A Chemo-enzymatic Approach to the Synthesis of Carbocyclic Nucleosides and Intermediates in the Biosynthesis of Carbocyclic Nucleosides by 
*Streptomyces citricolor*

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Doctor of Philosophy

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DECLARATION

This thesis is submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Edinburgh. Unless otherwise stated, the work described is original and has not been previously submitted, in whole or in part for any degree at this or any other university.
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Finally thank you to Mum and Dad for constant support and without whom, this PhD would never have been completed. For support, for always being there, for keeping me going in the right direction and for proof reading this report, a huge thank you to Alison.
ABSTRACT

The carbocyclic nucleosides aristeromycin I and neplanocin A II are produced from D-glucose by *Streptomyces citricolor*.

![Chemical structures of aristeromycin I and neplanocin A II]

To investigate the mechanism of the biosynthesis, an effective route to a putative intermediate III has been developed. By modifications to a known procedure, a single low yielding and unreliable reaction has been improved considerably.

![Chemical reaction pathway]

By using a tert-butyl-diphenyl silyl group in place of the acetyl group present in the original procedure, a more stable compound was obtained, which was tolerant to the insertion of a protected hydroxymethyl moiety. Yields for this reaction were increased from ≤ 20% to 76%.

An efficient route to enantiomerically pure 3-benzyloxymethylcyclopentene VI has been demonstrated. Using *Rhizopus arrhizus* ATCC 11145, ethyl cyclopentanone-2-carboxylate was reduced to give a single enantiomer of 2-hydroxy-cyclopentanecarboxylic acid ethyl ester VII which was converted in 5 steps to the desired substrate.

![Chemical reaction pathway]

It was observed that VI could be hydroxylated at C-5 by incubation with *Rhodococcus rhodochrous* NCIMB 9703, to give the corresponding alcohol VIII suitable for further elaboration to carbocyclic nucleosides.
# Abbreviations

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<thead>
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<tbody>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2'-azobisisobutyronitrile</td>
</tr>
<tr>
<td>Ala</td>
<td>alanine</td>
</tr>
<tr>
<td>AIDS</td>
<td>acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>bp</td>
<td>boiling point</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyl</td>
</tr>
<tr>
<td>CAL-B</td>
<td><em>Candida antarctica</em> lipase B</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicycl[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DDQ</td>
<td>2,3-dichloro-5,6-dicyano-1,4-benzoquinone</td>
</tr>
<tr>
<td>DEAD</td>
<td>diethyl azodicarboxylate</td>
</tr>
<tr>
<td>DHP</td>
<td>dihydropyran</td>
</tr>
<tr>
<td>DIBAL</td>
<td>diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DIPEA</td>
<td>diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4,4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td><em>N</em>,<em>N</em>-dimethylformamide</td>
</tr>
<tr>
<td>DMP</td>
<td>2,2-dimethoxypropane</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>e.e.</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>EEDQ</td>
<td>2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HMDS</td>
<td>hexamethyldisilazane</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>HMPA</td>
<td>hexamethyphosphoric triamide</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>IBX</td>
<td>o-iodobenzoic acid</td>
</tr>
<tr>
<td>Im</td>
<td>imidazole</td>
</tr>
<tr>
<td>LDA</td>
<td>lithium diisopropylamide</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MEM</td>
<td>methoxymethoxymethyl</td>
</tr>
<tr>
<td>MOM</td>
<td>methoxymethyl</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
<tr>
<td>Ms</td>
<td>methylsulfonyl (mesyl)</td>
</tr>
<tr>
<td>NCIMB</td>
<td>National Collection of Industrial and Marine Bacteria</td>
</tr>
<tr>
<td>NMO</td>
<td>4-methylmorpholine N-oxide</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NNRTI</td>
<td>non nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>nOe</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>NRTI</td>
<td>nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>PCC</td>
<td>pyridinium chlorochromate</td>
</tr>
<tr>
<td>PDC</td>
<td>pyridinium dichromate</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>PMB</td>
<td>p-methoxybenzyl</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>PPTS</td>
<td>pyridinium p-toluensulfonate</td>
</tr>
<tr>
<td>Pr</td>
<td>propyl</td>
</tr>
<tr>
<td>pTSA</td>
<td>p-toluensulfonic acid</td>
</tr>
<tr>
<td>RCM</td>
<td>ring closing metathesis</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>TBAF</td>
<td>tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert-butylmethylsilyl</td>
</tr>
<tr>
<td>TBDPS</td>
<td>tert-butylphenylsilyl</td>
</tr>
<tr>
<td>Tf</td>
<td>trifluoromethylsulfonyl (triflyl)</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TIPDS</td>
<td>tetraisopropylidisilane</td>
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<td>Description</td>
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<td>-------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilyl</td>
</tr>
<tr>
<td>TPAP</td>
<td>tetrapropylammonium perruthenate</td>
</tr>
<tr>
<td>Ts</td>
<td>$p$-tolylsulfonyl (tosyl)</td>
</tr>
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1. INTRODUCTION

1.1 AIDS and HIV treatment

It is estimated that around 40 million people globally are infected with HIV or AIDS. Of these people, it is thought that 95% live in the developing world. In many such nations, the impact of HIV on various social institutions, e.g. schools, health care, armed forces, is considerable. While clearly there is a need to prevent infection to avoid human suffering, it is also economically advantageous since preventative measures are less costly than treatment of infected individuals. Encouraging work is being done to identify effective HIV and AIDS prevention programs. There is a strong suggestion that the most effective strategies employ a combination approach in which multiple interventions are utilised. Senegal is reported to have one of the lowest rates of HIV infection in sub-Saharan Africa, and this is attributed to the adoption of various prevention strategies such as voluntary HIV counselling and testing, condom promotion among female sex workers and their clients, enhanced STI treatment programs, HIV education in schools and assistance by religious leaders in talking about HIV and AIDS in mosques.

In addition to preventative measures, HIV treatment is of great value. Where available, modern drug therapies can result in people living with HIV/AIDS having a longer and better life. HIV reverse transcriptase is the enzyme required for the formation of proviral DNA. The HIV RNA genome is converted to DNA that can be incorporated into a host cell's chromosome. The proviral DNA is then used to produce viral proteins and RNA which is assembled into viral particles in the presence of an HIV protease. These two enzymes - reverse transcriptase and the protease - have received the greatest attention as targets for inhibition. The World Health Organisation (WHO) Model List of Essential Medicines, April 2003 recommends seven reverse transcriptase inhibitors and five protease inhibitors. Of the reverse transcriptase inhibitors, five are nucleoside reverse transcriptase inhibitors (NRTIs) and two are non-nucleoside reverse transcriptase inhibitors (NNRTIs). Figure 1.1 shows the structures of the five NRTIs.
The most effective treatments use a combination of drugs from different classes, e.g., one protease inhibitor and two NRTIs. Combination therapy is more effective in disease progression, and the length of time in which the treatment continues to be effective is increased.

1.1.1 Abacavir

The only carbocyclic nucleoside recommended by the WHO or US Food and Drug Administration (FDA) for the treatment of HIV is abacavir. Carbocyclic nucleosides represent an important class of compounds in the treatment of viral infections. While similar to natural nucleosides, carbocyclic nucleosides differ in that the furanose oxygen is replaced with a methylene group. The result is a compound which is more resistant to metabolism in the body due to increased resistance to phosphorylase enzymes which cleave the glycosidic linkage of normal nucleosides. Abacavir was developed to address the shortcomings (bioavailability, toxicity and CNS penetration) of its predecessor carbovir. It is marketed as abacavir sulfate by GSK under the brand name of Ziagen or, along with lamivudine and zidovudine, as Trizivir®. For patients receiving abacavir in combination with other antiretroviral drugs, reductions in HIV RNA levels were greater and more prolonged than for patients receiving placebo in combination with the same antiretroviral drugs. In fact, in combination with lamivudine and zidovudine, viral
load was reduced below detectable levels for some patients. Although the greatest viral load reductions were observed for individuals with little or no previous antiretroviral treatment, useful responses were still sometimes achieved in heavily pretreated individuals. The increased effectiveness of including abacavir in combination with two other NRTIs is especially attractive since the option to use treatment involving protease inhibitors remains open. The inclusion of abacavir in combination with other NRTIs is effective because the anabolic pathway of abacavir is different to other NRTIs which are phosphorylated by nucleoside kinases or other phosphotransferases. The mechanism of conversion of abacavir 5 to the active metabolite is shown in scheme 1.2.

