz) and the β-anomeric proton at δ_H 6.16 ($J_{1,2}$ 3.2 Hz). A further point to note from the ^1H NMR spectrum was the large coupling between H-3 and H-4 ($J_{3,4}$ 7.0 Hz [α-anomer] and $J_{3,4}$ 6.4 Hz [β-anomer]), indicating an axial-axial relationship between the protons, and hence revealing the C-4 methoxy group to be in an equatorial configuration. As with acetylated lactol **91**, the flattening of the sugar ring by the 2,3-*O*-acetal group caused a decrease in the dihedral angle between H-3 and H-4 resulting in a lowering of the coupling constant.

Finally, the 2,3-*O*-isopropylidene group was hydrolysed with Dowex 50WX2-200 (H^+) in 95% methanol to give the methyl novioside **7** (see Scheme 1.2, page 10) as a 3:1 mixture of β- and α-anomers in 95% yield (Scheme 2.15), the ratio once again calculated from the anomeric protons in the ^{1}H NMR spectrum with the α-anomer resonating at δ_H 4.53 ($J_{1,2}$ 1.2 Hz) and the β-anomer at δ_H 4.67 ($J_{1,2}$ 3.0 Hz). The cleavage of the acetal group released the conformational strain in the sugar ring, as indicated by the coupling constant between H-2 and H-3 of $J_{2,3}$ 3.0 Hz returning to a typical equatorial-axial relationship as well as the coupling constant between H-3 and H-4 of $J_{3,4}$ 9.2 Hz, characteristic of an axial-axial relationship. A comparison of the ^{1}H and ^{13}C NMR data for the β-anomers of methyl 4-epi-novioside **92** and methyl novioside **7** is summarised in Table 2.7.

As illustrated, a large difference was observed between the coupling constants for H-3 and H-4. In **92** the coupling is small ($J_{3,4}$ 3.3 Hz), reflecting an axial-equatorial association, while in **7** the coupling is large ($J_{3,4}$ 9.2 Hz), indicative of an axial-axial relationship. Chemical shift differences were noted as well. H-2 resonated further downfield in **7** than in **92**, presumably as a result of their difference in spatial orientation, with H-2 in **7** being axial while in **92** it is equatorial. Similarly, H-3 resonated further downfield in **92** than in **7**.



92



7

Nucleus	**Signal ($CDCl_3$, ppm)**	
	92	**7**
H-1	4.70 (d, *J* 4.8 Hz)	4.67 (d, *J* 3.0 Hz)
H-2	3.52 (m)	3.94 (t, *J* 3.0 Hz)
H-3	4.23 (t, *J* 3.3 Hz)	4.03 (dd, *J* 3.0 and 9.2 Hz)
H-4	3.11 (dd, *J* 0.9 and 3.3 Hz)	3.25 (d, *J* 9.2 Hz)
C-1	100.9	101.2
C-2	71.2	71.2
C-3	65.5	68.6
C-4	85.3	84.4
C-5	76.3	74.8

Table 2.7: A comparison of the NMR data for **92** and **7**.

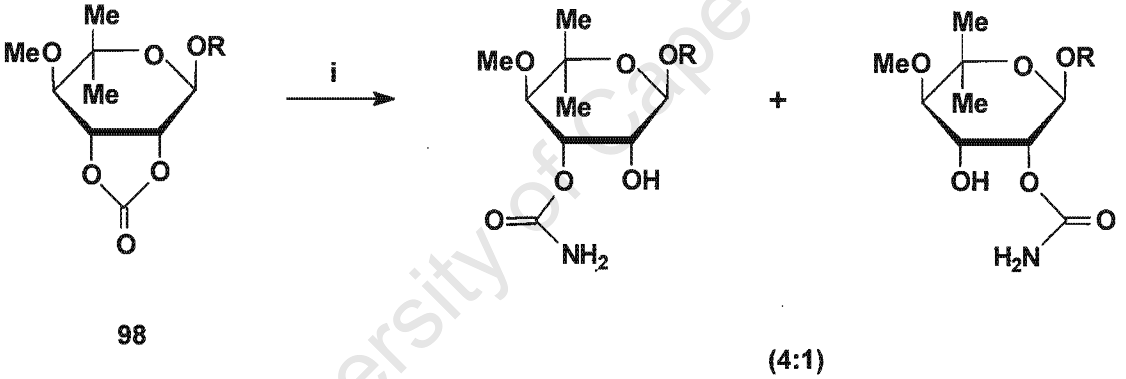
In summary, the synthesis of methyl novioside **7** has been achieved in 12 steps in an overall yield of 22% from D-ribose.[61] Table 2.8 summarises a comparison of the number of steps and yield of this synthesis (the first two entries in Table 2.7 from this work) with the syntheses of noviose and methyl novioside reported in Chapter 1. As can be seen, the number of steps for both targets compares favourably to the other syntheses while the overall yields are amongst the highest. Although the synthesis of noviose was achieved in only 7 steps in 33% overall yield from L-rhamnose, D-ribose (£UK62 for 100g, UK Aldrich catalogue 2004) is far less expensive than L-rhamnose (£UK138 for 100g, UK Aldrich catalogue 2004). Hence, the advantages of the synthesis from D-ribose are that it is both straightforward and efficient and does not require the use of expensive reagents or starting material. Its attraction lies in the fact that it is a divergent synthesis with both noviose and its C-4 epimer being achieved from a common derivative of D-ribose.

Starting material		**No. of steps**	**Yield**	**Product**
D-Ribose	**This work**	11	36%	Methyl 4-epi-novioside
D-Ribose	**This work**	12	22%	Methyl novioside
L-Arabinose		9	22%	Noviose
Methyl 3,5,6-tri-*O*-benzyl-2-*O*-methyl-D-glucofuranoside		11	2%	Methyl novioside
L-Rhamnose		7	33%	Noviose
D-Gulonolactone		11	12%	Noviose
(1*S*,4*R*)-4-acetoxy-5,5-dimethyl-cyclopent-2-en-1-ol		7	31%	D-Noviose
Enone **43** derived from furfural		15	23%	Noviose

Table 2.8: A comparative analysis of the syntheses of noviose and methyl novioside.

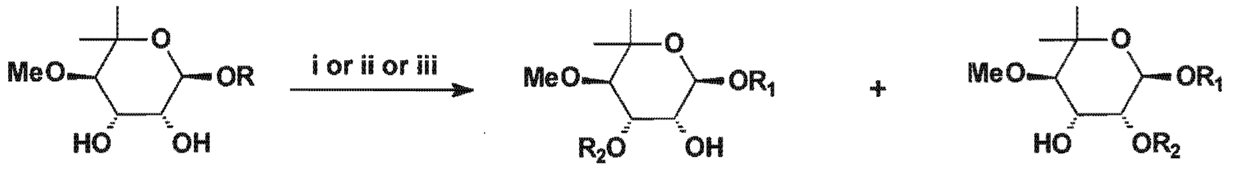
Chapter 3 : Introduction of the C-3 carbamoyl group

There is a plethora of reagents available for the protection of hydroxyl groups in synthetic organic chemistry, with newer types and useful modifications of existing ones continually being published.[54,67] Despite this, problems still arise in differentiating between hydroxyl groups in a molecule, particularly between secondary hydroxyls in a six-membered ring. In the synthesis of noviose, various efforts have been made to introduce the carbamoyl group regioselectively onto the C-3 hydroxyl group. The standard synthetic procedure employs opening of the 2,3-*O*-carbonate of 2,3-*O*-carbonyl-α-novioside **98** with NH_3 (Scheme 3.1).[68,19,22] However, the opening of the carbonate is not regioselective, leading to a mixture of the 3-substituted and 2-substituted derivatives (4:1 respectively), which are difficult to separate resulting in low yields of the 3-regioisomer.



Scheme 3.1: *Reagents and conditions:* (i) NH_3, MeOH, room temperature.

It has been well established that an equatorial OH group in a six-membered ring may be preferentially protected in the presence of a secondary, axial hydroxyl group (see Section 3.2 for indepth discussion). Musicki *et al.*[20] used this feature to regioselectively protect the equatorial 3-OH of α-novioside **99** with three different protecting groups, namely Et_3Si, MEM ($MeOCH_2CH_2OCH_2$-), and BOM ($PhCH_2OCH_2$-) protecting groups, the results of which are summarised in Scheme 3.2. In all three cases, the major product was the 3-regioisomer, which could be easily separated by chromatography from the 2'-regioisomer, although the yields were only moderate (66% for Et_3Si).



99

R_2 =	3'- OR_2	2'- OR_2
a. Et_3Si	66%	9%
b. MEM	60%	15%
c. BOM	60%	15%

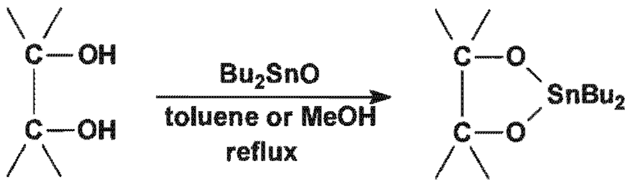
Scheme 3.2: *Reagents and conditions:* (i) Et_3SiCl, DIPEA, imidazole, CH_2Cl_2, room temperature, 66%. (ii) MEMCl, DIPEA, imidazole, CH_2Cl_2, room temperature, 60%. (iii) BOMCl, imidazole, CH_2Cl_2, room temperature, 60%.

In order to address this chemoselectivity problem further, two methods were investigated: (i) acylation of dialkystannylene acetals and (ii) the use of other protecting groups to exploit the different reactivities of the axial and equatorial hydroxyl groups.

3.1. Selective protection via dialkylstannylenes

3.1.1. Introduction

In 1985, David and Hanessian published a review on the use of dialkylstannylenes for the regioselective acylation and alkylation of hydroxyl groups, with applications in the field of carbohydrates.[69] Dialkylstannylenes may be prepared by treating a diol with an equimolar quantity of dibutyltin oxide in toluene or refluxing methanol to form an *O*-stannylene acetal (Scheme 3.3).



Scheme 3.3: Preparation of *O*-stannylene acetal.

The precise origin for the regioselectivity of acylation or alkylation of the dialkylstannylenes is poorly understood, although it has been hypothesised that one of the two oxygens in the stannylene ring is more nucleophilic than the other. David and Hanessian have suggested that chiral carbohydrate-derived stannylenes are dimeric in all physical states (except perhaps in polar solvents) as shown by ^{119}Sn NMR spectroscopy and mass spectrometry. Each of the tin atoms is in the centre of a trigonal bypyramid with the two butyl groups occupying the two equatorial positions. One of the O-atoms is in the apical position, while the other is in the equatorial position, and the two monomeric structures in the unit are joined as a parallelogram as seen in Figure 3.1.

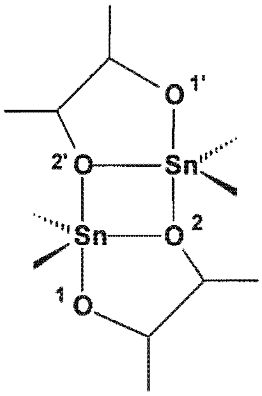


Figure 3.1: Dimeric nature of O-stannylene acetals.

Within one monomeric unit of the dimer, the apically bound O-atoms (O^1 and $O^{1'}$) are preferentially acylated and alkylated. David and Hanessian theorise that this regioselectivity is due to enhancement of the nucleophilicity, which may be the result of electron channelling from the tin atom towards the apically bound O-atoms. This results in preferential activation and bond rupture at the expense of the equatorially oriented and electronically less enriched O-atoms (O^2 and $O^{2'}$), as well as the fact that atoms O^2 and $O^{2'}$ are relatively protected by threefold coordination. Hence, they have postulated that the origin of the regioselectivity is inherent in the constitution of the dimer, and is not directly determined by the orientation (axial versus equatorial) of the OH groups in the carbohydrate ring. This dimer model was supported in a recent review (1998) by Grindley[70] who concurred that regioselectivity was directly related to the structure and reactivity of dimers in solution. He further states that if only a single symmetric dimer forms when a carbohydrate diol is converted to its stannylene acetal, as determined by ^{119}Sn NMR spectroscopy, highly regioselective acylation reactions are obtained, whereas if more than one type of dimer forms, then mixtures of products are observed. Although dimer structure is difficult to predict, Grindley observed some general trends with respect to carbohydrates which are as follows:

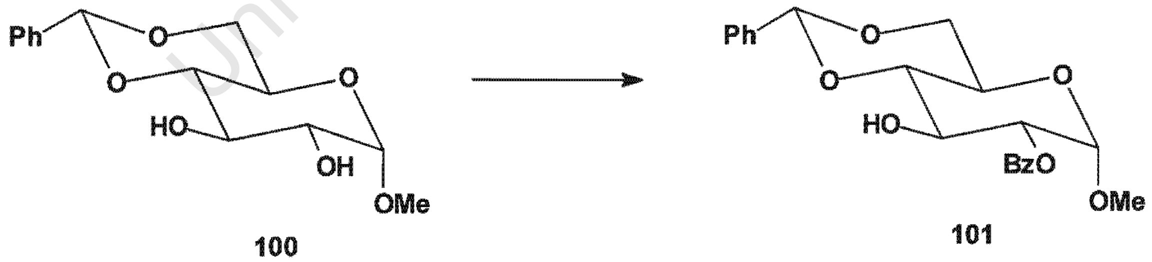
(i) In nonpolar solvents without added nucleophiles such as tetrabutylammonium halides, N-methylimidazole, and cesium fluoride, the dibutylstannylene acetals of *cis*-diols on pyranose rings react much faster than those of *trans*-diols and predominantly furnish the equatorial hydroxyl substitution product. An exception are those derived from mannopyranosides, which give varying amounts of reaction on the axial O-2. In the presence of added nucleophiles, the preference for equatorial substitution is enhanced.

(ii) Dibutylstannylene acetals of *trans*-diequatorial diols flanked by one axial substituent react on the oxygen atom adjacent to the axial substituent, but give mixtures if the adjacent substituents are either both equatorial or both axial.

(iii) If deoxy centres are present, reaction adjacent to the deoxy center is favoured.

These trends are illustrated in the following three examples, and where appropriate the different reactivities of the α- and β- anomers are noted.

1. (a) The stannylene acetal of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **100** undergoes exclusive benzoylation at the C-2 oxygen (Scheme 3.4),[71] which is in keeping with the X-ray crystal structure of the dimer of the 2,3-O-dibutylstannylene derivative of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, depicted schematically in Figure 3.2, revealing the C-2 oxygen in the apical position.



Scheme 3.4: *Reagents and conditions:* (i) (a) Dibutyltin oxide, benzene-methanol, reflux; (b) Et_3N, BzCl, dioxane, room temperature, 86% over 2 steps.

This example is also in accordance with the observation by Grindley in that the equatorial hydroxyl group of the *trans*-diol adjacent to the axial substituent reacts preferentially to furnish **101**, in 86% yield.

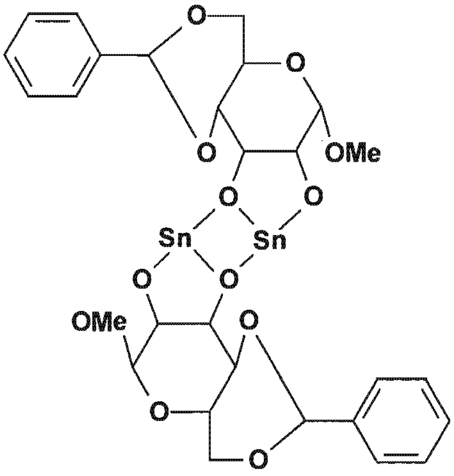
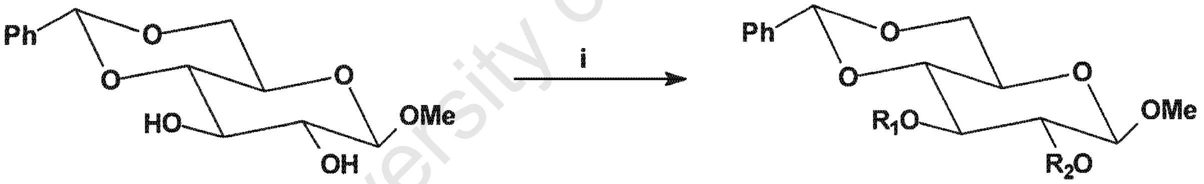


Figure 3.2: Schematic representation of the crystal structure of methyl 4,6-O-benzylidene-2,3-O-dibutylstannylene-α-D-glucopyranoside.

(b) Benzoylation of the stannylene acetal of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside **102** yielded a mixture of 2- and 3- benzoates **103** and **104** respectively (Scheme 3.5),[72] in agreement with the trend that *trans*-1,2-diols having both adjacent groups equatorial give mixtures of products.

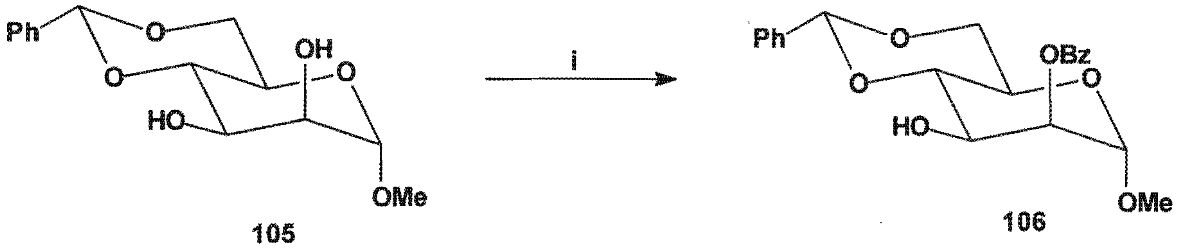


102

103 $R_1 = H, R_2 = Bz$ **49%**
104 $R_1 = Bz, R_2 = H$ **39%**

Scheme 3.5: *Reagents and conditions:* (i) (a) Dibutyltin oxide, benzene, reflux; (b) BzCl, room temperature, 88% over 2 steps.

2. The stannylene acetal of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **105** also undergoes selective benzoylation at the C-2 hydroxyl group to yield **106** in 85% yield, although this group is now in the axial configuration (Scheme 3.6),[73] suggesting that this oxygen is in the apical position. In contrast, benzylation of the stannylenated mannoside affords the 3-ether in 85% yield.



Scheme 3.6: *Reagents and conditions:* (i) (a) Dibutyltin oxide, benzene, reflux with removal of water; (b) BzCl, room temperature, 85% over 2 steps.

The crystal structure of methyl 4,6-*O*-benzylidene-2,3-O-dibutylstannylene-α-D-mannopyranoside revealed that it crystallizes as a pentamer (Figure 3.3).[74] The authors of this publication hypothesized that this compound exists in solution not only in a dimeric form, but also in several oligomeric states as evidenced by a complex ^{119}Sn NMR spectrum of a solution of the compound in deuteriobenzene. The existence of several tin-containing species in solution may account for the atypical behaviour of methyl 4,6-*O*-benzylidene-2,3-O-dibutylstannylene-α-D-mannopyranoside.

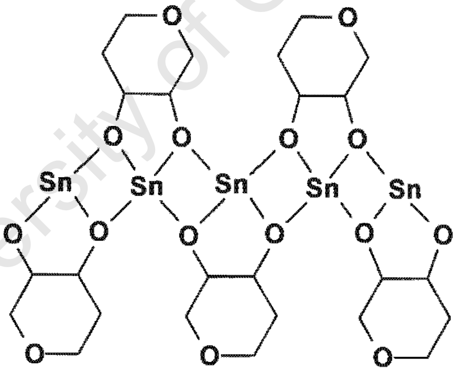
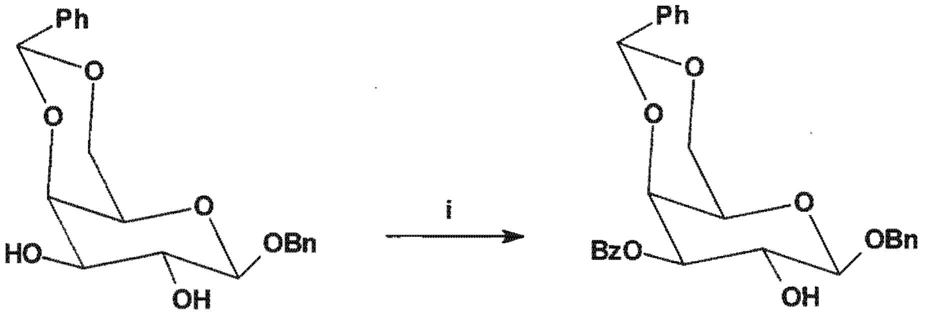


Figure 3.3: Schematic representation of the crystal structure of methyl 4,6-O-benzylidene-2,3-O-dibutylstannylene-α-D-mannopyranoside (for simplicity, only the ring structures are shown).

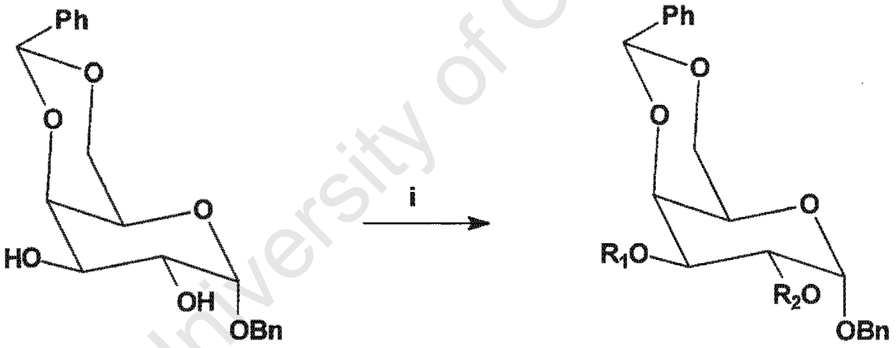
3. (a) In the final example and in contrast to the glucose series, the 2,3-*O*-stannylene acetal of benzyl 4,6-*O*-benzylidene-α-D-galactopyranoside **107** reacts at O-3 to give an almost quantitative yield of the monobenzoate **108** (Scheme 3.7).[75] The C-3 hydroxyl is adjacent to an axial group and hence is acylated preferentially, according to Grindley.



107 **108**

Scheme 3.7: *Reagents and conditions:* (i) (a) Dibutyltin oxide, benzene, reflux; (b) BzCl, molecular sieves, benzene, room temperature, 95% over 2 steps.

(b) Benzoylation of the 2,3-*O*-stannylene acetal of benzyl 4,6-*O*-benzylidene-α-D-galactopyranoside **109** yields a mixture of 3-O and 2-O benzoates **110** and **111** respectively (Scheme 3.8), again in keeping with the trend that *trans*-1,2-diols having both adjacent groups axial give mixtures of products.

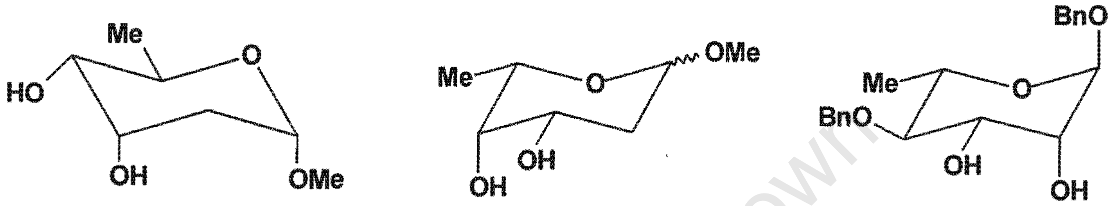


109

110 R_1 = **Bz**, R_2 = **H 46%**
111 R_1 = **H**, R_2 = **Bz 38%**

Scheme 3.8: *Reagents and conditions:* (i) (a) Dibutyltin oxide, benzene, reflux; (b) BzCl, room temperature, 84% over 2 steps.

Although stannylene chemistry has been employed in the regioselective hydroxyl group protection of hundreds of sugars as revealed in Grindley's review,[70] little has been reported in the literature with respect to L-lyxose or D-ribopyranose sugars. Reported syntheses involving these two sugars were published in 1987 and 1993 in which Monneret *et al.* reported the regioselective alkylation of the stannylene acetals of methyl 2,6-dideoxy-α-D-ribohexopyranoside (**112**) and methyl 2,6-dideoxy-L-lyxohexopyranoside (**113**).[76] Both sugars undergo exclusive alkylation at the C-3 hydroxyl group that is adjacent to the C-2 deoxy centre, which is one of the trends observed by Grindley.[70] Benzoylation of the stannylene acetal of benzyl 4-O-benzyl-α-L-rhamnopyranoside (**114**),[77] which is similar in configuration to noviose except that it lacks the additional axial methyl group on C-5, gave the 3-O and 2-O-benzoyl derivatives in 66 and 24% yields respectively, with equatorial substitution being dominant over axial substitution as predicted by Grindley.



112 **113** **114**

Figure 3.4: Methyl 2,6-dideoxy-α-D-ribohexopyranoside (**112**), methyl 2,6-dideoxy-L-lyxohexopyranoside (**113**) and benzyl 4-O-benzyl-α-L-rhamnopyranoside (**114**).

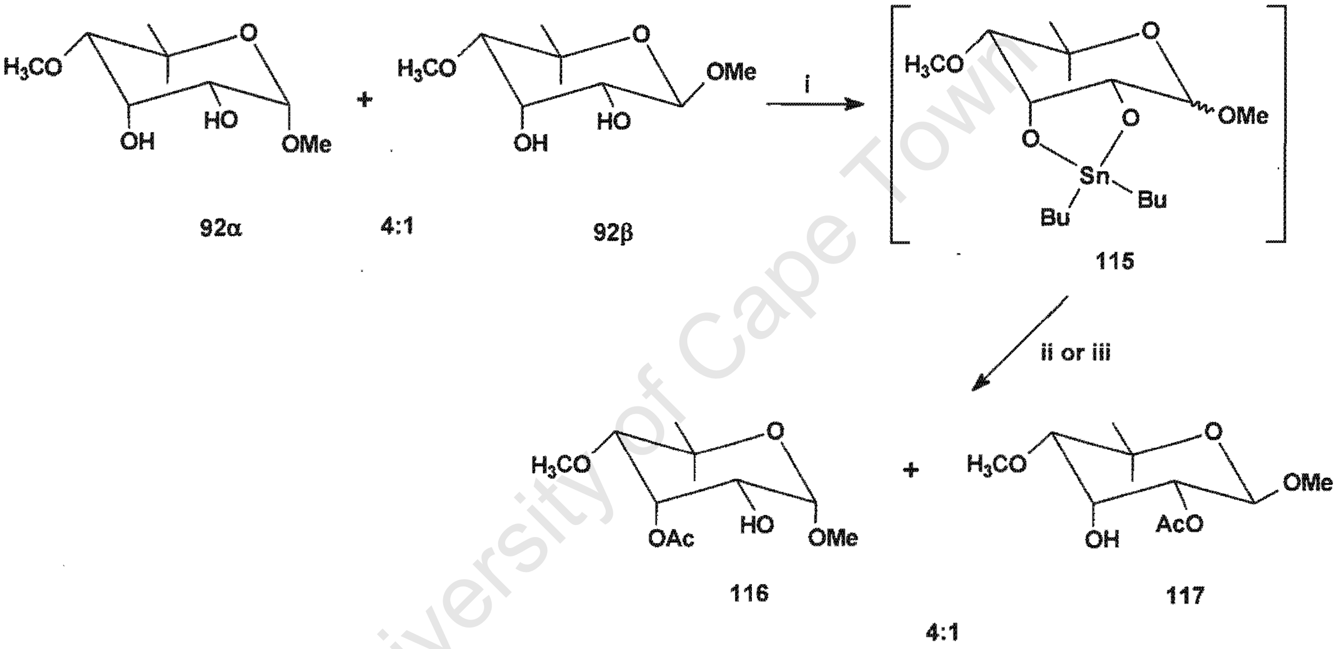
One notable omission in Grindley's observations is consideration of the role of conformation in observed selectivities. When vicinal diols are tethered as stannylene acetals, the conformation of the parent pyranose ring may well be distorted, with substituents no longer in true axial or equatorial orientation. This could affect both reactivity and steric accessibility.

As nothing had been reported concerning noviose and 4-epi-noviose, it seemed appropriate to explore the chemistry involving the stannylene acetals of methyl 4-O-methyl-5,5-C-dimethyl-D-ribopyranoside **92** and methyl 4-O-methyl-5,5-C-dimethyl-L-lyxopyranoside **7** in order to investigate which oxygen, *O*-2 or *O*-3, would be more nucleophilic and hence be selectively acylated.

In light of the trends observed by Grindley, it is clear that the configuration of the anomeric substituent plays a role in determining the stereoselective outcome of acylation of the stannylene acetals. However, as difficulty was experienced in separating the anomeric mixtures of both methyl novioside and methyl 4-epi-novioside, it was necessary to work with an anomeric mixture throughout.

3.1.2. Stannylene-mediated selective acylation of 4-epi-noviose

Following a literature procedure,[71] methyl 4-*O*-methyl-5,5-di-*C*-methyl-D-ribopyranoside **92**, as an inseparable mixture of anomers (α:β ~ 4:1), was refluxed with dibutyltin oxide in methanol until the milky solution turned clear (approximately 1 hour). After evaporating the solvent *in vacuo*, the resulting stannylene acetal **115** in the form of a syrup was dissolved in dichloromethane, cooled to 0°C and a slight excess of acetyl chloride was added. The reaction was complete within 30 minutes, with tlc suggesting the presence of a single product which was isolated in 85% yield (Scheme 3.9).



Scheme 3.9: *Reagents and conditions:* (i) Dibutyltin oxide, MeOH, reflux. (ii) AcCl, CH_2Cl_2, 0°C, 85%. (iii) AcCl, Et_3N, dioxane, room temperature, 90% over 2 steps.

However, the ^1H NMR spectrum of the product revealed that it was an inseparable mixture (4:1) of the methyl 3-*O*-acetyl-α-D-ribopyranoside (**116**) and the methyl 2-*O*-acetyl-β-D-ribopyranoside (**117**) respectively. The two sets of ^1H NMR signals were readily distinguishable as illustrated by an expanded section of the ^1H NMR spectrum in Figure 3.5, the signals of which are summarised in Table 3.1.

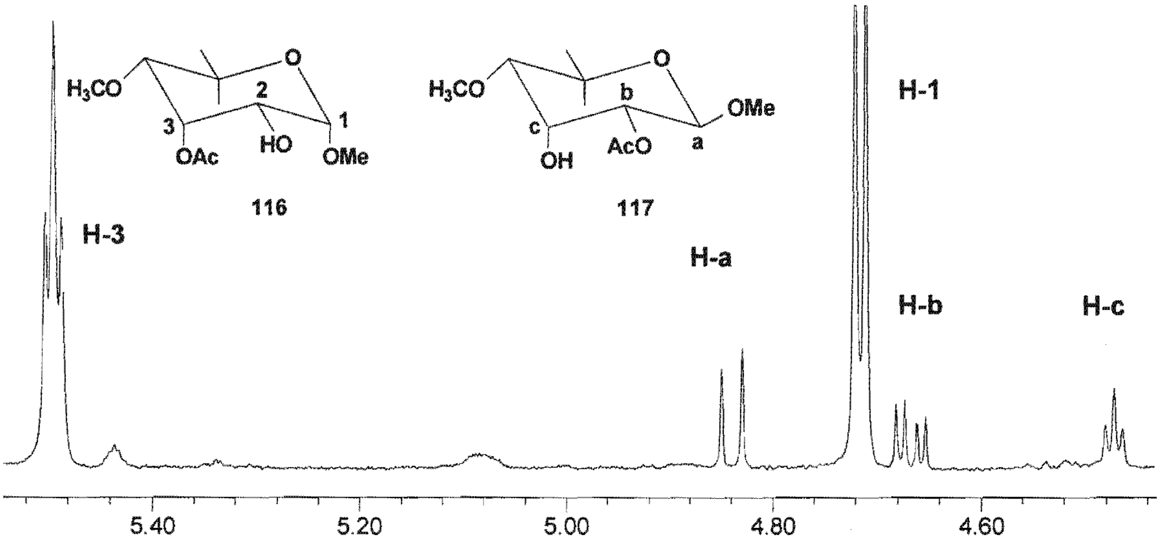
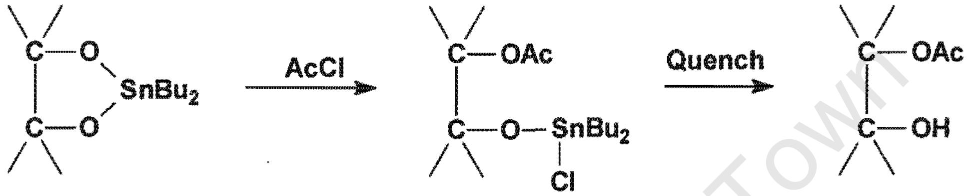


Figure 3.5: An expanded section of the ^{1}H NMR spectrum of the mixture of **116** and **117**.

^{1}H NMR	Signal (CDCl$_3$, ppm)	
	3-O-acetyl (α-anomer) 117	2-O-acetyl (β-anomer) 118
2 x CH$_3$	1.32, 1.38	1.25, 1.29
-COC$\underline{H}_3$	2.15	2.14
H-4	3.24 (d, *J* 3.0)	3.03 (d, *J* 3.6)
2 x -OMe	3.44, 3.48	3.43, 3.46
H-3	5.49 (t, *J* 3.0)	4.47 (t, *J* 3.6)
H-2	3.71 (dd, *J* 3.0 and 4.0)	4.67 (dd, *J* 3.6 and 8.0)
H-1	4.71 (d, *J* 4.0)	4.83 (d, *J* 8.0)

Table 3.1: ^{1}H NMR data for the inseparable mixture of **116** and **117**.

The ^{1}H NMR resonances for **116** in the spectrum of the mixture exhibited an expected downfield shift for H-3 (t, *J* 3.0 Hz) from δ_H 4.23 in **92α** to δ_H 5.49 as a result of the deshielding effect of the acetyl carbonyl group, while H-2 (dd, *J* 3.0 and 4.0 Hz) appeared slightly downfield from δ_H 3.52 in **92α** to δ_H 3.71. The anomeric proton revealed a coupling constant of 4.0 Hz, indicative of an axial-equatorial relationship and establishing **116** to be the α-anomer. The ^{1}H NMR resonances for **117** in the spectrum of the mixture showed a similar trend, with a downfield shift of H-2 from δ_H 3.52 in **92β** to δ_H 4.67, and H-3 as a triplet, shifted slightly downfield from δ_H 3.95 in **92β** to δ_H 4.47. The anomeric centre for **117** was assigned as the β-anomer owing to the large axial-axial coupling of 8.0 Hz for H-1. The above transformation was then repeated by treating the stannylene acetal **115** with AcCl and Et_3N in dioxane but the outcome was the same (Scheme 3.9). Although an excess of acetyl chloride was used in both reactions, no diacetylated product was observed. This is presumably the result of the formation of a dibutylchlorostannyl ether at the unacetylated hydroxyl group following the reaction with acetyl chloride, which was inert to further substitution (Scheme 3. 10).

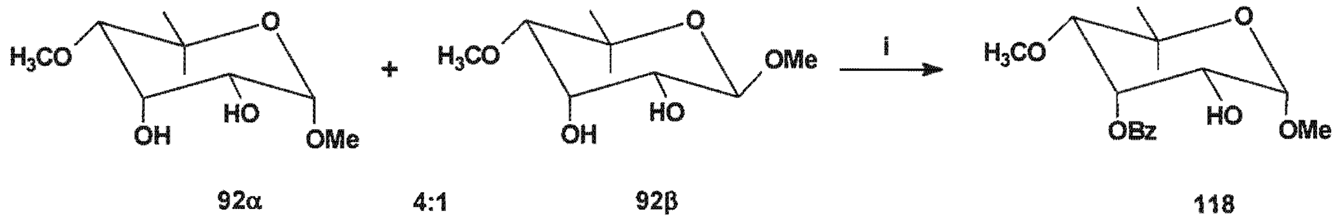


Scheme 3.10: Formation of a dibutylchlorostannyl ether post-acylation.

Since the reaction conditions could not promote epimerisation, regioselectivity appeared to be controlled by anomeric configuration with the α-anomer being C-3 selective and the β-anomer C-2 selective. According to Grindley's observed trends, *cis*-diols on pyranose rings predominantly furnish the equatorial hydroxyl substitution product. This was found to be the case for the β-anomer **92β**, where the equatorial hydroxyl group was substituted, but not for the α-anomer **92α**, where the axial hydroxyl group was substituted.

In view of the difficulty encountered in separating these regioisomers, introduction of a benzoyl group was considered as an alternative.

Thus the stannylene acetal **115**, prepared as before, was treated with benzoyl chloride and triethylamine in CH_2Cl_2, at 0°C (Scheme 3.11). The reaction proceeded rapidly, and tlc revealed the presence of a major and minor component. Only the major component was isolated by column chromatography (65% yield) as isolation of the minor product was complicated by the presence of stannylene salts.



Scheme 3.11: *Reagents and conditions:* (i) (a) dibutyltin oxide, MeOH, reflux. (b) BzCl, Et_3N, CH_2Cl_2, 0°C, 65% over 2 steps.

^{1}H NMR analysis (Figure 3.6) confirmed that the major product was the single regioisomer **118**, benzoylated at O-3, with H-3 (t, *J* 3.6 Hz) having shifted downfield from δ_H 4.23 in **92α** to δ_H 5.77.

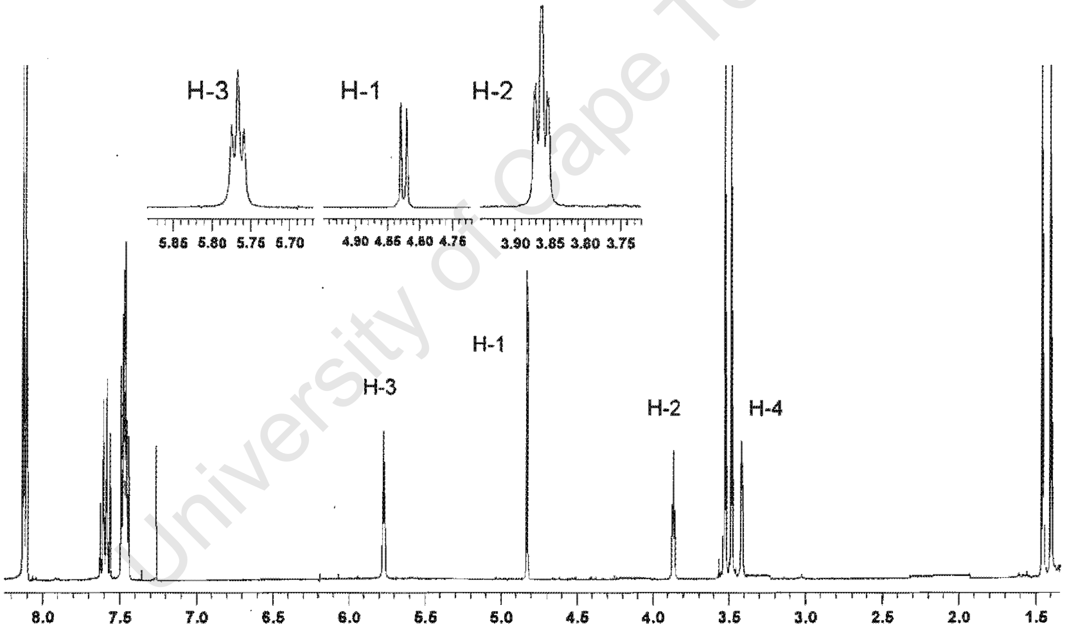
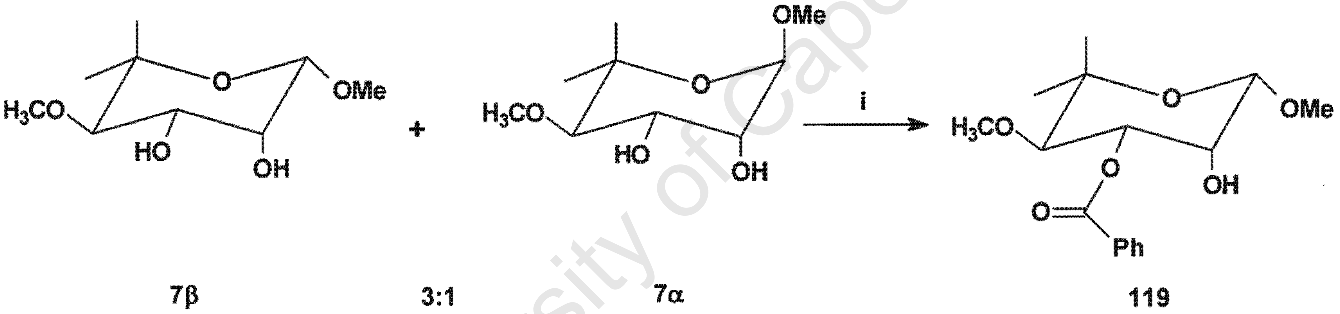


Figure 3.6: ^{1}H NMR spectrum of methyl 3-*O*-benzoyl-4-*O*-methyl-5,5-di-*C*-methyl-α-D-ribopyranoside (**118**).