Scheme 1.2
Postulated anabolic transformation of abacavir to the active metabolite in human cells.
INTRODUCTION

The abacavir enters erythrocytes and T-lymphoblastoid CD4+ CEM cells (white blood cells, also called “helper T lymphocytes” which lead the attack against infections) then binds to cytosolic protein. A sequence of phosphorylation, deamination and further phosphorylation then gives the active metabolite 8. The carbovir triphosphate 8 inhibits the activity of HIV-1 reverse transcriptase both by competing with the natural substrate 2'-deoxyguanosine triphosphate (dGTP) 9 (figure 1.3) and by its incorporation into the viral DNA. The lack of a 3' hydroxyl group in carbovir triphosphate 8 prevents formation of the 5' to 3' phosphodiester linkage, with the result that viral DNA growth is terminated.6

![Structure of 2'-deoxyguanosine triphosphate (dGTP)](image)

Figure 1.3
Structure of 2'-deoxyguanosine triphosphate (dGTP)

1.1.2 The need for continued development of anti-HIV treatment

Abacavir is not a complete solution to AIDS/HIV treatment however. The most common side effect for abacavir patients is nausea. Other side effects are include vomiting, malaise and fatigue, headache, diarrhoea, sleep disorders, cough, anorexia and rash. While the majority of symptoms are mild and transient, a number of patients (3-5%) cannot be treated with abacavir due to the development of a hypersensitivity reaction.5 Furthermore, no current HIV treatment is a complete cure. Current drugs have limited potency and time limited effectiveness.2 The benefit of these drugs decreases as drug resistance prevails. Drug resistance develops as mutations occur in the virus. While abacavir appears generally to be reasonably tolerant to single mutations, sensitivity was reduced in cases where multiple mutations were present.5 It is thought that in some patients, antiretroviral treatment can result in sustained suppression of plasma levels of HIV, but replication competent virus can still exist in latently infected resting CD4+ lymphocytes.7 The implication of this is that the provirus remains hidden from the immune system and inaccessible to antiretroviral drugs. It
was estimated that the half life of the latent virus in resting CD4+ lymphocytes is approximately 6 months. As such many years of effective HIV treatment would be required to eliminate the pool of HIV. Therefore it is necessary to continue development of NRTIs to increase potency, prolong effectiveness and at the same time reduce side effects. However, work is also required to establish a therapy which can deplete the reservoirs of latent HIV and ultimately eradicate HIV from the body entirely.
1.2 Aristeromycin and neplanocin A

1.2.1 Therapeutic utility

The importance of carbocyclic nucleosides in medicine extends beyond AIDS treatment however. The carbocyclic analogue of adenosine was first synthesised, in racemic form, by Shealy and Clayton in 1966.\textsuperscript{8} (-)-Aristeromycin \textsuperscript{10} and (-)-neplanocin A \textsuperscript{11} were then isolated from \textit{Streptomyces citricolor} and \textit{Ampullariella regularis} respectively. Neplanocin A was subsequently found to be co-produced along with aristeromycin by \textit{Streptomyces citricolor}.\textsuperscript{11} These compounds displayed antibiotic and antitumour activity and consequently ignited interest in carbocyclic nucleosides.\textsuperscript{4,12} The structures of aristeromycin and neplanocin A are shown in fig 1.4.

![Figure 1.4](image-url) Structures of (-)-aristeromycin and (-)-neplanocin A.

Borchardt \textit{et al.}\textsuperscript{13} observed, that as well as having antitumour activity against L1210 leukemia,\textsuperscript{10} neplanocin A is a potent inhibitor of S-adenosylhomocysteine (SAH) hydrolase. Inhibition of SAH hydrolase results in the accumulation of S-adenosylhomocysteine which in turn inhibits S-adenosylmethionine (SAM) transferase which is required for viral mRNA capping. mRNA must be capped and methylated at the 5' terminus to promote active translation of the corresponding proteins. As such, Borchardt deduced that the antiviral activity of neplanocin A arose from the inhibition of SAH hydrolase and reported that neplanocin A elicits potent antiviral activity against vaccina virus (WR). Despite considerable efforts and the discovery of promising antiviral agents, the need for new inhibitors of HIV, cytomegalovirus (CMV), herpes simplex virus types 1 and 2 (HSV 1 & 2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), hepatitis B virus (HBV)\textsuperscript{12} and West Nile virus (WNV)\textsuperscript{14} is undiminished.
1.2.2 Biosynthesis of aristeromycin and neplanocin A by *Streptomyces citricolor*

A complete understanding of the mechanism by which neplanocin A and aristeromycin are produced by *Streptomyces citricolor* could provide a valuable tool in the search for novel carbocyclic nucleosides. Many of the steps in this process are known, and a review by Turner and Jenkins\(^5\) provides much of this information. Key points will be outlined here, along with a discussion of the work carried out since this review.

Scheme 1.5 shows the known intermediates on the pathway of the biosynthesis of neplanocin A and aristeromycin.

\[
\begin{align*}
12 & \quad \text{D-glucose} \\
13 & \\
14 & \\
10 & \quad \text{aristeromycin} \\
11 & \quad \text{neplanocin A}
\end{align*}
\]

Scheme 1.5
Biosynthesis of (−)-aristeromycin and (−)-neplanocin A by *Streptomyces citricolor*. 
Formation of the carbocyclic ring

The carbocyclic ring was found, by Parry et al., to originate from D-glucose 12 by formation of a C—C bond between C2 and C6. Work by Turner used mutants of S. citricolor which were either unable to produce aristeromycin and neplanocin A (a secretor mutant) or able to rescue the synthesis (a converter mutant) by conversion of the supernatant from cultures of the secretor, to produce aristeromycin and neplanocin A. Such pairings could be used to identify intermediates in the biosynthetic pathway as illustrated in scheme 1.6.

Scheme 1.6
Cosynthesis and synthetic putative intermediate feeding experiments using Streptomyces citricolor

The mutant CC914 secreted a compound which supported production of neplanocin A and aristeromycin in the second mutant CC940. This compound was identified to be the tetrol 14. The strategy was then extended to the synthesis and feeding of other putative intermediates. In this way, the enone 13 was identified as an intermediate on the biosynthetic pathway.

Incorporation of the nucleoside base

Evidence for the origin of the adenine base was obtained by using a S. citricolor mutant in combination with isotopic labelling experiments. A mutant of S. citricolor (CC268) which required exogenous addition of adenine, was grown on a defined medium containing 8-13C-adenine as the only purine source. The aristeromycin produced contained the 13C label only at the C8 position in around 79% incorporation. This is
The alternative pathway for incorporation of the adenine base is a stepwise construction. There is evidence to suggest that the C2, 4 and 5 carbon atoms are derived from glycine, and an experiment similar to that described in scheme 1.7 using 1-$^{13}$C glycine as a precursor, resulted in production of 4-$^{13}$C labelled aristeromycin (ca. 20% incorporation). Nevertheless, the weight of evidence appeared to suggest that the major route to aristeromycin is direct incorporation. This in turn implied the involvement of a carbocyclic intermediate of the type 16, activated at C1 by a pyrophosphate group.

![Scheme 1.7](image)

Incorporation of labelled adenine base in (-)-aristeromycin

Figure 1.8
Phosphorylated carbocycle likely to be involved in a direct incorporation pathway

Relationship of aristeromycin to neplanocin A

The similarity of neplanocin A and aristeromycin suggested that they might be closely related on the biosynthetic pathway. It was possible that neplanocin A was a precursor to aristeromycin, or that they were produced along independent routes. Labelling studies by Parry showed that the 6-pro-R proton of glucose becomes the 6'-pro-S in aristeromycin and the 6-pro-S proton of glucose is lost. By feeding 6-$^3$H$_2$-glucose to a mutant of *S. citricolor* (CC1026) which produced neplanocin A but no aristeromycin, Turner obtained a sample of 6'-2H-neplanocin A. This sample was then administered
to a second mutant (CC826) which was able to convert neplanocin A to aristeromycin. The 6'-2H-aristeromycin obtained in this way was stereochemically identical to that derived from the equivalent 6-2H₂-glucose wild type S. citricolor experiment (scheme 1.9) thereby, suggesting that neplanocin A is the direct precursor to aristeromycin.

Scheme 1.9
Stereochemistry of deuterium incorporation into 6'-2H-neplanocin A and 6'-2H-aristeromycin using wild-type and mutant strains of S. citricolor.

1.2.3 Elucidation of remaining steps

Mechanism of cyclisation

Thus, the remaining steps in the sequence to be identified were those involved in the conversion of D-glucose to the enone 13. By comparison with known pathways of formation of 6-membered rings - the myo-inositol-1-phosphate synthase reaction, and shikimate biosynthesis Turner was able to propose 2 possible cyclisation pathways for 5 membered rings. Of these, Turner deduced that the shikimate-like route was more likely and this is shown in scheme 1.10. Subsequent work to determine the mode of cyclisation was based around this route and focused on the keto-tetrol 20.
Similar experiments to those already discussed involving a converter mutant in conjunction with the keto-tetrol 20 were envisaged. Scheme 1.10 shows the keto-tetrol 20 with undefined stereochemistry at Cl and therefore, both diastereomers of 20 were required for testing. Furthermore, in order to confirm the hypothesis that 16 was an intermediate on the pathway, and therefore, that the adenine base was incorporated intact, the synthesis and feeding of this activated carbocycle was also proposed. This work was carried out by Nicola Paterson in these labs. Paterson developed syntheses of compounds 14, (2R, 3S, 4R)-20, (2R, 3S, 4S)-20 and (2S, 3R, 4S)-20 (figure 1.11).

The tetrol 14 was required in order to establish that the S. citricolor strain was functioning as expected i.e. able to produce aristeromycin and neplanocin A from the known intermediate. The keto-tetrol (2S, 3R, 4S)-20, thought to be the 'wrong
enantiomer', was required to demonstrate that production of aristeromycin and neplanocin A would not be supported by an incorrect stereochemical configuration. The two diastereomers (2R, 3S, 4R)-\(\mathbf{20}\), (2R, 3S, 4S)-\(\mathbf{20}\) were synthesised in order to determine which, if either would support production of aristeromycin and neplanocin A in the \textit{S. citricolor} (CC940) mutant.

Production of aristeromycin and neplanocin A on feeding of the tetrol 14 indicated that the culture had been revived successfully. This procedure was run in parallel with subsequent experiments to identify erroneous results.

The first experiments demonstrated that keto-tetrol (2R, 3S, 4R)-\(\mathbf{20}\) was successfully converted to aristeromycin and neplanocin A, while the 'wrong enantiomer' (2S, 3R, 4S)-\(\mathbf{20}\) showed no signs of conversion to aristeromycin and neplanocin A, as expected. Since the diastereomeric pair (2R, 3S, 4R)-\(\mathbf{20}\) and (2R, 3S, 4S)-\(\mathbf{20}\) both eliminate to give the same product (13) the feeding of the (2R, 3S, 4S)-\(\mathbf{20}\) diastereomer was then carried out in order to determine whether or not the elimination process was entirely enzymatic. The (2R, 3S, 4S)-\(\mathbf{20}\) diastereomer was also found to be converted to aristeromycin and neplanocin A. These results are summarised in scheme 1.12.

\textbf{Scheme 1.12}

Results of feeding studies for all 3 keto-tetrols \(\mathbf{20}\).
These results suggested that spontaneous chemical elimination to tetrol 14 was possible, and it was unclear if either diastereomer was in fact an intermediate on the biosynthetic pathway. Subsequent experiments indicated that because chemical elimination was slow relative to the time scale of the conversion to aristeromycin, even at low pH, it was unlikely to be the pathway of formation of the enone. A small inseparable amount of the tetrol 14 resulting from the low pH required to remove the acetonide protection was known to be present in both samples of keto-tetrol 20. This added to the uncertainty surrounding these experiments and the ability of both diastereomers to support production of aristeromycin and neplanocin A could not therefore be understood fully. It was postulated that the synthesis of the 5-\(^{13}\)C (hydroxymethyl carbon) labelled keto-tetrols (2R, 3S, 4R)-20, (2R, 3S, 4S)-20 may provide greater insight into the feeding experiments. However, due to time constraints, and also, difficulties encountered in the synthesis of (2R, 3S, 4S)-20, Paterson was unable to verify this.

**Mechanism of incorporation of the base**

In order to examine more closely, the steps involved in the coupling of the purine base, compounds 14, 16 and 21, as shown in figure 1.13 were identified as key compounds for further feeding studies.

![Figure 1.13](image)

*Figure 1.13*

Synthetic targets for feeding studies using a mutant of *S. citricolor* to examine the mechanism of base coupling

In fact, tetrol 14 and triol 21 were successfully synthesised, but due to time constraints, Paterson was unable to synthesise the phosphorylated species 16 or carry out the feeding experiments. Therefore, some ambiguity remained surrounding the formation of the carbocyclic ring, and further information on the mechanism of base coupling was not obtained.
1.3 Synthesis of carbocyclic nucleosides

The therapeutic potential of carbocyclic nucleosides has resulted in the synthesis of these compounds receiving much attention. Below is a review of strategies towards the synthesis of carbocyclic nucleosides for the period 2000–2003. For an overview of work done prior to this time, papers by Borthwick and Crimmins provide thorough discussion, and Ferrero and Gotor detailed progress in the use of biocatalysis in this field. Furthermore, although there is some interest in four or six-membered carbocycles, conformationally locked carbocycles and racemic syntheses, only five membered optically active carbocycles will be discussed here.

Strategies for the synthesis of carbocyclic nucleosides can be categorised according to the method in which the nucleoside base is coupled to the carbocycle. Two possibilities exist: the linear approach and the convergent approach. This review will discuss how these couplings can be achieved, followed by recent examples of the synthesis of the required precursors.

1.3.1 Linear approaches

The linear approach entails the stepwise construction of the base on an appropriate amino precursor. This approach is less common than the convergent approach since it requires more steps and typically therefore lower overall yields. The linear approach does however allow facile variation in the base, making it attractive for the preparation of a series of compounds e.g. for biological screening. The techniques for construction of purine and pyrimidine bases are now well established. These will be described here, as well as some less common analogues.
Pyrimidines

Uridine and thymine derivatives

Santana et al.\textsuperscript{28} described the synthesis of uridine and thymine derivatives using methodology originally developed by Shaw and Warrener.\textsuperscript{29} The sequence is shown in scheme 1.14.

![Scheme 1.14](image)

**Scheme 1.14**

*Reagents and Conditions:* (a) O=C=N-CO-C(R)=CH-O-CH, benzene, DMF; (b) 2M H\textsubscript{2}SO\textsubscript{4}

After condensation of the amine 22 with the appropriate isocyanate, cyclisation in the presence of sulfuric acid gave the pyrimidines derivatives 25 and 26 in 69 and 55% overall yield respectively. Santana demonstrated that 25 could be halogenated at the uracil position 5 in acetic acid with N-bromosuccinimide or N-chlorosuccinimide, presumably with the intention of subsequent further elaboration.
Purines

Adenine derivatives

An approach by Katagiri et al. for the construction of adenine derivatives was applied to the synthesis of conformationally restricted bicyclic carbocyclic nucleosides by Bhushan and Vince as shown in scheme 1.15.

Scheme 1.15
Reagents and Conditions: (a) 5-amino-4,6-dichloropyrimidine, Et₃N, n-BuOH, reflux, 24h; (b) CH(OEt)₃, conc HCl, 24h; (c) liq NH₃, 48h.

Thus the amine 27 was coupled with 5-amino-4,6-dichloropyrimidine to give the pyrimidylamino derivative 28. Ring closure was effected with triethyl orthoformate in the presence of HCl to give the chloropurine 29. Subsequent displacement of the chloro group with liquid ammonia gave the adenine derivative 30.
Guanine derivatives

Santana and Vince both demonstrated the stepwise construction of guanine derivatives, according to the procedure described by Shealy and Clayton. The work by Vince is illustrated in scheme 1.16.

\[ \text{Scheme 1.16} \]
Reagents and Conditions: (a) 2-amino-4,6-dichloropyrimidine, Et,N, n-BuOH, reflux, 24h; (b) p-chloroaniline, 3N HCl, NaNO₂, AcOH, NaOAc, 24h; (c) Zn, AcOH, EtOH, reflux, 5h; (d) CH(OEt), conc HCl, 24h.

The amine was condensed with 2-amino-4,6-dichloropyrimidine to give the pyrimidylamino derivative 31. Introduction of the amino group at the 5 position was effected by diazotization with p-chlorobenezenediazonium chloride followed by reduction with zinc in the presence of acetic acid to give the amine 33. This was then cyclised using the same conditions as for adenine to give the 2-amino-6-chloropurine 34. Treatment with hydrochloric acid gave the guanine analogue 35. Formation of the diaminopurine 36 was effected by treatment of 34 with liquid ammonia. The propyl derivative 37 was obtained by displacement of the 6-chloro group with n-propanol in the presence of sodium hydride.
In addition to the construction of the diaminopurine and guanine bases discussed here, Santana described the synthesis of azapurine bases in which the carbon at the 8 position is replaced with a nitrogen\(^{28,32}\) (scheme 1.17).

\[
\begin{align*}
38 & \xrightarrow{(a)} 39 \\
& \xrightarrow{(b)} 60\% \\
& \xrightarrow{(c)} \\
39 & \xrightarrow{97\%} 40 \\
& \xrightarrow{90\%} 41
\end{align*}
\]

Scheme 1.17

*Reagents and Conditions:* (a) \(\text{NaNO}_2, \text{H}_2\text{O}, \text{AcOH}\); (b) \(\text{NH}_3/\text{MeOH}\); (c) 0.33M \(\text{NaOH}\).

Diazotization of the aminopyrimidine 38 with sodium nitrite in acetic acid and spontaneous cyclisation gave the 8-azapurinyl analog 39. Subsequent modifications to give the 8-aza-2,6-diaminopurinyl 40 or 8-azaguanine 41 derivatives were effected by treatment with liquid ammonia or sodium hydroxide respectively. Furthermore, using a similar modification to the cyclisation step, 8-azainosine and 8-azaadenine derivatives were constructed according to the Katagiri methodology.
Finally, Umezawa et al. described the construction of oxanosine derivatives. Although the oxanosine base is similar to guanine except that the 1-amino group is replaced with an oxygen, the synthesis is quite different and more alike to a procedure described by Birkett et al. The procedure is shown in scheme 1.18.

The amine 42 was reacted with ethyl N-[ethoxycarbonyl(cyano)methyl]-formimidate to give the imidazole 43. The thiourea 44 was then formed by reaction of 43 with ethoxycarbonyl isothiocyanate and this was transformed to the methylthio derivative 45 by treatment with iodomethane under basic conditions. The final ring formation was effected by sequential treatment with methanolic KOH and HCl to give the imidazo-oxazinone 46.
1.3.2 Carbocyclic precursors for the linear approach

Methods for the stepwise construction of nucleoside bases are relatively well established and much of the attention in recent times has focused on the search for improved routes to the carbocyclic moiety. Since the therapeutic activity of compounds of this type is associated with a single enantiomer, much attention has been paid to developing enantiomerically pure precursors. This report describes 4 possible means of achieving this.

1. Enzymatic resolution

A suitable enzyme can be used to resolve a racemic mixture or mediate the transformation of an achiral compound to give an optically active product.

2. Chiral starting materials

Some compounds can be bought as a single enantiomer. Carbohydrates are commonly used in this way.

3. Chiral auxiliary

A chiral compound can be attached to the achiral starting material to influence a subsequent transformation and give an optically active compound on its removal.

4. Enantioselective chemical transformation

Use of chiral reagents such as chiral reducing agents can be used to effect enantioselective transformations.
Synthesis of carbocycles using enzymatic resolution to introduce chirality

An enzyme can effect a transformation on a single enantiomer of a racemic mixture, thus giving a separable mixture of optically active starting material and product. Alternatively, where possible, the enzymatic transformation of a meso-type compound can afford a single enantiomer, without the loss associated with the resolution of a racemic mixture. However, Ogasawara et al.\textsuperscript{35} described a synthesis which included steps for chirality inversion of the undesired enantiomer to minimise this loss. The synthesis of the substrate for lipase resolution is shown in scheme 1.19.