The signal for H-2 (dt, *J* 1.2 and 3.6 Hz) had shifted only slightly downfield from δ_H 3.52 in **92α** to δ_H 3.86 and showed couplings with H-1, H-3 and -OH. The presence of this single regioisomer represents an 81% yield of mono-protected α-glycoside **118**. The anomeric status was confirmed as the α-anomer by the small coupling constant observed for H-1 (d, *J* 3.6 Hz) resonating at δ_H 4.82. No hydroxyl proton could be observed in the ^{1}H NMR spectrum although the IR spectrum did reveal the presence of a broad signal at 3670 cm^{-1} in the hydroxyl region for the C-2 hydroxyl group. The appearance of a carbonyl stretch at 1718 cm^{-1} in the IR spectrum, UV activity on tlc, and the presence of a carbonyl peak in the ^{13}C NMR spectrum at δ_C 166.1 provided supporting evidence for the presence of a benzoyl group. Once again, this result was in contradiction to the trends observed by Grindley,[70] as the C-3 hydroxyl group was substituted despite the fact that this group is axial, although conformational flexibility may play a role.

3.1.3. Stannylene-mediated selective acylation of noviose

In view of the high degree of chemoselectivity achieved for 4-epi-noviose, attention was turned to noviose. The stannylene acetal, prepared from anomeric mixture **7** (α:β ~ 1:3), was reacted with BzCl at room temperature. Despite the fact that base was omitted from the reaction, the starting material was consumed within 30 minutes (Scheme 3.12).



Scheme 3.12: *Reagents and conditions:* (i) (a) dibutyltin oxide, MeOH, reflux. (b) BzCl, CH_2Cl_2, room temperature, 75% over 2 steps.

Tlc indicated the presence of a UV-active major and minor component, with the major component **119** being isolated in 75% yield. The ^{1}H NMR spectrum of **119** revealed a downfield shift for H-3 (dd, *J* 3.0 and 9.0 Hz) from δ_H 4.03 in **7β** to δ_H 5.55 with a slight downfield shift for H-2 (t, *J* 3.0 Hz) from δ_H 3.94 in **7β** to δ_H 4.14, providing evidence for C-3 hydroxyl benzoylation. Similarly, H-1 resonated at δ_H 4.71 as a doublet (*J* 3.0 Hz) and H-2 resonated as a triplet (*J* 3.0 Hz) coupling with H-1 and with H-3. This demonstrated that both the H-1 and H-3 protons were axial so the anomeric centre was assigned as the β-anomer. The IR spectrum of **119** showed the presence of an –OH stretch at 3691 cm^{-1} and a carbonyl stretch at 1721 cm^{-1}, while the ^{13}C NMR spectrum revealed the presence of the benzoyl carbonyl carbon resonating at δ_C.165.7. This result is in keeping with Grindley's observations, as the equatorial C-3 hydroxyl group of the *cis*-diol in **7β** was preferentially substituted.

3.1.4. Conclusion

As can be seen from these results, acetylation and benzoylation of the stannylene acetals of α-D-ribopyranose and β-L-lyxose sugars **92α** and **7β** respectively, occurred preferentially at the C-3 hydroxyl group, regardless of whether that hydroxyl group was in an axial or equatorial configuration. According to the dimeric theory postulated by David and Hanessian,[69] it would suggest that the C-3 oxygen is in the apical position as illustrated in Figure 3.7.

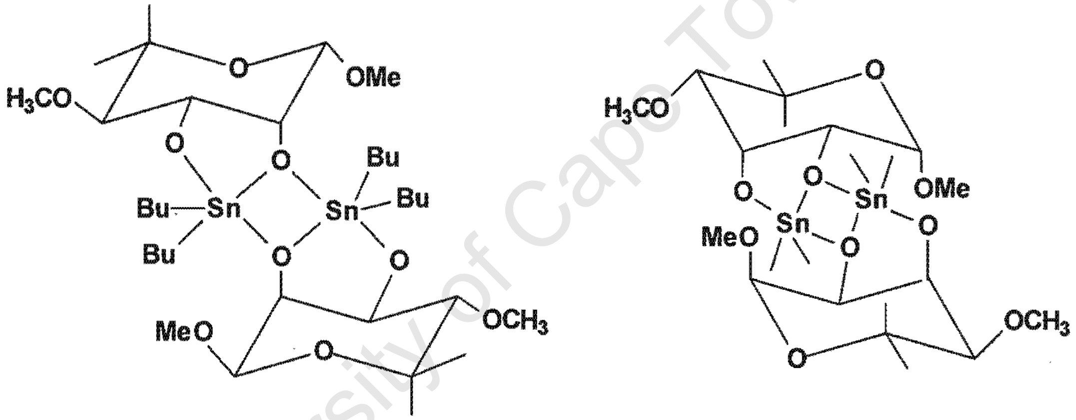


Figure 3.7: Theoretical dimeric nature of the stannylene acetals of noviose and 4-epi-noviose.

However, in the case of the acetylation of β-D-ribopyranose sugar **92β**, acetylation occurred at the C-2 hydroxyl group. As discussed in the previous sections, in the case of 4-epi-noviose, these results are against the trends observed by Grindley, while in the case of noviose, the equatorial 3-OH is preferentially substituted.[70]

The reasons for the difference in chemoselectivity of the α- and β-anomers of methyl 4-*O*-methyl-5,5-di-*C*-methyl-D-ribopyranoside **92** remain unclear. In 1976, Munavu observed that the α-anomer of methyl 4,6-O-benzylidene-D-glucopyranoside was selectively esterified at its 2-OH while the β-anomer failed to be selective, giving a mixture of 2-O and 3-O-esters.[71] Their explanation for this difference in behaviour was that coordination between the metal and the α-methoxy oxygen occurred, resulting in greater chemoselectivity of the α-anomer

(Figure 3.8). However, this was dismissed by David and Hanessian,[69] as the melting point for this stannylated compound was identical to that of the dimeric derivative prepared by David and Thieffry.[78] Even if coordination between tin and the α-methoxy oxygen in methyl 4-*O*-methyl-5,5-di-*C*-methyl-D-ribopyranoside **92** did occur, it would not explain why acylation of the α-anomer gives the 3-O-ester as the major product.

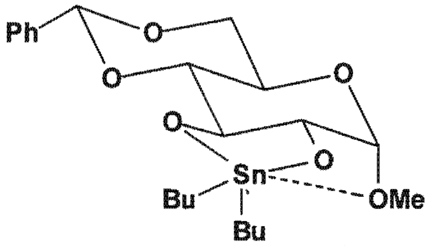


Figure 3.8: Stannylene acetal of methyl 4,6-O-benzylidene-α-D-glucopyranoside.

An explanation that could be surmised is simply that the α- and β-anomers formed different dimers when treated with dibutyltin oxide and hence gave different regioisomers post-acylation.

These results are intriguing and reveal that, despite the astute observations made by Grindley,[70] it is difficult to predetermine the chemoselectivity of stannylene acetals, particularly with respect to novel carbohydrates.

Although regioselective benzoylation of both noviose and 4-epi-noviose was achieved, they were frustrating by not being in the form of the desired 2-protected derivatives as was hoped.

3.2. Direct protection, exploiting differential reactivities of hydroxyl groups

Conformational analysis of substituted cyclohexanes, which can exist in two different chair conformers, has shown that, in almost all cases, the conformer with the substituent axial is higher in energy than that with the substituent is equatorial. There are two reasons for this increased energy. The first is that the axial conformer suffers from unfavourable 1,3-diaxial interactions as illustrated in Figure 3.9.

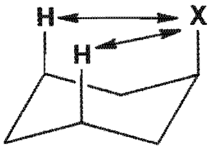
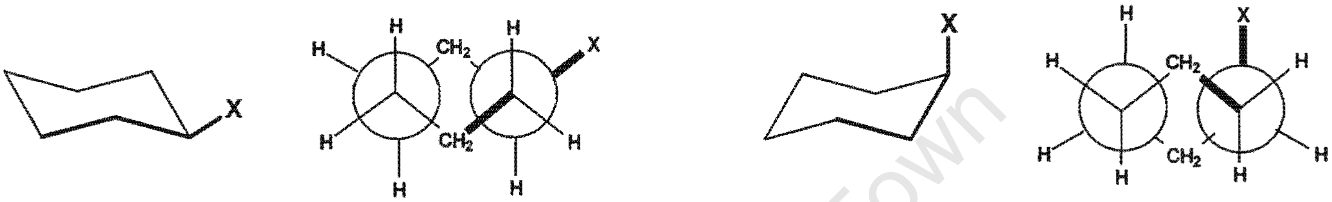


Figure 3.9: 1,3-Diaxial interactions (indicated by ←→) for the axial substituent of cyclohexane.

The second reason is that in the equatorial conformer the C-X bond is anti-periplanar to the two C-C bonds while for the axial conformer, the C-X bond is synclinal to two C-C bonds (Figure 3.10).



Equatorial substituted cyclohexane

axially substituted cyclohexane

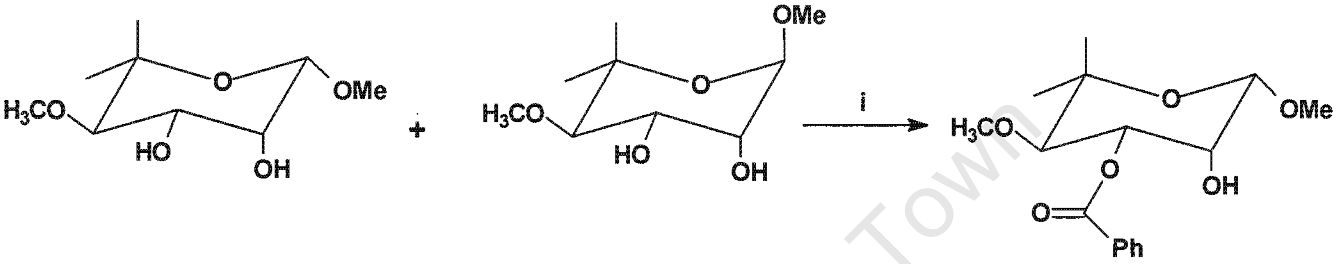
Figure 3.10: Illustration of the difference in bond alignment for equatorial substituted cyclohexane (black bonds) and anti-periplanar and axially substituted cyclohexane (black bonds are synclinal).

The introduction of an oxygen atom into a saturated six-membered ring does not alter its basic conformational characteristics since, with the exception of electronegative groups attached to the carbon atoms adjacent to the ring oxygen atom, substituents favour the equatorial orientation.

Functional group reactivity also depends significantly on conformation. As an example, axial hydroxyl groups of pyranoses are protected at a much lower rate than are their equatorial counterparts. Various factors have been reported to influence the nucleophilicity of individual OH groups of sugars,[79] including the axial or equatorial orientation of the OH groups, intramolecular H-bonds of equatorial OH groups to adjacent *cis*-OR substituents, the distance to the anomeric centre, and steric hindrance. This difference in reactivity was used by Musicki *et al.* to regioselectively protect the equatorial 3-OH of α-novioside **99** (Scheme 3.2)[20] and was used in this project as a means of regioselectively introducing the C-3 carbamoyl group onto noviose and its C-4 epimer.

3.2.1. Selective mono-acylation of noviose

Methyl 4-*O*-methyl-5,5-di-*C*-methyl-L-lyxopyranoside **7**, as a mixture of anomers (β:α ~ 3:1), was treated with BzCl, Et_3N and DMAP in CH_2Cl_2 at –20°C for 2 hours, followed by a standard aqueous work-up and column chromatography (Scheme 3.13). The major product, β-anomer **119**, was isolated in 77% yield. The NMR data and melting point (106–107°C) were identical to that of the product isolated from benzoylation of the stannylene acetal prepared from **7** (Scheme 3.12).



7β **3:1** **7α** **119**

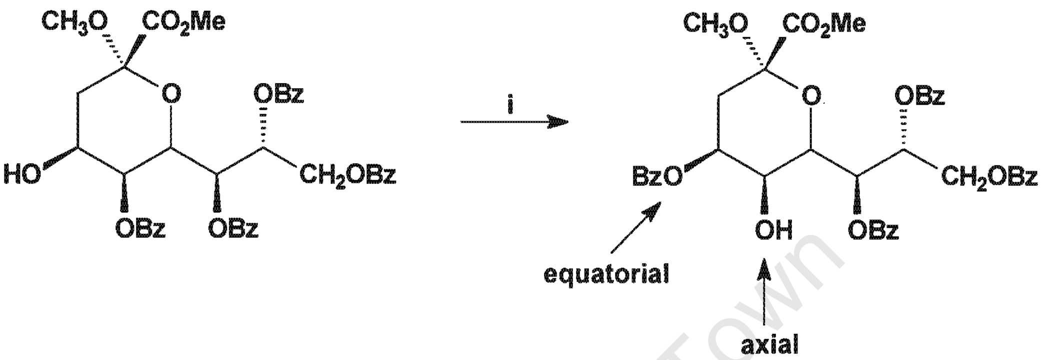
Scheme 3.13: *Reagents and conditions:* (i) BzCl, Et_3N, DMAP, CH_2Cl_2, -20°C, 77%.

This is consistent with the results of Musicki *et al.*, who regioselectively protected the C-3 equatorial hydroxyl group of α-novioside **99** as a triethylsilyl ether in 66% yield (see Scheme 3.2). These results confirm that the equatorial 3-OH is the more reative. With the emphasis in this study being on the total synthesis of a C-4 analogue of novenamine, the work on noviose itself was not taken further. However, this intermediate represented the successful synthesis of methyl novioside from D-ribose as well as the high-yielding regioselective protection of the equatorial C-3 hydroxyl group via either dialkylstannylenes (75% yield) or benzoyl chloride in base (77% yield).

3.2.2. Selective protection and subsequent carbamoylation of 4-epi-noviose

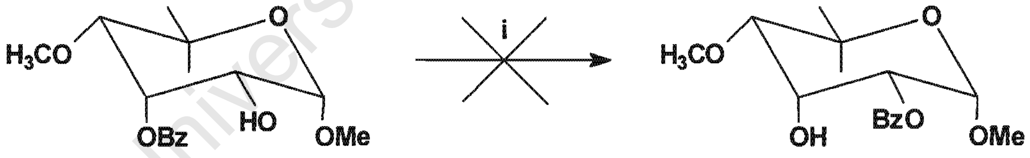
The findings on noviose reported above encouraged a similar study of 4-epi-noviose. Analysis of the conformational preference, as well as a crystal structure of a derivative (**90**), had suggested that the $^4C_1$ conformation was dominant, with the result that the 3-OH was axially-oriented and the 2-OH equatorially oriented. This difference in configuration was exploited in order to achieve regioselective protection.

We first considered the possibility of base-mediated migration of the 3-O-benzoyl product synthesized earlier via stannylene methodology. In the total synthesis of enantiomers of N-acetylneuraminic acid published in 1988, Danishefsky *et al.* used K_2CO_3 in CH_2Cl_2 to trigger benzoyl migration from an axial to an equatorial hydroxyl group (Scheme 3.14), as a thermodynamically driven process with an available migration mechanism.[80]



Scheme 3.14: *Reagents and conditions:* (i) K_2CO_3, CH_2Cl_2, room temperature, 90%.

In an attempt to force this migration, methyl 3-*O*-benzoyl-4-*O*-methyl-5,5-di-*C*-methyl-α-D-ribopyranoside **118** was mixed with finely ground and thoroughly dried K_2CO_3 in CH_2Cl_2 at room temperature overnight, followed by refluxing for a further 24 hours (Scheme 3.15).



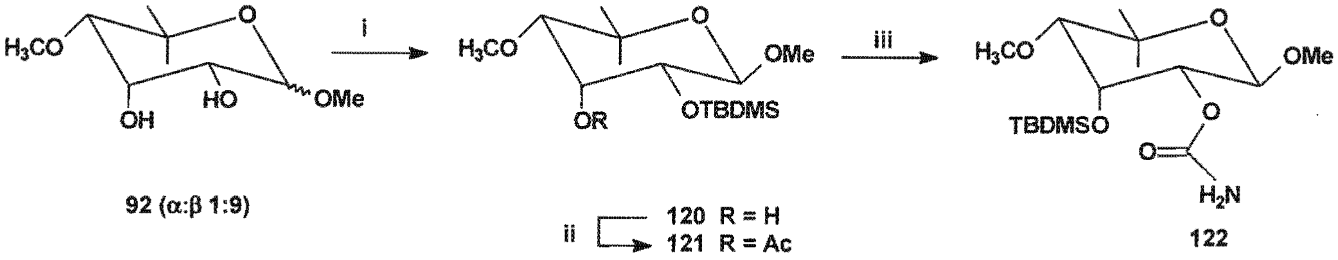
118

Scheme 3.15: *Reagents and conditions:* (i) K_2CO_3, CH_2Cl_2, room temperature – reflux.

Analysis of the reaction by tlc and ^1H NMR revealed this treatment to be unsuccessful, furnishing only starting material.

Attention was then turned to the possibility of selective mono-protection of the 2,3-diol, and protection as the *tert*-butyldimethylsilyl ether was first considered. It is a large bulky group, thus increasing the likelihood of chemoselectivity being achieved, and it is easily removed using mild conditions. Selective protection of secondary hydroxyl groups as TBDMS ethers has been extensively studied in the field of carbohydrates and often accomplished with

TBDMSCl and imidazole in DMF.[54,81,82] Accordingly, a mixture of diol **92** (1:9 mixture of α:β anomers), imidazole and TBDMSCl (one mole equivalent) in a minimum amount of DMF was stirred at room temperature overnight (Scheme 3.16) and the major product **120** was isolated by column chromatography in 79% yield.



Scheme 3.16: *Reagents and conditions:* (i) TBDMSCl, imidazole, DMF, room temperature, 79%. (ii) Ac_2O, Et_3N, DMAP, CH_2Cl_2, reflux, 50%. (iii) p-Nitrophenyl chloroformate, Et_3N, DMAP, CH_2Cl_2, reflux, 53%.

In the ^1H NMR spectrum of **120**, H-1, resonating at δ_H 4.49, showed a large coupling constant (*J* 7.5 Hz) indicating the β-anomer. This therefore, represents an 89% yield of mono-protected β-glycoside. Although the spectrum clearly showed the presence of a *t*-butyldimethylsilyl ether with singlets at δ_H 0.08 (6H, s) and δ_H 0.86 (9H, s), it was not clear from the data available which hydroxyl group (C-2 vs C-3) had been substituted. There was a slight upfield shift in H-2 (dd, *J* 3.3 and 7.5 Hz) from δ_H 3.52 in **92β** to δ_H 3.38 while H-3 (t, *J* 3.3 Hz) showed very little change in chemical shift. Interestingly, a literature survey failed to shed light on the effect a silyl ether has on the chemical shifts of the protons on a sugar ring. In the publications found regarding the monoprotection of a diol with a silyl protecting group, other means were used to determine which hydroxyl group had been silylated, the most common of these being the acetylation of the remaining hydroxl group.[80,81,83] The free hydroxyl of **120** was therefore acetylated to form fully protected **121** (Scheme 3.16). The ^1H NMR spectrum of **121** clearly showed a downfield shift for H-3 from δ_H 4.19 in **120** to δ_H 5.60, with little change observed for H-2. From these data it could be concluded that the C-3 hydroxyl group had been acetylated, and hence, that the equatorial C-2 hydroxyl group had been silylated.

This successful mono-silylation at O-2 prompted an immediate attempt to furnish the 3-carbamate, prepared by first reacting **120** with *p*-nitrophenyl chloroformate in CH_2Cl_2 in the presence of DMAP overnight at room temperature. No conversion to the carbonate was seen on tlc, so the mixture was refluxed for a further 12 hours, still with no effect. However, on addition of Et_3N and further refluxing, tlc showed that the starting material had been consumed. Following a standard aqueous work-up, the crude material was treated with ammonia in EtOH and the major product **122** isolated in 53% yield by column chromatography (Scheme 3.16). The ^1H NMR spectrum of **122** revealed the presence of a carbamoyl group as indicated by $-NH_2$ protons resonating as a broad singlet at δ_H 4.65, as well as by the appearance of a carbonyl carbon at δ_C 155.9 in the ^{13}C NMR spectrum. The signal for H-2 (dd, *J* 3.0 and 8.0 Hz) had shifted downfield from δ_H 3.38 in **120** to δ_H 4.45 while H-3 (t, *J* 3.0 Hz) appeared downfield from δ_H 4.19 in **120** to δ_H 4.48 in **122** (Table 3.2).

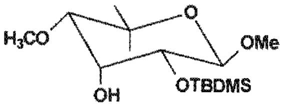
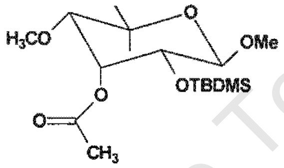
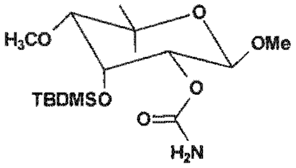
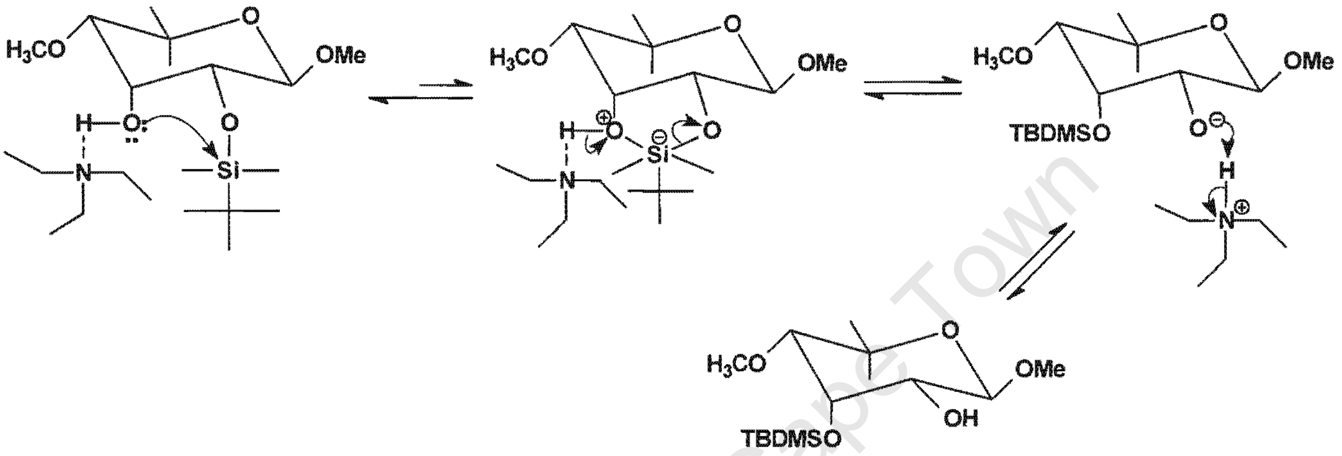
	 **120**	 **121**	 **122**
		Signal ($CDCl_3$, ppm)	
H-1	4.49	4.51	4.77
H-2	3.38	3.44	4.45
H-3	4.19	5.60	4.48
H-4	2.91	2.90	2.89

Table 3.2: A comparison of the ^1H NMR chemical shifts for **120**, **121** and **122**.

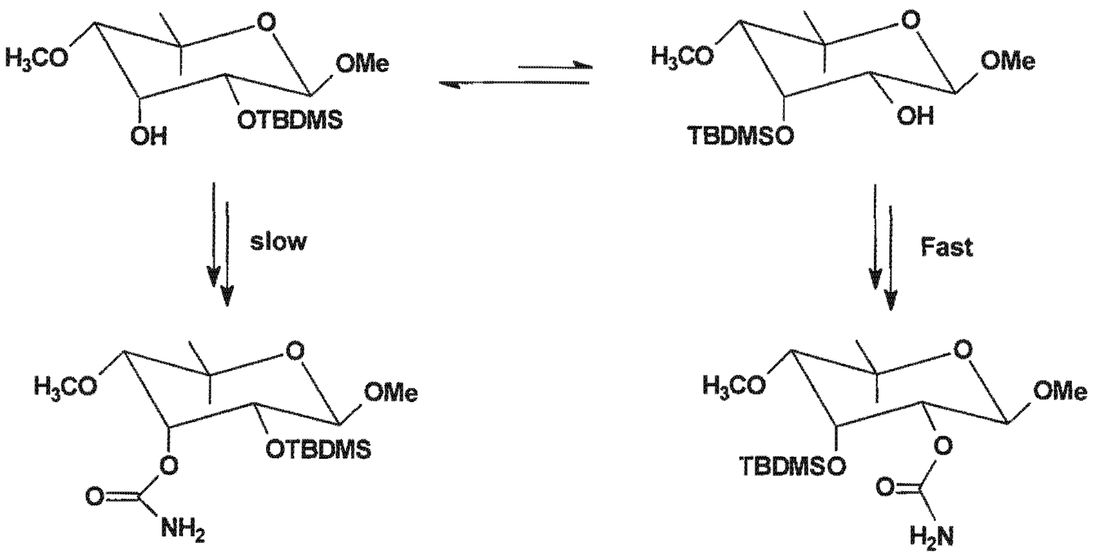
A comparison of the ^1H NMR spectrum of **122** to that of 3-O-acetate **121** is summarised in Table 3.2 and revealed that if carbamoylation of **120** had occurred on C-3 as expected, there would most likely have been a large downfield shift observed for H-3 as was seen for H-3 in **121**. However, a large downfield shift was observed for H-2 and not for H-3 in **122**.

This evidence led to the conclusion that the silyl group had migrated from the equatorial O-2 to the axial C-3 hydroxyl group during the reaction of **120** with *p*-nitrophenyl chloroformate and Et_3N, ultimately resulting in C-2 hydroxyl carbamoylation. It is known that TBDMS groups can migrate between suitably disposed hydroxyl groups under basic conditions.[81] Halmos and co-workers have described the migration of a TBDMS group in partially protected derivatives of methyl α- and β-aldohexopyranosides promoted by triethylamine in dipolar solvents,[82] while Arias-Pérez and Santos observed that, depending on the nature of the base and the reaction conditions, the migration of a TBDPS group from the equatorial O-3 to the axial O-2 of methyl α-D-mannopyranoside could be achieved.[83] Migration has been shown to proceed intramolecularly through a pentacoordinate silicon,[84] the base-catalyzed mechanism of which is depicted in Scheme 3.17, involving Et_3N interaction with the 3-OH proton, resulting in an increase in oxygen nucleophilicity.



Scheme 3.17: Mechanism for silyl migration.

The oxygen atom, through its lone pair of electrons, attacks silicon forming an intermediate five-membered ring. This reversible reaction is slow, with the equilibrium lying to the left. The ring then reopens, silicon is transferred from O-2 to O-3, and O-2 is protonated from the proton acquired by Et_3N. Silyl migration is a reversible reaction, resulting in thermodynamically equilibrated mixtures of the C-2 and C-3 regiosisomers. *p*-nitrophenyl chloroformate is a sterically demanding electrophile and so reacts with the axial C-3 hydroxyl group at a much lower rate than it would with the equatorial C-2 hydroxyl group (Scheme 3.18). Furthermore, the axial hydroxyl group (C-3) is *cis* to two vicinal groups, whereas the equatorial hydroxyl (C-2) is *cis* to only one. Thus, the observed product is produced via the kinetic product of an intermediate involved in a thermodynamic equilibrium.

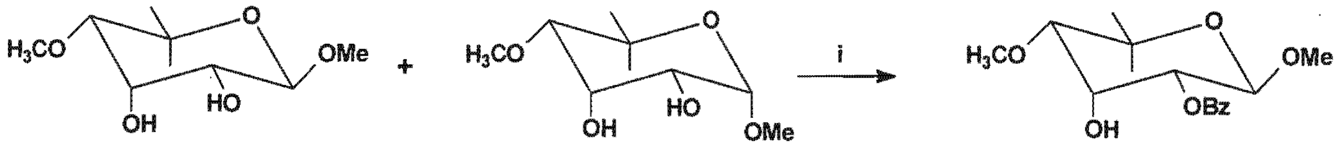


Scheme 3.18: Relative rates of silyl migration and subsequent carbamoylation.

Although protection with a TBDMS ether was regioselective and high-yielding, migration of the silyl ether from O-2 to O-3 was a complicating factor so attention was turned to benzoylation as a possible alternative. Regioselective *O*-benzoylation of monosaccharides has been the focus of many literature publications. Numerous nucleophilic catalysts have been used in order to increase the rate and chemoselectivity of the reaction, including DMAP (4-(dimethylamino)pyridine),[85] phosphines,[86] DMAP derivatives,[87] and TMEDA (N,N,N',N'-tetramethylethylenediamine).[88] Regioselective protection has also been achieved using lipase enzymes,[89] or by forming a copper or mercury chelate of the diol before introduction of the acylating agent.[90]

Once formed, benzoate esters are less easily hydrolyzed than acetate esters but their propensity for migration is not nearly as strong as either acetates or *t*-butyldimethylsilyl ethers.

Thus, diol **92**, as a 1:9 mixture of α:β anomers, was benzoylated using standard conditions of BzCl, pyridine, a catalytic amount of DMAP and co-solvent CH_2Cl_2 at 0°C (Scheme 3.19). After a standard aqueous work-up, monobenzoylated **123** was isolated as the major product in 83% yield, again representing very high conversion (90%) of the β-glycoside.



92β 9:1 **92α** **123**

Scheme 3.19: *Reagents and conditions:* (i) BzCl, pyridine, DMAP, CH_2Cl_2, 0°C, 83%.

The ^{1}H NMR spectrum of **123**, shown in Figure 3.11, revealed that H-2 (dd, *J* 3.3 and 7.5 Hz) had shifted downfield from δ_H 3.52 in **92β** to δ_H 4.92 and H-3 (t, *J* 3.3 Hz) had shifted from δ_H 4.23 in **92β** to δ_H 4.92, indicating benzoylation at O-2. A large coupling for H-1 (d, J 7.5 Hz) confirmed that **123** was the β-anomer as expected and the presence of additional signals in the aromatic region supported the presence of the benzoate ester. Further evidence for the presence of the benzoate ester was given in the ^{13}C NMR spectrum, with a carbonyl carbon observed at δ_C 165.7, and a carbonyl stretch in the IR spectrum at 1718 cm^{-1}.

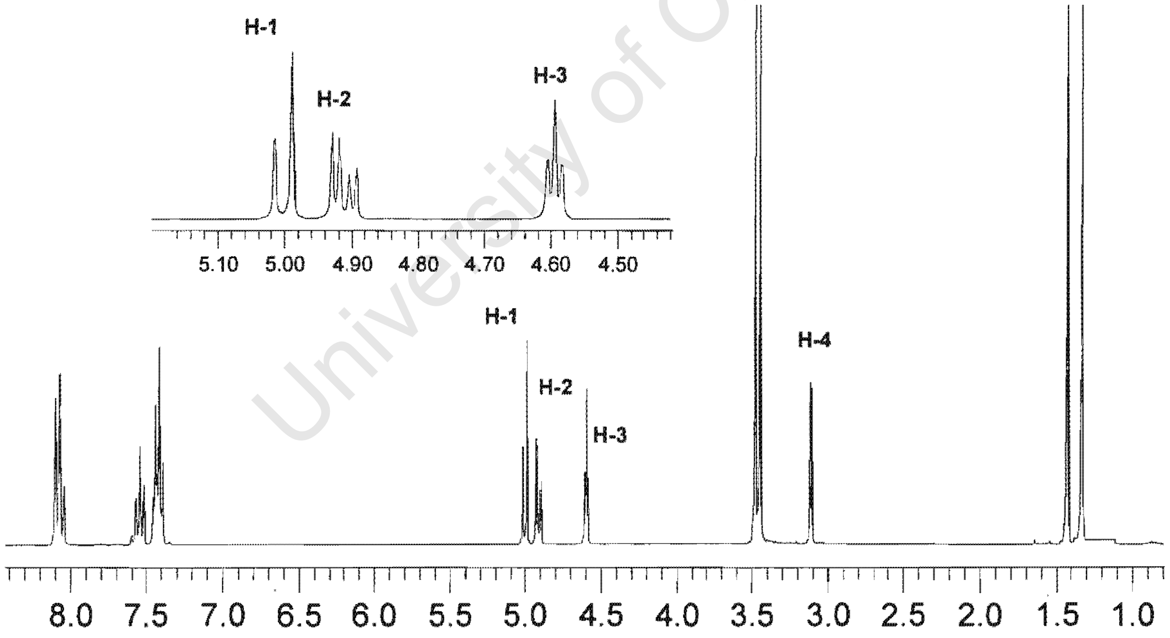
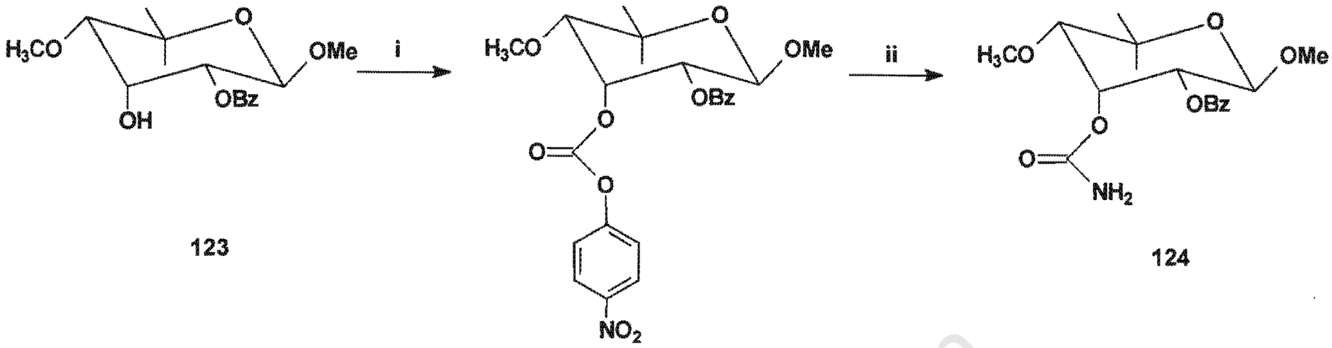


Figure 3.11: ^{1}H NMR of methyl 2-*O*-benzoyl-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranoside (**123**).

Once again, the selectively protected glycoside was immediately processed towards the 3-carbamate. The *p*-nitrophenyl carbonate was introduced by treating monobenzoylated **123** with *p*-nitrophenyl chloroformate, excess pyridine and DMAP in CH_2Cl_2 for 12 hours at room temperature. In contrast to the reaction with the mono-silylated derivative, the reaction was complete after this time and, following a standard aqueous work-up, the crude material was dissolved in ethanol and added to ethanol saturated with ammonia gas (Scheme 3.20).

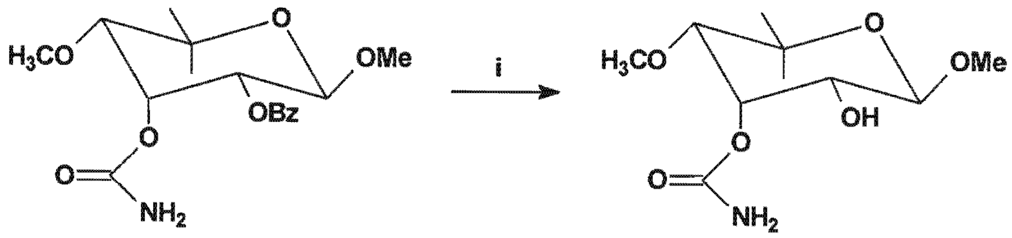


Scheme 3.20: *Reagents and conditions:* (i) *p*-nitrophenyl chloroformate, pyridine, DMAP, CH_2Cl_2, room temperature. (ii) EtOH, NH_3 gas, room temperature, 73% over 2 steps.

Ammonolysis proceeded rapidly with the reaction being complete within 10 minutes (monitored by tlc). The work-up simply involved the evaporation of the solvent *in vacuo* followed by column chromatography, and the major product **124** was isolated in 73% yield for the two steps.

The carbamoyl amino protons were observed in the ^{1}H NMR spectrum of **124** resonating as a singlet at δ_H 4.70 and the anomeric centre was confirmed as the expected β-anomer because of the large coupling observed for H-1 (d, J 8.0 Hz) resonating at δ_H 4.90. H-3 (t, *J* 3.3 Hz) experienced a large shift downfield from δ_H 4.59 in **123** to δ_H 5.74, while H-2 (dd, J 3.3 and 8.0 Hz) had shifted only slightly downfield from δ_H 4.92 in **123** to δ_H 5.00. Since the H-2 and H-3 shifts were very similiar, the data from the ^{1}H NMR were not conclusive evidence that the benzoate ester had not migrated from C-2 to C-3, so further evidence was required.

A small quantity of **124** was treated with sodium methoxide in methanol to furnish the 2-O-debenzoylated product **124a** (Scheme 3.21). The ^{1}H NMR spectrum of **124a** revealed a large upfield shift for H-2 from δ_H 5.00 to δ_H 4.18 and only a small upfield shift for H-3 from δ_H 5.74 to δ_H 5.29. This provided plausible evidence that carbamoylation of **123** had indeed occurred at C-3, with no migration of the C-2 benzoate observed.

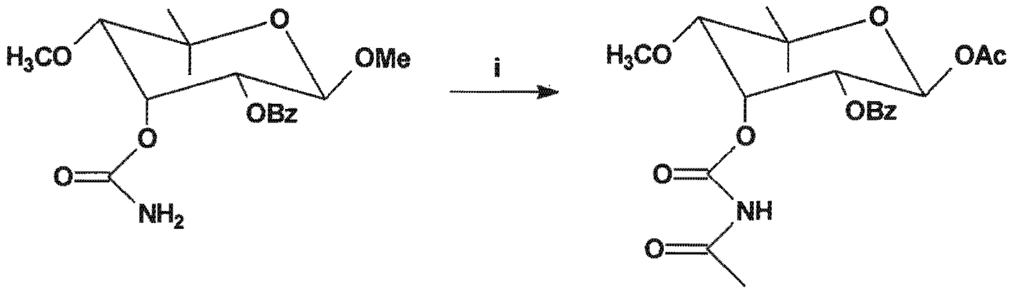


124 **124a**

Scheme 3.21: *Reagents and conditions:* (i) NaOMe, MeOH, room temperature, 26%.

3.2.3. Further functional group modifications of protected 4-epi-noviose

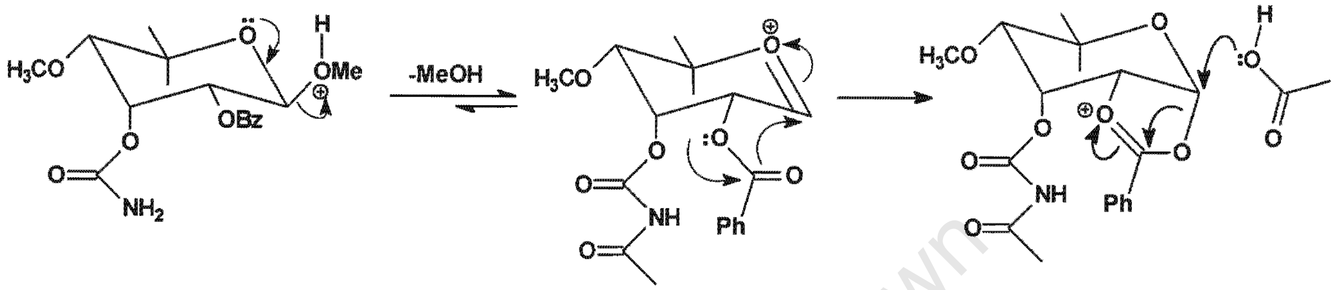
In preparation for glycosidation, the strategy involved conversion of methyl glycoside **124** to its anomeric acetate, followed by base-cleavage of both the anomeric acetate and 2-O-benzoate groups. It was decided to follow this two step procedure as direct hydrolysis of the methyl glycoside required harsher conditions than those required for cleavage of an ester group. Thus, conversion of **124** to its anomeric acetate was carried out with the standard method of Ac_2O in the presence of sulphuric and acetic acids at 0°C to afford **125** in 85% yield (Scheme 3.22).[91] Its ^1H NMR spectrum revealed the presence of two new acetyl methyl signals resonating characteristically at δ_H 2.04 and δ_H 2.20 indicating that N-acetylation of the carbamoyl group had also taken place. This was further corroborated by the presence of only one nitrogen proton (imide) resonating downfield at δ_H 8.34. Similarly, three carbonyl stretching bands were observed on the IR spectrum, as well as three carbonyl carbon signals in the ^{13}C NMR spectrum of **125**, providing satisfactory evidence for the acetylation of the anomeric centre as well as N-acetylation of the carbamoyl group.



124 **125**

Scheme 3.22: *Reagents and conditions:* (i) Ac_2O, H_2SO_4, AcOH, 0°C, 85%.