![Scheme 1.19](image)

\textbf{Scheme 1.19}

\textit{Reagents and Conditions:} (a) OsO\textsubscript{4}, trimethylamine N-oxide, t-BuOH, H\textsubscript{2}O, pyridine, reflux, 3h; (b) DMP, acetone, Dowex 50G-X8 cation-exchange resin, 4h; (c) PDC, pyridinium trifluoroacetate, silica gel, DCM, 24h; (d) LDA, THF, -78°C, 1h, then TMSCl, 0.5h; (e) Et\textsubscript{2}Zn, CH\textsubscript{3}I, hexane, DCM, 40°C, 30 minutes; (f) FeCl\textsubscript{3}, DBU, DMF, 0°C, 0.5h; (g) CeCl\textsubscript{3}.7H\textsubscript{2}O, NaBH\textsubscript{4}, MeOH, -78°C, 5 minutes; (h) Ac\textsubscript{2}O, DMAP, Et\textsubscript{3}N, DCM, 1h.

Conversion of the norborneneol 47 to the racemic triol (±)-49 was accomplished according to the procedure described by Cookson \textit{et al.}\textsuperscript{36} in 3 steps. Ring expansion was then accomplished by formation of the silyl enol ether, cyclopropanation, and oxidative ring expansion to give the enone (±)-52. Both the endo-alcohol (±)-53 and acetate (±)-54 formed by reduction of the enone, and subsequent acetylation respectively, were useful substrates for the lipase resolution. Scheme 1.20 illustrates the enzymatic resolution using \textit{Pseudomonas} sp., Amano (lipase PS).
INTRODUCTION

(±)-53 + OAc → (-)-54 + (+)-53
yield 49% e.e. 96%

(±)-54 → (-)-53 + (+)-54
yield 48% e.e. >99%

Scheme 1.20
Reagents and Conditions: (a) LipasePS, vinylacetate, t-BuOMe, 3.5d; (b) lipasePS, phosphate buffer, acetone, 2d.

Thus the alcohol (-)-53 could be obtained directly from the hydrolysis conditions (scheme 1.20(b)) or after deacetylation, using the transesterification route (scheme 1.20(a)). The remaining steps in the synthesis of the carbocyclic precursor are shown in scheme 1.21.

(-)-53 → 55 → 56
82% 85%

59 → 58 → 57
97% 78% 98%

Scheme 1.21
Reagents and Conditions: (a) i. O3, MeOH, -78°C, 20 minutes, ii. NaBH4, 0.5h, iii. NaNH, aq THF; (b) TPAP, NMO, DCM, mol sieves 4Å, 2h; (c) i. NH3, MeOH, 0°C, ii. Ac2O, pyridine, 16h; (d) t-BuOH, PbOAc4, Et3N, reflux, 5h; (e) 2M HCl, MeOH, reflux, 1h.
The alcohol (-)-53 was sequentially treated with ozone, sodium borohydride and finally sodium periodate to give the hydroxy aldehyde which after cyclisation gave the hemiacetal 55. Oxidation with TPAP gave the lactone (+)-56. The same enantiomer of this key lactone could also be obtained from alcohol (+)-53 after the chirality inversion (3 steps, 60% overall yield) which is not described here. The remaining steps are the conversion of this key lactone (+)-56 to the carbocyclic precursor (+)-59 as described by Ohno et al.37

Synthesis of carbocycles using optically active starting materials

Umezawa et al.33 described a short route using the commercially available (-)-2-azabicyclo[2.2.1]hept-5-en-3-one 60 (scheme 1.22).

![Scheme 1.22](image)

Scheme 1.22

Reagents and Conditions: (a) OsO₄, NMO, Me₂CO, 3h; (b) DMP, TsOH, DMF; (c) Boc₂O, DMAP, MeCN, 15h; (d) NaBH₄, MeOH, 0°C–R.T., 2h; (e) MOMCl, DIPEA, DCM, 5h, 88%; (f) H₂O, reflux, 6h.

The first 4 steps were performed according to the procedure described by Hutchinson et al.38 to give alcohol 63. The remaining protection/deprotection steps gave the precursor 64. It is noteworthy that Roberts et al.39 demonstrated the resolution of the lactam 60 using the whole cell systems ENZA-1 and ENZA-20 giving either enantiomer in >98% e.e.
Synthesis of carbocycles using enantioselective chemical transformation

The transformation of meso-compounds to give one enantiomer of the product can be achieved by chemical means using chiral reagents, as well as enzymatically. The example described here is the rearrangement of a meso-epoxide using a chiral lithium amide. The epoxide cis-71 was obtained according to the route shown in scheme 1.23.

\[
\text{Scheme 1.23}
\]
Reagents and Conditions: (a) Na$_2$CO$_3$, DCM, 40% peracetic acid, sodium acetate; (b) LiAlH$_4$, Et$_2$O, 0°C, 3h; (c) MsCl, Et$_3$N, DCM, 0°C, 0.5h; (d) NaN$_3$, DMF, 80°C, 16h; (e) PPh$_3$, THF, 1.5h, then H$_2$O, reflux 16h, then DCM, HCl, RT.; (f) BzCl, pyridine, Et$_3$N, CHCl$_3$; (g) mCPBA, CF$_3$CO$_2$I.

The alcohol 67 was obtained by epoxidation of cyclopentadiene and reduction, as described by Crandall et al.\textsuperscript{41} This was then transformed to the amine hydrochloride salt in 4 steps by the route described by Barrett et al.\textsuperscript{41} The remaining steps, benzoylation and epoxidation were carried out following work by Koga et al.\textsuperscript{42} The epoxidation gave the cis- and trans- isomers which could be separated by conventional chromatography. Asami had previously demonstrated that chiral lithium reagents could effectively mediate the rearrangement of such meso-epoxides. Although initially this required many equivalents of the chiral reagent,\textsuperscript{43} subsequent work demonstrated that in situ regeneration of the chiral lithium amide 72 using LDA allowed use of a catalytic quantity of the chiral lithium amide without significant decrease in the enantioselectivity of the reaction.\textsuperscript{44}
More recently, Asami et al.\textsuperscript{45} replaced the regenerating agent with a polymer bound lithium amide 73 which is less reactive than the solution phase counterpart (LDA) and was therefore less likely to participate in a non-enantioselective reaction. The reaction is illustrated in scheme 1.24.

\begin{center}
\begin{tikzpicture}
  \node[draw,shape=circle] (A) at (0,0) {cis-71};
  \node[draw,shape=circle] (B) at (1,0) {74};
  \draw[->] (A) -- (B);
  \node at (0.5,0.7) {89\%};
  \node at (0.5,0.3) {c.e. 97\%};
\end{tikzpicture}
\end{center}

Scheme1.24  

\textit{Reagents and Conditions:} 72 (0.2 eq), 73 (2.8 eq), THF, -15°C, 16h.

Therefore the benzoylamino-alcohol 74 was obtained in good yield and enantiomeric excess. The paper by Barrett\textsuperscript{41} went on to describe a similar transformation but with poorer e.e. and which required 3 equivalents of chiral amide.
Synthesis of carbocycles using a chiral auxiliary

The presence of a chiral centre in a molecule can influence the stereochemistry of transformations to that molecule. Therefore, it is possible to utilise a chiral moiety in a compound purely for the purpose of directing the generation of additional chiral centres, and remove this moiety once this is complete. Scheme 1.25 shows the route employed by Shireman and Miller\textsuperscript{46} for the synthesis of 79 using an amino acid as a chiral auxiliary.

![Scheme 1.25](image)

**Scheme 1.25**

*Reagents and Conditions:* (a) (COCl)	extsubscript{2}, DMSO, cyclopentadiene, DCM, then pyridine, -78°C to R.T.; (b) OsO	extsubscript{4}, NMO, THF, H	extsubscript{2}O, 0.5h; (c) DMP, p-TsOH, 20 minutes; (d) NaBH\textsubscript{4}, MeOH, 0.5h; (e) H	extsubscript{2}, Pd/C, MeOH, 0.5h.

The readily available hydroxamic acid 75 derived from D-Ala was oxidised under Swern conditions and the resulting acylnitroso intermediate was trapped by Diels-Alder cycloaddition with cyclopentadiene. The resulting bicyclic species 76 was generated as a mixture of diastereomers which could be separated by chromatography. After dihydroxylation which gave a single diastereomer and protection as the acetonide, the amino acid auxiliary was removed using sodium borohydride. Finally the amino-alcohol 79 was obtained by hydrogenation of the oxazabicyclic species 78. Conveniently by using the hydroxamic acid derived from L-Ala, the enantiomer of 79 was obtained in the same way.
1.3.3 Convergent approaches

The convergent approach involves the direct coupling of the base to the carbocyclic ring. This direct coupling has the advantage over the linear approach that fewer steps are required and generally therefore yields are higher. However regioselectivity for the coupling reaction can be problematic. Pyrimidines can have issues over N/O-coupling while purines can be attached at the N9, N7 and N3 nitrogens.

There are 4 commonly known methods for the coupling of the base with the carbocyclic precursor:\textsuperscript{4,12}:

1. Palladium catalysed displacement of an allylic ester or carbonate.

2. Nucleophilic displacement of a halide or activated hydroxyl group, including Mitsunobu coupling.

3. Nucleophilic ring opening of an epoxide.

4. Michael addition to an olefin activated by an electron withdrawing group - commonly a nitro group.
Palladium catalysed coupling

The palladium catalysed coupling of the base to the carbocycle was pioneered by Trost\textsuperscript{47} and proceeds with retention of configuration. Scheme 1.26 shows an example of the Trost methodology by Crimmins \textit{et al.}\textsuperscript{48}

\begin{equation}
\text{Scheme 1.26}
\end{equation}

\textit{Reagents and Conditions:} 2-amino-6-chloropurine, Pd(PPh\textsubscript{3})\textsubscript{4}, NaH, THF:DMSO 1:1, 45\textdegree C, 16h.