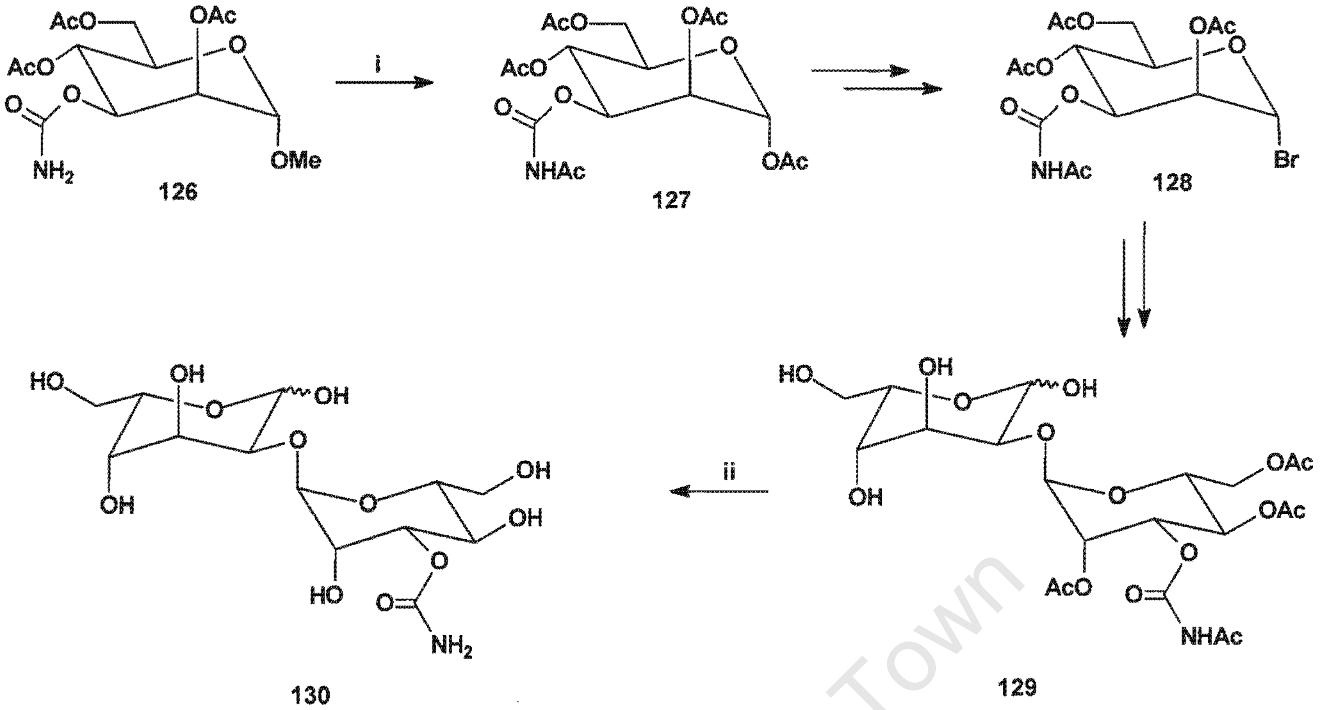
The mechanism for this stereoselective reaction is depicted in Scheme 3.23. The anomeric methoxy group is protonated and methanol is released via the formation of an oxocarbenium cation. Nucleophilic addition with acetic acid takes place on the β-face as a result of neighbouring group participation from the C-2 benzoate ester. Methanol reacts with acetic anhydride to form methyl acetate and acetic acid, thus pushing the equilibrium to the right.



Scheme 3.23: Mechanism for the acetylation of **124**.

Spectroscopic analysis of **125** using ^{1}H NMR spectroscopy revealed that H-2 appeared downfield at δ_H 5.11 from δ_H 5.00 in **124**, while H-3 showed a similar downfield shift from δ_H 5.74 in **124** to δ_H 5.85.

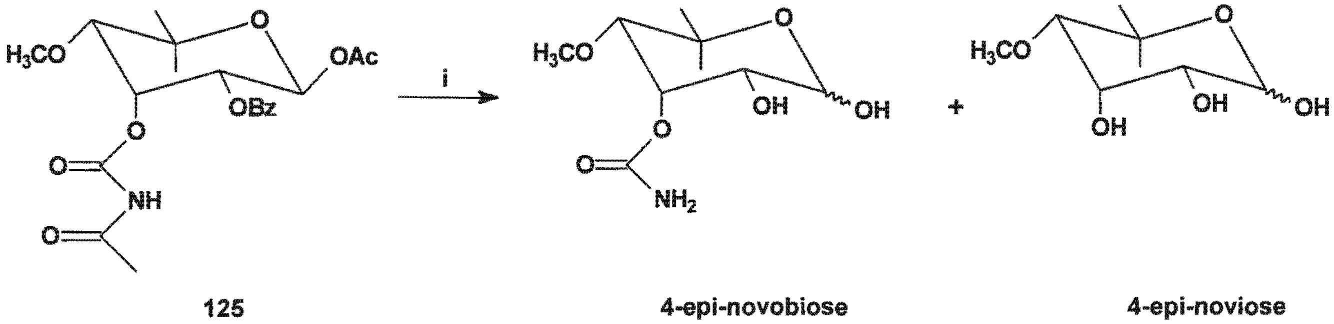
The final step in the synthesis of 4-epi-novobiose was the deprotection of the 1-O, 2-O and N-acetyl groups. In 1986, Hecht and co-workers published a synthesis of 2-O-(3-O-carbamoyl-α-D-mannopyranosyl)-L-gulose, the carbohydrate moiety of Bleomycin.[92] In this synthesis, they also found that the acid-catalysed conversion of methyl 2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranoside **126**, to the anomeric acetate **127**, resulted in N-acetylation of the carbamoyl group (Scheme 3.24). This followed several attempts to convert the methyl glycoside to a suitably reactive glycosyl donor without concomitant N-acylation. The use of hydrochloric or hydrobromic acids, $SOCl_2$, $ZnBr_2/Cl_2CHOCH_3$, or Lewis acids ($SnCl_4$, $TiCl_4$, $AlCl_3$) provided either complex reaction mixtures or modification of the carbamoyl unit. Treatment with trifluoroacetic anhydride in the presence of either sulfuric acid or trifluoromethanesulfonic acid resulted in extensive decomposition, while trifluoroacetic acid gave only N-acylation even at elevated temperatures.



Scheme 3.24: *Reagents and conditions:* (i) Ac_2O, concentrated H_2SO_4, quant. (ii) $NaOCH_3/CD_3OD$, 96%.

Compound **127** was converted to mannosyl bromide **128**, which was transformed to the coupled product **129**. Various methods were then employed in an attempt to N-deacetylate **129**. Treatment of **129** with alumina in benzene found to effect N-deacetylation in a related case[93] resulted in significant material loss but little detectable N-deacetylation. Treatment with $NaOCH_3/CH_3OH$ gave a mixture of **130** and the disaccharide lacking the carbamoyl moiety. However, when **129** was treated with catalytic sodium methoxide in CD_3OD and monitored by NMR, O- and N-deacetylation was achieved within 25 minutes to afford **130** in 96% yield. By closely monitoring this reaction by NMR, the deacetylated product could be isolated before the carbamoyl group was lost from the molecule.

In this project, the need to remove both the O- and N-acetyl groups necessitated the use of sodium methoxide, so **125** was treated with catalytic sodium methoxide (0.2 mol equivalents) in methanol at room temperature, and the reaction was carefully monitored by tlc (Scheme 3.25).



Scheme 3.25: *Reagents and conditions:* (i) $NaOCH_3$ (0.3 equiv.), MeOH, room temperature.

However, despite close monitoring of the reaction, a complex mixture of polar products was isolated from the reaction, which were inseparable by column chromatography. Presumably, based on the report by Katano *et al.*,[92] the mixture consisted of four products, namely the α- and β-anomers of 4-epi-novobiose as well as the α- and β-anomers of 4-epi-noviose (Scheme 3.25). However, this could not be confirmed by either the ^1H or ^{13}C NMR spectra and would require further investigation.

Literature reports have indicated that N-acetyl-carbamoyl groups are relatively labile.[94] There are two carbonyl groups in an N-acetylated carbamoyl group and as depicted by the resonance structures in Figure 3.12, N-acylation of the carbamoyl group of **i** to **ii**, makes the carbamate carbonyl group more electrophilic than that in **i**, thus making it more susceptible to attack by base.

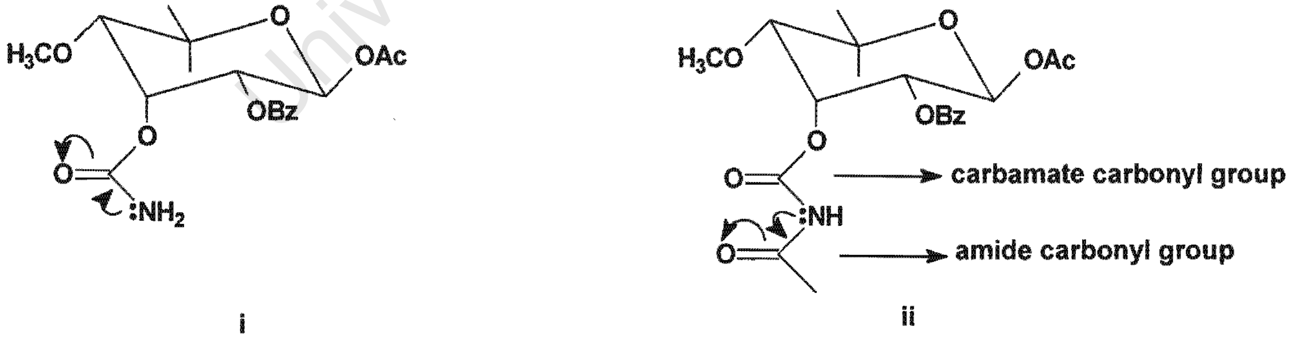


Figure 3.12: A comparison of the resonance structures of (i) an N-acetylated carbamoyl group, and (ii) a carbamoyl group.

Benzoate esters are less susceptible to base than acetate esters and hence require either harsher conditions or longer times to achieve cleavage. As a result of this, the time required for cleavage of the C-2 benzoate ester presumably also resulted in cleavage of the N-acetylated carbamoyl group.

3.2.4. Conclusion

The results of the observed selectivities of the various protecting groups using both dialkylstannylene acetal and direct protection methodologies are summarised in Table 3.3.

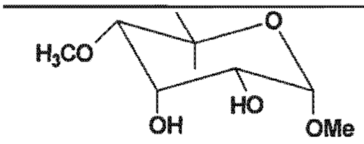
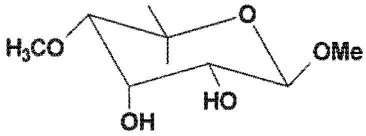
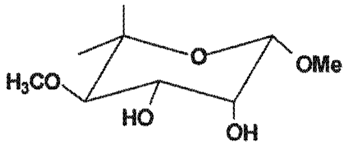
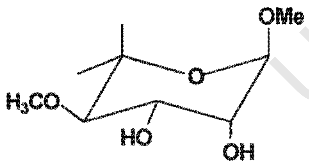
Compounds	Stannylene methodology		Direct protection	
	Position of substitution and observed yield			
	Acetylation	Benzoylation	TBDMS	Benzoylation
 92α	O-3 (axial) 90%	O-3 (axial) 81%	-	-
 92β	O-2 (eq.) 90%	-	O-2 (eq.) 89%	O-2 (eq.) 90%
 7β	-	O-3 (eq.)	O-3 (eq.)[a]	O-3 (eq.) 97%
 7α	-	-	-	-

Table 3.3: Observed selectivities of various protecting groups using both stannylene methodology and direct protection. [a] Results not shown.

As can be seen from these results, the use of stannylene methodology led to the α-anomer of methyl 4-epi-novioside (**92α**) being selectively acylated at the axial O-3, while the β-anomer (**92β**) was selectively protected at O-2. Using direct methodology, selective protection of the

C-2 hydroxyl group of the β-anomer of methyl 4-epi-novioside (**92β**) was achieved with both a TBDMS ether and benzoyl ester in high yield. Selective acylation of the β-anomer of methyl novioside (**7β**) was achieved at O-3 with both methodologies. In order to complete these results, the anomeric mixtures of both methyl novioside and its C-4 epimer would need to be separated and then subjected to the above mentioned protection methodologies.

Carbamoylation of TBDMS-protected methyl 4-epi-novioside led to silyl migration from the C-2 to the C-3 hydroxyl group, with carbamoylation resulting on the less hindered equatorial C-2 hydroxyl group. This complication was not observed for the benzoylated sugar, with carbamoylation occuring on the C-3 hydroxyl group as predicted. However, complications were experienced when the anomeric methoxy group of **124** was converted to the corresponding acetate group. The electrophilic acylating conditions of the reaction led to N-acetylation of the carbamoyl group as well as anomeric O-acetylation. As seen in Figure 3.11, the carbamate carbonyl group of **125** is more electrophilic than its non-acetylated form and hence is more susceptible to base-catalysed cleavage. Thus, treatment of **125** with catalytic sodium methoxide presumably resulted in loss of the N-acetylated carbamoyl group as well as the C-2 benzoate and anomeric acetate groups.

From these latter results, it was clear that the strategy employed for the introduction of the carbamoyl group to C-3 would require some minor modifications. Suggestions for these modifications are listed below:

1. The conditions employed for the conversion of the anomeric methoxy group of **124** to its corresponding acetate ester included acetic anhydride, acetic acid and sulphuric acid. The mechanism for this reaction suggests that acetic acid is the acylating agent, while acetic anhydride removes methanol produced during the reaction as well as N-acetylating the carbamoyl group. A modification to the synthesis possibly could be to find conditions to achieve acetylation of the anomeric methoxy group without concomitant N-acylation. Examples could possibly include acetic acid/$BF_3 \cdot Et_2O$ or acetic acid/$Cu(OAc)_2$.[91]

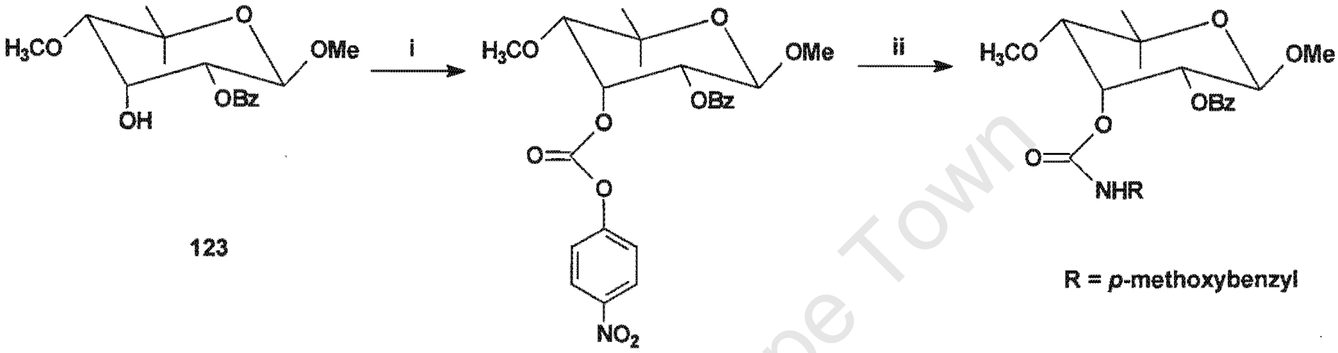
2. Carbamoylation of the C-2 benzoylated sugar **123** was achieved by converting the C-3 hydroxyl group to its *p*-nitrophenyl carbonate followed by ammonolysis with NH_3. At this step, the use of a protected amine could be used in place of ammonia gas (Scheme 3.26). An example of such an amine is *p*-methoxybenzylamine which is commercially available and the *p*-methoxybenzyl group is easily cleaved by hydrogenolysis or with ceric ammonium nitrate in

aqueous acetonitrile. The advantages of using an N-benzylated amine are envisaged to include:

(i) the N-benzylated amino group would not be susceptible to N-acetylation when the anomeric methoxy group is converted to its acetate ester;

(ii) N-benzylation would not enhance carbamate electrophilicity as much as N-acylation;

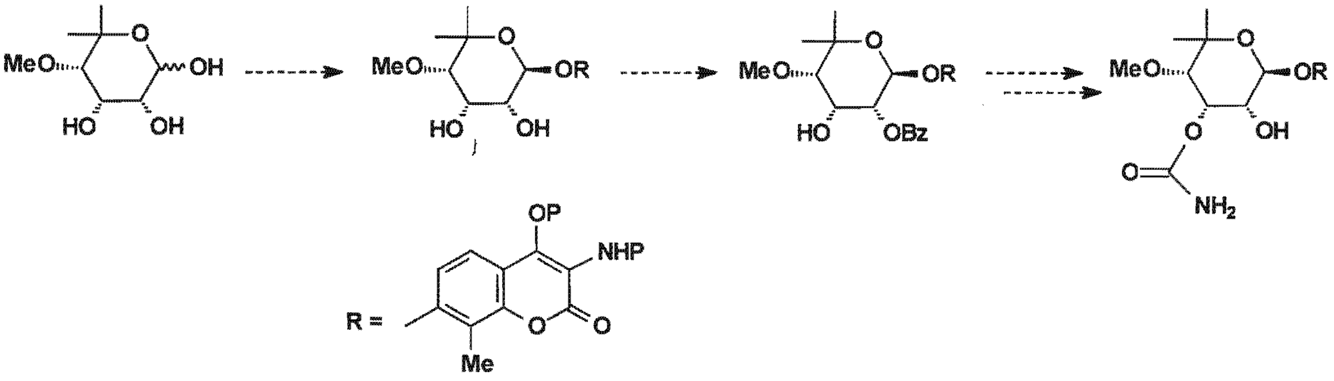
(iii) the protecting group could simply be cleaved before or after glycosidic coupling.



123

Scheme 3.26: *Reagents and conditions:* (i) *p*-nitrophenyl chloroformate, pyridine, DMAP, CH_2Cl_2, room temperature. (ii) NH_2R, e.g. R = *p*-methoxybenzyl.

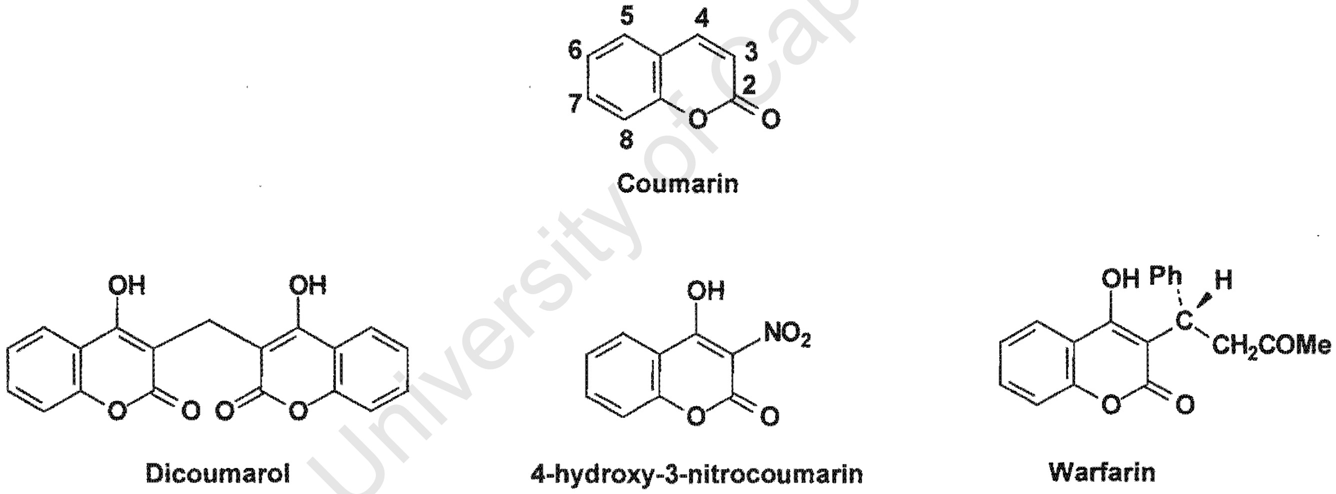
3. In both syntheses of novobiocin discussed in Section 1.5, the carbamoyl group was introduced after the sugar molecule had been coupled to the coumarin component. Although it was anticipated in this project to introduce the carbamoyl group prior to glycosidic coupling, perhaps the better strategy would be to introduce the carbamoyl group after coupling, while still employing the regioselective introduction of the C-3 carbamoyl group via protection of the C-2 hydroxyl group as the benzoate ester, which was achieved in high yield (Scheme 3.27). However, it would have to be ascertained experimentally as to whether conditions could be found to hydrolyse the C-2 benzoate ester without subsequent loss of the carbamoyl group.



Scheme 3.27: Introduction of the carbamoyl group post-glycosidation via regioselective protection of the C-2 hydroxyl group as the benzoate ester.

Chapter 4 : Synthesis of the coumarin unit

Natural coumarin (Figure 4.1) was first isolated by Vogel in 1820 from the tonka bean,[95] and its name originates from the Caribbean word '*coumarou*' for the tonka tree, which was known botanically as *Coumarouna odorata.* Since then, hundreds of natural coumarins have been isolated and their structures elucidated. Interest in naturally occurring 4-hydroxycoumarins has been limited to three key compounds, namely 3,3'-methylenebis(4-hydroxycoumarin) commonly known as Dicoumarol (Figure 4.1), and the coumarin antibiotics novobiocin and coumermycin. Dicoumarol was first isolated in 1924 from mouldy sweet clover (*Melilotus* spp.) hay and is best known for its anticoagulant effect on blood. Aside from the naturally occurring compounds, numerous 4-hydroxycoumarin analogues have been synthesized, including 4-hydroxy-3-nitrocoumarin (Figure 4.1), which shows strong antiallergic activity as well as Warfarin (Figure 4.1), which is a recognized anti-coagulant drug.



Coumarin

Dicoumarol

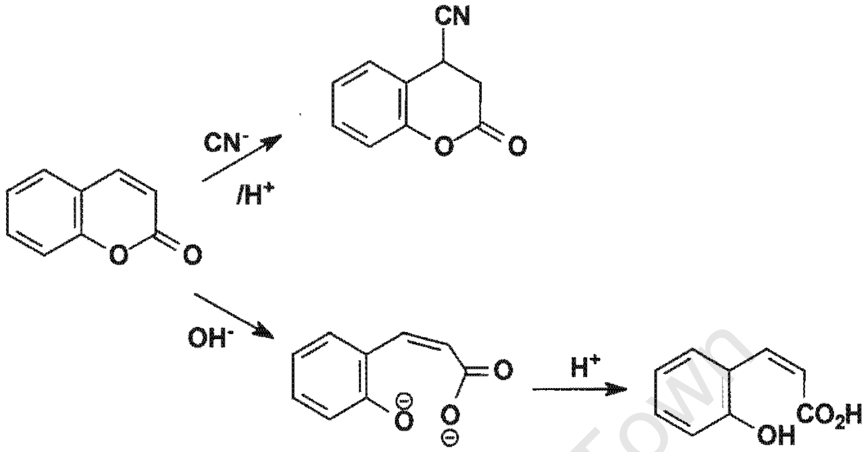
4-hydroxy-3-nitrocoumarin

Warfarin

Figure 4.1

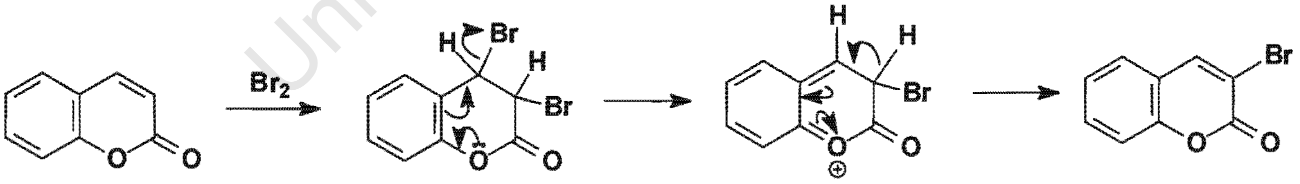
A number of methods are available for the synthesis of 4-hydroxycoumarins. These include treating a phenol with an equimolar amount of malonic acid in the presence of excess anhydrous zinc chloride and phosphoryl chloride at 60-75°C;[96] Hoesch condensation of phenols with ethyl cyanoacetate followed by acid hydrolysis;[97] condensation of *o*-hydroxyacetophenones with diethyl carbonate and sodium;[27] and an internal Claisen condensation of methyl *o*-acetoxybenzoate with sodium in liquid paraffin at 240°C.[98]

Many of the reactions of coumarins are analogous to those of monocyclic 2*H*-pyran-2-one (α-pyrone). Thus, coumarin is attacked by nucleophiles at C-4 and C-2. Attack at C-4 by 'soft' nucleophiles such as CN$^{\ominus}$ leads to the formation of addition products, while attack at C-2 causes ring-opening (Scheme 4.1).



Scheme 4.1: Nucleophilic attack on coumarin.

By comparison, electrophilic methodologies such as nitration and sulphonation take place in the benzene ring, at C-6. Coumarin can also be substituted by electrophiles at the 3-position, the latter being activated by the ring oxygen atom. An example is bromination which proceeds via an addition – elimination mechanism, via addition of bromine across the 3,4-bond followed by the loss of hydrogen bromide (Scheme 4.2).



Scheme 4.2: Mechanism for bromination of coumarin.

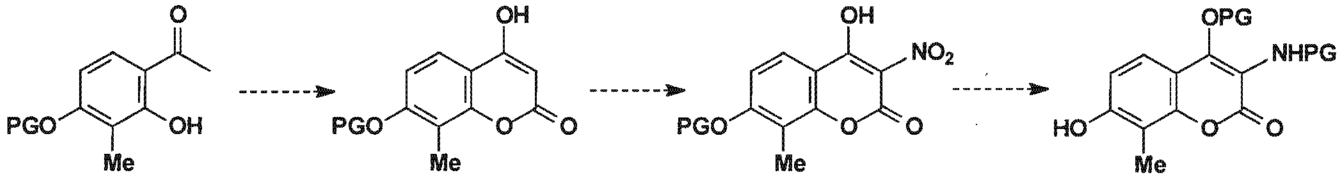
All these chemical properties provide evidence that the heterocyclic ring system is that of an unsaturated lactone rather than aromatic in nature.

The strategy for the synthesis of the coumarin component is summarised in Scheme 4.3 and involves:

i. Formation of the 4-hydroxycoumarin unit;

ii. Selective introduction of the amino group at C-3;

iii. Developing an appropriate protecting group strategy to allow for glycosylation at O-7.

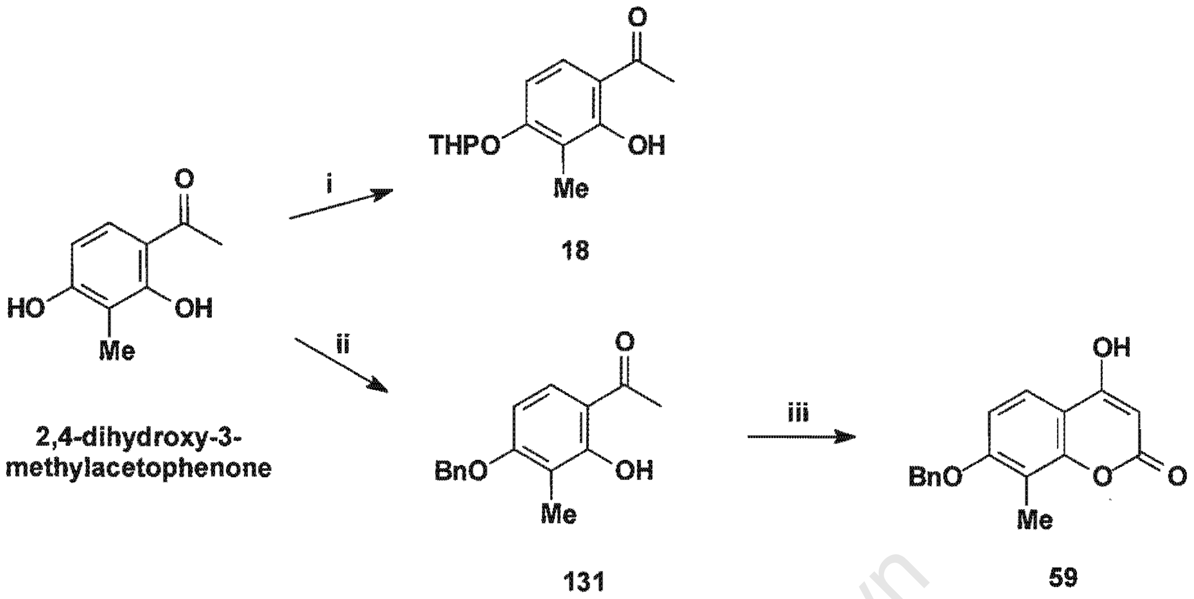


PG = protecting group

Scheme 4.3: Strategy for the synthesis of the coumarin component.

The commercial availability of 2,4-dihydroxy-3-methylacetophenone has resulted in the application of the Barker methodology[27] involving diethyl carbonate and sodium for the synthesis of 7,8-disubstituted-4-hydroxycoumarin in which the 4-hydroxyl group is first selectively protected. The 2-hydroxyl group of 2,4-dihydroxy-3-methylacetophenone is both hydrogen-bonded to and hindered by the adjacent acetyl group, so the 4-hydroxyl group tends to be regioselectively protected. In 1999, Laurin *et al.*[16] achieved regioselective 4-protection in 83% yield with a THP protecting group. However, attempts in this work to repeat this reaction gave the THP acetal **18** (see Scheme 1.6, page 13) in only 68% yield (Scheme 4.4).[**] The reaction was very sluggish, failed to go to completion even after 3 days, and isolation of the product was complicated by the formation of the 2-O-protected isomer. This disappointing result led to an alternative protecting group to be sought.

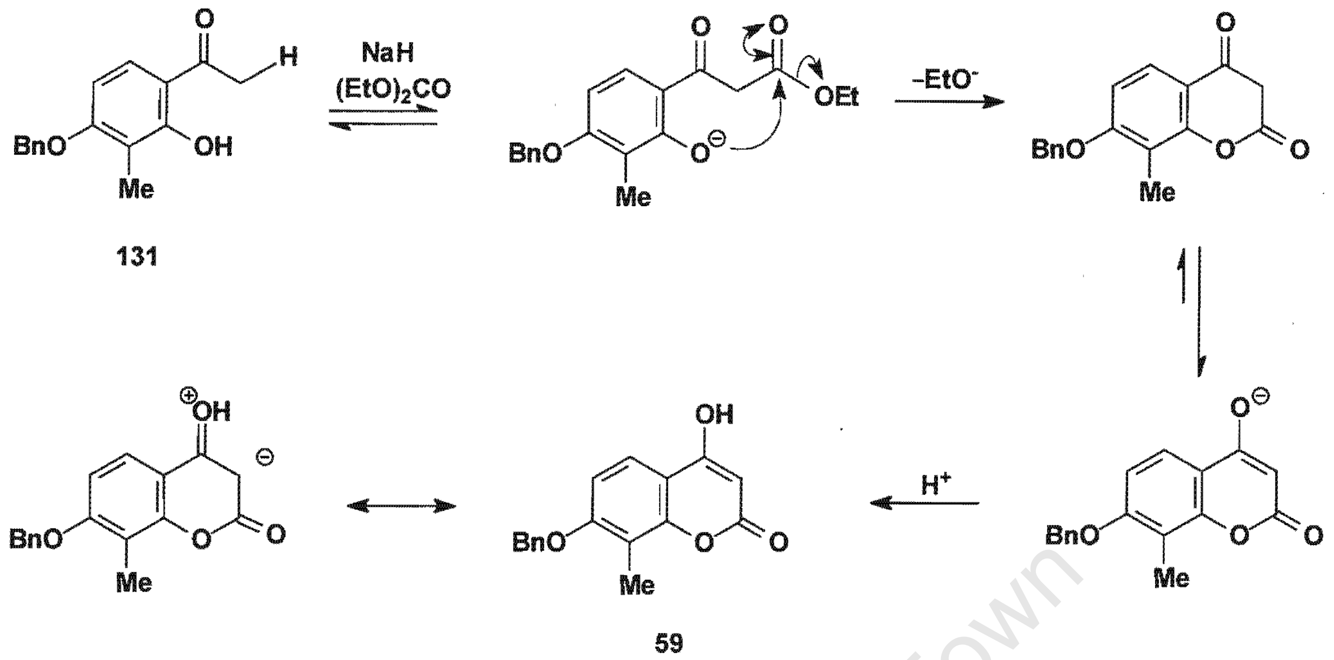
[**] This was the highest recorded yield. The yields were typically 50-60%.



Scheme 4.4: *Reagents and conditions*: (i) DHP, *p*-TsOH, Et_2O, room temperature, 68%. (ii) BnCl, K_2CO_3, KI, acetone, reflux, 88%. (iii) NaH, $CO(OEt)_2$, toluene, reflux, 76%.

In 1988, Raphael *et al.* selectively benzylated the 4-hydroxyl group of 2,4-dihydroxy-3-methylacetophenone in 81% yield with BnCl, K_2CO_3 and KI in acetone.[99] Application of this method in this project gave the 4-O-benzyl ether **131** in 88% yield (Scheme 4.4). Isolation of the product simply involved filtration, evaporation of the solvent *in vacuo* followed by recrystallization of the residue. The ^1H NMR spectrum and the melting point corresponded to that published.[99]

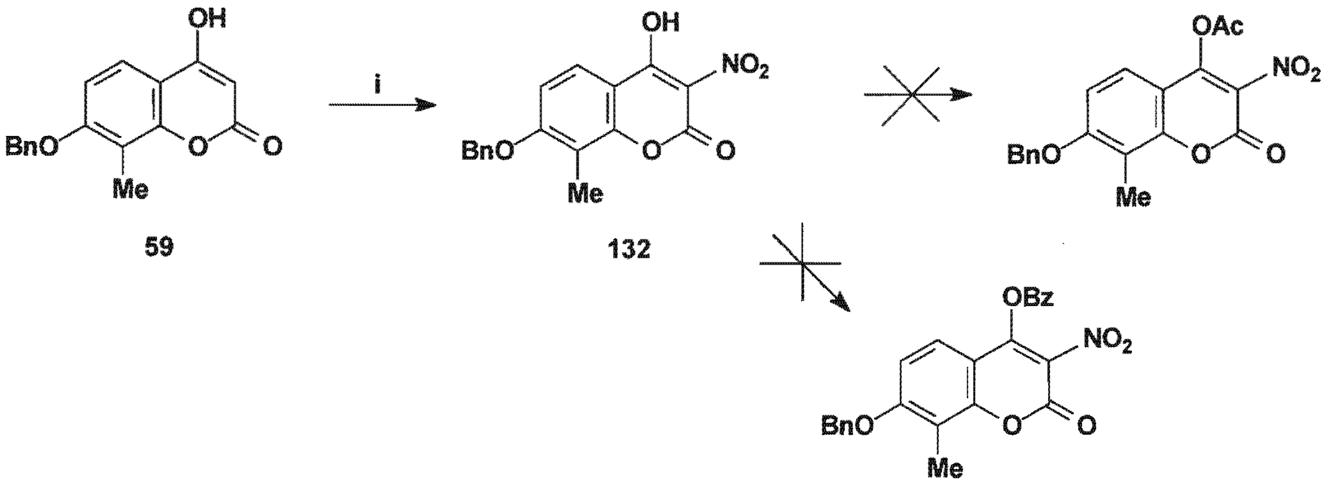
Following the method descibed by Barker *et al.*,[27] acetophenone **131** was converted directly into the 4-hydroxycoumarin **59** (see Scheme 1.15, page 23), in 76% isolated yield, with diethyl carbonate and two equivalents of NaH in refluxing toluene (Scheme 4.4). Work-up of this reaction involved quenching with water and extracting with ethyl acetate to remove starting material and by-products. The aqueous phase was then acidified with 1 M HCl and the coumarin extracted with ethyl acetate and recrystallized. The mechanism is depicted in Scheme 4.5 and involves C-acylation of the acetophenone **131** with diethyl carbonate followed by ring closure to yield a β-ketolactone which forms a stable enolate under the basic conditions of the reaction. An acidic work-up furnishes coumarin **59**.



Scheme 4.5: Mechanism for synthesis of **59**.

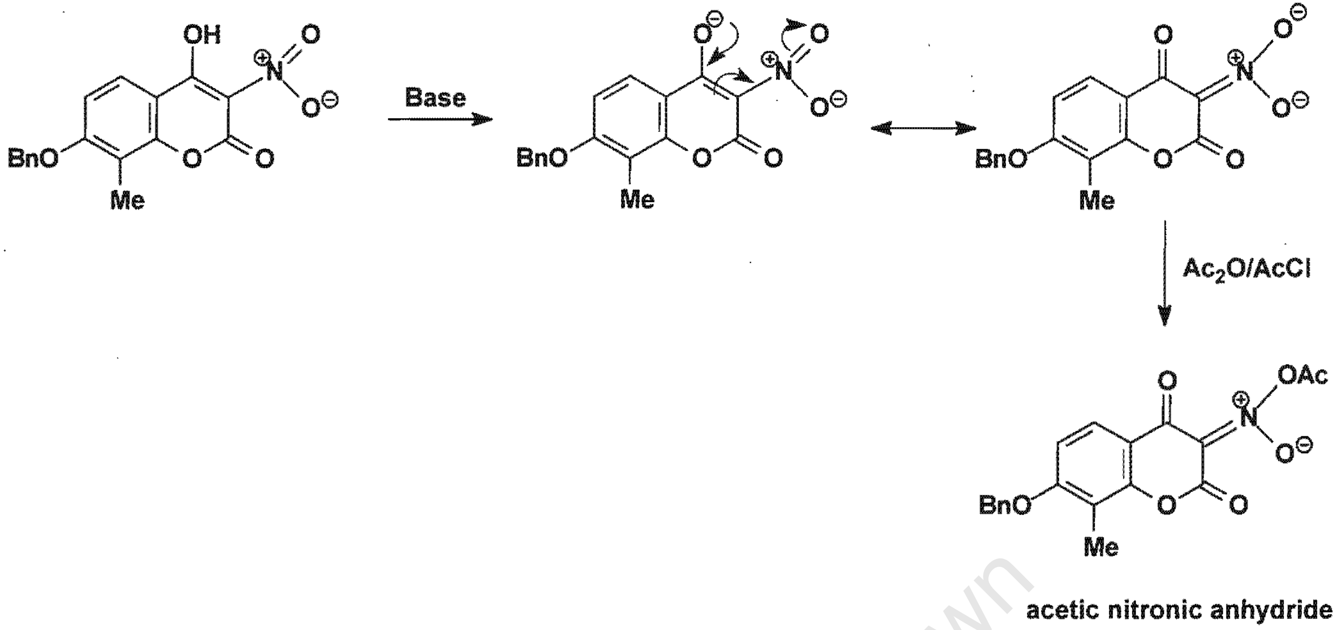
The ^1H NMR spectrum of **59** revealed the presence of the characteristic vinyl H-3 proton as a singlet downfield at δ_H 5.77. As can be seen by the resonance structure for coumarin **59** in Scheme 4.5, the H-3 proton is shielded by mesomeric release from the hydroxyl group and hence is shifted slightly upfield from that which would be expected for an α-enone. The carbonyl carbon in the ^{13}C NMR spectrum of **59** was seen at δ_C 166.1, and the melting point and ^1H and ^{13}C NMR data for **59** were identical to those for the reported compound.[38]

The introduction of the nitro group onto C-3 of **59** was achieved using a procedure described by Klosa in 1953, involving the generation of a nitronium ion from the reaction between nitric and sulfuric acids.[37] The simplicity of the nitration method made it an attractive choice in that the product could be simply filtered from the reaction mixture and recrystallized with glacial acetic acid to afford **132** in 93% yield (Scheme 4.6). The ^1H NMR spectrum of **132** revealed the absence of the H-3 proton observed at δ_H 5.77 in **59** and the presence of the aromatic H-5 and H-6 protons resonating as two doublets (*J* 9.0 Hz) at δ_H 7.06 and δ_H 7.77. This verified that nitration had occurred regioselectively at C-3 and not in the benzene ring, presumably as a result of resonance assistance from the enol. The presence of two bands at 1530 and 1525 cm^{-1} were clearly seen in the IR spectrum, indicative of the symmetrical and asymmetrical NO_2 stretching bands. The IR spectrum also showed a carbonyl signal at 1754 cm^{-1}, and a broad OH signal at 3540 cm^{-1}.



Scheme 4.6: *Reagents and conditions*: (i) HNO_3, H_2SO_4, $CHCl_3$, room temperature, 93%.