Under the conditions shown, the reaction displays only moderate selectivity for N9 over N7. In the same paper, Crimmins demonstrated that by replacing the chlorine in the base with a more bulky group the N9/N7 ratio could be improved. By using 2-amino-6-(cyclopropylamino)purine, the ratio increased to 95:5. Crimmins and Zuercher\textsuperscript{49} also demonstrated that by carrying out the coupling reaction with a resin bound allylic benzoate carbocycle 179, the N7 product was not observed at all. As such this method was more generally applicable.
While these reactions depend on an enantioselective synthesis of the carbocyclic precursor to provide a single enantiomer of the carbocyclic nucleoside, Trost reported a coupling method using a bis-benzoate 84 which gave e.e.'s as high as 96% using by chiral ligands (83) on the palladium. The synthesis is shown in scheme 1.27.

Scheme 1.27
Reagents and Conditions: (a) \([\eta^1-C_5H_5PdCl]_n, 83, 2\text{-acetamido-6-(N,N-diphenyl)carbamoyloxypurine}, \text{pemipidine, THF, DMSO, 0°C, 8h}\); (b) Ph$_3$P, Pd$_2$(dba).CHCl$_3$, phenylsulfonyl(nitro)methane, Et$_3$N, THF, 8h; (c) Me$_2$NC(NH)NMe$_2$, tetrabutylammonium-oxone, Na$_2$CO$_3$, MeOH, DCM, 16h.

Some formation of the N7 isomer was observed for the coupling reaction, and also, some formation of the doubly alkylated species occurred. The key steps for the introduction of the hydroxymethyl side chain are shown in scheme 1.27. The nitrosulfone 86 was obtained as a 1:1 diastereomeric mixture by a second Pd(0)-catalysed reaction, this time with phenylsulfonyl(nitro)methane. Oxidation of the nitrosulfone using TBA-oxone gave the methyl ester 87 which could easily be converted to a hydroxymethyl group.
Nucleophilic displacement of a halide or activated hydroxyl group

In contrast to the palladium coupling the direct displacement of activated hydroxyl groups and halides proceeds with inversion. Howarth et al.\textsuperscript{5} used this approach to synthesise all 4 diastereomers of an amino-carboxylic acid carbocyclic nucleoside 88. The brosylate 89 and iodo derivative 90 were both obtained from the same 1S, 3S alcohol and treated as shown in scheme 1.28.

\begin{equation}
\begin{align*}
(1S, 3S)-89 & \xrightarrow{(a) \; 57\% \quad (b) \; 71\%} (1S, 3R)-88 \\
(1S, 3R)-90 & \xrightarrow{(a) \; 49\% \quad (b) \; 71\%} (1S, 3S)-88
\end{align*}
\end{equation}

Scheme 1.28

Reagents and Conditions: (a) $N^\text{a}$-benzoylthymine, NaH, DMF, 40°C; (b) NaOEt, EtOH.

By carrying out the same reactions for the brosylate and iodo derivatives obtained from the 1R, 3S rather than 1S, 3S alcohol the remaining 2 diastereomers were obtained.
A common method for the coupling of the base to the carbocyclic precursor is the Mitsunobu reaction. The hydroxyl group is activated using a triphenylphosphine/DEAD complex and displaced by the heterocyclic base. Scheme 1.29 shows an example of the coupling of a guanine base using Mitsunobu conditions as reported by Chu et al.\textsuperscript{52}

\[
\begin{align*}
\text{TBDMSO—OH} & \quad \text{N} & \quad \text{CI} \\
\text{TBDMSO—N,N—OH} & \quad \text{N} & \quad \text{HAc}
\end{align*}
\]

Scheme 1.29
Reagents and Conditions: DEAD, PPh\textsubscript{3}, N\textsuperscript{2}-acetylamino-6-chloropurine, THF.

In this example, the product 92 formed as an inseparable mixture with reduced DEAD and could not be purified until the desilylation had taken place. The yield over these 2 steps was 63%. While this procedure appears to be free of the problem over N9/N7 selectivity, formation of O-alkylated products is common for pyrimidine coupling. For this reason, and problems separating by-products, Chu et al. were prompted to carry out a Mitsunobu coupling using solid phase synthesis.\textsuperscript{54} The key steps are illustrated in scheme 1.30.

\[
\begin{align*}
\text{THPO—OH} & \quad \text{OBz} & \quad 
\text{HO—O—OBz} & \quad 
\text{OBz} & \quad 
\text{OBz} & \quad 
\text{OBz} & \quad 
\text{OBz}
\end{align*}
\]

Scheme 1.30
Reagents and Conditions: (a) DMAP, DIPEA, p-nitrophenylcarbonate resin, DCM, 40°C, 24h; (b) PPTS, 1-butanol, 1,2-dichloroethane, 60°C, 16h; (c) DEAD, PPh\textsubscript{3}, N\textsuperscript{2}-benzoyluracil, DMF, 24h; (d) K\textsubscript{2}CO\textsubscript{3}, THF, MeOH, 24h.
The ability to wash away excess reagents and by-products meant that separation from reduced DEAD was no longer an issue. Comparison of \( N/O \) selectivity for the solution phase and solid phase syntheses revealed that for all the common pyrimidine bases, the solid phase method was superior. In the example shown for uracil (scheme 1.30), the reported solution phase \( N/O \) ratio was 64/36 compared to 98/2 for solid phase albeit with a slightly reduced yield of 74% cf. 79%. In the case of purine bases, a ratio of 100/0 was reported for solid and solution phase, but yields were increased in the case of solid phase syntheses.

Two examples used silylated bases to couple to the carbocyclic moiety. The first example, by Bianco et al.\textsuperscript{55} uses Vorbrüggen\textsuperscript{56} methodology as shown in Scheme 1.31.

\[
\begin{align*}
\text{Reagents and Conditions:} & \quad \text{(a)} \ N^\text{o}-\text{benzoyl-adenine, MeCN, TMSCl, HMDS, reflux, 7h, \ ii. C}_7\text{F}_5\text{Si(CH}_3)_2\text{, DCM, reflux, 8h, 20%; (b) DIBAL, DCM, -78\textdegree C, 95\%.}
\end{align*}
\]

Similarly, Perez and Gordillo used silylated bases to couple to iodo compounds formed in situ, as illustrated in scheme 1.32.

\[
\begin{align*}
\text{Reagents and Conditions:} & \quad \text{(a) I}_2, \text{ DCM, 72h; (b) 1N NaOH, EtOH; (c) H}_2, \text{ Pd/C, MeOH, AcOH.}
\end{align*}
\]
Nucleophilic ring opening of an epoxide

Introduction of the heterocyclic base by coupling with an epoxide is a convenient route to useful carbocyclic nucleosides, particularly in view of the fact that a hydroxyl group results from the ring opening, on the opposite side of the ring to the base. Kuang et al. investigated this technique in the synthesis of carbocyclic ribavirin \(105\). Scheme 1.33.

\[
\begin{align*}
\text{BnO} & \quad \text{BnO} \\
\text{BnO} & \quad \text{BnO} \\
\text{103} & \quad \text{104} \quad \text{40}\% \\
\end{align*}
\]

\[
\begin{align*}
\text{2 steps} & \\
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{NH}_2 \\
\text{105} & \\
\end{align*}
\]

Scheme 1.33

Reagents and Conditions: (a) 1,2,4-triazol-3-carboxylic acid ethylester, NaH, DMF, 110°C, 40%.

The low yield of 40% for the ring opening is likely to be in part due to formation of the undesired N2 isomer. In fact Kuang noted that the linear approach was the preferred route to this compound.

A limited number of examples of coupling by Michael addition to an olefin activated by a nitro group exist but none fall within the time period of this review and will not be discussed further.
1.3.4 Carbocyclic precursors for the convergent approach

As with the linear approach, methods for the introduction of the base are well known, and attention has been focused more on novel enantiospecific syntheses of the carbocyclic moiety.

Cyclopentenone 106

Synthesis of carbocyclic nucleotides from cyclopentenone 106

A number of syntheses involve the key cyclopentenone intermediate (-)-106. Scheme 1.34 shows how this intermediate can be converted to (-)-aristeromycin analogues according to Bestmann and Roth.

\[
\begin{align*}
(-)-106 \quad &\xrightarrow{(a)} \text{MOMO}^\cdot \text{O}^\cdot \quad &\xrightarrow{(b)} \text{MOMO}^\cdot \text{O}^\cdot \quad &\xrightarrow{(c)} \text{O}^\cdot \text{OH} \\
&\xrightarrow{89\%} 107 \quad &\xrightarrow{92\%} 108 \\
\text{NH}_2 \quad &\xrightarrow{(d, c)} \text{MOMO}^\cdot \text{N}^\cdot \quad &\text{Cl} \\
&\xrightarrow{77\%} (-)-10 \quad &\xrightarrow{58\%} 109
\end{align*}
\]

Scheme 1.34

Reagents and Conditions: (a) (2-thienyl)(MOMOCH)CuCNLi, THF; (b) NaBH\textsubscript{4}, CeCl\textsubscript{3}·7H\textsubscript{2}O, MeOH; (c) 6-chloropurine, PPh\textsubscript{3}, DEAD, THF; (d) NH\textsubscript{3}, MeOH; (e) HCl, MeOH.

Conjugate addition of the protected hydroxymethyl moiety was followed by reduction to give a single diastereomer. The introduction of the base is another example of a Mitsunobu type coupling and the final steps are ammonolysis and deprotection to give (-)-aristeromycin.
Similarly, Chu et al. discussed the transformation of the enantiomer of this key intermediate (+)-106 to Neplanocin A (scheme 1.35). In fact, the same paper described the conversion of (-)-106 to L-(+)-neplanocin A using a similar route (not shown here).