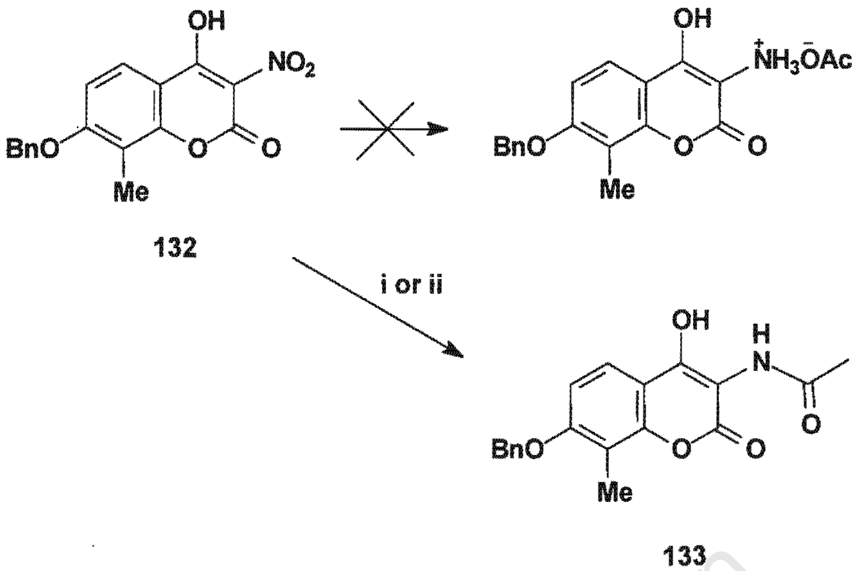
Originally, the strategy was to protect the C-4 hydroxyl group of **132** as an ester, deprotect the 7-O-benzyl ether, couple the coumarin unit to both the noviose and 4-epi-noviose sugars and finally reduce the nitro group to the corresponding amine and deprotect the C-4 ester. However, protection of the C-4 hydroxyl group as either an acetate or benzoate ester proved difficult. Standard conditions for acylation such as (i) acetic anhydride in pyridine; (ii) acetic anhydride, triethylamine and DMAP in dichloromethane; (iii) acetyl chloride, triethylamine and DMAP in dichloromethane and (iv) benzoyl chloride in pyridine did not result in conversion of the starting material **132** to the 4-O-ester (Scheme 4.6). The use of the stronger base NaH in the presence of acetyl chloride in both THF and DMF was unsuccessful as was the use of 10% KOH, either acetyl chloride or benzoyl chloride and a phase transfer catalyst (t-$Bu_4N^+HSO_4^-$). Two plausible explanations could account for the failure of the acylation. The first of these was the insolubility of the nitro compound **132** in standard solvents such as CH_2Cl_2, THF, DMF, and acetonitrile. **132** was even insoluble in methanol and water. Obtaining suitable ^1H and ^{13}C NMR data was also difficult as the compound was only sparingly soluble in deuterated DMSO, requiring heat for complete dissolution. Secondly, deprotonation of the hydroxyl group of **132** may result in resonance delocalization of the electrons into the coumarin ring as depicted in Scheme 4.7, thereby decreasing the nucleophilicity of the resulting oxygen anion and leading to the formation of a nitronate ion. Alkyl nitronate anions have been reported to react with acid chlorides or anhydrides to form acetic nitronic anhydrides.[100] However, these were not isolated from the reation of **132** with acetic anhydride or benzoyl chloride.



Scheme 4.7: Resonance structure for the anion of **132**.

The next sequence considered involved first reducing the nitro group of **132** to the amine, followed by protection of the hydroxyl and amine functionalities as an oxazole. As with the protection step, reduction of **132** to the corresponding amine was complicated by insolubility. Potanay *et al.*[38] has shown that reduction of substituted 4-hydroxy-3-phenylazocoumarins to the corresponding amines can be achieved with aqueous sodium dithionite in pyridine without cleaving the benzyl ether. Unfortunately, the treatment of the nitro compound **132** with these reagents was unsuccessful, with only starting material being recovered from the reaction. Various other reductions were attempted including (i) Fe and 1 M HCl in MeOH; (ii) Fe and 1 M HCl in THF; (iii) Zn dust and 1 M NaOH in EtOH, all of which were unsuccessful. In 1962, Okumura reduced 4,7-dihydroxy-3-phenylazocoumarin to the corresponding 3-acetamido-4,7-dihydroxycoumarin with Zn dust, acetic acid and acetic anhydride at room temperature in 56% yield.[40]

Accordingly, nitro compound **132**, zinc and 1 equivalent of acetic anhydride were refluxed in acetic acid until the colour of the solution changed from red to colourless (approximately 1 hour), and while the solution was still hot, the zinc salts were removed by filtration. The filtrate was allowed to cool and the precipitate collected. Recrystallization from ethyl acetate gave acetamide **133** in 86% yield (Scheme 4.8).

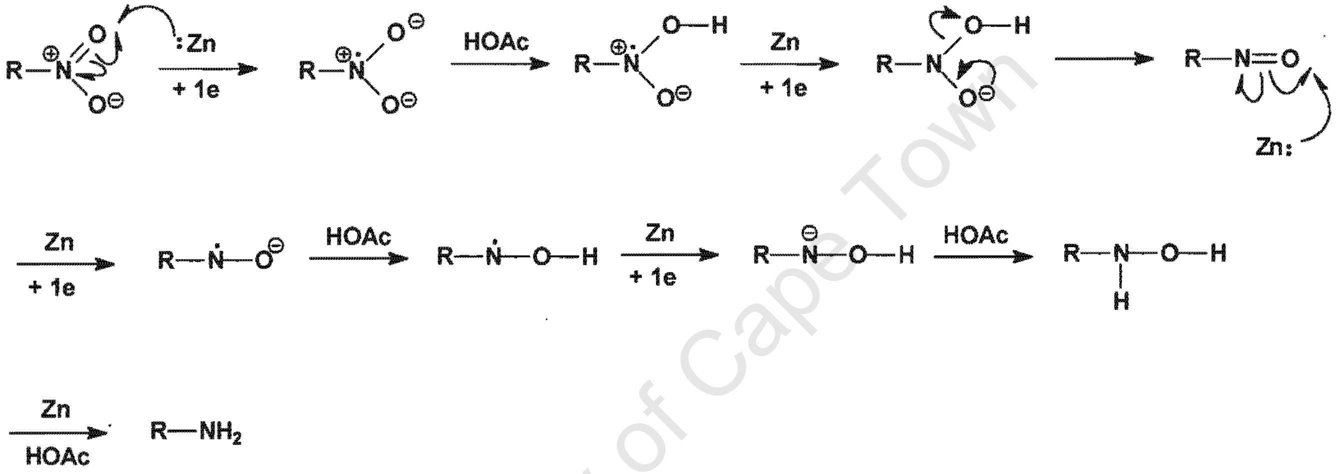


Scheme 4.8: *Reagents and conditions*: (i) Zn, AcOH, Ac_2O (1 equiv.), 160°C, 86%. (ii) Zn, AcOH, 160°C, 85%.

The characteristic methyl signal of the acetamide of **133** was clearly seen at δ_H 2.11 in its ^1H NMR spectrum as well as the presence of two broad singlets, one at δ_H 9.42 corresponding to the amide proton and the other at δ_H 12.2 indicative of the hydroxyl proton. The carbonyl carbon of the acetamide was seen at δ_C 171.2 in the ^{13}C NMR spectrum, the chemical shift of which was distinctive from that of the lactone carbonyl carbon which was observed at δ_C 160.3. The IR spectrum clearly showed the presence of an acetamide with an N-H stretch and bend observed at 3288 cm^{-1} and 1572 cm^{-1} respectively, and the carbonyl of the amide at 1632 cm^{-1}. The lactone carbonyl was observed at 1686 cm^{-1}. The formation of acetamide **133** was corroborated by elemental microanalysis.

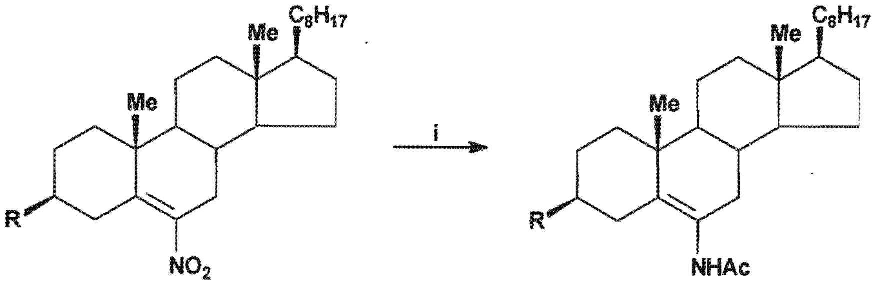
Interestingly, when the reduction was repeated with the exclusion of acetic anhydride, the product was again the acetamide and not the acetic acid salt of the amine as predicted (Scheme 4.8). The ^1H and ^{13}C NMR spectra of both the acetamide and the amine salt would be similar as both compounds have an acetyl group making distinction between the two compounds difficult. A distinguishing feature would be the number of protons attached to the nitrogen atom, which should be observable in the ^1H NMR spectrum. However, the acetamide should be clearly observable in the IR spectrum, and definitive confirmation should be provided by the molecular mass of the product. In the event, an elemental microanalysis was obtained for **133** and provided conclusive evidence that the product was an amide and not the acetate salt of the amine. The yield for this reaction (85%) was comparable to the former reaction (86%) and hence, both reactions (with or without one equivalent of acetic anhydride) would be applicable in this case.

The mechanism for the reduction of a nitro group with metals in acid has been given very little attention, although it is presumed that nitroso compounds and hydroxylamines are intermediates. The mechanistic path that has been suggested is illustrated in Scheme 4.9 where R is an aromatic or an aliphatic residue that does not contain an α hydrogen.[101] Nitro compounds that contain an α hydrogen can be reduced to oximes with Zn dust and acetic acid.[102]



Scheme 4.9: Proposed mechanism for the reduction of a nitro group to an amine.

The reaction conditions as well as the nucleophilicity of the resulting amine determine whether N-acetylation of the amine with acetic acid will take place. It is well documented that reduction of aromatic nitro groups with zinc and acetic acid at both room temperature and reflux afford the corresponding amine and not the acetamide. As an example, in 1910 Heller published the reduction of *o*-nitrocinnamic acid to *o*-aminocinnamic acid using Zn dust and acetic acid at 0°C in 80% yield without N-acylation taking place.[103] However, in 1987, Ahmad *et al.* observed that reduction of steroidal nitroolefins with zinc in acetic acid at room temperature gave the N-acylated enamine as the major product as shown in Scheme 4.10.[104]



Scheme 4.10: *Reagents and conditions*: (i) Zn, AcOH, room temperature, 65%.

In both examples, the amines are conjugated. However, in the case of the cinnamic acid, there is an electron-withdrawing group *ortho* to the nitro group, thereby decreasing the nucleophilicity of the resulting amine. It is presumably for this reason that reduction of deactivated aromatic nitro groups with zinc in acetic acid fails to result in the N-acylation of the resulting amine. As disubstituted 4-hydroxy-3-nitrocoumarin **133** is insoluble in acetic acid at room temperature, reduction with zinc and acetic acid was performed at reflux temperature. The resulting 3-amino group was readily N-acylated as a result of the high temperatures and the greater nucleophilicity of the amine (not directly conjugated to the lactone carbonyl group) when compared to the aromatic amines.

The next step in the synthesis was protection of both phenolic hydroxyl and amino groups by formation of an oxazole heterocycle. The oxazole ring is formally related to furan by replacement of the CH group at position 3 by nitrogen. During the last three decades, numerous oxazole-containing secondary metabolites have been isolated and characterised. Examples include Ulapualide A,[105] a macrocycle that is known to inhibit leukaemia cell proliferation, as well as Calcimycin[106] and Cezomycin,[107] which are both polycyclic antibiotics that contain the 4-carboxybenzoxazole ring system. As a consequence, numerous methods are available for the synthesis of oxazoles.

2-Substituted benzoxazoles can be synthesized by:

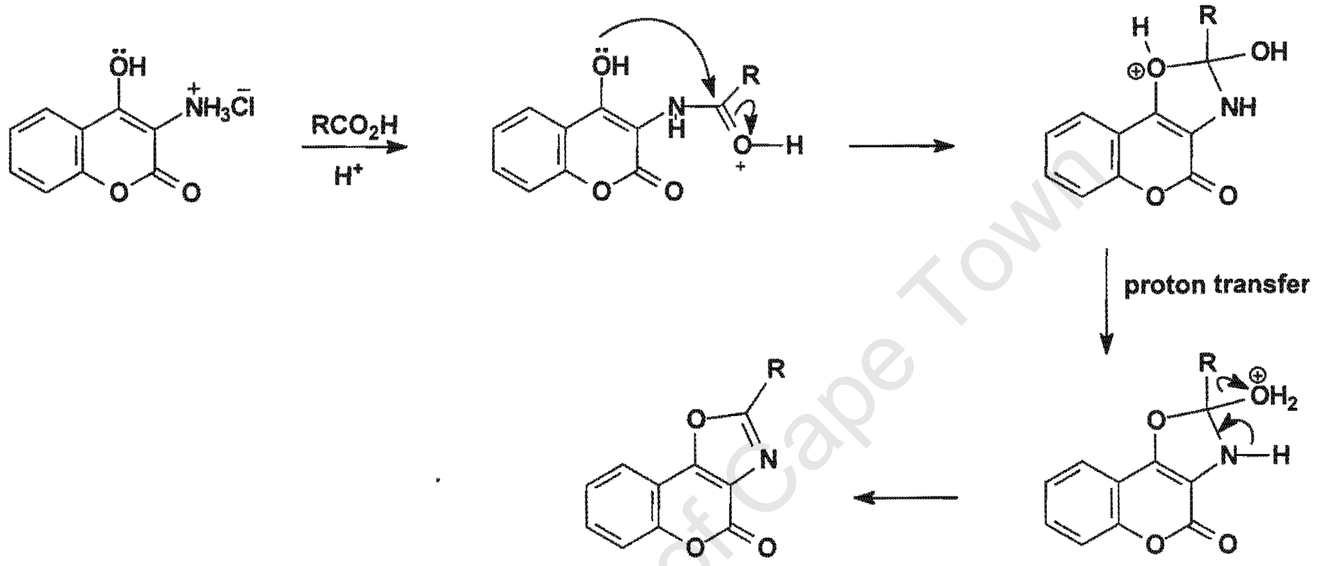
(i) Reacting 2-aminophenol in xylene with an acylating agent and trialkylamine base in the presence of catalytic pyridinium p-toluenesulfonate at reflux for 16 hours.[108]

(ii) Condensation of orthoesters with 2-aminophenol in the presence of toluene and KSF clay as the acid-catalyst at reflux for 12 hours.[109]

(iii) Beckmann rearrangement of *o*-acylphenol oximes with phosphoryl chloride and N,N-dimethylacetamide.[110]

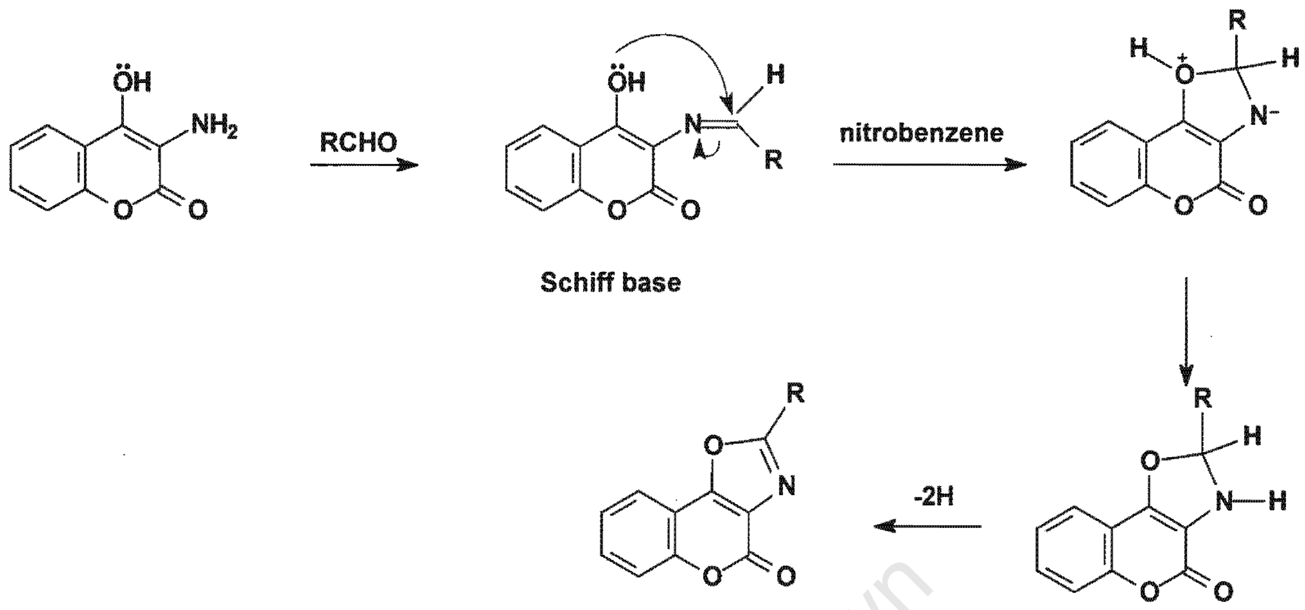
Of even closer relevance to the challenge at hand, benzopyrano[3,4-d]oxazol-4-ones have been prepared by:

(i) Heating 3-amino-4-hydroxycoumarin hydrochloride with the appropriate carboxylic acid in polyphosphoric acid for 3.5 hrs at 160-200°C, the proposed mechanism of which is depicted in Scheme 4.11. Following amide formation, the amide carbonyl centre undergoes acid-assisted nucleophilic attack from the C-4 hydroxyl oxygen and dehydration ensues.[111]



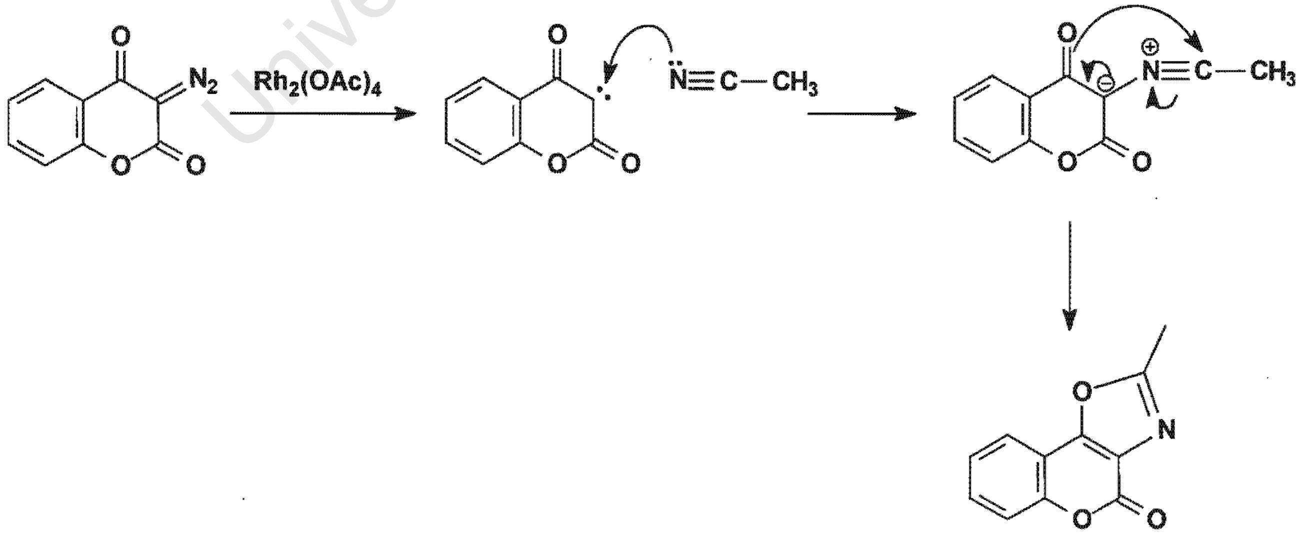
Scheme 4.11: Proposed mechanism for oxazole formation when 3-amino-4-hydroxycoumarin is treated with a carboxylic acid in polyphosphoric acid.

(ii) Condensing 3-amino-4-hydroxycoumarin derivatives with aromatic aldehydes to yield the corresponding Schiff bases which, by heating in nitrobenzene, underwent cyclization and H_2 elimination to afford the corresponding oxazole derivatives, the proposed mechanism of which is illustrated in Scheme 4.12.[112]



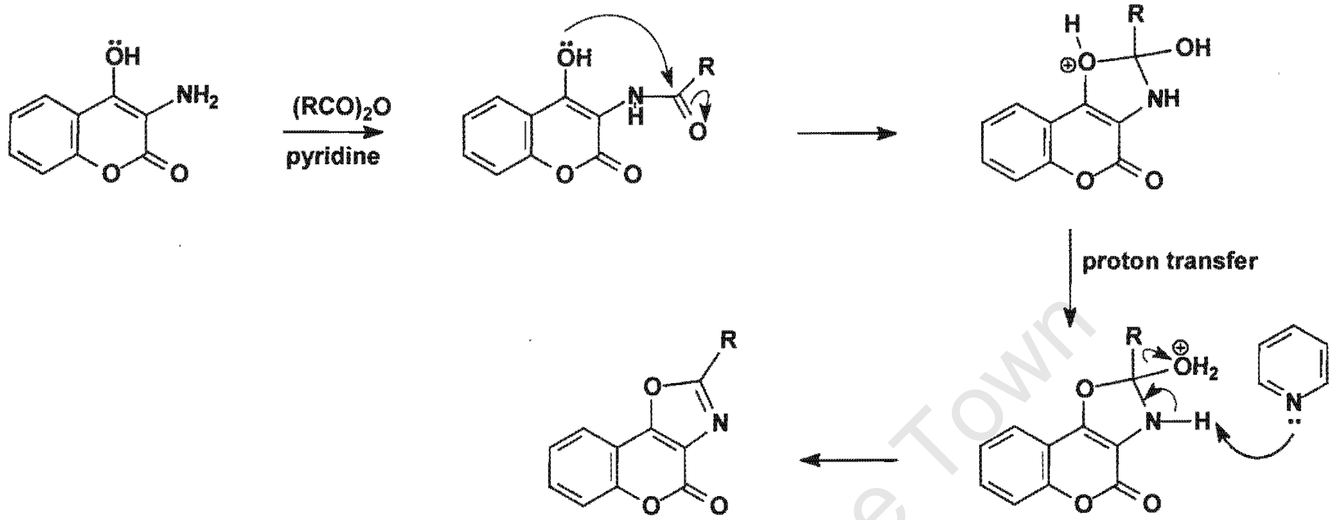
Scheme 4.12: Proposed mechanism for oxazole formation when 3-amino-4-hydroxycoumarin is treated with an aromatic aldehyde followed by refluxing in nitrobenzene.

(iii) Heating 3-diazo-2,4-chromenedione with acetonitrile in the presence of $Rh_2(OAc)_4$ for 5 hours at 60°C.[113] The presence of $Rh_2(OAc)_4$ presumably results in the formation of a carbenoid species that attacks the lone pair of electrons on the nitrogen of acetonitrile leading to ring closure (Scheme 4.13).



Scheme 4.13: Proposed mechanism for oxazole formation when 3-diazo-2,4-chromenedione is treated with $Rh_2(OAc)_4$ in acetonitrile.

(iv) Heating 3-amino-4-hydroxycoumarin derivatives with the appropriate anhydride in pyridine. The proposed mechanistic details of this reaction, illustrated in Scheme 4.14, are similar to Scheme 4.11, but in this case, cyclization and dehydration are base-assisted.[114]



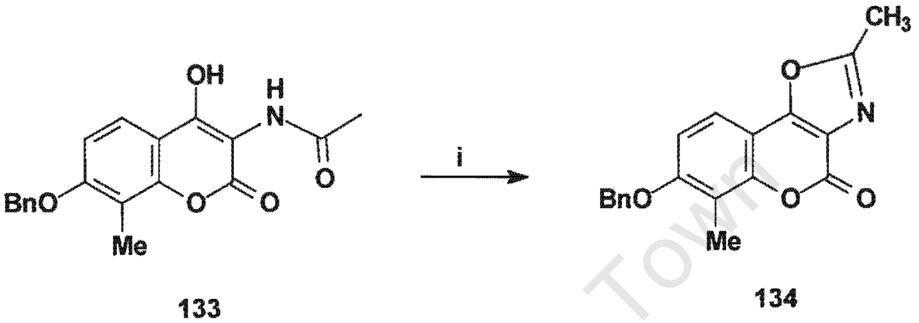
Scheme 4.14: Proposed mechanism for oxazole formation when 3-amino-4-hydroxycoumarin is treated with an acid chloride in pyridine.

The preparation of benzopyrano[3,4-d]oxazol-4-ones by the above methods either involved high temperatures and long reaction times or unsatisfactory yields (≤ 50%) so an alternative method was sought.

Phosphorous oxychloride is an electrophilic phosphorylating agent that is widely used in synthesis, particularly in reactions with N,N-dimethylformamide, which leads to the formation of chloromethyleneiminium salts (Vilsmeier reagents). These salts are highly versatile intermediates and are involved in numerous important reactions, including the Vilsmeier-Haack and Bischler-Napieralski reactions. The combination of phosphoryl oxychloride and pyridine is commonly used as an effective dehydrating agent for alcohols, and as can be seen from Schemes 4.11 and 4.14, this is a common mode of action for oxazole formation.

Taking this into consideration, N-(7-benzyloxy-4-hydroxy-8-methyl-2-oxo-(2H)-chromen-3-yl)-acetamide **133** was suspended in THF to which was added $POCl_3$ and excess pyridine and the mixture was refluxed (Scheme 4.15). Pleasingly, the reaction was complete within 5 minutes and work-up simply involved evaporation of both THF and pyridine, the latter by azeotropic removal with toluene, followed by recrystallization to give 7-benzyloxy-2,6-

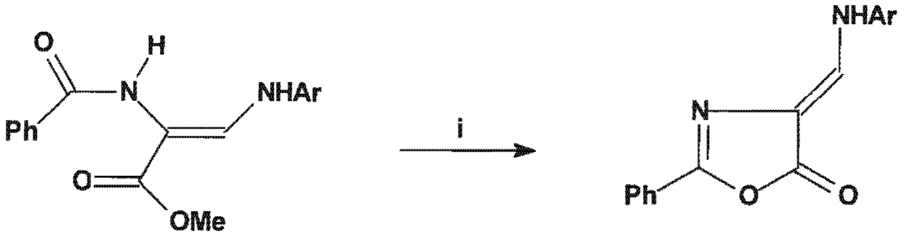
dimethyl-chromeno[3,4-d]oxazol-4-one **134** in 87% yield. The observable changes in the ^{1}H NMR spectrum of **134** included a slight downfield shift in one of the methyl singlets from δ_H 2.11 in **133** to δ_H 2.56 as well as the disappearance of the amide proton, observed at δ_H 9.42 in **133**. The methyl signal at δ_H 2.56 was assigned to the methyl substituent on the oxazole ring, which was more deshielded than the methyl of the acetamide **133** as it is positioned on a double bond attached to two heteroatoms.



Scheme 4.15: *Reagents and conditions*: (i) $POCl_3$, pyridine, THF, reflux, 87%.

In the ^{13}C NMR spectrum of **134**, the oxazole carbon C-2 resonated at δ_C 162.6 whereas the carbonyl carbon of the acetamide of **133** resonated at δ_C 171.2, revealing an upfield shift for this carbon. This is in keeping with oxazole formation because the carbonyl group of the acetamide of **133** is more deshielding that the C=N bond of the oxazole group as a result of the greater electronegativity of oxygen compared to nitrogen. Hence, the carbonyl carbon of the acetamide of **133** experiences a greater deshielding effect than the corresponding oxazole C-2 carbon. The IR spectrum of **134** revealed the absence of a signal in the NH region as well the amide I and II bands, a further indication that oxazole formation had taken place.

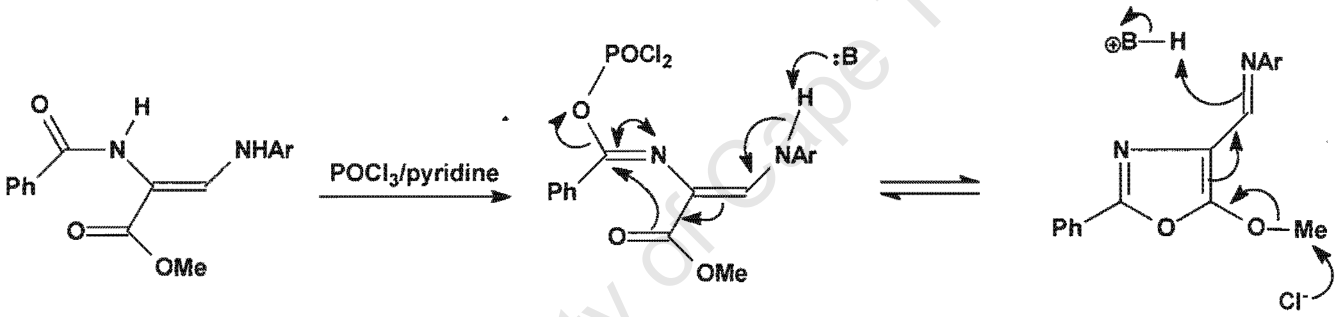
Only one literature analogy for the use of this reagent combination was found whereby methyl 3-arylamino-2-benzoylaminopropenoate **135** was converted with phosphorous oxychloride and pyridine to 4-arylaminomethylene-2-phenyl-2-oxazolin-5-one **136** (Scheme 4.16).[115] This conversion was realised without THF as a co-solvent and in high yields (83%) in only 2 minutes at 70°C.



135 **136**

Scheme 4.16: *Reagents and conditions*: (i) $POCl_3$, pyridine, 70°C, 83%.

The tentative mechanism suggested by the authors for this reaction is depicted in Scheme 4.17 whereby oxazolinone formation is assisted by resonance stabilisation from the arylamino group.



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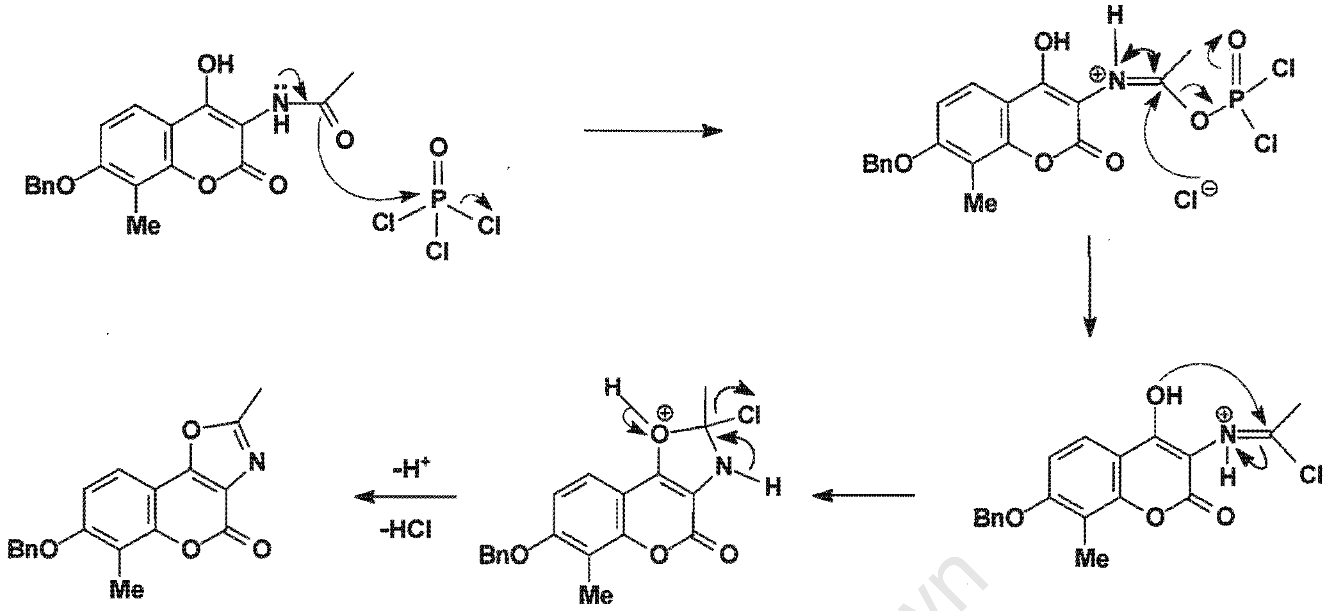
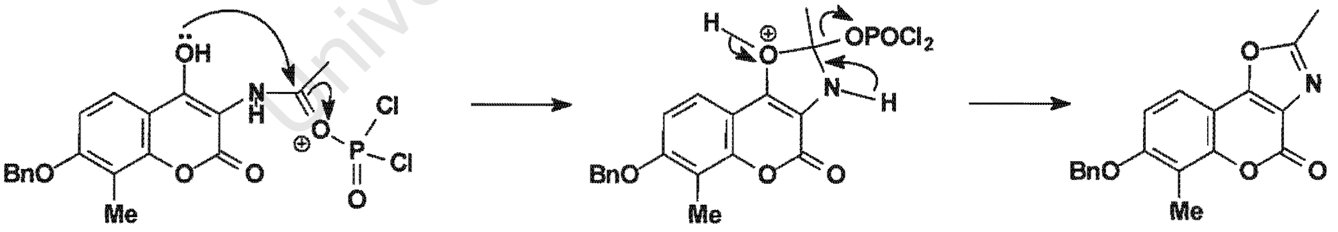
Scheme 4.17: Proposed mechanism for oxazolinone formation.

In this project, no resonance activator (i.e. –NHAr) was present on the coumarin ring so this mechanism was not applicable. As no further literature analogies could be found, it seemed appropriate to explore the scope of the methodology for general oxazole formation. The very high rate at which the reaction occurred (5 minutes at 66°C) compared to other literature cases also prompted a closer examination of the mechanistic pathway. Three mechanisms were considered for oxazole formation using $POCl_3$ and pyridine. Mechanism 1, depicted in Scheme 4.18, involves the formation of an imidoyl chloride intermediate that undergoes nucleophilic attack from the coumarin hydroxyl group. In principle, this mechanism is similar to that of Scheme 4.17.



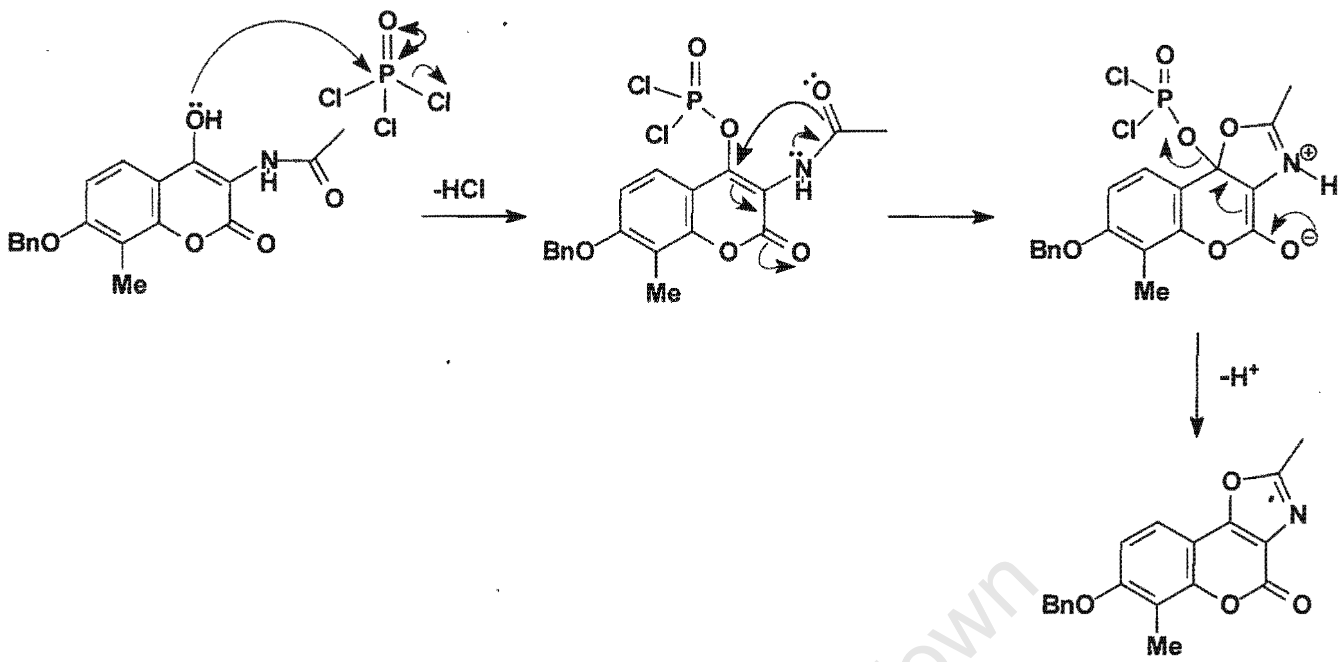
Scheme 4.18: Proposed mechanism for oxazole formation via the formation of an imidoyl chloride intermediate.

The second mechanism proposed (Scheme 4.19) is simply a variation on mechanism 1 involving the formation of a complex between $POCl_3$ and the carbonyl carbon of the amide. The carbonyl centre undergoes nucleophilic attack by the hydroxyl oxygen and dehydration ensues.



Scheme 4.19: Proposed mechanism for oxazole formation via dehydration of $HOPOCl_2$.

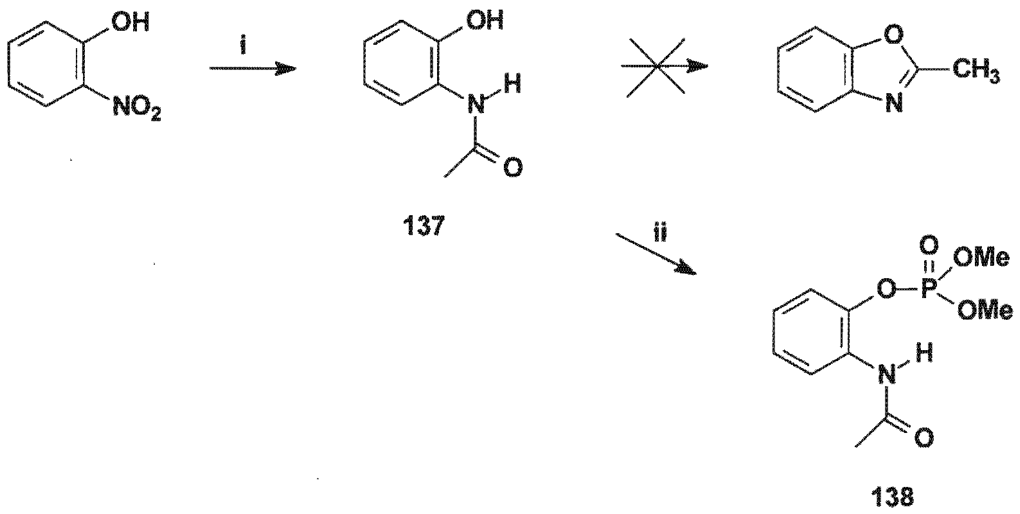
Nucleophilic substitution at the 4-hydroxy centre was proposed for the final mechanism, depicted in Scheme 4.20. In this case, a dichlorophosphate ester forms between $POCl_3$ and the hydroxyl oxygen, turning the hydroxyl group into a good leaving group. This sets up an addition-elimination reaction that is assisted by resonance stabilization into the coumarin ring.



Scheme 4.20: Proposed mechanism for oxazole fomation via nucleophilic substitution.

Two model compounds were selected for the study, namely *o*-nitrophenol and methyl 4-hydroxy-3-nitrobenzoate. The former was considered to be a poor candidate for the reaction according to mechanism 3, whereas the latter compound was selected based on the electron-withdrawing effect of the benzoate at the para position to the hydroxyl group which was anticipated to provide evidence for the nucleophilic substitution mechanism (mechanism 3).

To prepare for oxazole formation, *o*-nitrophenol was reduced with Zn and acetic acid in the presence of acetic anhydride to give the corresponding amide **137** in 71% yield (Scheme 4.21). Interestingly, the treatment of **137** with $POCl_3$ and pyridine in THF did not furnish the benzoxazole. However, the addition of methanol to the reaction mixture after 1 hour gave the dimethoxyphosphate ester **138** which was isolated in 18% yield, providing evidence that the dichlorophosphate ester was an intermediate in the reaction (Scheme 4.21).



Scheme 4.21: *Reagents and conditions*: (i) Zn, AcOH, Ac_2O, room temperature, 71%. (ii) (a) $POCl_3$, pyridine, THF, reflux, 1 hour; (b) MeOH, reflux, 18% over 2 steps.

The characterization of **138** was achieved by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.[116] The proton-phosphorous and carbon-phosphorus coupling constants, as determined from the ^{1}H and ^{13}C NMR spectra of **138** respectively, are summarised in Figure 4.2.