**Scheme 1.35**

*Reagents and Conditions:* (a) (CH$_3$)$_2$COCH$_3$, t-BuOK, sec-BuLi, -78°C, 3h; (b) Ac$_2$O, Et$_3$N, DMAP, DCM, 24h; (c) PdCl$_2$(CH$_3$CH)$_2$, p-benzoquinone, THF, reflux, 24h; (d) K$_2$CO$_3$, MeOH, 1h.

The hydroxymethyl moiety was introduced in protected form to the least hindered face of the ring, to give alcohol 110. This alcohol was acylated and subsequent palladium mediated rearrangement gave the acetate 112. Removal of the acetate gave the alcohol 113 which was then converted to (-)-neplanocin A using routine techniques.
Synthesis of cyclopentenone 106 with ring closing metathesis (RCM)

The synthesis of both enantiomers of cyclopentenone 106 was described in 1990 by Borchardt et al., from D-ribose (for (+)-106) or D-lyxose (for (-)-106), in three steps both in around 40% yield. Recent approaches to these compounds will be discussed here.

A large number of syntheses, including those for the synthesis of cyclopentenone 106 employ RCM for formation of the cyclopentane ring. This is an attractive approach since the formation of the ring results in the presence of an endocyclic double bond which is desirable for many carbocyclic nucleosides. Grubbs' ruthenium complex114 is commonly used, and one example using Schrock's molybdenum complex115 will be reported.

Figure 1.36
Ring closing metathesis catalysts.
Jeong et al. described the synthesis of both enantiomers of cyclopentenone 106 from D-ribose, developed from their previous work. Synthesis of the (-)-enantiomer is illustrated in scheme 1.37.

Scheme 1.37
Reagents and Conditions: (a) acetone, H₂SO₄, 2.5h; (b) vinylmagnesium bromide, THF, -78-0°C, 3h; (c) NaIO₄, DCM, H₂O, 0°C-R.T.; (d) NaH, DMSO, CH₃PPh₃Br, THF, 0°C-reflux, 16h; (e) Grubbs' catalyst, CHCl₃, 3h; (f) MnO₂, DCM, 6h.

Thus the protected D-ribose 117 was treated with vinylmagnesium bromide to give the triol 118 which was oxidatively cleaved using sodium metaperiodate to give the lactol 119. Wittig reaction to give the diene 120 was followed by the ring closing metathesis step using Grubbs' catalyst. Finally oxidation with manganese dioxide gave the desired cyclopentenone (-)-106.
Using a similar strategy, the (+)- enantiomer was synthesised (scheme 1.38).

![Chemical structure](image)

**Scheme 1.38**

Reagents and Conditions: (a) CH$_3$PPh$_3$Br, t-BuOK, THF, 16h; (b) NaIO$_4$, DCM, H$_2$O; (c) vinylmagnesium bromide, THF, -78°C, 1h; (d) Grubbs' catalyst, CHCl$_3$; (e) MnO$_2$, DCM, 16h.

The protected D-ribose 117 was transformed by Wittig reaction to the olefin 122 which without being isolated was converted to the vinyl aldehyde 123. Treatment of 123 with vinylmagnesium bromide gave diene 120 and subsequent transformations as for the (-)-enantiomer gave cyclopentenone (+)-106.

Jin and Chu$^{65}$ described an almost identical synthesis of the (-)-enantiomer (-)-106, but with a slightly higher overall yield (56% cf. 45%) despite involving two additional steps. The key changes were that the hydroxymethyl group of the D-ribose acetonide 117 was protected as the TBDMS ether for the insertion of the Grignard reagent and subsequently deprotected, in overall yield of 81% for the three steps (cf. 81% for the free hydroxy group, single step). The yield for the oxidative cleavage (118–119) was improved from 85% to 97% by using water as solvent instead of a mixture of water and DCM. Finally, (-)-106 was obtained from the diene 120 in 2 steps with overall yield 93% (cf. 80% for the same two steps). This increase was the result of using the volatile cyclopentenol 121 (obtained from RCM) without purification, and using PCC for the oxidation rather than manganese dioxide.
Synthesis of other Mitsunobu precursors using RCM

Agrofoglio et al.\textsuperscript{66} reported the synthesis of an unusual L-carbanucleoside precursor, similar to 113 with the only example of a ring closing metathesis which does not utilise Grubbs’ catalyst (scheme 1.39).

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=0.8\textwidth]{scheme.png}};
\end{tikzpicture}
\end{center}

Scheme 1.39

Reagents and Conditions: (a) CH\textsubscript{3}PPh\textsubscript{3}Br, n-BuLi, THF, -78°C–RT.; (b) PCC, AcONa, mol sieves 4Å, DCM; (c) CH\textsubscript{3}PPh\textsubscript{3}Br, n-BuLi, THF, -78°C–R.T.; (d) Schrock’s catalyst, C\textsubscript{6}F\textsubscript{16}, drybox, 80°C; (e) 1M BCl\textsubscript{3}, DCM, -78°C–R.T.; (f) acetone, p-TsOH; (g) TBDMSI, pyridine, 0°C.

Therefore, the while most steps are generally similar, the key difference is the use of Schrock’s catalyst.\textsuperscript{62} The RCM step was attempted using Grubbs’ catalyst, but low yields (10%) made it unsuitable. Agrofoglio noted that Grubbs’ catalyst is known to be ineffective for producing trisubstituted alkenes. The use of Schrock’s molybdenum complex catalyst proved to be more effective for this sterically congested olefin, with a yield of 85%. Agrofoglio observed that the precursor 129 was suitable for Mitsunobu or Trost type coupling and demonstrated the coupling of a thymine base under Mitsunobu conditions.
Lastly, Chu et al.\textsuperscript{53} described the synthesis of the Mitsunobu precursor (1R, 3S)-91 as shown in scheme 1.40.

![Scheme 1.40](image)

**Scheme 1.40**

*Reagents and Conditions:* (a) (EtO)\textsubscript{2}P(O)CHFCO\textsubscript{2}Et, NaHMDS; (b) i. conc. HCl, EtOH; ii. TBDMSCI; (c) i. DIBAL; ii. NaBH\textsubscript{4}, CeCl\textsubscript{3}; iii. TBDMSCI; (d) (EtO)\textsubscript{2}CCH\textsubscript{3}, propionic acid, 130°C; (e) LiAlH\textsubscript{4}, THF, -40 to -35°C, 1.5 h; (f) PCC, mol sieves 4Å, DCM, 2 h; (g) vinylmagnesium bromide, THF, -78°C, 1.5 h; (h) Grubbs' catalyst, DCM, 0.5 h; (i) H\textsubscript{2}, Pd/C, cyclohexane, 2 h; (j) PhCOOH, DEAD, PPh\textsubscript{3}, THF, 6 h; (k) LiAlH\textsubscript{4}, THF, -40 to -35°C.

The synthesis of ester 135 had been reported previously by the Chu group.\textsuperscript{67,68} Reduction of the ester followed by oxidation of the resulting alcohol gave the aldehyde 136. Grignard reaction with vinylmagnesium bromide gave the diene RCM substrate 137. After successful RCM reaction, the unstable cyclopentenol 138 was hydrogenated to reduce the double bond, giving two separable diastereomers. The undesired diastereomer (1S, 3S)-91 was converted to the required (1R, 3S)-91 diastereomer in 2 steps.
Synthesis of Mitsunobu type precursors without RCM

Chu et al.\textsuperscript{54} demonstrated the synthesis of the precursor for Mitsunobu coupling on solid phase from the alcohol 140 which can be obtained from the cyclopentenone (+)-106 in 2 steps.\textsuperscript{69} The synthesis is illustrated in scheme 1.41.

This rather lengthy synthesis consists mainly of protecting group manipulations. Synthesis of the alcohol 93 for demonstration of the solid phase techniques was the priority for this sequence.
Tomioka et al.\textsuperscript{70} described the synthesis of (-)-neplanocin A using a lithium thiolate initiated Michael-Aldol tandem cyclisation reaction. The route is shown in scheme 1.42.

Scheme 1.42

Reagents and Conditions: (a) acetone, H\textsubscript{2}SO\textsubscript{4}; (b) AcOH, H\textsubscript{2}O; (c) NaIO\textsubscript{4}, MeOH, H\textsubscript{2}O; (d) NaBH\textsubscript{4}, MeOH, H\textsubscript{2}O; (e) PMBCI, KOH, C\textsubscript{6}H\textsubscript{5}, reflux, 9h; (f) (COCl)\textsubscript{2}, DCM, DMSO, -60°C, 15 minutes, then Et\textsubscript{3}N, 60°C–R.T.; (g) (EtO\textsubscript{2})\textsubscript{2}POCH\textsubscript{3}CO\textsubscript{2}Et, NaH, THF, -20°C, 1h; (h) 2N HCl, EtOH, 40°C, 1.5h; (i) TBDMSOTI, Et\textsubscript{3}N, DCM, 0°C, 0.5h; (j) DDQ, aq CH\textsubscript{3}CN, 80 minutes; (k) (COCl)\textsubscript{2}, DCM, DMSO, -60°C, 15 minutes, then Et\textsubscript{3}N, 60°C–R.T.; (l) BnSLi, THF, -20°C, 0.5h; (m) aq HF, CH\textsubscript{3}CN, 3h; (n) p-TsOH, acetone, 4h; (o) NaIO\textsubscript{4}, aq EtOH; (p) decalin, 180°C; (q) PDC, DCM, 3h; (r) DIBAL, toluene, 1h; (s) TBDMSCl, Et\textsubscript{3}N, DMAP, DCM, 4h.