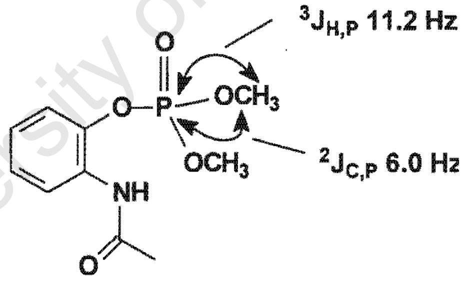
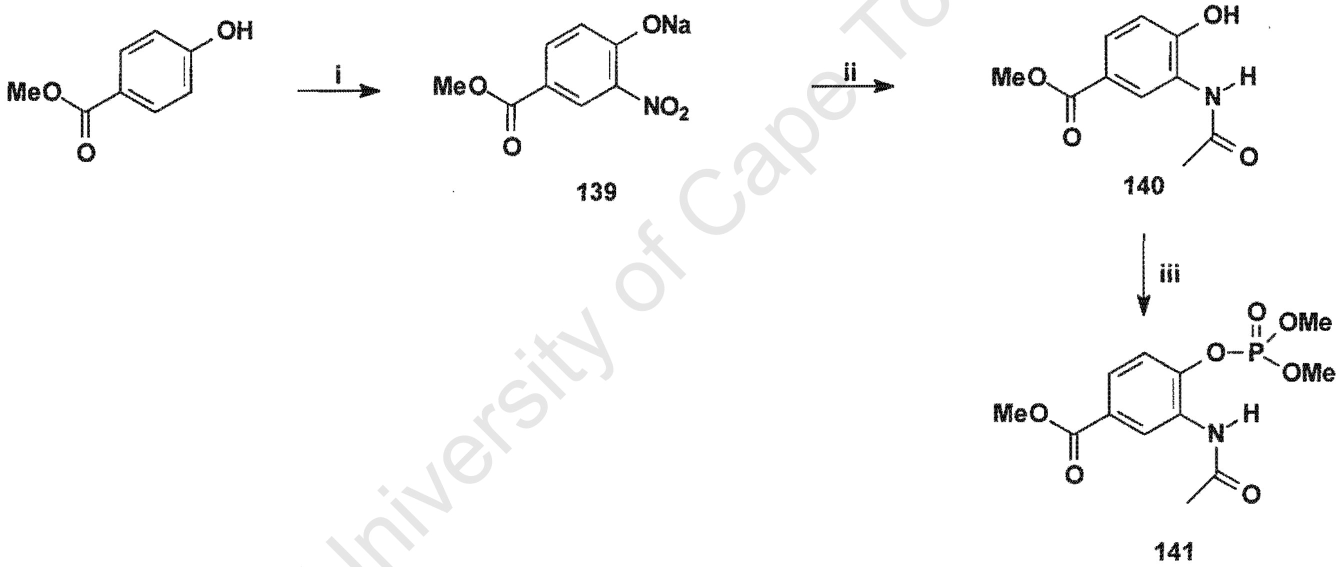


Figure 4.2: Proton-phosphorus and carbon-phosphorus coupling constants for **138**.

The ^{1}H NMR spectrum of **138** clearly indicated the appearance of equivalent enantiotopic methoxy signals resonating as a doublet at δ_H 3.79 ($^3J_{H,P}$ 11.2 Hz). The large coupling constant of 11.2 Hz is indicative of a ^3J phosphorus-proton coupling. Similarly, the methoxy carbons were observed as a doublet at δ_C 55.7 ($^2J_{C,P}$ 6.0 Hz) in the ^{13}C NMR spectrum, the coupling constant indicating a ^2J carbon-phosphorus coupling.

The second model compound was prepared by mono-nitration of methyl 4-hydroxybenzoate with concentrated HNO_3 in acetic acid to furnish **139** as the sodium salt in 62% yield (Scheme 4.22). The reaction was quenched with sodium carbonate, which resulted in deprotonation of the phenol group and hence, sodium salt formation. Mono-nitration at C-3 was achieved because the ester group of methyl 4-hydroxybenzoate is *meta* directing while the 4-hydroxy is *ortho/para* directing, and since the ester group is *para* to the hydroxyl group, nitration could only take place *ortho* to the hydroxyl group. Nitro compound **139** was then reduced with zinc in acetic acid to give amide **140** in 77% yield (Scheme 4.22). As with amide **137**, treatment of **140** with $POCl_3$ and pyridine in THF did not furnish the benzoxazole and the addition of methanol to the reaction mixture after 1 hour at reflux similarly afforded the dimethoxyphosphate ester **141** in 42% yield (Scheme 4.22). Once again, ^1H, ^{13}C and ^{31}P NMR spectroscopy were employed to identify **141**, with the proton-phosphorus and carbon-phosphorus coupling constants consistent with the formation of a dimethyl phosphoric acid ester.[116]

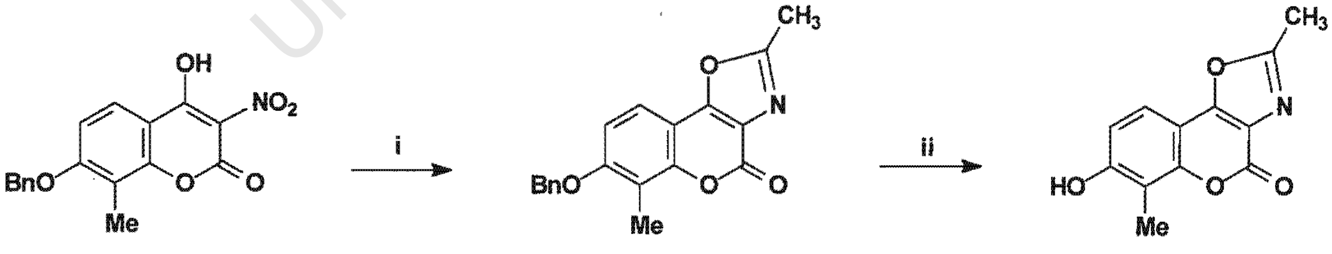


Scheme 4.22: *Reagents and conditions*: (i) HNO_3, acetic acid, room temperature, 62%. (ii) Zn, AcOH, Ac_2O, room temperature, 77%. (iii) (a) $POCl_3$, pyridine, THF, reflux, 1 hour; (b) MeOH, reflux, 42% over 2 steps.

Although oxazole formation in the aromatic series was not achieved with $POCl_3$ and pyridine, some insight into the mechanism of the reaction was revealed. In both aromatic substrates, a stable phosphate ester could be formed by quenching, which was isolated and characterized as the mixed dimethyl phosphoric acid ester. Mechanism 1 postulates that the key step involved the formation of an imidoyl chloride intermediate while mechanism 2, a variation on mechanism 1, suggests elimination of $HOPOCl_2$. The failure of the reaction to promote oxazole formation in the aromatic series would suggest that these two mechanisms are incorrect as they should be applicable to both the aromatic and coumarin series. The rapidity with which the reaction occurs in the coumarin series would also suggest that dehydration is not the mode of action, as the previous syntheses of benzopyrano[3,4-d]oxazol-4-ones that occurred via dehydration (Schemes 4.11 and 4.14) required high temperatures and long reaction times. Mechanism 3 postulates that nucleophilic substitution is the mode of action, which would depend on having a strong electron-withdrawing group in close proximity to the reaction site. The intrinsic nature of the structure of coumarin **133** provides this condition. Presumably neither of the aromatic model compounds satisfy this requirement, as seemingly the benzoate ester of methyl 4-hydroxybenzoate is too far away from the hydroxyl group to be effective. The exclusion of mechanisms 1 and 2 on the grounds that they should be applicable to both the coumarin and aromatic systems as well as the rapidity with which oxazole formation occurred with $POCl_3$ and pyridine, provides a plausible explanation that nucleophilic substitution is the mode of action, i.e. mechanism 3. It would be of interest to determine whether this methodolgy could be applied to methyl 2-amino–3-hydroxybenzoate as, in this case, the electron-withdrawing group is in closer proximity to both the amino and hydroxyl groups so mechanism 3 might be applicable.

In order to establish the mechanism accurately, either the coumarin hydroxyl oxygen or the amide oxygen would need to be isotopically labelled, thereby determining which of these oxygens becomes incorporated into the oxazole ring.

Interestingly, when nitro compound **132** was refluxed with zinc, acetic acid and a large excess of acetic anhydride, oxazole **134** was isolated directly in 87% yield (Scheme 4.23).



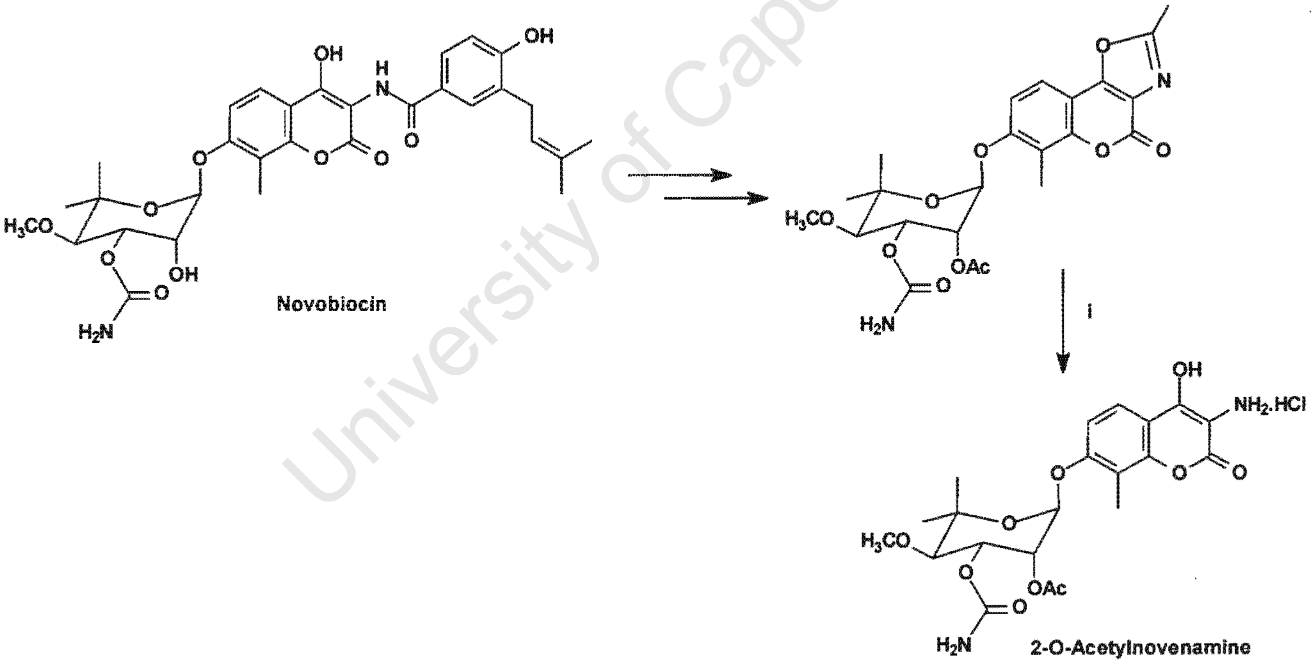
132 **134** **142**

Scheme 4.23: *Reagents and conditions*: (i) Zn, AcOH, Ac_2O, 160°C, 87%. (ii) 10% Pd/C, H_2, THF/CH_2Cl_2, room temperature, 74%.

In 1959, Stammer found that refluxing 3-acetamido-7-acetoxy-4-hydroxy-8-methylcoumarin in acetic anhydride gave the corresponding oxazole in 66% yield.[117] Acetic anhydride, like $POCl_3$, has been employed as a dehydrating agent. However, in this case, the coumarin hydroxyl group most likely undergoes acid-catalyzed acetylation, with ring closure following a similar nucleophilic substitution mechanism as described above.

Finally, oxazole **134** was debenzylated with 10% Pd/C and H_2 gas in a mixture of THF and CH_2Cl_2 to furnish **142** in 74% yield (Scheme 4.23).

The cleavage of the oxazole moiety in noviosylcoumarin has been achieved by Ueda *et al.* in 1988.[118] In this publication, novobiocin was treated with acetic anhydride in refluxing pyridine, whereby the amide bond of novobiocin was selectively cleaved to form oxazole-protected 2-O-acetylnovenamine (Scheme 4.24). This oxazole-protected product was then treated with acetyl chloride in refluxing ethanol (generation of HCl *in situ*) to afford 2-O-acetylnovenamine in 97% yield.



Scheme 4.24: *Reagents and conditions*: (i) AcCl, EtOH, reflux, 97%.

As with the majority of organic syntheses, each step in this synthesis presented a new challenge, despite all the published data available on these compounds and as a result, this synthesis took a lot longer than anticipated. Despite this, the synthesis of the oxazole-protected coumarin fragment of Novenamine was accomplished in only 5 steps in 40% overall yield, the highlights of which are listed below:

1. Only one of the five steps required an aqueous extraction work-up, the remaining steps simply involved either filtration or evaporation of solvent.
2. The purification of each product was achieved in high yield by recrystallization, with the avoidance of column chromatography, which is both expensive and time consuming.
3. New methodogy was developed for oxazole formation which was particularly applicable to the coumarin series. Although this methodology could not be extended to the aromatic series, insight was obtained as to the possible mechanism for oxazole formation. Further insight into the mechanism could be achieved by extending the work to aromatic systems with electron-withdrawing groups *ortho* or *meta* to a phenolic hydroxyl group.
4. Table 4.1 summarises a comparison of the number of steps and yield of the various coumarin aglycons that have been synthesized, with Entry 1 being from this study. 4-*O*-Protected coumarins **10** and **20** (Entries 2 and 3) were coupled to noviose prior to the introduction of the C-3 amino group, whereas oxazole-protected coumarin **142**, synthesized in this work in a higher yield than that of **10** and **20**, already included the 3-amino substituent, thus minimizing post-glycosylation manipulations.

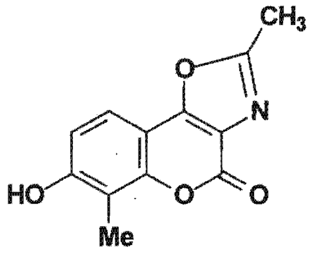
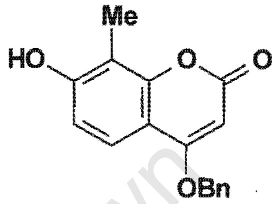
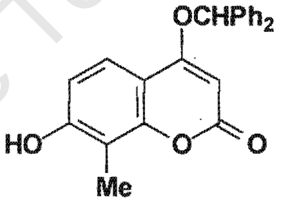
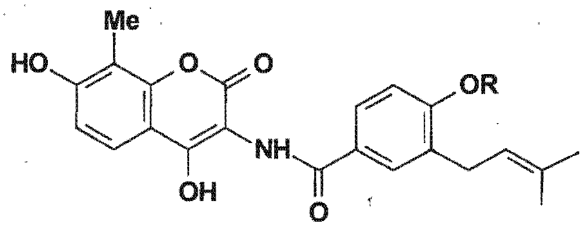
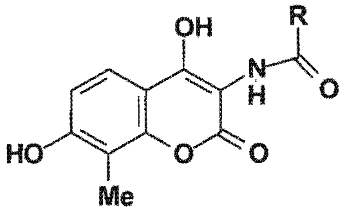
Starting Material	No. of steps	Yield	Product
2,4-Dihydroxy-3-methylacetophenone **THIS WORK**	5	40%	 **142**
2-Methylresorcinol	3	13%	 **10**
2,4-Dihydroxy-3-methylacetophenone	4	37%	 **20**
2-Methylresorcinol	7	<7%	 **Novobiocinic acid**
4-Benzyloxy-2-hydroxy-3-methylacetophenone	5	20%	 **63** **R = 3,4,5-trimethoxyphenyl**

Table 4.1: A comparative analysis of the syntheses of coumarin aglycons.

Chapter 5

5.1. Towards the synthesis of a C-5 analogue of 4-epi-noviose

Noviose preferentially adopts the $^1C_4$ chair conformation, which is assumed to be vital for enzyme binding to DNA-gyrase. Based on our earlier analysis, it is likely that 4-epi-noviose adopts the $^4C_1$ conformation and, hence, is unlikely to have a similiar biological activity to noviose because its conformation would prevent it binding to the DNA-gyrase active site. One of the aims of this project was to bias the $^4C_1$ chair conformation of 4-epi-noviose to the $^1C_4$ conformation by stereoselectively substituting one of the 5,5-dimethyl groups with a bulky alkyl group. The stereoselective introduction of different alkyl groups at the 5- position has been achieved in the synthesis of C-5 analogues of noviose from L-arabinose.[119] However, this work only probed the noviose series, while nothing has been published regarding the 4-epi-noviose series.

As shown in Figure 5.1, 4-epi-noviose would adopt the $^1C_4$ conformation if the A-value of R_2 were sufficiently large to force the C-4 methoxy, the C-5 methyl and the C-1 and C-2 hydroxyl substituents to adopt an axial orientation and hence flip the ring to the $^1C_4$ conformation. Adding up the A-values of the axial substituents on the $^1C_4$ chair gives a total of 17.5 kJ/mole (OR ~ 3.1 kJ/mole, OH = 4.0 kJ/mole, OMe = 3.1 kJ/mole, CH_3 = 7.3 kJ/mole[120]). This would require R_2 to have an A-value of at least 17.5 kJ/mole in order to counterbalance the axial substituents and the only alkyl group that satisfies this requirement is *tert*-butyl which has an A-value of 21 kJ/mole.

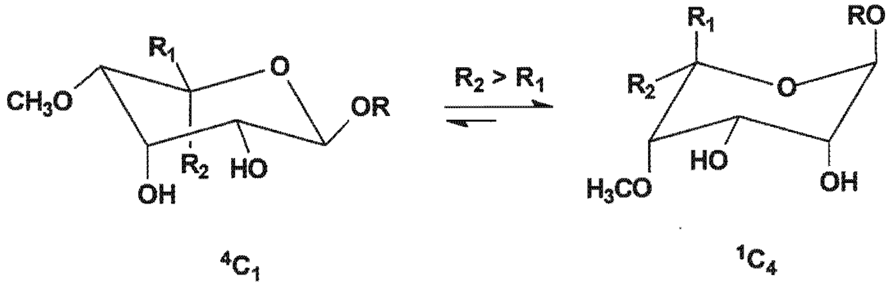


Figure 5.1: If R_2 is sufficiently large, 4-epi-noviose would flip from a $^4C_1$ to a $^1C_4$ conformation.

Hence, *tert*-butyl was selected to replace the pro-*S* C-5 methyl group to create a 5*S* centre (Figure 5.2).

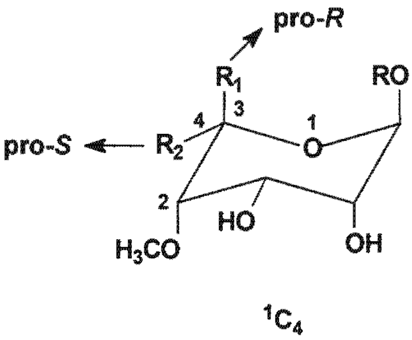
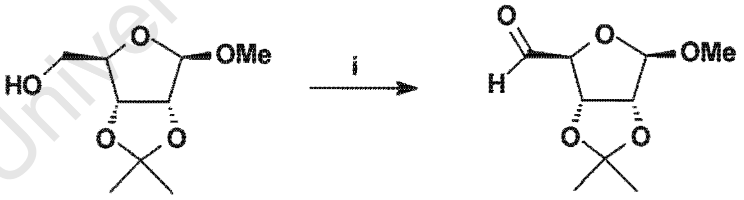


Figure 5.2: Illustration of assignment of R_1 and R_2 as pro-*R* and pro-*S* respectively. Numbers 1-4 indicate functional group priority.

In order to achieve this stereoselectively, a new strategy was developed that would allow for the introduction of the two alkyl groups at different stages in the synthesis.

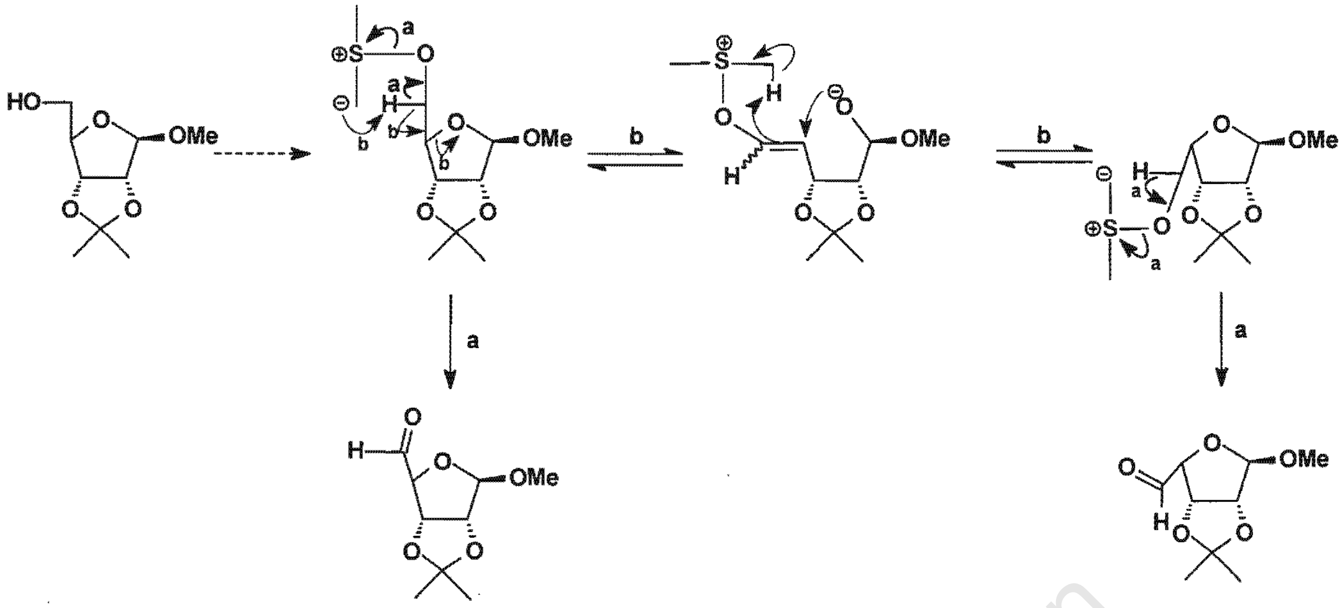
Thus, methyl 2,3-O-isopropylidene-β-D-ribofuranose **64β**, prepared in Section 2.1, was oxidized to its corresponding aldehyde **143**, using standard Swern conditions (Scheme 5.1). Swern oxidation was selected on the basis that it was applied so successfully in the oxidative ring closure of diol **88** to lactol **90** (Scheme 2.8, page 42), an intermediate in the synthesis of of methyl 4-epi-novioside. However, aldehyde **143** was only isolated in 22% yield after column chromatography, with many side products observed on tlc. The data for **143** corresponded to those reported in the literature.[121]



64β **143**

Scheme 5.1: *Reagents and conditions*: (i) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -78°C, 22%.

Further insight into the course of events was provided by a literature survey on the oxidation of **64β**, which revealed that Swern oxidation results in the epimerization of H-4 furnishing a mixture of methyl 2,3-O-isopropylidene-β-D-ribo-pentodialdo-1,4-furanoside and methyl 2,3-O-isopropylidene-β-D-lyxo-pentodialdo-1,4-furanoside. The mechanism for this epimerization was proposed by Kjær *et al.* and is depicted in Scheme 5.2, whereby 4-epimerization chiefly takes place via an equilibrium process involving a vinyloxysulfonium ion.[122]

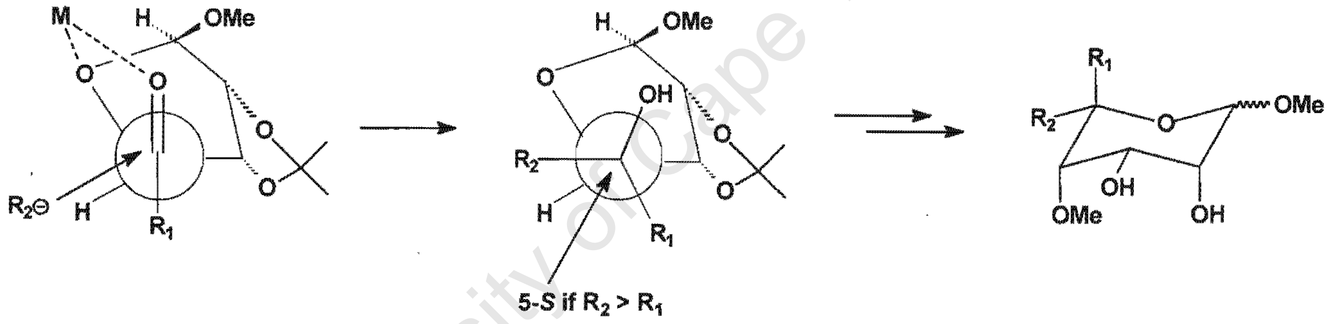


Scheme 5.2: Proposed mechanism for 4-epimerization of **64β** under Swern conditions.

Returning to the ^{1}H NMR spectrum of **143**, no coupling was observed between H-1 and H-2 ,which confirmed the β-anomer as expected (see discussion in Section 2.1). Similarly, no coupling was observed between H-3 and H-4 which provided evidence that 4-epimerization had not taken place and that **143** was the ribose sugar derivative. This was verified by the observation by Kjær *et al.* of a coupling between H-3 and H-4 for methyl 2,3-O-isopropylidene-β-D-lyxo-pentodialdo-1,4-furanoside which was isolated after the Swern oxidation of **64β** along with methyl 2,3-O-isopropylidene-β-D-ribo-pentodialdo-1,4-furanoside, for which no coupling was observed between H-3 and H-4.[122]

A more detailed investigation of the literature revealed that oxidation of **64β** to its corresponding aldehyde has been effected with Sarrett's reagent of chromium trioxide and pyridine in dichloromethane in 60% yield,[123] pyridinium dichromate (70% yield),[124] and alkaline potassium permanganate in 56% yield.[121] None of these reactions lead to 4-epimerization and hence would be the methods of choice, but which were not investigated in this project.

The synthesis of aldehyde **143** set the scene for the introduction of the first C-5 alkyl group. Since stereoinduction occurred in the second addition step, a decision had to be made as to which alkyl group, the methyl or *tert*-butyl group, was to be introduced first. The Felkin-Ahn stereoselective induction model was employed as an aid to reach this decision. After introduction of the first alkyl group to the aldehyde, the resulting secondary hydroxyl group would be oxidized to form a ketone. The introduction of the second alkyl group was anticipated to be via nucleophilic addition of an organometallic compound onto this ketone. As shown schematically in Figure 5.3, if chelation between the ring oxygen and the carbonyl carbon could be achieved with the metal used for the nucleophilic addition, then the largest group, namely C-3, would assume a perpendicular orientation to the carbonyl group, and the second alkyl group would be delivered to the least hindered face. This would result in a 5-*S* stereogenic centre as required if R_2 were sufficiently large, resulting in a 5S-axial configuration when the five-membered ribofuranose sugar was converted into a six-membered ribopyranose sugar, thereby forcing the ring to flip to the $^1C_4$ conformation (Figure 5.1).



5-*S* if $R_2 > R_1$

Figure 5.3: Felkin-Ahn chelation model for the introduction of the second alkyl group onto a ketone.

However, as depicted in Figure 5.4, if chelation could not be achieved, the electronegative C4-ring oxygen bond would now assume a perpendicular orientation with respect to the carbonyl group and R_2 would be delivered to the least hindered face. In this case, the 5-*S* stereogenic centre would now be achieved if R_2 were smaller than R_1.

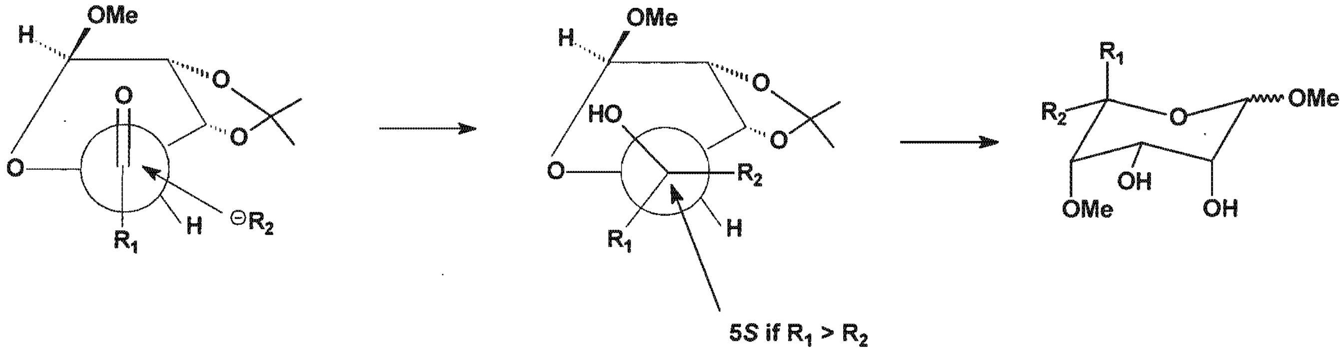
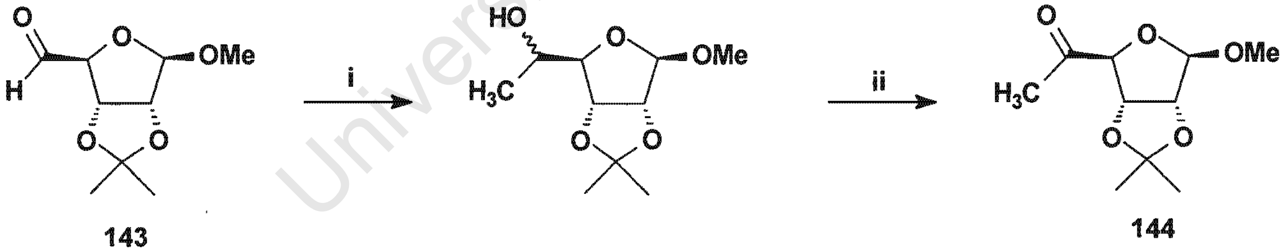


Figure 5.4: Felkin-Ahn non-chelation model for the introduction of the second alkyl group onto a ketone.

As the use of organometallics with chelating metals for nucleophilic addition to a carbonyl is common-place in organic synthesis, the decision reached was to introduce the smaller methyl group to the aldehyde and then introduce the larger *tert*-butyl group to the ketone.

Hence, **143** was treated with MeMgI in ether, and the crude material from this reaction was treated immediately with chromium trioxide and pyridine in CH_2Cl_2 to give ketone **144** in 81% yield (Scheme 5.3). This oxidant was used instead of a Swern oxidation in order to avoid 4-epimerisation as was observed in the synthesis of aldehyde **143**. In addition, no ketal hydrolysis was observed owing to the presence of the basic pyridine.

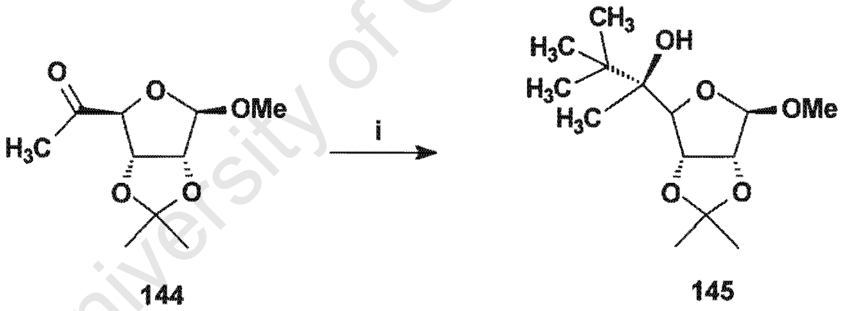


Scheme 5.3: *Reagents and conditions*: (i) MeMgI, Et_2O, reflux. (ii) CrO_3, pyridine, CH_2Cl_2, room temperature, 81% over 2 steps.

The data for ketone **144** were in accordance with those reported in the literature.[125]

The next step was the stereoselective introduction of a *tert*-butyl group to ketone **144**, which proved to be a significantly non-trivial step. The introduction of a *tert*-butyl group to a ketone has been achieved with *tert*-butyl Grignard reagents.[126] However, the treatment of **144** with the Grignard reagents *t*-BuMgBr or *t*-BuMgCl in refluxing THF gave no reaction, which led to the use of *t*-BuLi as a possible alternative. However, there were inherent problems in using *t*-BuLi, which included the fact that it is a powerful base and may possibly result in the formation of a C-4 enolate instead of acting as a nucleophile and that if nucleophilic addition did occur, the stereoselective outcome would be difficult to predict as lithium is not a strong chelating metal. In 1984, Imamoto et al. prepared organocerium reagents from organolithiums and anhydrous cerium (III) chloride, and demonstrated that they react cleanly with easily enolizable ketones to afford the addition products in good to excellent yields.[127] Cerium chloride has strong oxophilicity, and is capable of activating carbonyl components by coordination so Imamoto proposed that this is the driving force for cerium chloride promoted carbonyl additions. Another important aspect is that organocerium reagents are distinctly less basic than lithium reagents.

Following the method described by Imamoto, a *t*-butylcerium reagent was prepared at –78°C, to which ketone **144** was added (Scheme 5.4).[127] Pleasingly, after only 30 minutes, tlc revealed the development of a less-polar spot, as well as a small amount of starting material and numerous polar spots of insignificant quantity. Compound **145**, as a single diastereomer, was eventually isolated in 65% yield after column chromatography.



Scheme 5.4: *Reagents and conditions*: (i) $CeCl_3.7H_2O$, *t*-BuLi (1.7M in pentane), THF, -78°C, 65%.

The incorporation of the *t*-butyl methyl groups into **144** was clearly seen in the ^1H NMR of **145**, in which they resonated as a singlet at δ_H 1.04 and at δ_C 26.5 in the ^{13}C NMR spectrum. A very small coupling was observed between H-3 and H-4 ($J_{3,4}$ 1.2 Hz), which was not observed for **144**, possibly as a result of the added bulk at C-5 causing the substituents on C-4 and C-1 to move as far apart as possible, resulting in a slight puckering of the C-4 – O – C-1 bond. No coupling was observed between H-1 and H-2, once again confirming the β-anomer. Interestingly, the signal for H-3 (δ_H 5.12) appeared more downfield than that for H-1 (δ_H 4.92). This is in contrast to the corresponding 5,5-dimethyl compound **66** (Scheme 2.4, page 36), where the H-1 signal (δ_H 4.95) was downfield from the H-3 signal (δ_H 4.88). A comparison of H-4 signals revealed a downfield shift for H-4 (δ_H 4.38) which was seen at δ_H 4.09 in **66**.

The diastereoselective outcome of the reaction was based on the Felkin-Ahn chelation model as shown in Figure 5.5 involving cerium chloride chelation to the carbonyl oxygen causing C-3 to assume a perpendicular orientation, and the *t*-butyl group delivery to the least hindered face. This model predicts formation of the derived 5-*S* centre. X-ray structure analysis would ultimately confirm the configuration of **145** and if this is not possible, the use of NOE experiments could be employed

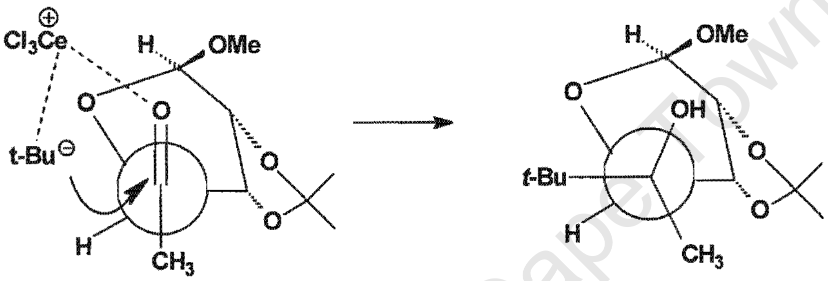
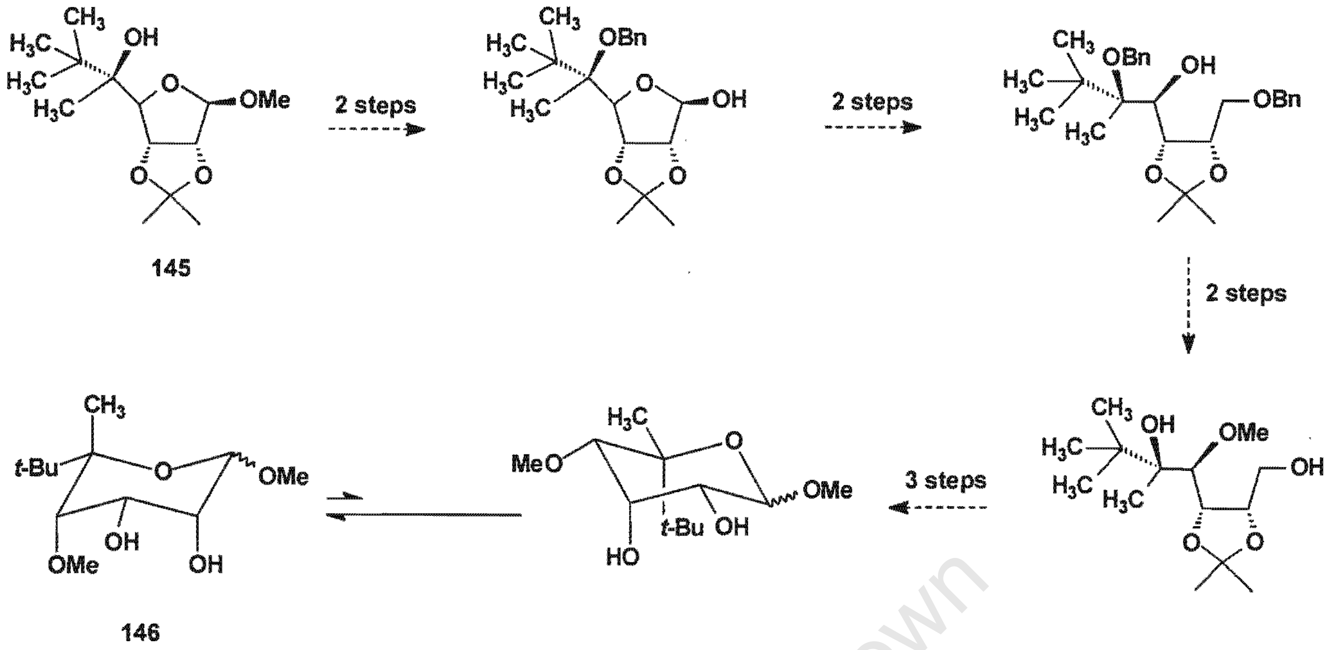


Figure 5.5: Felkin-Ahn chelation model for the nucleophilic addition of *t*-butyl to ketone **144**.

Since the completion of this synthesis was not within the timescale of this project, the synthetic effort was suspended at **145**. This intermediate represented the successful stereoselective introduction of two different alkyl groups, namely a methyl and *t*-butyl group, onto C-5. The strategy envisioned for the synthesis of methyl 5-*C*-*tert*-butyl-4-*O*-methyl-5-*C*-methyl-D-ribopyranoside **146** is depicted in Scheme 5.5. Following the strategy developed for the synthesis of 4-epi-noviose, tertiary alcohol **145** is predicted to be converted to **146** in nine predetermined steps.



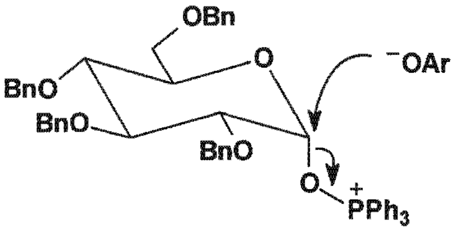
Scheme 5.5: Strategy for the synthesis of methyl 5-C-*tert*-butyl-4-*O*-methyl-5-*C*-methyl-D-ribopyranoside **146**.

5.2. Glycosidic coupling on model compounds

In Chapter 1, two methods employed in the glycosidic coupling of noviose to a coumarin moeity were introduced. The first of these methods was the coupling of glycosyl chloride **11** to 4-benzyloxy-7-hydroxy-8-methylcoumarin **10** with silver oxide and quinoline (Scheme 1.4, page 11).[21] The formation of β-glycosides by halide displacements from α-glycosyl halides with alcohols or phenols in the presence of insoluble silver carbonate or silver oxide is the basis of the well-established Koenigs-Knorr synthesis. Kinetic studies have indicated that displacement of the glycosyl halide occurs via the formation of an oxocarbenium ion, in which the silver oxide acts as a halogen-selective Lewis acid.[8] However, since the Walden inversion product predominates, it is probable that cleavage of the carbon-halide bond and delivery of the alcohol take place synchronously on the surface of the silver oxide, leading to configurational inversion.[8] Quinoline acts as both a solvent as well as a base that neutralizes HCl generated during glycosidation.

The second of these methods made use of Mitsunobu conditions to couple noviose to 4-benzhydryl-7-hydroxy-3-methylcoumarin **20** (Scheme 1.7, page 14).[16] As was discussed in Section 1.5, under these conditions, the free anomeric hydroxyl group was displaced by the

7-hydroxyl group of the coumarin moiety with inversion of configuration. As depicted in Scheme 5.6, the reaction presumably proceed via S_N2 displacement of an anomeric oxyphosphonium salt intermediate.[128]

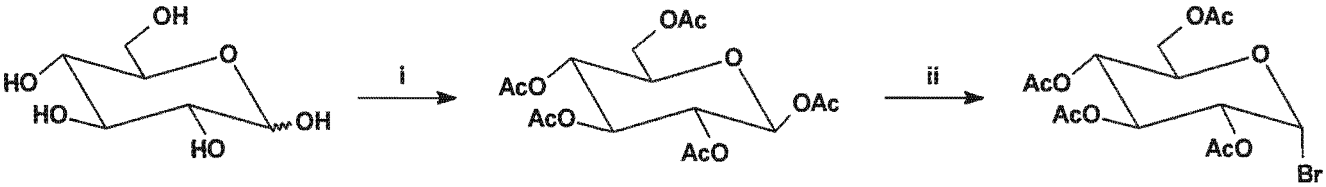


Scheme 5.6: Proposed mechanism for displacement of the anomeric hydroxyl group with a phenolic hydroxyl group.

The stereochemical outcome of the reaction is thus dependent on the anomeric configuration of the starting sugar. The advantages of using the Mitsunobu reaction are that the anomeric centre does not require activation prior to coupling, and that the sugar hydroxyl groups do not have to be completely protected since the anomeric hydroxyl group is regioselectively displaced over other hydroxyl groups on the sugar ring.

In order to become accustomed with these reaction conditions, a model study was performed in which D-glucose, protected either with acetate esters or benzyl ethers, was coupled to methyl salicylate. Methyl salicylate was selected on the grounds both of its availability and that the ester group is ortho to a phenolic hydroxyl group, thus providing steric and electron-withdrawing effects, features that are common to the coumarin moiety.

In preparation for glycosidic coupling, D-glucose was per-acetylated following a standard procedure[129] to furnish **147** in 74% yield, and converted into its α-glycosyl bromide **148** using HBr in acetic acid[130] in 71% yield (Scheme 5.7). The ^{1}H and ^{13}C NMR data, as well as the melting points for both **147** and **148**, corresponded to that published in the literature.[129,130]

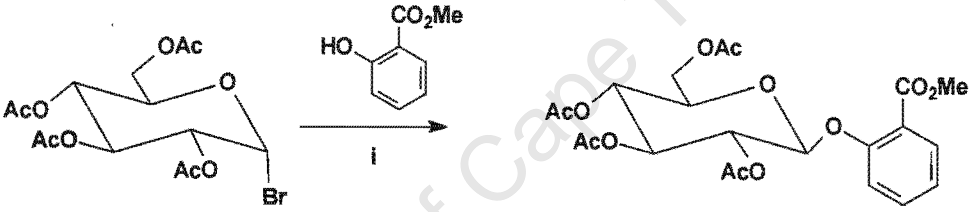


147

148

Scheme 5.7: *Reagents and conditions*: (i) CH_3CO_2Na, Ac_2O, reflux, 74%. (ii) HBr (30% in acetic acid), CH_2Cl_2, room temperature, 71%.

The coupling was achieved by dissolving glycosyl bromide **148** and methyl salicylate in freshly distilled quinoline and adding silver oxide, freshly prepared from silver nitrate and aqueous sodium hydroxide (Scheme 5.8).



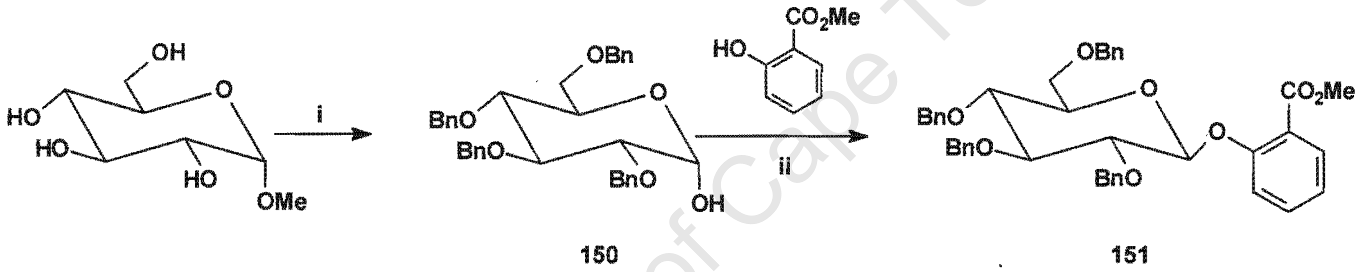
148

149

Scheme 5.8: *Reagents and conditions*: (i) Ag_2O, quinoline, room temperature, 43%.

After stirring for one hour at room temperature, acetic acid was added, and the silver salts removed by filtration. The filtrate was diluted with water and the solid product was simply filtered from the solution and recrystallized to afford **149** in 43% yield. On the grounds that this was a model study, the recrystallized yield was not fully optimised; however it could perhaps have been improved by extracting the organic matter from the aqueous phase and subjecting it to column chromatography. Further modifications could also include changing the temperature, time and solvent system. Tlc revealed the development of a single product with an R_f different to that of the starting materials and the ^1H NMR spectrum of **149** clearly revealed signals in both the aliphatic and aromatic regions as well as an upfield shift for H-1 from δ_H 6.60 in **149** to δ_H 5.12. These data provided plausible evidence that coupling had taken place. Compound **144** was designated as the β-anomer based on the observation that H-1, resonating as a doublet, exhibited a large coupling constant of 7.2 Hz. This is in keeping with Walden inversion of configuration which may be a result of neighbouring group participation from the C-2 acetate ester, which forces delivery of the nucleophile to the β-face, or as stated earlier, cleavage of the carbon-halide bond and delivery of the alcohol may take place synchronously on the surface of the silver oxide, leading to configurational inversion. The melting point and ^{1}H and ^{13}C NMR data corresponded to that published. [131]

The sugar donor selected for Mitsunobu coupling was tetra-benzylated glucopyranose **150**, which was prepared following a known literature procedure[132] and in 72% yield, by benzylating methyl α-D-glucoside, followed by hydrolysis of the anomeric methoxy group (Scheme 5.9). Tetra-benzylated **150** was assigned as the α-anomer on the basis of a small coupling constant of 3.0 Hz observed for H-1 resonating at δ 5.24 in the ^{1}H NMR spectrum of **150**. The melting point of **150** corresponded to that reported.[132]



Scheme 5.9: *Reagents and conditions*: (i) (a) NaH, BnBr, ^{n}Bu$_4$NI, 1,2-dimethoxyethane, reflux. (b) AcOH, 1 M H_2SO_4, reflux, 72% over 2 steps. (ii) DIAD, Ph$_3$P, CH_2Cl_2, 0°C, 53%.

The Mitsunobu reaction was performed with diisopropyl azodicarboxylate and triphenylphosphine in dichloromethane at 0°C (Scheme 5.9). The reaction was monitored by tlc and was typically complete within 30 minutes. Coupling product **151** was isolated by column chromatography in 53% yield. Once again, the yield was not fully optimised. As with coupling product **149**, evidence for coupling was provided by tlc and the ^{1}H NMR spectrum of **151**. A large coupling constant was observed for H-1 (d, $J_{1,2}$ 7.6 Hz) in the ^{1}H NMR spectrum, so **151** was assigned as the β-anomer. The S_N2 mechanism of the Mitsunobu reaction ensures inversion of configuration of C-1, and this was indeed observed. The ^{1}H and ^{13}C NMR data as well as the melting point were consistent with those published. [133]

In summary, 1-O-(2-carbomethoxyphenyl)-2,3,4,6-tetra-O-protected-α-D-glucopyranosides **149** and **151** were synthesized, in reasonable but unoptimised yields from two different methods, demonstrating the possibility of employing both for the coupling of novobiose or 4-epi-novobiose to oxazole-protected coumarin **142** (See Scheme 4.23, page 110). The Mitsunobu reaction would be the method of choice as coupling can take place without prior protection of the hydroxyl groups on the sugar moiety, and the anomeric hydroxyl group does not have to be activated by conversion to a halide group.

Chapter 6 : Summary and Conclusion

In conclusion, this thesis presents the significant progress made towards the synthesis of analogues of novenamine with modifications at C-4' and C-5'. The main achievements include (a) the synthesis of methyl novioside in an overall yield which is an improvement on all but one of the published syntheses, (b) the development of a synthetic route to the novel methyl 4-epi-novioside yielding the product in 36% overall yield in 11 steps from D-ribose, and also permitting access to noviose and its C-4 analogues, (c) establishing decisive procedures for the selective protection of the C-2 hydroxyl group of methyl 4-epi-novioside and subsequent introduction of the C-3 carbamoyl group, and (d) an improved synthesis of the coumarin moiety with new methodology introduced for the simultaneous protection of the 4-hydroxyl and 3-amino groups as an oxazole,

In the course of this work it was realized that the choice of methyl 2-O-benzoyl-3-O-carbamoyl-4-epi-novioside as the target precursor to the glycosyl donor required for synthesis of 4-epi-novenamine represented a severe limitation of the overall synthetic strategy. At a crucial stage the α- and β-methyl glycosides were not separable, and had to be "carried" through subsequent steps, thus adding unnecessary complications. In addition, selective anomeric deprotection was difficult, and attempts to circumvent this problem compromised the newly introduced carbamoyl group. The current anomeric protecting group strategy needs to be revised, and one alternative is to retain the anomeric acetate group of 2,3-O-isopropylidene-β-4-epi-noviosyl acetate (**91**, Scheme 2.9) by employing other methods for selective removal of the isopropylidene acetal. Another alternative is to exploit the easy availability of anomerically pure 2,3-O-isopropylidene-β-4-epi-noviose (**90**) by subjecting it to careful protection as an allyl, benzyl, or pentenyl ether, avoiding conditions that might lead to epimerization at C-1. Each of these could be selectively removed in the latter stages of the synthesis.

Progress was also made in this work in the synthesis of a C-5 analogue of 4-epi-noviose, whereby a methyl and *tert*-butyl group were introduced sequentially and stereoselectively to C-5 of D-ribose. This is a promising result, suggesting scope for synthesis of a range of unusual sugars, branched at their non-reducing terminus. Future work in this regard would include the completion of the synthesis of this C-5 analogue using a sequence similar to that established for 4-epinoviose, and evaluating its conformation from NMR and X-ray crystallographic data. The synthesis of these C-5 analogues of 4-epi-novenamine does, however, admittedly involve a rather lengthy procedure, requiring approximately 20 steps from D-ribose and may only be vindicated by a significant enhancement of biological activity and selectivity. This suggests that a molecular modelling approach to evaluating binding interactions in the active site of the target gyrase subunit should be embarked on at this stage in order to further validate this line of thinking.

Chapter 7 : Experimental Section

7.1. General methods

Thin layer chromatography (tlc) was performed on aluminium-backed plates coated with Merck silica gel F_{254}. Compounds on tlc plates were observed by a combination of a UV lamp, iodine vapour, or by spraying with a 2.5% anisaldehyde/sulphuric acid solution and baking at 180°C. Column chromatography was carried out using silica gel 60 mesh (Merck 7734), using ethyl acetate and hexane as eluents unless otherwise stated. For reactions that required extractive work-up the organic layers were dried by stirring with anhydrous magnesium sulphate follwed by filtration.

Melting points were measured on a Reichert Jung hot-stage microscope and are uncorrected. Infrared spectra were recorded either in chloroform solutions on sodium chloride plates or as KBr pellets on a Perkin-Elmer Paragon 1000 FT-IR spectrometer over the range 4000 to 600 cm^{-1}. Optical rotations were obtained using a Perkin Elmer 141 polarimeter at 20°C. The concentration *c* refers to g/100 ml. Elemental analyses for C and H were carried out using a Fisons EA 110 CHN elemental analyser. High-resolution mass spectrometry were obtained using VG70-SEQ micromass spectrometer.

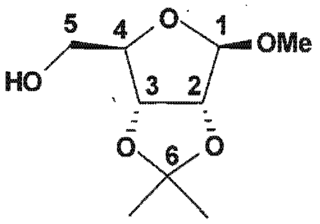
^1H or ^{13}C NMR were recorded on either a Varian VXR-300 (at 300.08 MHz for ^1H and 75.5 MHz for ^{13}C) or a Varian Unity 400 (at 399.95 MHz for ^1H and 100.58 MHz for ^{13}C) spectrometer in deuteriochloroform unless otherwise stated. Chemical shifts are quoted using residual chloroform (δ 7.24 in ^1H NMR and δ 77.00 in ^{13}C NMR) or residual DMSO ((δ 2.50 in ^1H NMR and δ 39.52 in ^{13}C NMR) as internal standards. All chemical shifts are reported in ppm and *J* values are quoted in Hz.

All solvents were freshly distilled. Diethyl ether and tetrahydrofuran were distilled under nitrogen and dried over sodium wire with benzophenone. Dichloromethane was dried and distilled over phosphorous pentoxide under nitrogen. Other reagents were purified according to standard procedures.[134]

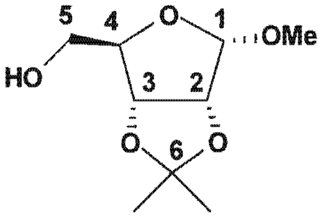
All reagents were purchased from Aldrich or Merck. Low temperature reactions were carried out using dry ice in acetone or liquid nitrogen in either chloroform or acetone.

7.2. Compounds

Methyl 2,3-*O*-isopropylidene-D-ribofuranoside: β-anomer (64β) and α-anomer (64α)



64β



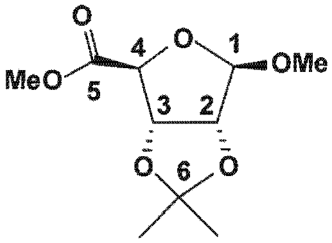
64α

D-Ribose (10.00 g, 66.6 mmol) was dissolved in methanol (200 ml) and cooled to 0°C. H_2SO_4 (3.60 ml, 66.6 mmol) was added dropwise and the mixture stirred at 0°C for 4 hours. Acetone (100 ml) was then added and the mixture brought slowly to room temperature with stirring over 2 hours. The reaction was neutralized with KOH (14 g) dissolved in a minimum volume of MeOH, filtered through Celite and the filtrate evaporated under reduced pressure. The resulting oil was dissolved in EtOAc, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude material (12.99 g) was chromatographed (150 g silica) using 60% ethyl acetate in hexane as eluent, to afford first the β- then the α-anomer as colourless oils.

β-Anomer: (**64β**) (10.99 g; 81%); $[\alpha]_D$ -68° (*c* 1.00, $CHCl_3$) (lit.[46] $[\alpha]_D$ –82°C (c 2.00, $CHCl_3$)); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3441 (-OH stretch); δ_H (400 MHz, $CDCl_3$): 1.31, 1.47 (6H, 2 x s, 2 x CH_3), 3.05 (1H, br, -OH), 3.42 (3H, s, -OMe), 3.58, 3.70 (2H, m, H-5), 4.41 (1H, t, *J* 2.8 Hz, H-4), 4.57 (1H, d, *J* 6.0 Hz, H-2), 4.82 (1H, d, *J* 6.0 Hz, H-3), 4.96 (1H, s, H-1); δ_C (100 MHz, $CDCl_3$): 24.7, 26.4 (2 x CH_3), 55.5 (-OMe), 64.0 (C-5), 81.5 (C-3), 85.8 (C-2), 88.4 (C-4), 110.0 (C-1), 112.1 (C-6); HRMS: *m/z* 189.0764 [(M^+- CH_3), $C_8H_{13}O_5$ requires 189.0763].

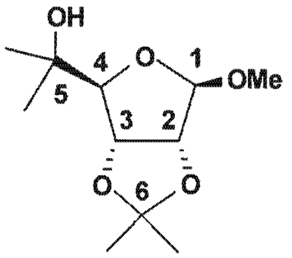
α-Anomer: (**64α**) (0.55 g; 4%); $[\alpha]_D$ +88° (*c* 1.20, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3600 (-OH stretch); δ_H (400 MHz, $CDCl_3$, D_2O wash): 1.32 (3H, s, CH_3), 1.53 (3H, s, CH_3), 3.43 (3H, s, -OMe), 3.66 (1H, dd, *J* 4.0 and 12.0 Hz, H-5), 3.77 (1H, dd, *J* 3.6 and 12.0 Hz, H-5), 4.13 (1H, m, H-4), 4.63 (2H, m, H-3 and H-2), 4.89 (1H, m, H-1); δ_C (100 MHz, $CDCl_3$): 25.7, 25.9 (2 x CH_3), 55.2 (-OMe), 62.7 (C-5), 80.3, 80.9 (C-2, C-3), 81.6 (C-4), 102.8 (C-1), 115.4 (C-6); HRMS: *m/z* 189.0756 [(M^+- CH_3), $C_8H_{13}O_5$ requires 189.0763].

Methyl (methyl-2,3-*O*-isopropylidene-β-D-ribofuranosid)uronate (65)



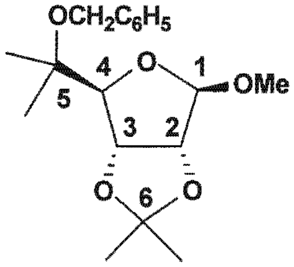
A solution of **64β** (8.00 g, 39.2 mmol) in CCl_4/acetonitrile (1:1, 120 ml) was added to a vigorously stirred aqueous solution of $NaIO_4$ (10% w/v, 250 ml) and $RuO_4.H_2O$ (12 mg; 0.1 mmol), and the black biphasic mixture stirred overnight at room temperature.[52] The reaction was extracted with EtOAc (x 3), the combined organic extracts dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The black residue was dissolved in DMF (10 ml) and treated with MeI (12.3 ml, 195.9 mmol) and anhydrous K_2CO_3 (10.83 g, 78.4 mmol). After 1 hour the reaction mixture was neutralized with 1M HCl, and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography (60 g silica; 10% EtOAc in hexane) to yield the title compound (**65**) (8.47 g, 93%) as a colourless oil. A sample for analysis was obtained by vacuum distillation: bp 90°C, 0.1 mm Hg; $[\alpha]_D$ –67.8° (*c* 1.28, $CHCl_3$), (lit.[135,53] bp 116-118°C, 0.3 mm Hg; $[\alpha]_D$ –72.7° (*c* 2.00, $CHCl_3$)); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1735 (C=O stretch); δ_H (300 MHz, $CDCl_3$): 1.30 (3H, s, CH_3), 1.46 (3H, s, CH_3), 3.36 (3H, s, -COOMe), 3.74 (3H, s, -OMe), 4.51 (1H, dd, *J* 0.3 and 6.0 Hz, H-2), 4.58 (1H, s, H-4), 5.00 (1H, s, H-1), 5.19 (1H, dd, *J* 1.2 and 6.0 Hz, H-3); δ_C (75.5 MHz, $CDCl_3$): 24.9, 26.3 (2 x CH_3), 52.1 (-O$\underline{C}H_3$), 55.2 (COO$\underline{C}H_3$), 82.1 (C-3), 83.5 (C-4), 84.3 (C-2), 109.3 (C-1), 112.7 (C-6), 170.6 (C-5); HRMS: *m/z* 231.0852 [(M^+-H), $C_{10}H_{15}O_6$ requires 231.0869]; Found: C, 51.67%; H, 7.00%; $C_{10}H_{16}O_6$ requires C, 51.72%; H, 6.94%.

Methyl 2,3-*O*-isopropylidene-5,5-di-*C*-methyl-β-D-ribofuranoside (66)



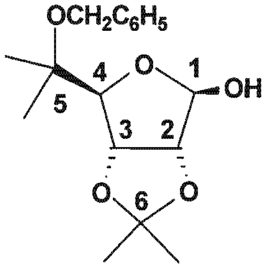
MeI (11.4 ml, 182.3 mmol) was added dropwise to a slurry of magnesium (3.56 g, 146.5 mmol) in diethyl ether (200 ml) at 0°C. The mixture was refluxed for 1 hour, then cooled to 0°C. A solution of **65** (8.50 g, 36.6 mmol) in diethyl ether (100 ml) was added dropwise and the mixture refluxed for 4.5 hours. The mixture was once again cooled to 0°C and quenched with aqueous NH_4Cl. The excess diethyl ether was evaporated under reduced pressure, and the remaining crude material extracted with EtOAc (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography (100 g silica, 50% EtOAc in hexane) to yield **66** (7.83 g, 92%) as a colourless oil. A sample for analysis was obtained by vacuum distillation: bp 92°C, 0.3 mm Hg, mp. 33-34°C; $[\alpha]_D$ –54.9° (*c* 1.00, $CHCl_3$), (lit.[43] bp 90°C, 0.1 mm Hg; mp. 35.5-36.5; $[\alpha]_D$ –64°C (c 3.750, $CHCl_3$)); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3442 (-OH stretch); δ_H (300 MHz, $CDCl_3$): 1.24 (6H, s, 2 x CH_3), 1.31, 1.46 (6H, 2 x s, 2 x CH_3), 3.33 (1H, br, -OH), 3.46 (3H, s, -OMe), 4.09 (1H, s, H-4), 4.56 (1H, d, *J* 6.0 Hz, H-2), 4.88 (1H, d, *J* 6.0 Hz, H-3), 4.95 (1H, s, H-1); δ_C (75.5 MHz, $CDCl_3$): 24.7, 25.2, 26.4, 27.0 (4 x CH_3), 55.9 (-O$\underline{C}H_3$), 70.1 (C-5), 80.9 (C-3), 85.8 (C-2), 95.2 (C-4), 110.6 (C-1), 112.0 (C-6); HRMS: *m/z* 217.1077 [(M^+-CH_3), $C_{10}H_{17}O_5$ requires 217.1076].

Methyl 5-O-benzyl-2,3-*O*-isopropylidene-5,5-di-*C*-methyl-β-D-ribofuranoside (82)



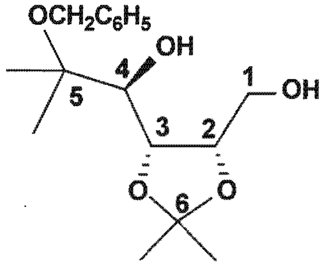
A solution of **66** (12.00 g, 51.7 mmol) in THF (80 ml) was added to a slurry of NaH (60% in oil, 2.48 g, 62.0 mmol) in THF (20 ml) and the resulting mixture refluxed for 1 hour. After cooling to room temperature, BnBr (8.37 ml, 70.4 mmol) and $^{n}Bu_4NI$ (2.10 g, 5.7 mmol) were added and the mixture was refluxed for a further 2 hours. The excess NaH was quenched with MeOH, the organic solvents evaporated under reduced pressure and the remainder extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was chromatographed on silica gel (150 g) using 10% EtOAc in hexane as eluent to afford **82** (16.40 g, 98%) as a colourless oil. A sample for analysis was obtained by vacuum distillation: bp 133°C, 0.6 mm Hg; $[\alpha]_D$ –40.2° (*c* 1.40, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1497 (C=C aromatic stretch); δ_H (300 MHz, $CDCl_3$): 1.30, 1.31, 1.34, 1.53 (12H, 4 x s, 4 x CH_3), 3.39 (3H, s, -OMe), 4.14 (1H, d, *J* 2.4 Hz, H-4), 4.50 – 4.58 (3H, m, H-2 and $-OC\underline{H}_2C_6H_5$), 4.87 (1H, dd, *J* 2.4 and 6.0 Hz, H-3), 5.03 (1H, d, *J* 1.2 Hz, H-1), 7.22 – 7.40 (5H, m, $-OCH_2C_6\underline{H}_5$); δ_C (75.5 MHz, $CDCl_3$): 21.3, 22.6, 25.3, 27.0 (4 x CH_3), 55.2 ($-O\underline{C}H_3$), 64.1 ($-O\underline{C}H_2C_6H_5$), 75.6 (C-5), 80.6 (C-3), 85.7 (C-2), 93.3 (C-4), 110.6 (C-1), 112.4 (C-6), 127.0, 127.1, 128.2, 139.8 ($-OCH_2\underline{C}_6H_5$); HRMS: *m/z* 307.1539 [(M^+-CH_3), $C_{17}H_{23}O_5$ requires 307.1546].

5-O-Benzyl-2,3-*O*-isopropylidene-5,5-di-*C*-methyl-β-D-ribofuranose (83)



HCl (1M, 73 ml) was added to a stirred solution of **82** (4.69 g, 14.5 mmol) in 1,4-dioxane (30 ml) and the mixture heated at 60°C for 3 hours. After cooling to room temperature the mixture was neutralized with aqueous $NaHCO_3$ and extracted with EtOAc (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The oily residue was dissolved in acetone (130 ml) and cooled to 0°C. Concentrated H_2SO_4 (0.77 ml, 14.5 mmol) was added dropwise and the mixture stirred at 0°C for 1 hour. The excess H_2SO_4 was neutralized with aqueous Na_2CO_3 and the acetone evaporated under reduced pressure. The resultant slurry was extracted with EtOAc (x 3), the combined organic extracts dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography (100 g silica; 20% EtOAc in hexane) to yield **83** (3.60 g, 81%) as colourless crystals: mp. 55-57°C (hexane); $[\alpha]_D$ +25.5° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3392 (-OH stretch); δ_H (300 MHz, $CDCl_3$): 1.31, 1.41, 1.44, 1.47 (12H, 4 x s, 4 x CH_3), 4.01 (1H, d, *J* 0.9 Hz, H-4), 4.35 (1H, d, *J* 6.0 Hz, H-2), 4.48 (2H, s, $-OC\underline{H}_2C_6H_5$), 4.56 (1H, d, *J* 12.3 Hz, -OH), 4.89 (1H, dd, *J* 0.9 and 6.0 Hz, H-3), 5.20 (1H, d, *J* 12.3 Hz, H-1), 7.26 – 7.40 (5H, m, $-OCH_2C_6\underline{H}_5$); δ_C (75.5 MHz, $CDCl_3$): 24.7, 25.2, 26.4, 27.0 (4 x CH_3), 55.9 ($-O\underline{C}H_2C_6H_5$), 76.6 (C-5), 80.6 (C-3), 87.5 (C-2), 94.6 (C-4), 103.7 (C-1), 111.9 (C-6), 128.1, 128.3, 128.8, 137.0 ($-OCH_2\underline{C}_6H_5$); HRMS: *m/z* 293.1389 [(M^+-CH_3), $C_{16}H_{21}O_5$ requires 293.1389]; Found: C, 66.31%; H, 7.99%; $C_{17}H_{24}O_5$ requires C, 66.21%; H, 7.85%.

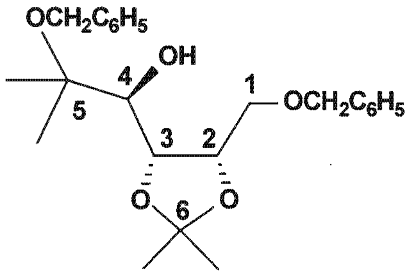
5-*O*-Benzyl-2,3-*O*-isopropylidene-5,5-di-*C*-methyl-D-ribitol (84)[††]



A mixture of **83** (3.00 g, 9.7 mmol) in THF (50 ml) was added to a slurry of $LiAlH_4$ (0.55 g, 14.6 mmol) in THF (50 ml) at 0°C. The mixture was stirred for 1 hour at 0°C before H_2O (0.55 ml) was added. After the addition of 1M NaOH (1.10 ml) and further H_2O (1.10 ml), the mixture was stirred at room temperature for 1 hour. After removing the lithium salts by filtration through Celite, the organic solvent was evaporated under reduced pressure. The crude material (3.15 g) was chromatographed on silica gel (80 g) using 20% ethyl acetate in hexane to afford **84** (2.84 g, 94%) as a colourless oil. A sample for analysis was obtained by vacuum distillation: bp 155°C, 0.5 mm Hg; $[\alpha]_D$ –20.2° (*c* 1.29, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3484 (-OH stretch); δ_H (400 MHz, $CDCl_3$): 1.31, 1.35 (6H, 2 x s, 2 x CH_3), 1.43 (6H, s, 2 x CH_3), 3.10 (2H, br. s, 2 x -OH), 3.68 (1H, dd, *J* 4.8 and 11.6 Hz, H-1), 3.71 (1H, d, *J* 9.3 Hz, H-4), 3.89 (1H, dd, *J* 7.6 and 11.6 Hz, H-1), 4.19 (1H, dd, J 6.2 and 9.3 Hz, H-3), 4.36 (1H, m, H-2), 4.49 (2H, s, $-OC\underline{H}_2C_6H_5$), 7.26 – 7.37 (5H, m, $-OCH_2C_6\underline{H}_5$); δ_C (100 MHz, $CDCl_3$): 19.3, 23.0, 25.3, 27.9 (4 x CH_3), 61.1 (C-1), 63.5 ($-O\underline{C}H_2C_6H_5$), 73.9 (C-4), 76.3 (C-3), 77.7 (C-2), 78.6 (C-5), 108.5 (C-6), 127.5, 127.6, 128.5, 138.6 ($-OCH_2\underline{C}_6H_5$); HRMS: *m/z* 295.1546 [(M^+-CH_3), $C_{16}H_{23}O_5$ requires 295.1546]; Found: C, 65.52%; H, 8.37%; $C_{16}H_{23}O_5$ requires C, 65.78%; H, 8.44%.

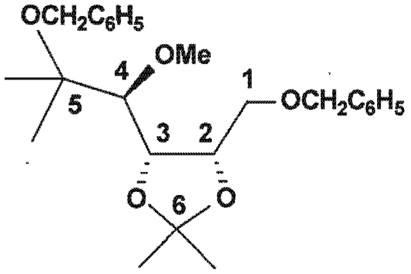
[††] The nomenclature for the open chain derivatives is based on the parent sugar based on "Tentative Rules for Carbohydrate Nomenclature, Part I, 1969, published by the IUPAC Commission on the Nomenclature of Organic Chemistry and the IUPAC-IUB Commission on Biochemical Nomenclature.

1,5-Di-*O*-benzyl-2,3-*O*-isopropylidene-5,5-di-*C*-methyl-D-ribitol (86)



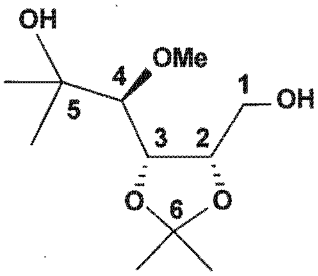
Diol **84** (7.72 g, 24.9 mmol) was dissolved in THF (100 ml), added to NaH (60% in oil, 1.10 g, 27.4 mmol) in THF (20 ml) and the mixture stirred for 30 minutes at room temperature. BnBr (3.25 ml, 27.4 mmol) was added and the reaction stirred at room temperature for a further 12 hours before being neutralized with aqueous NH_4Cl, diluted with water and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was chromatographed on silica gel (150 g) starting with hexane and increasing the polarity to 20% ethyl acetate in hexane to afford **86** (9.27 g, 94%) as a colourless oil: $[\alpha]_D$ –20.9° (*c* 1.30, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3540 (-OH stretch); δ_H (300 MHz, $CDCl_3$): 1.31, 1.36, 1.39, 1.44 (4 x 3H, 4 x s, 4 x CH_3), 3.32 (1H, d, *J* 3.3 Hz, –OH), 3.57 (1H, dd, *J* 6.3 and 9.6 Hz, H-1), 3.61 (1H, dd, *J* 3.3 and 9.6 Hz, H-4), 3.83 (1H, dd, *J* 5.3 and 9.6 Hz, H-1), 4.22 (1H, dd, *J* 5.7 and 9.6 Hz, H-3), 4.44 (1H, quart, *J* 5.3, 5.7 and 6.3 Hz, H-2), 4.50 (2H, s, -$O\underline{CH_2}C_6H_5$), 4.60 (2H, d, *J* 1.8 Hz, -$O\underline{CH_2}C_6H_5$), 7.34 (10H, m, 2 x -$OCH_2C_6\underline{H_5}$); δ_C (75.5 MHz, $CDCl_3$): 20.5, 22.9, 25.6, 28.1 (4 x CH_3), 63.7 (-$O\underline{C}H_2C_6H_5$), 69.2 (C-1), 73.5 (-$O\underline{C}H_2C_6H_5$), 73.6 (C-4), 76.6 (C-3), 77.2 (C-2), 77.6 (C-5), 108.8 (C-6), 127.0, 127.1, 127.5, 127.6, 128.2, 128.3, 138.0, 139.5 (-$OCH_2\underline{C_6}H_5$); HRMS: *m/z* 400.2224 [(M^+), $C_{24}H_{32}O_5$ requires 400.2249].

1,5-Di-*O*-benzyl-2,3-*O*-isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-D-ribitol (87)



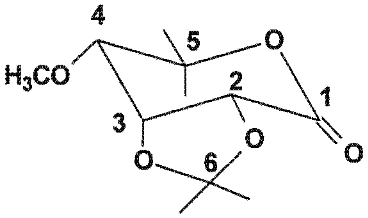
Compound **86** (2.90 g, 7.2 mmol) was dissolved in THF (80 ml), added to NaH (60% in oil, 0.87 g, 14.4 mmol) and the mixture refluxed for 2 hours. After cooling to room temperature, MeI (0.91 ml, 14.4 mmol) was added and the mixture refluxed for a further 1.5 hours. The excess NaH was quenched with MeOH, the organic solvents evaporated under reduced pressure and the remainder extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material (3.44 g) was chromatographed on silica gel (60 g) using ethyl acetate - hexane (10 – 20%) as eluent to afford **87** (2.93 g, 98%) as a colourless oil: $[\alpha]_D$ –44.6° (*c* 1.10, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1602 (C=C aromatic stretch); δ_H (300 MHz, $CDCl_3$): 1.29, 1.33, 1.35, 1.49 (4 x 3H, 4 x s, 4 x CH_3), 3.46 (3H, s, -OMe), 3.47 (1H, d, *J* 4.2 Hz, H-4), 3.57 (1H, dd, *J* 8.1 and 10.6 Hz, H-1), 3.78 (1H, dd, *J* 2.7 and 10.6 Hz, H-1), 4.31 – 4.41 (2H, m, H-2 and H-3), 4.48 (2H, s, $-OC\underline{H}_2C_6H_5$), 4.52 (2H, s, $-OC\underline{H}_2C_6H_5$), 7.34 (10H, m, 2 x $-OCH_2C_6\underline{H}_5$); δ_C (75.5 MHz, $CDCl_3$): 22.1, 22.5, 25.6, 28.3 (4 x CH_3), 61.3 (-OMe), 63.8 ($-O\underline{C}H_2C_6H_5$), 70.4 (C-1), 73.1 ($-O\underline{C}H_2C_6H_5$), 76.7, 78.0 (C-2, C-3), 78.5 (C-5), 85.3 (C-4), 107.4 (C-6), 127.1, 127.2, 127.3, 127.7, 128.1, 128.2, 138.6, 139.5 ($-OCH_2\underline{C}_6H_5$); HRMS: *m/z* 399.2161 [(M^+-CH_3), $C_{24}H_{31}O_5$ requires 399.2171].

2,3-*O*-Isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-D-ribitol (88)



10% Pd/C (0.99 g, 0.9 mmol) was added to compound **87** (2.93 g, 7.1 mmol) in EtOH (100 ml) and the solution stirred overnight at room temperature in a positive pressure of H_2 using a balloon. The excess solvent was evaporated after removing the Pd/C by filtration through Celite. The crude material (1.83 g) was chromatographed on silica gel (17 g) using ethyl acetate - hexane (60%) as eluent to afford **88** (1.65 g, 99%) as a colourless oil: ($[\alpha]_D$ -20.1° (*c* 1.20, $CHCl_3$; IR ν_{max} (CH_2Cl_2) /cm^{-1} 3521 (-OH stretch); δ_H (300 MHz, $CDCl_3$): 1.25, 1.26, 1.36, 1.48 (4 x 3H, 4 x s, 4 x CH_3), 3.27 (1H, d, *J* 7.7 Hz, H-4), 3.54 (3H, s, -OMe), 3.70 (1H, dd, *J* 7.0 and 11.4 Hz, H-1), 3.77 (1H, dd, *J* 5.2 and 11.4 Hz, H-1), 4.22 (1H, dd, *J* 5.9 and 7.7 Hz, H-3), 4.33 (1H, m, H-2); δ_C (75.5 MHz, $CDCl_3$): 25.1, 25.4, 27.3, 28.0 (4 x CH_3), 61.2 (-OMe), 61.6 (C-1), 73.4 (C-5), 77.2 (C-3), 78.1 (C-2), 84.6 (C-4), 107.4 (C-6); HRMS: *m/z* 219.1231 [(M^+-CH_3), $C_{10}H_{19}O_5$ requires 219.1233].

2,3-*O*-Isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-D-ribolactone (89)



1. Via TPAP oxidation[136]

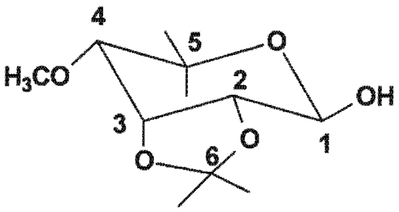
A solution of tetrapropylammonium perruthenate (0.01 g, 0.04 mmol), N-methylmorpholine-N-oxide (0.16 g, 1.2 mmol), 4Å molecular sieves (0.21 g) and **88** (0.10 g, 0.4 mmol) in CH_2Cl_2 (10 ml) was stirred for 4 hours at room temperature. The excess solvent was evaporated and the crude material filtered through a short pad of silica (5 g), eluting with 50% EtOAc in hexane to give **89** (0.07 g, 72%) as colourless crystals: mp. 113-115°C (ethyl acetate/hexane);

2. Via Swern oxidation

A solution of DMSO (1.10 ml, 15.4 mmol) in CH_2Cl_2 (10 ml) was cooled to –78°C and oxalyl chloride (0.67 ml, 7.7 mmol) was added. After 5 minutes, **88** (0.82 g, 3.5 mmol), dissolved in CH_2Cl_2 (30 ml), was added and the mixture stirred at –78°C for 30 minutes, after which triethylamine (4.87 ml, 34.9 mmol) was added and the mixture kept at –78°C for 5 minutes before being brought to room temperature. The reaction was then quenched with aqueous Na_2CO_3 and extracted with CH_2Cl_2 (x 3). The organic extract was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was chromatographed on silica gel (60 g) using ethyl acetate - hexane (20%) as eluent to afford **89** (0.65 g, 81%) as colourless crystals: mp. 113-115°C (ethyl acetate/hexane);

$[\alpha]_D$ +79.6° (*c* 1.10, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1748 (C=O stretch); δ_H (300 MHz, $CDCl_3$): 1.38, 1.40, 1.50, 1.55 (4 x 3H, 4 x s, 4 x CH_3), 3.41 (1H, d, *J* 3.9 Hz, H-4), 3.57 (3H, s, -OCH_3), 4.63 (1H, d, *J* 8.7 Hz, H-2), 4.80 (1H, dd, *J* 3.9 and 8.7 Hz, H-3); δ_C (75.5 MHz, $CDCl_3$): 24.8, 25.4, 25.8, 25.8 (4 x CH_3), 61.1 (-OCH_3), 71.3 (C-3), 74.0 (C-2), 78.4 (C-4), 82.9 (C-5), 111.4 (C-6), 169.0 (C-1); HRMS: *m/z* 231.1250 [$(M+H)^+$, $C_{11}H_{19}O_5$ requires 231.1233]; Found: C, 57.63%; H, 7.82%; $C_{11}H_{18}O_5$ requires C, 57.38%; H, 7.88%.

2,3-*O*-Isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranose (90)



1. From **88**:

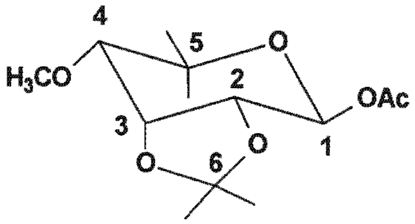
A solution of DMSO (1.10 ml, 15.5 mmol) in CH_2Cl_2 (70 ml) was cooled to –78°C and oxalyl chloride (0.67 ml, 7.8 mmol) was added. After 5 minutes, **88** (1.65 g, 7.0 mmol), dissolved in CH_2Cl_2 (70 ml), was added and the mixture stirred at –78°C for 15 minutes, after which triethylamine (4.91 ml, 35.2 mmol) was added and the mixture kept at –78°C for 5 minutes before being brought slowly to 0°C. The reaction was quenched with aqueous Na_2CO_3, brought to room temperature, and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material (2.10 g) was chromatographed on silica gel (60 g) using ethyl acetate - hexane (30%) as eluent to afford **90** (1.33 g, 81%) as the major product as colourless crystals: mp 91-93°C (ethyl acetate/hexane);

2. From **89**:

Lactone **89** (0.64g, 2.8 mmol) was dissolved in toluene (20 ml) and cooled to –78°C. DIBALH (1M in toluene, 3.34 ml) was added slowly over a period of 15 minutes and the mixture left for a further 15 minutes at –78°C. The reaction was quenched with aqueous NH_4Cl, brought to room temperature, and extracted with EtOAc (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material (0.45 g) was chromatographed on silica gel (15 g) using ethyl acetate - hexane (50%) as eluent to afford **90** (0.44 g, 68%) as the major product as colourless crystals: mp 91-93°C (ethyl acetate/hexane).

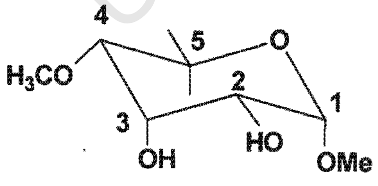
$[\alpha]_D$ +1.6° (*c* 1.10, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3392 (-OH stretch); δ_H (300 MHz, $CDCl_3$): 1.31, 1.37, 1.39, 1.54 (4 x 3H, 4 x s, 4 x CH_3), 3.32 (1H, d, *J* 4.1 Hz, H-4), 3.40 (1H, d, *J* 6.0 Hz, -OH), 3.50 (3H, s, -OCH_3), 3.97 (1H, t, *J* 6.0 Hz, H-2), 4.63 (1H, dd, *J* 4.1 and 6.0 Hz, H-3), 5.02 (1H, t, *J* 6.0 Hz, H-1); δ_C (75.5 MHz, $CDCl_3$): 21.3, 25.3, 27.3, 29.6 (4 x CH_3), 58.7 (-OMe), 72.1 (C-3), 75.4 (C-2), 77.7 (C-5), 80.8 (C-4), 92.2 (C-6), 110.5 (C-1); HRMS: *m/z* 232.1304 [(M^+), $C_{11}H_{20}O_5$ requires 232.1311]; Found: C, 57.30%; H, 8.64%; $C_{11}H_{20}O_5$ requires C, 56.88%; H, 8.68%.