42
The protected diol 147 was synthesised in 4 steps according to the procedure by Terashima et al.\textsuperscript{7} The acetonide protected analogue of 151 was found to be unsuitable for the cyclisation reaction. Consequently the protecting group modifications made to acetonide 149 shown here were necessary. Thus Michael-Aldol tandem cyclisation of aldehyde 151 with BnSLi was reasonably effective, with cyclisation products 152 and 153 both utilised after further protecting group modifications. Synthesis of (−)-neplanocin A from alcohol 129 was completed in 2 steps.

In addition to the synthesis of precursors to D-carbocyclic nucleosides already described, Chu et al.\textsuperscript{53} demonstrated the synthesis of some L-carbocyclic nucleosides. The procedure, from ester 135 is shown in scheme 1.43.

Scheme1.43

Reagents and Conditions: (a) LiAlH\textsubscript{4}, THF, -40°C, 1.5h; (b) NaH, BnBr, THF, 16h; (c) O\textsubscript{3}, MeOH, -78°C then DMS, 2h; (d) (EtO\textsubscript{2})\textsubscript{P(O)}CH\textsubscript{2}CO\textsubscript{2}Et, NaHMDS, THF, -78°C, 1h; (e) H\textsubscript{2}, Pd/C, cyclohexane, 24h; (f) MsCl, pyridine, DCM, 24h; (g) NaH, THF, reflux, 16h; (h) NaOH, H\textsubscript{2}O, EtOH, 5h; (i) Pb(OAc)\textsubscript{4}, CCl\textsubscript{4}, hv, reflux, 15minutes, I\textsubscript{2}, CCl\textsubscript{4}, hv, reflux 2h; (j) NaHCO\textsubscript{3}, 15% (v/v) water/HMPA, 100°C, 16h.
Gallos et al.\textsuperscript{72} demonstrated a ring forming method using mercury acetate for the formation of Mitsunobu type precursors. The starting material 163 was obtained by periodate cleavage of 3,4-\textit{O}isopropylidene-D-arabinopyranose\textsuperscript{73} and then transformed as shown in scheme 1.44.

\begin{center}
\begin{tikzpicture}

\node (163) at (0,0) {\includegraphics[width=0.3\textwidth]{scheme1.png}};
\node (164) at (3,0) {\includegraphics[width=0.3\textwidth]{scheme1.png}};
\node (165) at (6,0) {\includegraphics[width=0.3\textwidth]{scheme1.png}};
\node (166) at (9,0) {\includegraphics[width=0.3\textwidth]{scheme1.png}};
\node (167) at (12,0) {\includegraphics[width=0.3\textwidth]{scheme1.png}};

\draw[->] (163) -- (164) node[above] {\textit{(a)}};
\draw[->] (164) -- (165) node[above] {\textit{(b, c)}};
\draw[->] (165) -- (166) node[above] {\textit{(d, e)}};
\draw[->] (166) -- (167) node[above] {\textit{(f)}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 1.44}

\textit{Reagents and Conditions:} (a) \textit{Ph}_3\text{P}=\textit{CH}_2; (b) \textit{(COCl)}_2, DMSO, \textit{Et}_3\text{N}, DCM, -50 to 20\textdegree\text{C}, 0.5h; (c) \textit{Ph}_3\text{P}=\textit{CHCO}_2\text{Et}, \textit{EtOH}, \textit{PhCO}_2\text{H}, 0-20\textdegree\text{C}, 24h; (d) \textit{Hg(OAc)}_2, \textit{AcOH}, 20\textdegree\text{C}, 12h; (e) \textit{NaBH(O\text{Me})}_3, DCM, 20\textdegree\text{C}, 24h; (f) \textit{LiAlH}_4, THF, reflux, 5h.

Therefore conversion of hemiacetal 163 to the alkene 164 was achieved by Wittig reaction with methylene triphenylphosphorane.\textsuperscript{74} Subsequent oxidation of the alcohol and a second Wittig reaction gave the diene 165. This then underwent the ring forming procedure, without isolation after the mercuration step to give the acetate 166, which on treatment with lithium aluminium hydride was reduced to the diol (-)-167. (+)-167 was obtained by a similar procedure, starting from D-ribose.
Synthesis of Trost type precursors using RCM

Crimmins et al. reported the synthesis of enantiomerically pure carbocyclic precursors using a chiral auxiliary. Initial attempts using Evans' auxiliary were successful, but required the expensive lithium borohydride for removal of the oxazolidinone auxiliary. Therefore, the route shown (scheme 1.45) shows the modified procedure which was devised, using an oxazolidinethione auxiliary 168.

![Chemical reaction diagram](attachment:image.png)

**Scheme 1.45**

*Reagents and Conditions: (a) n-BuLi, THF, -78°C then 0°C, 0.5h; (b) TiCl₄, (-)-sparteine, crotonaldehyde, DCM, 20 minutes; (c) NaBH₄, THF, H₂O; (d) Ac₂O, Et₃N, DCM; (e) Grubbs' catalyst, DCM.*

Attempts to carry out the RCM reaction on the diene 171 resulted in low yields due to poor conversion. This was thought to be due to a stabilising effect of coordination of the thiocarbonyl to the metal centre. Changing the order of the sequence to that shown was found to be more effective. Crimmins went on to describe a procedure in which the opposite enantiomer of the same auxiliary was used to synthesis the non-Evans syn aldol adduct, which in contrast to the Evans syn aldol adduct 171, underwent efficient RCM. The same procedure was used in the synthesis of a carbocycle for use in solid phase coupling reactions. This is illustrated in scheme 1.46.
The diene 174 was formed in much the same way though with acrolein instead of crotonaldehyde and DIPEA rather than (-)-sparteine as base. As discussed, the ring closing was highly effective despite the thiocarbonyl. Apparently there is a difference in the ability of the thiocarbonyl to coordinate to the metal in the intermediate alkylidene in the Evans syn and non-Evans syn diastereomers 171 and 174. Reductive removal of the auxiliary was followed by protecting group manipulations to give the desired allylic benzoate 178.
Gurjar and Maheshwar described the synthesis of an unsaturated carbocycle with a tertiary hydroxy group, starting from 1,2,5,6-di-O-isopropylidene-a-D-glucofuranose. The procedure is illustrated in scheme 1.47.

Scheme 1.47
Reagents and Conditions: (a) oxidation (b) allylmagneesium bromide, THF, 0°C, 2h then reflux 1h; (c) 4-methoxyphenylmethyl bromide/NaH, THF, 6h; (d) H₂SO₄, MeOH, 10h; (e) MeSO₂Cl, Et₃N, DMAP, DCM, 1h; (f) NaI, EtCOMe, reflux, 8h; (g) Grubbs' catalyst, DCM, 6h; (h) H₂SO₄, dioxane, reflux; (i) NaIO₄, DCM, SiO₂, 1h; (j) NaBH₄, MeOH, 1h; (k) Im-CO-Im, C₆H₆, reflux, 4h.

The olefin 181 was obtained according to the procedure described by Mandal et al., followed by protection of the free alcohol. This was then converted to the diene 182 in three steps. Ring closing metathesis then gave the bicyclic 183 which was transformed to the diol 184 by acetonide hydrolysis, oxidative cleavage and reduction. Cyclisation to the Trost precursor 185 was then achieved by refluxing with carbonyl diimidazole.
Similarly, Hong et al. described a route to various carbocyclic nucleosides containing a tertiary hydroxyl group. The synthesis of the coupling precursor is shown in scheme 1.48.

The paper by Hong discussed the elaboration of carboxylic acid obtained as shown in 5 steps from D-lactose. Having unsuccessfully attempted direct vinylation of the Weinreb amide, the amide was first reduced to the aldehyde. Treatment with vinylmagnesium bromide then gave the diene which underwent efficient RCM to give the separable diastereomers of alcohol. The carbocyclic precursor was then obtained by reaction of alcohol with ethyl chloroformate. The base coupled product could then be dihydroxylated using osmium tetroxide or hydrogenated to remove the double bond, thus giving 3 carbocycles from the same route.
RESULTS AND DISCUSSION

2. SYNTHESIS OF PUTATIVE INTERMEDIATES IN THE BIOSYNTHESIS OF ARISTEROMYCIN BY STREPTOMYCES CITRICOLOR

2.1 Synthesis of keto-tetrol (2R, 3S, 4S)-20

The synthesis of keto-tetrol (2R, 3S, 4S)-20 was based on a synthesis previously attempted in the group. Work by Ian Archer\textsuperscript{82}, based on a procedure by Johnson \textit{et al.}\textsuperscript{83} had shown that the acetate 197 could be obtained from cyclopentadiene. It was envisaged that the desired (2R, 3S, 4S)-keto-tetrol-20 could be obtained according to the retrosynthetic plan shown in scheme 2.1.

![Scheme 2.1](image)

Scheme 2.1

Retrosynthesis of keto-tetrol (2R, 3S, 4S)-20.

The resulting 11 step route developed was largely satisfactory,\textsuperscript{18} but a single low yielding step late in the synthesis meant that obtaining significant quantities of the desired keto-tetrol would be very difficult. This key step was the incorporation of a \textit{p}-methoxybenzyloxymethyl moiety\textsuperscript{84} into the ketone 197 to give alcohol 196, which resulted in considerable decomposition of the product and starting material (scheme 2.2).

Evidence for the elimination products 106, 199 and the retro aldol compound 198 had been found by NMR spectroscopy. The decomposition was thought to be due to the intolerance of the acetate moiety to the conditions employed for this reaction. Therefore it was postulated that exchanging the acetate for a more robust protecting group may provide a compound more stable to the reaction conditions and therefore afford higher yields. Silyl protecting groups are used routinely and known to be stable to a variety of reaction conditions and therefore, the \textit{tert}-butyl-dimethyl silyl ether was chosen as the replacement for the acetyl group. Although a simple change of protecting group for the acetate 197 would have been ideal, attempts to remove the acetate resulted in formation
RESULTS AND DISCUSSION: SYNTHESIS OF PUTATIVE INTERMEDIATES

Scheme 2.2
Reagents and Conditions: (a) Bu₃SnCH₂OPMB, BuLi, THF, -78°C.

of the same decomposition products 106 and 198. As such the modifications would have to be carried out prior to the formation of the carbonyl responsible for the acidity of the C–5 protons.