1-*O*-Acetyl-2,3-*O*-isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranose (91)

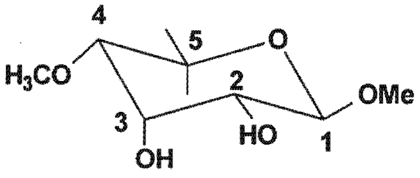


Compound **90** (1.31 g, 5.7 mmol) was dissolved in CH_2Cl_2 and acetic anhydride (0.80 ml, 8.5 mmol), triethylamine (1.18 ml, 8.5 mmol) and DMAP (0.07 g, 0.6 mmol) added. The reaction was stirred at room temperature for 20 minutes, quenched with aqueous NH_4Cl and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography (100 g silica, 20% EtOAc/hexane) to yield **91** (1.22 g, 79%) as a colourless oil; $[\alpha]_D$ –21.8° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1750 (C=O stretch); δ_H (300 MHz, $CDCl_3$): 1.30, 1.38, 1.46, 1.56 (12H, 4 x s, 4 x CH_3), 2.11 (3H, s, $-OCOCH_3$), 3.30 (1H, d, *J* 3.9 Hz, H-4), 3.52 (3H, s, -OMe), 4.11 (1H, t, *J* 6.0 Hz, H-2), 4.69 (1H, dd, *J* 3.9 and 6.0 Hz, H-3), 5.93 (1H, d, *J* 6.0 Hz, H-1); δ_C (75.5 MHz, $CDCl_3$): 21.0 ($-CO\underline{C}H_3$), 21.2, 25.4, 27.2, 29.3 (4 x CH_3), 58.6 ($-O\underline{C}H_3$), 72.0 (C-3), 75.4 (C-2), 76.4 (C-5), 80.6 (C-4), 90.7 (C-6), 110.9 (C-1), 169.3 ($-O\underline{C}OCH3$); HRMS: *m/z* 259.1162 [(M^+-CH_3), $C_{12}H_{19}O_6$ requires 259.1182]

Methyl 4-*O*-methyl-5,5-di-*C*-methyl-D-ribopyranoside (92)



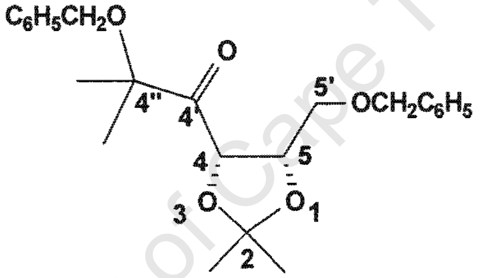
92α



92β

Dowex 50WX2-200 (0.48 g, 110% w/w) was added to compound **91** (0.44 g, 1.6 mmol) dissolved in 95% methanol (20 ml) and the mixture refluxed for 12 hours. The ion-exchange resin was filtered off and rinsed with methanol. The filtrate was evaporated under reduced pressure and the residue purified by chromatography (17 g silica, 80% EtOAc/hexane) to yield **92** (0.31 g, 95%) as an inseparable 14:1 mixture of α and β anomers respectively: IR ν_{max} (CH_2Cl_2) /cm^{-1} 3572 (-OH stretch); δ_H: major α -anomer (300 MHz, $CDCl_3$, D_2O wash): 1.31, 1.33 (6H, 2 x s, 2 x CH_3), 3.11 (1H, d, *J* 3.3 Hz, H-4), 3.43 (3H, s, -OMe), 3.51 (3H, s, -OMe), 3.52 (1H, dd, *J* 3.3 and 4.8 Hz H-2), 4.23 (1H, t, *J* 3.3 Hz, H-3), 4.70 (1H, d, *J* 4.8 Hz, H-1); δ_C: major α -anomer (75.5 MHz, $CDCl_3$): 23.7, 27.8 (2 x CH_3), 55.9, 60.4 (2 x $-O\underline{C}H_3$), 65.5 (C-3), 71.2 (C-2), 76.3 (C-5), 85.3 (C-4), 100.9 (C-1); δ_H: minor β-anomer ((300 MHz, $CDCl_3$, D_2O wash): 1.26, 1.38 (6H, 2 x s, 2 x CH_3), 3.07 (1H, d, *J* 3.0 Hz, H-4), 3.49 (3H, s, -OMe), 3.58 (3H, s, -OMe), 3.76 (1H, dd, *J* 3.0 and 7.5, H-2), 3.95 (1H, t, *J* 3.0 Hz, H-3), 4.50 (1H, d, *J* 7.5 Hz, H-1); HRMS: *m/z* 175.0964 [(M^+-OCH_3), $C_8H_{15}O_4$ requires 175.0970].

(*4S,5S*)-5-Benzyloxymethyl-4-(2-benzyloxy-2-methyl-1-oxopropyl)-2,2-dimethyl-1,3-dioxolane (93)[‡‡]

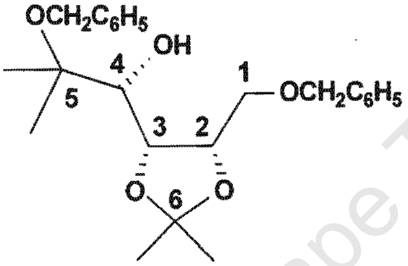


DMSO (0.39 ml, 5.5 mmol) was dissolved in CH_2Cl_2 (10 ml) and cooled to –78°C before adding oxalyl chloride (0.24 ml, 2.7 mmol). After 5 minutes, **86** (1.00 g, 2.5 mmol), dissolved in CH_2Cl_2 (20 ml), was added and the mixture stirred at –78°C for 30 minutes, after which time triethylamine (1.74 ml, 12.5 mmol) was added. The mixture was kept at –78°C for 5 minutes before being brought to room temperature. The reaction was diluted with ether and washed with brine (x 2). The organic extract was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material (1.07 g) was chromatographed on silica gel (15 g) using ethyl acetate - hexane (20%) as eluent to afford **93** (0.99 g, 99%) as colourless crystals: mp. 43-45°C (hexane); $[\alpha]_D$ –31.7° (*c* 1.20, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1732 (C=O stretch); δ_H (300 MHz, $CDCl_3$): 1.34, 1.36, 1.3, 1.54 (12H, 4 x s, 4 x CH_3), 3.33

[‡‡] As the stereochemistry of C-4 of the open chain derivative **86** is no longer defined, the carbonyl compound **93** cannot be named after the parent sugar, D-ribose, and so is named as a substituted heterocycle according to the IUPAC Commission on Nomenclature of Organic Chemistry.

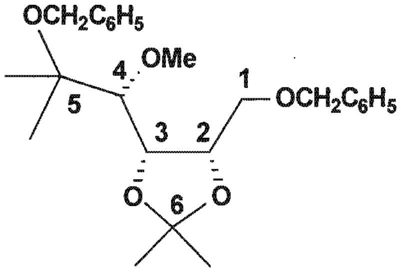
(1H, dd, *J* 6.0 and 9.6 Hz, H-5'), 3.44 (1H, dd, *J* 6.3 and 9.6 Hz, H-5'), 4.44 (4H, m, 2 x -OC<u>H</u>$_2$C$_6$H$_5$), 4.55 (1H, q, *J* 6.3 Hz, H-5), 5.30 (1H, d, *J* 6.3 Hz, H-4), 7.26 – 7.35 (10H, m, 2 x -OCH$_2$C$_6$<u>H</u>$_5$); δ_C (75.5 MHz, CDCl$_3$): 21.6, 23.3, 25.6, 27.7 (4 x CH$_3$), 65.0 (-O<u>C</u>H$_2$C$_6$H$_5$), 68.8 (C-5'), 73.1 (-O<u>C</u>H$_2$C$_6$H$_5$), 76.7 (C-4), 76.9 (C-5), 82.0 (C-4"), 109.6 (C-2), 127.5, 127.6, 127.7, 127.9, 128.3, 128.4, 137.8, 138.3 (-OCH$_2$<u>C</u>$_6$H$_5$), 207.3 (C-4'); HRMS: *m/z* 398.2094 [(M$^+$), $C_{24}H_{30}O_5$ requires 398.2093]; Found: C, 72.56%; H, 7.65%; $C_{24}H_{30}O_5$ requires C, 72.34%; H, 7.59%.

1,5-di-*O*-Benzyl-2,3-*O*-isopropylidene-5,5-di-*C*-methyl-L-lyxitol (94)



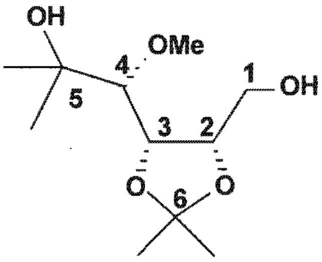
Ketone **93** (0.50 g, 1.3 mmol) was dissolved in toluene (10 ml) under N$_2$ and cooled to 0°C. K-Selectride© (1M in THF, 2.51 ml, 2.5 mmol) was added dropwise and the solution warmed to room temperature. After 2 hours, a mixture of 1M NaOH (3.5 ml) and 30% H$_2$O$_2$ (4.0 ml) was added and the mixture stirred for an additional 1 hour before being extracted with CH$_2$Cl$_2$ (x 3). The combined organic extracts were dried over anhydrous MgSO$_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography (60 g silica, 10% EtOAc/hexane) to yield **94** (0.41 g, 82%) as a colourless oil: $[\alpha]_D$ –18.2° (*c* 1.30, CHCl$_3$); IR ν_{max} (CH$_2$Cl$_2$) /cm^{-1} 3535 (-OH stretch); δ_H (400 MHz, CDCl$_3$): 1.24, 1.36, 1.46, 1.47 (4 x 3H, 4 x s, 4 x CH$_3$), 2.76 (1H, d, *J* 8.8 Hz, –OH), 4.47 (1H, d, *J* 8.8 Hz, H-4), 3.60 (2H, m, H-1), 4.25 (2H, m, H-2 and H-3), 4.49 (2H, s, -OC<u>H</u>$_2$C$_6$H$_5$), 4.54 (2H, s, -OC<u>H</u>$_2$C$_6$H$_5$), 7.30 (10H, m, 2 x -OCH$_2$C$_6$<u>H</u>$_5$); δ_C (100 MHz, CDCl$_3$): 20.5, 22.8, 26.9, 27.31 (4 x CH$_3$), 63.6 (-O<u>C</u>H$_2$C$_6$H$_5$), 70.1 (C-1), 73.5 (-O<u>C</u>H$_2$C$_6$H$_5$), 74.2 (C-4), 76.0 (C-3), 77.4 (C-5), 77.6 (C-2), 109.7 (C-6), 126.9, 127.1, 127.5, 127.6, 128.2, 128.3, 138.0, 139.6 (-OCH$_2$<u>C</u>$_6$H$_5$); HRMS: *m/z* 385.2018 [(M$^+$- CH$_3$), $C_{23}H_{29}O_5$ requires 385.2015].

1,5-di-O-Benzyl-2,3-*O*-isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-L-lyxitol (95)



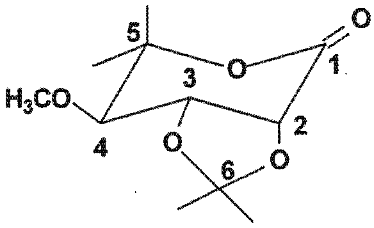
Compound **94** (0.36 g, 0.9 mmol), dissolved in THF (10 ml) was added to NaH (60% in oil, 0.07 g, 1.8 mmol) and the mixture refluxed for 2 hours. After cooling to room temperature, MeI (0.11 ml, 1.8 mmol) was added and the mixture refluxed for a further 1 hour. The excess NaH was quenched with aqueous NH_4Cl, the organic solvents evaporated under reduced pressure and the remainder extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was chromatographed on silica gel (15 g) using ethyl acetate - hexane (10%) as eluent to afford **95** (0.36 g, 95%) as a colourless oil: $[\alpha]_D$ –11.1° (*c* 1.30, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1590 (C=C aromatic stretch); δ_H (300 MHz, $CDCl_3$): 1.27, 1.38, 1.41, 1.46 (4 x 3H, 4 x s, 4 x CH_3), 3.17 (1H, d, *J* 3.2 Hz, H-4), 3.54 – 3.64 (5H, m, H-1 and -OMe), 3.14 (1H, dd, *J* 3.2 and 8.4 Hz, H-3), 4.25 (1H, m, H-2), 4.43 (1H, d, *J* 11.6 Hz, $-OC\underline{H}_2C_6H_5$), 4.48 (1H, d, *J* 11.6 Hz, $-OC\underline{H}_2C_6H_5$), 4.52 (2H, s, $-OC\underline{H}_2C_6H_5$), 7.30 (10H, m, 2 x $-OCH_2C_6\underline{H}_5$); δ_C (75.5 MHz, $CDCl_3$): 21.0, 22.9, 26.9, 27.1 (4 x CH_3), 62.1 (-OMe), 63.4 ($-O\underline{C}H_2C_6H_5$), 70.7 (C-1), 73.4 ($-O\underline{C}H_2C_6H_5$), 78.2 (C-2), 78.8 (C-3), 80.0 (C-5), 85.4 (C-4), 108.8 (C-6), 127.1, 127.2, 127.4, 127.5, 128.1, 128.2, 138.1, 139.6 ($-OCH_2\underline{C}_6H_5$); HRMS: *m/z* 399.2166 [(M^+-CH_3), $C_{24}H_{31}O_5$ requires 399.2171].

2,3-*O*-Isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-L-lyxitol (17)



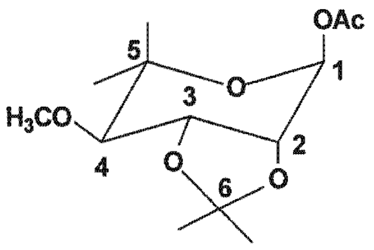
Pd/C (10%, 0.14 g, 0.1 mmol) was added to compound **95** (0.36 g, 0.9 mmol) dissolved in EtOH (10 ml) and the solution stirred overnight at room temperature in an atmosphere of H_2 using a balloon. After removing the Pd/C by filtration through Celite, the excess solvent was evaporated under reduced pressure. The crude residue was chromatographed on silica gel (15 g) using ethyl acetate - hexane (50%) as eluent to afford **17** (0.19 g, 95%) as a colourless oil: ($[\alpha]_D$ –7.6° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3523 (-OH stretch); δ_H (400 MHz, $CDCl_3$, D_2O wash): 1.25, 1.27, 1.38, 1.42 (4 x 3H, 4 x s, 4 x CH_3), 2.93 (1H, d, *J* 2.0 Hz, H-4), 3.53 (3H, s, -OMe), 3.70 (1H, dd, *J* 3.2 and 11.4 Hz, H-1), 3.80 (1H, dd, *J* 3.2 and 11.4 Hz, H-1), 4.14 – 4.17 (2H, m, H-2 and H-3); δ_C (100 MHz, $CDCl_3$): 26.3, 26.7, 27.0, 27.5 (4 x CH_3), 62.0 (-OMe), 62.3 (C-1), 73.8 (C-5), 77.4 (C-3), 77.8 (C-2), 84.8 (C-4), 109.1 (C-6); HRMS: *m/z* 219.1229 [(M^+-CH_3), $C_{10}H_{19}O_5$ requires 219.1233].

2,3-*O*-Isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-L-lyxolactone (96)

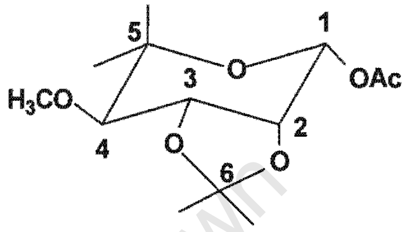


Diol **17** (100 mg, 0.4 mmol) was dissolved in CH_2Cl_2 (5 ml) and cooled to 0°C. TPAP (8 mg, 0.02 mmol), NMO (0.15 g, 1.2 mmol) and 4 Å molecular sieves were added and the mixture was warmed to room temperature and stirred overnight.The crude material was filtered through a 5 g plug of silica gel, rinsing with 20% EtOAc in hexane to yield **96** (89 mg, 91%) as colourless crystals: mp. 83-85°C (ethyl acetate/hexane); $[\alpha]_D$ +95.8° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1768 (C=O stretch); δ_H (300 MHz, $CDCl_3$): 1.39, 1.48, 1.49, 1.53 (4 x 3H, 4 x s, 4 x CH_3), 3.54 (3H, s, -OCH_3), 3.56 (1H, d, *J* 9.3 Hz, H-4), 3.95 (1H, dd, *J* 9.3 and 10.8 Hz, H-3), 4.04 (1H, d, *J* 10.8 Hz, H-2); δ_C (75.5 MHz, $CDCl_3$): 24.5, 26.4, 26.9, 30.1 (4 x CH_3), 59.5 (-OCH_3), 73.5 (C-2), 76.6 (C-3), 85.0 (C-4), 88.1 (C-5), 113.8 (C-6), 166.5 (C-1); HRMS: *m/z* 215.0938 [(M^+-CH_3), $C_{10}H_{15}O_5$ requires 215.0920].

1-*O*-Acetyl-2,3-*O*-isopropylidene-4-*O*-methyl-5,5-di-*C*-methyl-L-lyxopyranose (97)



97α

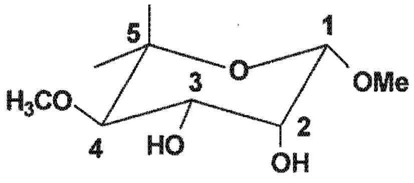


97β

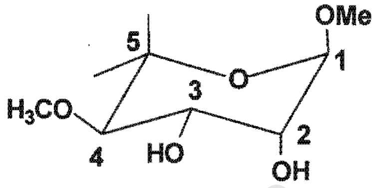
A solution of DMSO (0.13 ml, 1.8 mmol) in CH_2Cl_2 (10 ml) was cooled to –78°C and oxalyl chloride (0.08 ml, 0.9 mmol) was added. After 5 minutes, **17** (0.20 g, 0.8 mmol), dissolved in CH_2Cl_2 (10 ml), was added and the mixture stirred at –78°C for 15 minutes, after which triethylamine (0.58 ml, 4.2 mmol) was added and the mixture kept at –78°C for 5 minutes before being brought slowly to 0°C. The reaction was quenched with aqueous Na_2CO_3, brought to room temperature, and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material (0.22 g) was dissolved in CH_2Cl_2, to which was added Ac_2O (0.12 ml, 1.3 mmol), Et_3N (0.18 ml, 1.3 mmol) and DMAP (0.01 g, 0.1 mmol). After stirring for 1 hour, the mixture was quenched with aqueous NH_4Cl and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure The crude material was chromatographed on silica gel (25 g) using ethyl acetate - hexane (10%) as eluent to afford **97** (0.16 g, 69%) as an inseparable 2:1 mixture of β and α anomers respectively: IR ν_{max} (CH_2Cl_2) /cm^{-1} 1749 (C=O stretch); β -anomer (**97β**), δ_H (400 MHz, $CDCl_3$): 1.19, 1.33, 1.36, 1.53 (12H, 4 x s, 4 x CH_3), 2.08 (3H, s, -$OCOCH_3$), 3.14 (1H, d, *J* 7.0 Hz, H-4), 3.55 (3H, s, -OMe), 4.21 (1H, dd, *J* 3.2 and 7.0 Hz, H-3), 4.30 (1H, m, H-2), 6.08 (1H, d, *J* 3.2 Hz, H-1); δ_C (100 MHz, $CDCl_3$): 22.7, 25.5, 26.9, 27.5 (4 x CH_3), 55.4, (-$OCO\underline{C}H_3$), 61.6 (-$O\underline{C}H_3$), 68.6, 71.2 (C-2 and C-3), 74.8 (C-5), 84.4 (C-4), 101.2 (C-1), 169.3 (-$O\underline{C}OCH_3$); α-anomer (**97α**), δ_H (400 MHz, $CDCl_3$):1.15, 1.32, 1.36, 1.49 (12H, 4 x s, 4 x

CH_3), 2.11 (3H, s, $-OCOCH_3$), 3.52 (1H, d, *J* 6.4 Hz, H-4), 3.55 (3H, s, -OMe), 4.30 (2H, m, H-2 and H-3), 6.16 (1H, d, *J* 3.2 Hz, H-1); δ_C (100 MHz, $CDCl_3$): 21.1, 25.0, 26.6, 28.9 (4 x CH_3), 56.5 ($-OCO\underline{C}H_3$), 61.8 ($-O\underline{C}H_3$), 70.8, 71.5 (C-2 and C-3), 76.2 (C-5), 84.5 (C-4), 96.5 (C-1), 169.2 ($-O\underline{C}OCH_3$); HRMS: *m/z* 259.1199 [(M^+-CH_3), $C_{12}H_{19}O_6$ requires 259.1182].

Methyl 4-*O*-methyl-5,5-di-*C*-methyl-L-lyxopyranoside (7)



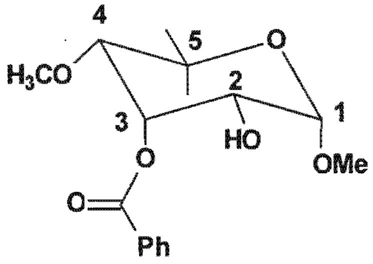
7β



7α

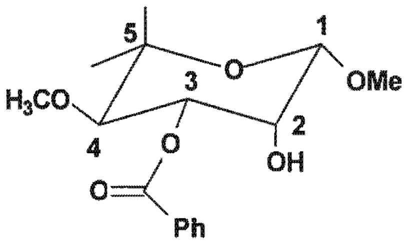
Dowex 50WX2-200 (0.33 g, 110% w/w) was added to compound **97** (0.30 g, 1.6 mmol) dissolved in 95% methanol (10 ml) and the mixture refluxed for 12 hours. The ion-exchange resin was filtered off and rinsed with methanol. The filtrate was evaporated under reduced pressure and purified by chromatography (15 g silica, 80% EtOAc/hexane) to yield **7** (0.22 g, 97%) as an inseparable 3:1 mixture of β and α anomers respectively: IR ν_{max} (CH_2Cl_2)/cm^{-1} 3601 (-OH stretch); β -anomer (**7β**), δ_H (400 MHz, $CDCl_3$): 1.30, 1.37 (6H, 2 x s, 2 x CH_3), 2.60 (2H, br, -OH), 3.25 (1H, d, *J* 9.2 Hz, H-4), 3.41 (3H, s, -OMe), 3.57 (3H, s, -OMe), 3.94 (1H, t, *J* 3.0 Hz, H-2), 4.03 (1H, dd, *J* 3.0 and 9.2 Hz, H-3), 4.67 (1H, d, *J* 3.0 Hz, H-1); δ_C (100 MHz, $CDCl_3$): 22.6, 28.8 (2 x CH_3), 55.4, 61.6 (2 x $-O\underline{C}H_3$), 68.6, 71.2 (C-2 and C-3), 74.8 (C-5), 84.4 (C-4), 101.2 (C-1); α-anomer (**7α**), δ_H (400 MHz, $CDCl_3$):1.17, 1.37 (6H, 2 x s, 2 x CH_3), 1.65 (2H, br, -OH), 3.20 (1H, d, *J* 9.2 Hz, H-4), 3.51 (3H, s, -OMe), 3.61 (3H, s, -OMe), 3.54 (1H, dd, *J* 3.6 and 9.2 Hz, H-3), 3.99 (1H, dd, *J* 1.2 and 3.6 Hz, H-2), 4.53 (1H, d, *J* 1.2 Hz, H-1); δ_C (100 MHz, $CDCl_3$): 18.7, 28.5 (2 x CH_3), 56.5, 61.8 (2 x $-O\underline{C}H_3$), 70.8, 71.5 (C-2 and C-3), 76.2 (C-5), 84.5 (C-4), 96.5 (C-1); HRMS: *m/z* 175.0965 [(M^+-OCH_3), $C_8H_{15}O_4$ requires 175.0970].

Methyl 3-*O*-benzoyl-4-*O*-methyl-5,5-di-*C*-methyl-α-D-ribopyranoside (118)



Dibutyltin oxide (0.25 g, 1.0 mmol) was added to diol **92** (0.19 g, 0.9 mmol) dissolved in MeOH (10 ml) and the mixture refluxed for 1 hour. After cooling to room temperature, the solvent was evaporated under reduced pressure. The sticky oil remaining was dissolved in CH_2Cl_2 (5 ml) and cooled to 0°C. Et_3N (0.26 ml, 1.8 mmol) and benzoyl chloride (0.21 ml, 1.8 mmol) were added and the mixture stirred at 0°C for 3 hours after which the solvent was evaporated under reduced pressure and the residue purified by chromatography (15 g silica, 20% EtOAc/hexane) to yield **118** (0.19 g, 65%) as a colourless oil: $[\alpha]_D$ –13.5° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1718 (C=O stretch), 3670 (-OH stretch); δ_H: (400 MHz, $CDCl_3$): 1.40, 1.45 (6H, 2 x s, 2 x CH_3), 3.41 (1H, d, *J* 3.6 Hz, H-4), 3.48 (3H, s, -OMe), 3.52 (3H, s, -OMe), 3.86 (1H, dt, $J_{2,OH}$ 1.2 and $J_{(1,2),(2,3)}$ 3.8 Hz, H-2), 4.82 (1H, d, *J* 4.0 Hz, H-1), 5.77 (1H, t, *J* 3.6 Hz, H-3), 7.40 – 8.12 (5H, m, -$OCOC_6H_5$); δ_C: (100 MHz, $CDCl_3$): 24.4, 27.5 (2 x CH_3), 55.8, 60.6 (2 x -$O\underline{C}H_3$), 68.1 (C-3), 69.9 (C-2), 76.7 (C-5), 83.9 (C-4), 102.2 (C-1), 128.4, 129.4, 129.8, 133.1 (-$OCO\underline{C}_6H_5$), 166.1 (-$O\underline{C}OC_6H_5$); HRMS: *m/z* 279.1249 [(M^+-OCH_3), $C_{15}H_{19}O_5$ requires 279.1233].

Methyl 3-*O*-benzoyl-4-*O*-methyl-5,5-di-*C*-methyl-α-L-lyxopyranoside (119)



Via two methods:

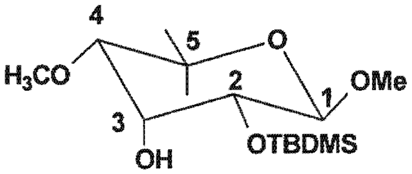
1. Diol **7** (0.06 g, 0.3 mmol) and dibutyltin oxide (0.09 g, 0.4 mmol) were refluxed in methanol (5 ml) for 1 hour until the solution became clear and then the solvent was removed in vacuo.

The resulting syrup was dissolved in CH_2Cl_2 (5 ml), to which was added BzCl (0.07 ml, 0.6 mmol). After stirring for 30 minutes, the undissolved salts were filtered and washed with CH_2Cl_2 and the filtrate evaporated under reduced pressure. The residue was purified by chromatography (17 g silica, 5% - 20% EtOAc/hexane) to yield the β-anomer **119** (0.07 g, 75%) as the major product as colourless crystals: mp. 105-107°C (EtOAc/hexane);

2. Diol **7** (0.13 g, 0.6 mmol) was dissolved in CH_2Cl_2 (10 ml) and cooled to –20°C. BzCl (0.08 ml, 0.7 mmol), Et_3N (0.09 ml, 0.7 mmol) and DMAP (0.01 g , 0.1 mmol) were added and the mixture stirred at –20°C for 2 hours, quenched with aqueous NH_4Cl and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was chromatographed on silica gel (15 g) using ethyl acetate - hexane (10%) as eluent to afford the β-anomer **119** (0.15 g, 77%) as the major product as colourless crystals: mp. 106-107°C (EtOAc/hexane);

$[\alpha]_D$ +4.1° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3691 (-OH stretch), 1721 (C=O stretch); δ_H (300 MHz, $CDCl_3$): 1.38, 1.39 (6H, 2 x s, 2 x CH_3), 3.44, 3.51 (6H, 2 x s,2 x -OMe), 3.56 (1H, d, *J* 9.0 Hz, H-4), 4.14 (1H, t, *J* 3.0 Hz, H-2), 4.71 (1H, d, *J* 3.0 Hz, H-1), 5.55 (1H, dd, *J* 3.0 and 9.0 Hz, H-3), 7.45 - 8.10 (5H, m, $-OCOC_6\underline{H}_5$); δ_C (75.5 MHz, $CDCl_3$): 23.0, 28.3 (2 x CH_3), 55.6, 61.4 (2 x $-O\underline{C}H_3$), 70.2 (C-2), 72.2 (C-3), 77.7 (C-5), 81.7 (C-4), 101.4 (C-1), 128.5, 129.7, 130.0, 133.2 ($-OCO\underline{C}_6H_5$), 165.7 ($-O\underline{C}OC_6H_5$); HRMS: *m/z* 279.1226 [(M^+-OCH_3), $C_{15}H_{19}O_5$ requires 279.1233]

Methyl 2-*O*-*tert*-butyldimethylsilyloxy-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranoside (120)

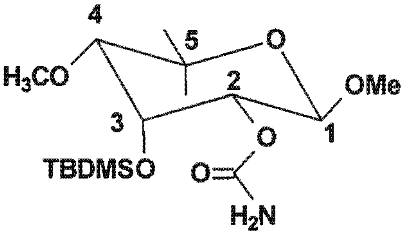


Diol **92** (0.133 g, 0.6 mmol) was dissolved in DMF (2 ml) to which TBDMSCl (0.11 g, 0.7 mmol) and imidazole (0.07 g, 1.0 mmol) were added. The mixture was stirred at room temperature overnight, diluted with EtOAc and washed with water (x 3). The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure.The crude material was purified by chromatography (12 g silica, 5% EtOAc/hexane) to yield **120** (0.13 g, 79%) as the major product as a colourless oil; δ_H: (300 MHz, $CDCl_3$): 0.08 (6H, s, -$OSi(C\underline{H}_3)_2C(CH_3)_3$), 0.86 (9H, m, -$OSi(CH_3)_2C(C\underline{H}_3)_3$), 1.24 (3H, s, -$CH_3$), 1.36 (3H, s, -$CH_3$), 2.51 (1H, s, -OH), 2.91 (1H, d, *J* 3.3 Hz, H-4), 3.38 (1H, dd, *J* 3.3 and 7.5 Hz, H-2), 3.42 (6H, s, 2 x –OMe), 4.19 (1H, t, *J* 3.3 Hz, H-3), 4.49 (1H, d, *J* 7.5 Hz, H-1); δ_C: (75.5 MHz, $CDCl_3$): -5.2, -4.4 (-$OSi(\underline{C}H_3)_2C(CH_3)_3$), 18.0 (-$OSi(CH_3)_2\underline{C}(CH_3)_3$), 20.3 (-$CH_3$), 25.7 (-$OSi(CH_3)_2C(\underline{C}H_3)_3$) 29.4 (-$CH_3$), 56.5, 57.4 (2 x -$O\underline{C}H_3$), 68.4 (C-3), 72.3 (C-2), 75.3 (C-5), 83.2 (C-4), 97.6 (C-1).

Silylated **120** was characterized as its C-3 acetate **121**:

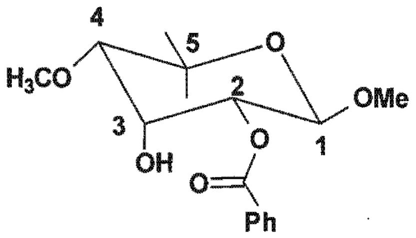
Compound **120** (0.13 g, 0.4 mmol), acetic anhydride (0.06 ml, 0.6 mmol), Et_3N (0.08 ml, 0.6 mmol) and DMAP (0.01 g, 0.04 mmol) were dissolved in CH_2Cl_2 (5 ml) and the mixture refluxed for 2 days, quenched with aqueous NH_4Cl and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was chromatographed on silica gel (15 g) using ethyl acetate - hexane (10%) as eluent to afford the β-anomer **121** (0.07 g, 49%) as the major product as colourless crystals: mp. 60-62°C (EtOAc/hexane); $[\alpha]_D$ –25.1° (*c* 1.30, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1748 (C=O stretch); δ_H: (300 MHz, $CDCl_3$): 0.06 (6H, s, -$OSi(C\underline{H}_3)_2C(CH_3)_3$), 0.84 (9H, m, -$OSi(CH_3)_2C(C\underline{H}_3)_3$), 1.26 (3H, s, -$CH_3$), 1.31 (3H, s, -$CH_3$), 2.08 (3H, s, -$COCH_3$), 2.90 (1H, d, *J* 3.6 Hz, H-4), 3.35 (3H, s, -OMe), 3.44 (1H, dd, *J* 3.6 and 7.5 Hz, H-2), 3.49 (3H, s, –OMe), 4.51 (1H, d, *J* 7.5 Hz, H-1), 5.60 (1H, t, *J* 3.6 Hz, H-3); δ_C: (75.5 MHz, $CDCl_3$): -5.3, -4.6 (-$OSi(\underline{C}H_3)_2C(CH_3)_3$), 18.1 (-$OSi(CH_3)_2\underline{C}(CH_3)_3$), 19.8 (-$CO\underline{C}H_3$), 21.0 (-$CH_3$), 25.6 (-$OSi(CH_3)_2C(\underline{C}H_3)_3$) 29.4 (-$CH_3$), 56.6, 57.8 (2 x -$OCH_3$), 69.0 (C-3), 70.6 (C-2), 75.5 (C-5), 82.2 (C-4), 98.2 (C-1), 170.0 (-$\underline{C}OCH_3$); HRMS: *m/z* 331.1917 [(M^+-OCH_3), $C_{16}H_{31}O_5Si$ requires 331.1941].

Methyl 3-*O-tert*-butyldimethylsilyloxy-2-O-carbamoyl-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranoside (122)



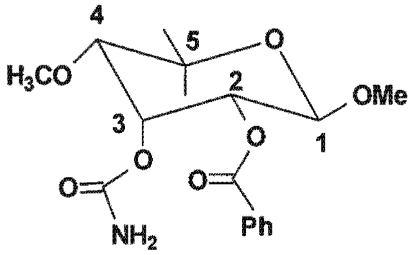
Silylated **120** (0.14 g, 0.4 mmol) was dissolved in CH_2Cl_2 (20 ml), *p*-nitrophenyl chloroformate (0.18 g, 0.8 mmol), DMAP (0.01 g, 0.1 mmol), and Et_3N (0.12 ml, 0.8 mmol) were added and the mixture refluxed overnight. After cooling, the reaction was quenched with aqueous ammonium chloride and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude product (0.35 g) was dissolved in EtOH (5 ml) and added to a solution of EtOH (2 ml) saturated with NH_3 gas. After stirring at room temperature for 1 hour, the solvent was evaporated under reduced pressure and the crude material purified by chromatography (15 g silica, 20% EtOAc/Hexane) to yield **122** (0.08 g, 53%) as colourless crystals: mp 150-152°C (EtOAc/hexane); $[\alpha]_D$ –27.2° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2)/cm^{-1} 1603 (C=O stretch); δ_H: (300 MHz, $CDCl_3$): 0.07, 0.08 (6H, 2 x s, $-OSi(C\underline{H}_3)_2C(CH_3)_3$), 0.92 (9H, m, $-OSi(CH_3)_2C(C\underline{H}_3)_3$), 1.27 (3H, s, $-CH_3$), 1.36 (3H, s, $-CH_3$), 2.89 (1H, d, *J* 3.0 Hz, H-4), 3.38 (3H, s, –OMe), 3.46 (3H, s, –OMe), 4.45 (1H, dd, *J* 3.0 and 8.0 Hz, H-2), 4.48 (1H, t, *J* 3.0 Hz, H-3), 4.65 (2H, br, NH_2), 4.77 (1H, d, *J* 8.0 Hz, H-1); δ_C: (75.5 MHz, $CDCl_3$): -5.1, -4.8 ($-OSi(\underline{C}H_3)_2C(CH_3)_3$), 18.6 ($-OSi(CH_3)_2\underline{C}(CH_3)_3$), 20.8 ($CH_3$), 26.0 ($-OSi(CH_3)_2C(\underline{C}H_3)_3$), 29.8 ($CH_3$), 56.2, 58.3 (2 x $-O\underline{C}H_3$), 68.5 (C-3), 73.7 (C-2), 76.4 (C-5), 84.0 (C-4), 95.4 (C-1), 155.9 ($-CONH_2$); HRMS: *m/z* 364.2189 [(M^+ + H), $C_{16}H_{34}NO_6Si$ requires 364.2155]; Found: C, 52.64%; H, 9.07%; N, 3.89%; $C_{16}H_{33}NO_6Si$ requires C, 52.86%; H, 9.15%; N, 3.85%.

Methyl 2-*O*-benzoyl-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranoside (123)



Diol **92** (0.10 g, 0.5 mmol) was dissolved in CH_2Cl_2 (3 ml) and cooled to 0°C. Benzoyl chloride (0.06 ml, 0.5 mmol), pyridine (0.04 ml, 5.3 mmol), and DMAP (6 mg, 0.05 mmol) were added, the mixture stirred at 0°C for 3 hours before being quenched with aqueous NH_4Cl and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography (5 g silica, 5% EtOAc/hexane) to yield **123** (0.13 g, 83%) as a colourless oil: $[\alpha]_D$ –31.2° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1718 (C=O stretch), 3670 (-OH stretch); δ_H: (300 MHz, $CDCl_3$): 1.33, 1.43 (6H, 2 x s, 2 x CH_3), 3.11 (1H, d, *J* 3.3 Hz, H-4), 3.45 (3H, s, -OMe), 3.48 (3H, s, -OMe), 4.59 (1H, t, *J* 3.3 Hz, H-3), 4.92 (1H, dd, *J* 3.3 and 7.5 Hz, H-2), 4.99 (1H, d, *J* 7.5 Hz, H-1), 7.41 – 8.10 (5H, m, $-OCOC_6\underline{H}_5$); δ_C: (75.5 MHz, $CDCl_3$): 20.9, 29.8 (2 x CH_3), 56.4, 58.3 (2 x $-O\underline{C}H_3$), 66.1 (C-3), 72.4 (C-2), 75.8 (C-5), 83.1 (C-4), 95.5 (C-1), 128.3, 129.8, 129.9, 133.1 ($-OCO\underline{C}_6H_5$), 165.7 ($-O\underline{C}OC_6H_5$); HRMS: *m/z* 279.1247 [(M^+-OCH_3), $C_{15}H_{19}O_5$ requires 279.1233].

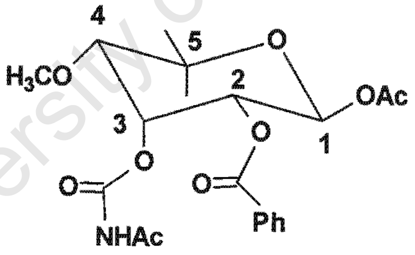
Methyl 2-*O*-benzoyl-3-*O*-carbamoyl-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranoside (124)



p-nitrophenyl chloroformate (0.12 g, 0.6 mmol), pyridine (0.04 ml, 0.5 mmol) and DMAP (0.05 g, 0.04 mmol) were added to compound **123** (0.12 g, 0.4 mmol) dissolved in CH_2Cl_2 (10 ml) and the mixture stirred overnight. After quenching with aqueous NH_4Cl and extracting with

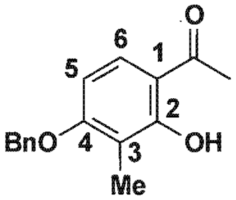
CH_2Cl_2 (x 3), the combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material (0.29 g) was dissolved in ethanol (5 ml) and added to ethanol (2 ml) saturated with ammonia gas. After stirring for one hour, the solvent was evaporated and purified by chromatography (5 g silica, 20% EtOAc/hexane) to yield **124** (0.10 g, 73%) as colourless crystals: mp 170-171°C (ethyl acetate/hexane); $[\alpha]_D$ –26.6° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2)/cm^{-1} 1601, 1737 (C=O stretch); δ_H: (300 MHz, $CDCl_3$): 1.35, 1.39 (6H, 2 x s, 2 x CH_3), 3.18 (1H, d, *J* 3.3 Hz, H-4), 3.41 (3H, s, -OMe), 3.48 (3H, s, -OMe), 4.70 (2H, s, $-NH_2$), 4.90 (1H, d, *J* 8.0 Hz, H-1), 5.00 (1H, dd, *J* 3.3 and 8.0 Hz, H-2), 5.74 (1H, t, *J* 3.3 Hz, H-3), 7.41 – 8.03 (5H, m, $-OCOC_6\underline{H}_5$); δ_C: (75.5 MHz, $CDCl_3$): 20.3, 29.3 (2 x CH_3), 56.2, 58.1 (2 x $-O\underline{C}H_3$), 67.9 (C-3), 70.2 (C-2), 76.2 (C-5), 81.7 (C-4), 95.7 (C-1), 128.3, 129.8, 130.0, 133.0 ($-OCO\underline{C}_6H_5$), 155.7 ($-O\underline{C}ONH_2$), 165.7 ($-O\underline{C}OC_6H_5$); HRMS: *m/z* 322.1322 [(M^+-OCH_3), $C_{16}H_{20}NO_6$ requires 322.1291]; Found: C, 57.83%; H, 6.41%; N, 3.95%; $C_{17}H_{23}O_7N$ requires C, 57.78%; H, 6.56%; N, 3.96.

Methyl 2-*O*-benzoyl-3-*O*-(*N*-acetylcarbamoyl)-4-*O*-methyl-5,5-di-*C*-methyl-β-D-ribopyranoside (125)



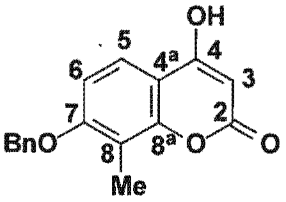
Compound **124** (0.13 g, 0.4 mmol) was dissolved in acetic anhydride (2.0 ml) and cooled to 0°C. A 2% solution of H_2SO_4 in AcOH (0.5 ml) was added dropwise and the mixture stirred at 0°C for 1 hour. After quenching with aqueous $NaHCO_3$ and extracting with EtOAc (x 3), the combined organic extracts were dried over anhydrous $MgSO_4$, filtered, evaporated under reduced pressure, and azeotroped with toluene (x 3). The crude material (0.29 g) was purified by chromatography (5 g silica, 10% EtOAc/hexane) to yield **125** (0.14 g, 85%) as colourless crystals: mp 59-61°C (ethyl acetate/hexane); $[\alpha]_D$ –25.4° (*c* 1.29, $CHCl_3$); IR ν_{max} (CH_2Cl_2)/cm^{-1} 1602, 1723, 1766 (C=O stretch); δ_H: (300 MHz, $CDCl_3$): 1.35, 1.44 (6H, 2 x s, 2 x CH_3), 2.04, 2.20 (6H, 2 x s, 2 x $-OCOC\underline{H}_3$), 3.24 (1H, d, *J* 3.6 Hz, H-4), 3.42 (3H, s, -OMe), 5.11 (1H, dd, *J* 3.6 and 8.4 Hz, H-2), 5.85 (1H, t, *J* 3.6 Hz, H-3), 6.32 (1H, d, *J* 8.4 Hz, H-1), 7.39 – 7.97 (5H, m, -OCOC$_6$H$_5$), 8.34 (1H, br, -NHCOCH$_3$); δ$_C$: (75.5 MHz, CDCl$_3$): 19.5, 23.8 (-COCH$_3$), 20.9, 29.1 (2 x CH$_3$), 58.2 (-OCH$_3$), 68.6 (C-3), 69.3 (C-2), 77.6 (C-5), 81.1 (C-4), 86.8 (C-1), 128.5, 129.1, 129.6, 133.5 (-OCOC$_6$H$_5$), 151.5 (-OCONHAc), 165.0 (-OCOC$_6$H$_5$), 169.4, 172.4 (-OCOCH$_3$ and –OCONHCOCH$_3$); HRMS: *m/z* 365.1089 [(M$^+$-CH$_3$ and –COCH$_3$), C$_{17}$H$_{19}$NO$_8$ requires 365.1111].

4-Benzyloxy-2-hydroxy-3-methylacetophenone (131)



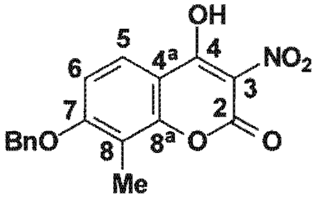
A mixture of 2,4-dihydroxy-3-methylacetophenone (1.00 g, 6.0 mmol), benzyl chloride (0.76 ml, 6.6 mmol), potassium iodide (0.10 g, 0.6 mmol) and anhydrous potassium carbonate (0.92 g, 6.6 mmol) in acetone (30 ml) was stirred and heated under reflux overnight, cooled, filtered, and the solvent evaporated under reduced pressure. The brown residue was crystallized from methanol to give the benzylated acetophenone **131** as needles (1.36 g, 88%): mp. 88-90°C; (lit.[99] mp 87-88°C); IR ν_{max} (CH$_2$Cl$_2$)/cm^{-1} 1499 (aromatic), 1628 (C=O stretch); δ$_H$ (400 MHz, CDCl$_3$): 2.19 (3H, s, ArCH$_3$), 2.55 (3H, s ,-COCH$_3$), 5.16 (2H, s, -CH$_2$C$_6$H$_5$), 6.50 (1H, d, *J* 9.0 Hz, H-5), 7.34 - 7.45 (5H, m, -CH$_2$C$_6$H$_5$), 7.57 (1H, d, *J* 9.0 Hz, H-6), 12.79 (1H, s, -OH); δ$_C$ (100 MHz, CDCl$_3$): 8.0 (ArCH$_3$), 26.4 (-COCH$_3$), 70.4 (-CH$_2$C$_6$H$_5$), 103.4 (C-5), 114.3, 114.5 (C-1 and C-3), 127.2 (C-6), 128.3, 128.9, 130.0, 136.8 (-CH$_2$C$_6$H$_5$), 162.4, 162.8 (C-2 and C-4), 203.1 (-COCH$_3$); HRMS: *m/z* 256.1103 [(M$^+$), C$_{16}$H$_{16}$O$_3$ requires 256.1099].

7-Benzyloxy-4-hydroxy-8-methylchromen-2-one (59)



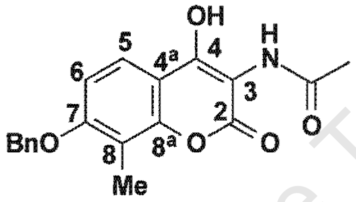
Acetophenone **131** (0.50 g, 1.9 mmol) was dissolved in toluene (10 ml) and added to NaH (0.17 g, 4.3 mmol) suspended in toluene (10 ml). $CO(OEt)_2$ (0.31 ml, 2.5 mmol) was added and the mixture refluxed for 2 hours before being cooled to 0°C, quenched with water and extracted with EtOAc (x 1). The aqueous phase was acidified with 1M HCl and extracted with EtOAc (x 3). The combined organic extracts from the acidic extractions were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was crystallized from MeOH to give **59** as colourless crystals (0.42 g, 76%): mp. 233-234°C; (lit.[38] mp 233 - 236°C); IR ν_{max} (CH_2Cl_2)/cm^{-1} 1514, 1587 (aromatic C=C), 1723, 1667 (C=O stretch); δ_H (300 MHz, DMSO): 2.19 (3H, s, $ArCH_3$), 5.22 (2H, s, $-C\underline{H}_2C_6H_5$), 5.44 (1H, s, H-3), 7.07 (1H, d, *J* 8.7 Hz, H-6), 7.31 - 7.47 (5H, m, $-CH_2C_6\underline{H}_5$), 7.62 (1H, d, *J* 8.7 Hz, H-5), 12.20 (1H, br, -OH); δ_C (75.5 MHz, DMSO): 8.2 ($ArCH_3$), 69.9 ($-\underline{C}H_2C_6H_5$), 88.5 (C-3), 108.3 (C-6), 109.3 (C-4^a), 112.6 (C-8), 121.3 (C-5), 127.3, 127.8, 128.4, 136.7 ($-CH_2\underline{C}_6H_5$), 152.5 (C-8^a), 159.3 (C-4), 162.2 (C-7), 166.1 (C-2); HRMS: *m/z* 282.0883 [(M^+), $C_{17}H_{14}O_4$ requires 282.0892]. Found: C, 72.16%; H, 4.82%; $C_{17}H_{14}O_4$ requires C, 72.33%; H, 5.00%.

7-Benzyloxy-4-hydroxy-8-methyl-3-nitrochromen-2-one (132)



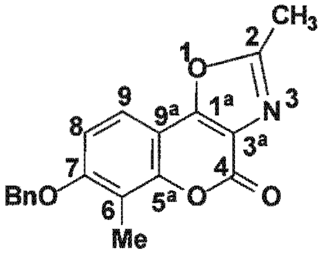
Concentrated H_2SO_4 (1.61 ml, 22.1 mmol) and concentrated HNO_3 (1.00 ml, 18.4 mmol) were mixed slowly at 0°C and then added, over a period of 15 minutes, to **59** (2.08 g, 7.4 mmol) suspended in $CHCl_3$. After an additional 1 hour, the solvent was evaporated under reduced pressure and 1M HCl (30 ml) was added. The yellow solid was filtered, rinsed well with MeOH, and crystallized from glacial acetic acid to give **132** as yellow plates (2.23 g, 93%): mp. 205-208°C; IR ν_{max} (KBr pellet)/cm^{-1} 1325 and 1530 (NO stretch), 1754 (C=O stretch), 3540 (OH stretch); δ_H (300 MHz, DMSO): 2.18 (3H, s, ArCH$_3$), 5.07 (1H, br, -OH), 5.22 (2H, s, -C$\underline{H}_2$C$_6$H$_5$), 7.06 (1H, d, *J* 9.0 Hz, H-6), 7.30 - 7.48 (5H, m, -CH$_2$C$_6\underline{H}_5$), 7.77 (1H, d, *J* 9.0 Hz, H-5); δ_C (75.5 MHz, DMSO): 8.2 (ArCH$_3$), 69.9 (-$\underline{C}$H$_2$C$_6$H$_5$), 108.1 (C-6), 112.2 (C-3), 113.9 (C-4^a), 119.5 (C-8), 123.9 (C-5), 127.3, 127.8, 128.5, 136.8 (-CH$_2\underline{C}_6$H$_5$), 151.3 (C-8^a), 157.0 (C-4), 159.7 (C-7), 166.4 (C-2). Found: C, 62.14%; H, 3.88%; N, 4.31%; $C_{17}H_{13}NO_6$ requires C, 62.39%; H, 4.00%; N, 4.28%.

N-(7-Benzyloxy-4-hydroxy-8-methyl-2-oxo-(2H)-chromen-3-yl) acetamide (133)



Compound **132** (1.00 g, 3.1 mmol) and Zn (1.00 g, 15.5 mmol) were refluxed in acetic acid (10 ml) for 1 hour, in which time the solution turned deep purple and then colourless. The Zn salts were filtered and rinsed well with hot acetic acid. The filtrate was cooled to room temperature, allowing the amide to precipitate. The product was filtered and crystallized with EtOAc to yield **133** as colourless crystals (0.87 g, 86%): mp. 240-243°C; IR ν_{max} (CH$_2$Cl$_2$)/ cm^{-1} 1500 (aromatic C=C), 1572 (amide I), 1598 (aromatic C=C), 1632 (amide II), 1686 (C=O stretch), 3288 (NH stretch), 3500 (OH stretch); δ_H (300 MHz, CDCl$_3$): 2.11 (-NHCOC$\underline{H}_3$), 2.23 (3H, s, ArCH$_3$), 5.24 (2H, s, -C$\underline{H}_2$C$_6$H$_5$), 7.16 (1H, d, *J* 9.0 Hz, H-6), 7.38 - 7.48 (5H, m, -CH$_2$C$_6\underline{H}_5$), 7.68 (1H, d, *J* 9.0 Hz, H-5), 9.42 (1H, s, -NH), 12.2 (1H, br, -OH); δ_C (75.5 MHz, CDCl$_3$): 8.1 (ArCH$_3$), 22.6 (-NHCO$\underline{C}$H$_3$), 69.9 (-$\underline{C}$H$_2$C$_6$H$_5$), 101.4 (C-3), 108.9 (C-6), 109.7 (C-4^a), 112.5 (C-8), 121.7 (C-5), 127.3, 127.9, 128.4, 136.7 (-CH$_2\underline{C}_6$H$_5$), 150.1 (C-8^a), 157.4 (C-4), 158.9 (C-7), 160.3 (C-2), 171.2 (-$\underline{C}$OCH$_3$). Found: C, 67.27%; H, 4.94%; N, 4.07%; $C_{19}H_{17}NO_5$ requires C, 67.25%; H, 5.05%; N, 4.13%.

7-Benzyloxy-2,6-dimethylchromeno[3,4-d]oxazol-4-one (134)



1. From **133**

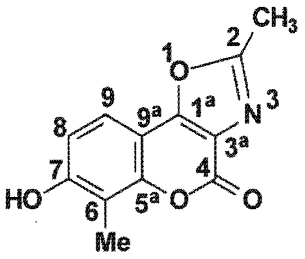
Amide **133** (2.06 g, 6.1 mmol), pyridine (1.72 ml, 18.3 mmol) and $POCl_3$ (2.96 ml, 30.4 mmol) were suspended in THF (50 ml) and refluxed for 30 minutes. After cooling to room temperature, the mixture was filtered, the excess solvent evaporated under vacuum and the remaining slurry azeotroped with toluene (x3) to remove traces of pyridine. The product was crystallized with EtOAc to yield **134** as colourless crystals (1.70 g, 87%): mp. 206-207°C;

2. From **132**

A mixture of **132** (0.80 g, 2.4 mmol), Zn (0.80 g, 12.2 mmol), acetic acid (10 ml) and acetic anhydride (5 ml) were refluxed for 1 hour. The Zn salts were filtered and rinsed with hot acetic acid. The filtrate was left to stand overnight and the crystals were collected and recrystallized with EtOAc to yield **134** as colourless crystals (0.68 g, 87%): mp. 206-207°C;

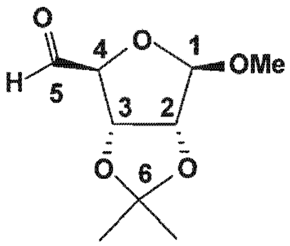
IR ν_{max} (CH_2Cl_2)/cm^{-1} 1503, 1605 (aromatic C=C), 1647 (coumarin C=C), 1748 (C=O stretch); δ_H (400 MHz, $CDCl_3$): 2.33 (3H, s, $ArCH_3$), 2.56 (3H, s, $-CH_3$), 5.10 (2H, s, $-C\underline{H}_2C_6H_5$), 6.88 (1H, d, *J* 8.8 Hz, H-8), 7.29 - 7.34 (5H, m, $-CH_2C_6\underline{H}_5$), 7.48 (1H, d, *J* 8.8 Hz, H-9); δ_C (100 MHz, $CDCl_3$): 8.8 ($ArCH_3$), 14.1 ($-\underline{C}H_3$), 70.79 ($-\underline{C}H_2C_6H_5$), 105.1 (C-9a), 108.9 (C-8), 115.8 (C-6), 118.9 (C-9), 122.4 (C-3a), 127.1, 128.2, 128.7, 136.3 ($-CH_2\underline{C}_6H_5$), 152.1 (C-5^{a}), 156.2 (C-1^{a}), 156.3 (C-7), 159.3 (C-4), 162.6 (C-2). Found: C, 70.96%; H, 4.56%; N, 4.34%; $C_{19}H_{15}NO_4$ requires C, 71.02%; H, 4.71%; N, 4.36%.

7-Hydroxy-2,6-dimethylchromeno[3,4-d]oxazol-4-one (142)



10% Pd/C (0.50 g, 0.05 mmol) was added to compound **134** (0.15 g, 0.5 mmol) in a mixture of THF (10 ml) and CH_2Cl_2 (5 ml) and the solution stirred for 3 hours at room temperature in an atmosphere of H_2 using a balloon. The excess solvent was evaporated after removing the Pd/C by filtration (rinsing with hot MeOH). The product was recrystallized with MeOH to yield **142** as colourless crystals (0.08 g, 74%): mp. 330-332°C (decomp.) (lit.[117] mp 295-303°C (decomp.) from H_2O/DMF); IR ν_{max} (CH_2Cl_2)/cm^{-1} 1503, 1584, 1604 (aromatic C=C), 1647 (coumarin C=C), 1749 (C=O stretch), 3150 (OH stretch); δ_H (400 MHz, $CDCl_3$): 2.17 (3H, s, $ArCH_3$), 2.59 (3H, s, $-CH_3$), 6.91 (1H, d, J 8.4 Hz, H-8), 7.48 (1H, d, J 8.4 Hz, H-9), 10.55 (1H, s, -OH); δ_C (100 MHz, $CDCl_3$): 9.0 ($ArCH_3$), 14.4 ($\underline{C}H_3$), 103.5 (C-9^a), 112.5 (C-6), 113.3 (C-8), 119.8 (C-9), 120.8 (C-3^a), 152.8 (C-5^a), 156.2 (C-1^a), 157.0 (C-7), 159.5 (C-4), 163.0 (C-2). Found: C, 62.13%; H, 3.99%; N, 5.98%; $C_{12}H_9NO_4$ requires C, 62.34%; H, 3.92%; N, 6.06%.

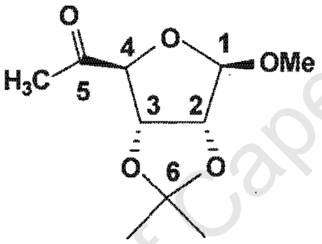
Methyl 2,3-*O*-isopropylidene-β-D-pentodialdo-1,4-furanoside (143)



A solution of DMSO (10.40 ml, 146.5 mmol) in CH_2Cl_2 (100 ml) was cooled to –78°C and oxalyl chloride (6.4 ml, 73.3 mmol) was added. After 5 minutes, **64β** (12.5 ml, 61.1 mmol), dissolved in CH_2Cl_2 (100 ml), was added and the mixture stirred at –78°C for 15 minutes, after which triethylamine (42.5 ml, 305.3 mmol) was added and the mixture kept at –78°C for 5 minutes before being brought slowly to 0°C. The reaction was quenched with aqueous

Na_2CO_3, brought to room temperature, and extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was chromatographed on silica gel (200 g) using ethyl acetate - hexane (40%) as eluent to afford **143** (2.73 g, 22%) as the major product as colourless crystals: mp 61-62°C (hexane) (lit.[123] mp 60 – 61°C); $[\alpha]_D$ –191.5° (*c* 1.10, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 1733 (C=O stretch); δ_H (400 MHz, $CDCl_3$): 1.30, 1.46 (2 x 3H, 2 x s, 2 x CH_3), 3.42 (3H, s, -OCH_3), 4.44 (1H, s, H-4), 4.47 (1H, d, *J* 6.0 Hz, H-2), 5.02 (1H, d, *J* 6.0 Hz, H-3), 5.06 (1H, s, H-1), 9.56 (1H, s, -CHO); δ_C (100 MHz, $CDCl_3$): 24.8, 26.1 (2 x CH_3), 55.6 (-OMe), 80.7 (C-3), 83.9 (C-2), 89.5 (C-4), 109.1 (C-1), 112.6 (C-6), 200.6 (C-5).

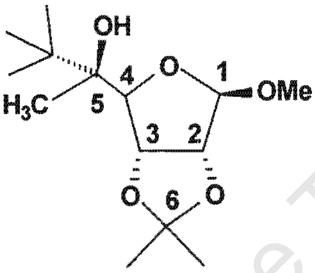
Methyl 2,3-*O*-isopropylidene-5-*C*-methyl-β-D-ribofuranosid-5-ulose (144)



MeI (1.86 ml, 29.7 mmol) was added dropwise to a slurry of magnesium (0.66 g, 27.0 mmol) in diethyl ether (50 ml) at 0°C. The mixture was refluxed for 1 hour, then cooled to 0°C. A solution of **143** (2.73 g, 13.5 mmol) in diethyl ether (50 ml) was added dropwise and the mixture refluxed for 4 hours. The mixture was once again cooled to 0°C and quenched with aqueous NH_4Cl. The excess diethyl ether was evaporated under reduced pressure, and the remaining crude material extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to give the crude alcohol (2.69 g) as an oil. CrO_3 (14.8 g, 147.9 mmol) was dissolved in pyridine (24 ml) and stirred for 20 minutes. The crude material was suspended in CH_2Cl_2 (200 ml) and added to the chromium mixture. After stirring overnight, cold $NaHCO_3$ was added and the mixture poured into a separating funnel, extracting the remaining tar with diethyl ether which was added to the separating funnel. The organic material was extracted with CH_2Cl_2 (x 3), the combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography (60 g silica, 50% EtOAc in hexane) to yield **144** (2.38 g, 81%) as a colourless oil; $[\alpha]_D$ –115° (*c* 1.00, $CHCl_3$), (lit.[125] $[\alpha]_D$ –120° (c 0.8, $CHCl_3$)); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3442 (-OH stretch); δ_H (400 MHz,

$CDCl_3$): 1.31, 1.47 (2 x 3H, 2 x s, 2 x CH_3), 2.23 (3H, s, -$COCH_3$), 3.41 (3H, s, -OCH_3), 4.43 (1H, s, H-4), 4.49 (1H, d, *J* 6.0 Hz, H-2), 5.00 (1H, s, H-1), 5.19 (1H, d, *J* 6.0 Hz, H-3); δ_C (100 MHz, $CDCl_3$): 24.9, 26.0, 26.3 (2 x CH_3 and –$CO\underline{C}H_3$), 56.1 (-OMe), 80.4 (C-3), 84.4 (C-2), 90.3 (C-4), 110.0 (C-1), 112.4 (C-6), 206.6 (–$\underline{C}OCH_3$).

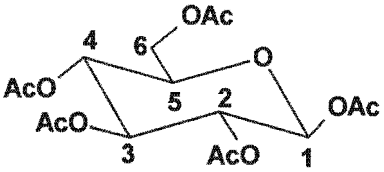
(5*S*)-Methyl 5-*C*-*tert*-butyl-2,3-*O*-isopropylidene-5-*C*-methyl-β-D-ribofuranoside (145)



Cerium chloride ($CeCl_3{\cdot}7H_2O$) (1.0 g, 2.8 mmol) was quickly finely ground to a powder using a mortar and pestle and placed in a two-necked flask. The flask was immersed in an oil bath and heated gradually to 135-140°C with evacuation. After maintenance of the cerium chloride at a constant temperature for 1 hour, a magnetic stirrer bar was placed in the flask and the cerium chloride was completely dried in vacuo by stirring at the same temperature for an additional 1 hour. While the flask was still hot, nitrogen gas was introduced and the flask was cooled to 0°C. THF (5 ml) was added with vigorous stirring and the suspension was stirred overnight under nitrogen at room temperature. The flask was then cooled to –78°C and *t*-butyllithium (1.7M in pentane, 1.22 ml, 2.1 mmol) was added. After stirring for 1.5 hours at –78°C a solution of ketone **144** (0.30 g, 1.4 mmol) in THF (5 ml) was added and the stirring continued for 2 hours. The reaction mixture was quenched with water and and the product extracted with CH_2Cl_2 (x 3). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography (17 g silica, 20% EtOAc in hexane) to yield **145** (0.25 g, 65%) as a colourless oil; $[\alpha]_D$ –60° (*c* 1.00, $CHCl_3$); IR ν_{max} (CH_2Cl_2) /cm^{-1} 3461 (-OH stretch); δ_H (300 MHz, $CDCl_3$): 1.04 (9H, s, 3 x CH_3), 1.16 (3H, s, CH_3), 1.30 (3H, s, CH_3), 1.47 (3H, s, CH_3), 2.73 (1H, s, -OH), 3.46 (3H, s, -OCH_3), 4.38 (1H, d, *J* 1.2 Hz, H-4), 4.49 (1H, d, *J* 6.0 Hz, H-2), 4.92 (1H, s, H-1), 5.12 (1H, dd, *J* 1.28 and 6.0 Hz, H-3); δ_C (75.5 MHz, $CDCl_3$): 21.4, 24.9

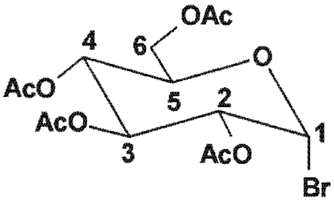
(2 x CH_3), 26.5 (3 x CH_3), 26.6 (1 x CH_3), 56.3 (-OMe), 76.5 (C-5), 81.8 (C-3), 85.3 (C-2), 92.5 (C-4), 109.7 (C-1), 111.8 (C-6), . HRMS: *m/z* 259.1542 [(M^+-CH_3), $C_{13}H_{23}O_5$ requires 259.1545].

1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranose (147)



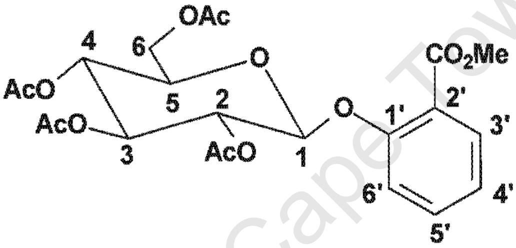
A suspension of anhydrous sodium acetate (2.7 g, 33.3 mmol) and D-glucose (5.0 g, 27.8 mmol) in acetic anhydride (35 ml) was heated at reflux for 1 hour. The clear solution was allowed to cool to room temperature and then poured onto crushed ice (20 g). After standing for 3 hour with occasional stirring, the resultant crystalline material was filtered, washed several times with cold water and dried over phosphorous pentoxide to afford **147** (8.0 g, 74%) as colourless crystals: mp 131-132°C (ethanol) (lit.[129] mp 132°C); δ_H (300 MHz, $CDCl_3$): 1.99, 2.00, 2.01, 2.07, 2.09 (15H, 5 x s, 5 x -$OCOCH_3$), 3.85 – 3.79 (1H, m, H-5), 4.10 (1H, dd, *J* 2.3 and 12.5 Hz, H-6_b), 4.26 (1H, dd, *J* 4.7 and 12.5 Hz, H-6_a), 5.15 - 5.08 (2H, m, H-2 and H-4), 5.24 (1H, t, *J* 9.5 Hz, H-3), 5.70 (1H, d, *J* 8.1 Hz, H-1); δ_C (75.5 MHz, $CDCl_3$): 20.5, 20.5, 20.5, 20.6, 20.7 (5 x -$OCO\underline{C}H_3$), 61.5 (C-6), 67.8 (C-4), 70.3 (C-2), 72.7 (C-3), 72.8 (C-5), 91.7 (C-1), 168.9, 169.2, 169.3, 170.0, 170.5 (5 x –$O\underline{C}OCH_3$).

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (148)



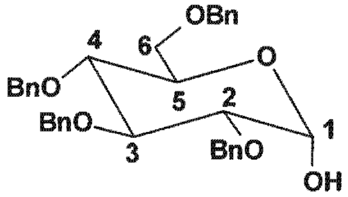
To a solution of **147** (23.0 g, 58.9 mmol) in CH_2Cl_2 (24 ml) was added HBr in acetic acid (30%, 23 ml, 388 mmol). The round-bottomed flask was tightly stoppered and kept at room temperature for 2 hour. Azeotropic distillation with toluene (300 ml x 3) and diethyl ether (80 ml x 2) resulted in a white slurry, which was dissolved in dry diethyl ether (200 ml) and diluted with petroleum ether (300 ml). The semi-crystalline material was left at 0°C for 18 hour and filtered to give compound **148** (17.26 g, 71%) as crystals: mp 87-89°C (diethyl ether/hexane) (lit.[130] 88-89°C); δ_H (300 MHz, $CDCl_3$): 2.02, 2.04, 2.08, 2.09 (12H, 4 x s, 4 x $-OCOC\underline{H}_3$), 4.12 (1H, dd, *J* 1.9 and 12.0 Hz, H-6_b), 4.28 (1H, m, H-5), 4.32 (1H, dd, *J* 4.2 and 12.0 Hz, H-6_a), 4.83 (1H, dd, *J* 4.0 and 9.9 Hz, H-2), 5.15 (1H, dd, *J* 9.6 and 10.2 Hz, H-4), 5.55 (1H, t, *J* 9.6 Hz, H-3), 6.60 (1H, d, *J* 4.0 Hz, H-1); δ_C (75.5 MHz, $CDCl_3$): 20.5, 20.6, 20.6, 20.6 (4 x $-OCO\underline{C}H_3$), 61.0 (C-6), 67.2 (C-4), 70.2 (C-3), 70.6 (C-2), 72.2 (C-5), 86.6 (C-1), 169.4, 169.7, 169.8, 170.4 (4 x $-O\underline{C}OCH_3$).

2,3,4,6-Tetra-O-acetyl-1-O-(2-carbomethoxyphenyl)-β-D-glucopyranoside (149)



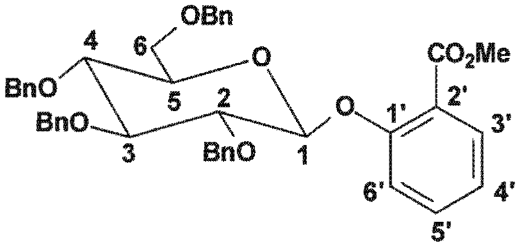
Methyl salicylate (0.07 g, 0.5 mmol) and compound **148** (0.2 g, 0.5 mmol) were suspended in quinoline (0.2 ml) and cooled to 0°C. Ag_2O was added and the mixture stirred at room temperature for 1 hour. Acetic acid (3 ml) was added, the silver salts filtered and the filtrate added to water (50 ml) and the mixture left overnight. The crystals were collected by filtration and recrystallized with methanol to give **149** (0.1 g, 43%): mp 161-162°C (lit.[131] mp 158 – 160°C); δ_H (400 MHz, $CDCl_3$): 2.02, 2.03, 2.05, 2.06 (12H, 4 x s, 4 x $-OCOCH_3$), 3.84 (3H, s, $-OCOC\underline{H}_3$), 3.90 (1H, ddd, *J* 2.4 and 5.2 and 9.2 Hz, H-5), 4.18 (1H, dd, *J* 2.4 and 12.2 Hz, H-6_a), 4.28 (1H, dd, *J* 5.2 and 12.2 Hz, H-6_b), 5.10 (1H, d, *J* 7.2 Hz, H-1), 5.17 (1H, t, *J* 9.2 Hz, H-4), 5.28 (1H, t, *J* 9.2 Hz, H-3), 5.34 (1H, dd, *J* 7.2 and 9.2 Hz, H-2), 7.11 (2H, m, H-4' and H-6'), 7.43 (1H, ddd, J 2.0 and 7.2 and 8.4 Hz, H-5'), 7.74 (1H, dd, J 1.2 and 7.2 Hz, H-3'); δ_C (100 MHz, $CDCl_3$): 20.7, 20.8 (4 x $-OCO\underline{C}H_3$), 52.3 ($-OCO\underline{C}H_3$), 62.2 (C-6), 68.6 (C-4), 71.1 (C-2), 72.3 (C-5), 72.9 (C-3), 99.7 (C-1), 117.1 (C-6'), 122. 7 (C-2'), 123.3 (C-4'), 131.5 (C-3'), 133.2 (C-5'), 155.7 (C-1'), 166.7, 169.5, 169.6, 170.4, 170.7 (5 x $-O\underline{C}OCH_3$).

2,3,4,6-Tetra-O-benzyl-α-D-glucopyranose (150)



Methyl-α-D-glucopyranoside (5.0 g, 25.7 mmol) was added to a suspension of NaH (60% in oil, 8.2 g, 206 mmol) in 1,2-dimethoxyethane (glyme) (25 ml) and refluxed for 2 hours. BnBr (15.3 ml, 129 mmol) and $^{n}Bu_4NI$ (0.95 g, 0.3 mmol) were added and the mixture refluxed for a further 1 hour before being cooled, quenched with methanol and extracted with CH_2Cl_2 (x 3). The crude material was chromatographed (60 g silica, hexane - 20% EtOAc/hexane) to give a colourless oil (13.3 g) which was dissolved in a mixture of acetic acid (200 ml) and H_2SO_4 (1M, 54 ml), and the solution refluxed for 4 hours. A second portion of H_2SO_4 (1M, 54 ml) was added and the mixture refluxed for a further hour. Water (1000 ml) was added and the mixture cooled to 4°C and left for 2 days. The resultant solid was collected and recrystallized with methanol to give **150** (10.0 g, 72%) as colourless crystals: mp. 149-151°C (lit.[132] mp 151-152°C from MeOH); δ_H (300 MHz, $CDCl_3$): 3.06 (1H, d, *J* 3.0 Hz, -OH), 3.65 (3H, m, H-5 and H-6), 4.00 (2H, m, H-3 and H-4), 4.47 – 4.79 (7H, m, -$C\underline{H}_2C_6H_5$), 4.84 (1H, dd, *J* 3.0 and 11.1 Hz, H-2), 4.96 (1H, d, *J* 10.8 Hz, -$C\underline{H}_2C_6H_5$), 5.24 (1H, t, *J* 3.0 Hz, H-1), 7.15 –7.34 (20H, m, -$CH_2C_6\underline{H}_5$); δ_C (75.5 MHz, $CDCl_3$): 68.6 (C-6), 70.4, 73.3, 73.5, 74.9 (-$\underline{C}H_2C_6H_5$), 75.7 (C-4), 77.7 (C-5), 80.1 (C-2), 81.7 (C-3), 91.3 (C-1), 127.58, 127.65, 127.83, 127.89, 127.92, 127.96, 128.02, 128.12, 128.35, 128.37, 128.49, 128.51, 137.86, 137.91, 138.24, 138.71 (-$CH_2\underline{C}_6H_5$).

2,3,4,6-Tetra-O-benzyl-1-O-(2-carbomethoxyphenyl)-β-D-glucopyranoside (151)



Compound **150** (0.30 g, 0.6 mmol), methyl salicylate (0.08 g, 0.6 mmol), and Ph_3P (0.15 g, 0.6 mmol) were dissolved in CH_2Cl_2 (10 ml) and cooled to 0°C. Diisopropyl azodicarboxylate (0.1 ml, 0.6 mmol) was added slowly via syringe and the mixture stirred for 30 minutes. The solvent was evaporated under reduced pressure and the crude material purified by chromatography (15 g silica, 10% EtOAc/hexane) to give **151** (0.2 g, 53%) as colourless crystals: mp 113-114°C (MeOH); $[\alpha]_D$ -32° (*c* 0.99, $CHCl_3$) (lit.[133] mp: 111-112°C (MeOH); $[\alpha]_D$ –34°C (c 1.00, $CHCl_3$)); δ_H (400 MHz, $CDCl_3$): 3.67 (3H, m, H-4 and H-5 and H-6_a), 3.80 (5H, m, H-3 and H-6_b and $-OCOC\underline{H}_3$), 3.86 (1H, dd, *J* 7.4 and 9.0, H-2), 4.56 (3H, m, $-C\underline{H}_2C_6H_5$), 4.86 (3H, m, $-C\underline{H}_2C_6H_5$), 4.99 (1H, d, *J* 10.6, $-C\underline{H}_2C_6H_5$), 5.12 (1H, d, *J* 7.6, H-1), 4.16 (1H, *J* 10.6, $-C\underline{H}_2C_6H_5$), 7.09 (1H, t, *J* 7.6 Hz, H-4'), 7.17 (1H, d, *J* 8.2 Hz, H-6'), 7.20 – 7.35 (20H, m, $-CH_2C_6\underline{H}_5$), 7.42 (1H, ddd, *J* 2.0 and 7.6 and 8.2 Hz, H-5'), 7.83 (1H, dd, *J* 1.6 and 7.6 Hz, H-3'); δ_C (100 MHz, $CDCl_3$): 52.2 ($-OCO\underline{C}H_3$), 69.2 (C-6), 73.7, 75.2, 75.3, 75.9 ($-\underline{C}H_2C_6H_5$), 75.6 (C-4), 77.9 (C-5), 82.3 (C-2), 84.9 (C-3), 101.0 (C-1), 116.1 (C-6'), 121.6 (C-2'), 122.1 (C-4'), 127.75, 127.79, 127.85, 127.88, 128.05, 128.09, 128.19, 128.35, 128.55, 128.58, 128.61, 128.64, ($-CH_2\underline{C}_6H_5$), 131.7 (C-3'), 133.6 (C-5'), 138.26, 138.37, 138.69, 138.80 ($-CH_2\underline{C}_6H_5$), 156.4 (C-1'), 166.7 ($-O\underline{C}OCH_3$).

7.3. Crystal Structure Determinations

A single crystal was covered in a small amount of paratone oil and mounted on a glass fibre. X-ray intensity data were collected at 173 K using a Nonius Kappa CCD with 1.5 kW graphite monochromated Mo radiation. The strategy for the data collection was evaluated using the *COLLECT* Software. The detector to crystal distance was 40 mm. Exposure times of 20 s per frame and scan widths of 1° were used throughout the data collection. Three sets of data were collected: a 181° phi scan and two omega scans to collect cusp data. The three sets of data were scaled and reduced using DENZO-SMN. Unit cell dimensions were refined on all data. The space group $P2_12_12_1$ was chosen on the basis of the systematic absences. The structure was solved and refined using *SHELX97*. Hydrogen atoms were placed in calculated positions and included in the model as riding atoms during later stages of the refinement.

2,3-*O*-Isopropylidene-4-*O*-methyl-5,5-dimethyl-β-D-ribopyranose (90)

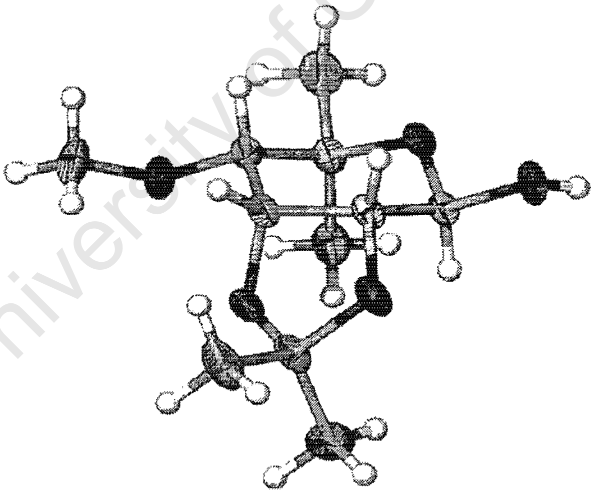


Table 7.1: Crystal data and structure refinement for **90**

Empirical formula	$C_{22}H_{40}O_{10}$
Formula weight	464.54
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic

Space group	$P\,2_1\,2_1\,2_1$
Unit cell dimensions	a = 9.2000 (18) Å α = 90°
	b = 10.697 (2) Å β = 90°
	c = 12.807 (3) Å γ = 90°
Volume	1260.4(4) $Å^3$
Z	2
Density (calculated)	1.224 Mg/m^3
Absorption coefficient	0.096 mm^{-1}
F(000)	504
Crystal size	0.59 x 0.55 x 0.53 mm^3
Theta range for data collection	2.48 to 25.68°
Index ranges	-11<=h<=11, -12<=k<=13, -15<=l<=15
Reflections collected	2386
Independent reflections	2386 [R(int) = 0.0000]
Completeness to theta = 25.68°	99.8 %
Max. and min. transmission	0.9510 and 0.9457
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2386 / 0 / 152
Goodness-of-fit on F^2	1.038
Final R indices [I>2sigma(I)]	R1 = 0.0266, wR2 = 0.0673
R indices (all data)	R1 = 0.0297, wR2 = 0.0695
Absolute structure parameter	-0.1(7)
Extinction coefficient	0.029(3)
Largest diff. peak and hole	0.144 and -0.125 e.$Å^{-3}$

Table 6.2: Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for **90**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	**y**	**z**	**U(eq)**
O(1)	798(1)	9810(1)	5849(1)	30(1)
O(2)	2573(1)	9330(1)	3360(1)	29(1)
O(3)	2635(1)	8814(1)	6694(1)	35(1)
O(4)	-314(1)	10027(1)	3135(1)	38(1)
O(5)	3759(1)	8140(1)	4574(1)	35(1)
C(6)	3920(1)	8690(1)	3549(1)	34(1)
C(4)	111(1)	9412(1)	4071(1)	29(1)
C(3)	1487(1)	8650(1)	3924(1)	27(1)
C(1)	2242(1)	9346(1)	5742(1)	27(1)
C(5)	209(1)	10409(1)	4929(1)	30(1)
C(2)	2302(1)	8322(1)	4924(1)	28(1)
C(5')	-1322(2)	10776(2)	5252(1)	49(1)
C(6')	4148(2)	7659(2)	2757(1)	48(1)
C(14)	-714(2)	9201(2)	2319(1)	54(1)
C(5")	1080(2)	11556(1)	4607(1)	38(1)
C(6")	5115(2)	9644(2)	3554(1)	49(1)

Table 6.3: Selected bond lengths for **90**

Bond	Length (Å)	Bond	Length (Å)
O(1)-C(1)	1.4252(15)	C(6)-C(6")	1.500(2)
O(1)-C(5)	1.4463(15)	C(6)-C(6')	1.513(2)
O(2)-C(3)	1.4316(15)	C(4)-C(3)	1.5173(17)
O(2)-C(6)	1.4364(15)	C(4)-C(5)	1.5347(18)
O(3)-C(1)	1.3922(14)	C(3)-C(2)	1.5249(17)
O(4)-C(14)	1.4173(17)	C(1)-C(2)	1.5173(16)
O(4)-C(4)	1.4222(14)	C(5)-C(5')	1.5189(19)
O(5)-C(2)	1.4265(16)	C(5)-C(5")	1.5222(19)
O(5)-C(6)	1.4465(14)		

Table 6.4: Selected torsion angles for **90**

Bonds	Angle (°)	Bonds	Angle (°)
C(1)-O(1)-C(5)	115.20(9)	C(4)-C(3)-C(2)	115.48(10)
C(3)-O(2)-C(6)	105.93(9)	O(3)-C(1)-O(1)	107.48(9)
C(2)-O(5)-C(6)	109.04(9)	O(3)-C(1)-C(2)	107.43(9)
O(2)-C(6)-O(5)	104.97(9)	O(1)-C(1)-C(2)	110.57(9)
O(4)-C(4)-C(3)	111.94(10)	O(1)-C(5)-C(4)	107.29(10)
O(4)-C(4)-C(5)	107.34(10)	O(5)-C(2)-C(1)	110.49(10)
C(3)-C(4)-C(5)	114.40(10)	O(5)-C(2)-C(3)	103.24(9)
O(2)-C(3)-C(4)	111.80(9)	C(1)-C(2)-C(3)	113.31(10)
O(2)-C(3)-C(2)	101.42(10)		

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