Synthesis of the chiral alcohol 202

The initial steps employed were the same as in the original synthetic route. Freshly cracked cyclopentadiene 65 was epoxidised using peracetic acid to give cyclopentadiene monooepoxide 66 (scheme 2.3) as described by Knapp et al.⁴⁰,⁸⁵ Although yields as high as 43% were achieved, the reaction was typically low yielding (15–20%, based on peracetic acid) and only by using a large quantity of cyclopentadiene could sufficient quantities of the epoxide be obtained. However, dicyclopentadiene is relatively inexpensive and cyclopentadiene reasonably easy to produce in large quantities so a low yield in this instance was considered acceptable. The product was isolated by

Scheme 2.3
Reagents and Conditions: (a) Peracetic acid (36–40 wt% soln in aqueous acetic acid), sodium acetate, sodium carbonate, DCM, 0°C; (b) Pd(PPh₃)₄, acetic anhydride, THF, 0°C.
distillation under low vacuum.
Palladium catalysed diacetylation with acetic anhydride gave the meso diacetate 200 in up to 64% yield (scheme 2.3).

Although not especially high yielding, this procedure was straightforward and the product could be isolated by routine flash chromatography. Scheme 2.4 shows the mechanism for the diacetylation reaction.

Scheme 2.4
Mechanism of palladium catalysed diacetylation.

Ring opening of the epoxide by coordination of the palladium and attack at the carbonyl group of acetic anhydride gave the intermediate 201. The palladium was then displaced by the acetate anion on the same side as the existing acetate to give the meso-diacetate 200.

The final step, prior to the protecting group modifications, was an enzyme catalysed asymmetric hydrolysis. This was a crucial step in the synthesis, since chirality is introduced at this point. The transformation was carried out cleanly and efficiently with Candida antarctica lipase B (CAL-B) SP-435 in phosphate buffer (scheme 2.5). Thus the 1S acetate was hydrolysed giving the alcohol 202 in quantitative yield and e.e. >99% (by Chiral GC), without the need for purification.

Scheme 2.5
Reagents and Conditions: Candida antarctica lipase B Novozyme SP-435, phosphate buffer.
2.1.1 Protecting group modifications

Replacement of acetyl protection with tert-butyldimethylsilyl ether protection

The immediate goal was then to exchange the acyl protecting group for a tert-butyldimethylsilyl ether. This meant that an orthogonal protecting group had to be chosen to temporarily protect the free alcohol. To this end, methoxyethoxymethyl protection appeared to be suitable. This ether protecting group is tolerant to silyl protection conditions, chemical deacetylation and can be removed without effecting the silyl ether. Thus, the alcohol 202 was treated with methoxyethoxymethyl chloride in the presence of diisopropylethylamine in DCM (scheme 2.6). This gave the MEM ether 203 in quantitative yield, often without the need for purification, which was easily achieved by flash chromatography if required.

\[
\begin{align*}
\text{HO...çy.aOAc} & \quad \text{(a)} \quad \text{MEMO..çOAc} & \quad \text{(b)} \quad \text{MEMO..çOH} \\
202 & \quad \text{quant} & \quad \text{203} & \quad \text{89\%} \\
\text{HO...çy.OAc} & \quad \text{(c)} & \quad \text{MEMO..çOAc} & \quad \text{(d)} & \quad \text{MEMO..çOAc} \\
206 & \quad \text{205} & \quad \text{89\%}
\end{align*}
\]

Scheme 2.6

*Reagents and Conditions:* (a) MEMCl, diisopropylethylamine, DCM; (b) ammonia saturated methanol; (c) TBDMSCI, imidazole, DCM; (d) ZnBr$_2$, DCM.

The remaining acetate group was removed by stirring in ammonia saturated methanol (scheme 2.6) to give the 1R alcohol 204 in 89\% yield. This reaction went cleanly and required no purification. The acetate was then replaced with tert-butyldimethylsilyl protection by treating the alcohol 204 with tert-butyldimethylsilyl chloride in the presence of imidazole, giving the doubly protected species 205 in 89\% yield, without the need for purification (scheme 2.6). However, it was necessary to wash the product with saturated sodium bisulfite to remove traces of salts which were detrimental to subsequent reactions. Removal of the MEM protection would now complete the protecting group manipulations and give the silyl ether analogue 206 of the acetate 202.
RESULTS AND DISCUSSION: SYNTHESIS OF PUTATIVE INTERMEDIATES

However, stirring the MEM ether in DCM with zinc bromide resulted in the formation of a mixture of compounds, and after work up, NMR spectroscopy showed that there were no significant quantities of the desired product. Therefore after 2 further unsuccessful attempts, this reaction was abandoned.

It was thought that the presence of the double bond in the ring may be facilitating removal of the entire OMEM fragment. Scheme 2.7 shows how this might occur, taking into account a possible cleavage mechanism, although compounds 208 and 209 were not isolated. It appeared therefore that it would not be profitable to pursue the MEM removal further until the double bond had been removed.

![Scheme 2.7](image)

*Reagents and Conditions:* (a) ZnBr₂, DCM (b) H₂O.

Therefore the original synthetic route was continued using the MEM ether 205. Dihydroxylation of the double bond at the less hindered side was effected by catalytic osmium tetroxide with excess N-methylmorpholine N-oxide (NMO)³ to give the cis-diol 210 as a single diastereomer in 80% yield (scheme 2.8).

The diol 210 was then converted to the corresponding acetonide by stirring in DCM with pTSA and dimethoxypropane to give the fully protected species 211 (scheme 2.8). This reaction was clean and high yielding (96%). The alternative method for acetonide protection - stirring in acetone with pTSA, tended to proceed less cleanly, generally required chromatography, and was lower yielding (74%).

These steps gave a compound thought to be better suited to the removal of the MEM
RESULTS AND DISCUSSION: SYNTHESIS OF PUTATIVE INTERMEDIATES

Scheme 2.8
Reagents and Conditions: (a) \( \text{OsO}_4 \) (4% wt(aq) soln), NMO, THF, water, acetone; (b) DMP, \( \rho \text{TSA} \), DCM.

Stirring the MEM ether 211 with 5 equivalents of zinc bromide in DCM gave unsatisfactory results. The reaction was performed under anhydrous conditions, non-anhydrous conditions, with finely powdered or granular zinc bromide. Under most conditions the reaction was not effective in consuming starting material, or a mixture of compounds was observed on work up, amongst which the product would represent a small portion. The most effective conditions were found to be the use of toluene instead of DCM and 6 equivalents of finely powdered zinc in non-anhydrous conditions. After stirring overnight, the reaction was incomplete and the zinc bromide was replaced with a fresh portion. In this way, after stirring for 3 more days the starting material had been almost entirely consumed and after work up and purification, the deprotected species 212 was obtained in 39% yield (scheme 2.9).

Scheme 2.9
Reagents and Conditions: ZnBr\textsubscript{2}, toluene, 39%.
It was crucial that the protecting group manipulations were highly efficient, since the overall aim was to increase the yield of the route, and therefore a maximum yield of 39% was considered unacceptable. Other conditions investigated for the removal of the MEM ether included treatment of compound 211 with sodium iodide and trimethylsilyl chloride in acetonitrile,\textsuperscript{80, 90} titanium tetrachloride in DCM,\textsuperscript{88} and trifluoroacetic acid in DCM.\textsuperscript{92} In each case the reaction was unsuccessful. Furthermore, attempts to synthesise the deprotection reagents dimethylboronbromide\textsuperscript{93} and 2-chloro-1,3,2-dithioborola\textsubscript{nl}\textsuperscript{94, 95} were unsuccessful and the MEM deprotection had to be abandoned. However, sufficient quantities of the deprotected species 212 had been synthesised to allow investigation of the effectiveness of the incorporation of the PMBOCH\textsubscript{2} moiety in the presence of the silyl ether.

The free alcohol 212 was oxidised using Jones reagent in acetone\textsuperscript{83} (scheme 2.10). The relatively low yield (30\%) may be a result of the small quantities used in this reaction. Hence, the compound required for the PMBOCH\textsubscript{2} insertion had been synthesised. Accordingly, the ketone 213 was added to a solution of Bu\textsubscript{3}SnCH\textsubscript{2}OPMB 230 which had been treated with \textit{n}-butyllithium. After work up, and purification by chromatography, the desired compound 214 was obtained in 16\% yield (cf. 0–20\% for acetate) (scheme 2.10).

\begin{equation}
\text{HO} \quad \text{OTBDMS} \quad \text{212} \quad \text{(a)} \quad \text{O} \quad \text{OTBDMS} \quad \text{213} \quad \text{30\%} \quad \text{PMBO} \quad \text{OTBDMS} \quad \text{214} \quad \text{16\%}
\end{equation}

\textbf{Scheme 2.10}

\textit{Reagents and Conditions:} (a) Jones reagent (CrO\textsubscript{3}, H\textsubscript{2}SO\textsubscript{4}, H\textsubscript{2}O), acetone, 0°C–RT; (b) Bu\textsubscript{3}SnCH\textsubscript{2}OPMB, BuLi, THF, -78°C.

This result suggested that replacing the acetate with silyl protection had no impact on the effectiveness of the reaction. However, work was already underway with an alternative to the MEM ether strategy.
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Replacement of acetyl protection with tert-butyldiphenylsilyl ether protection

Having established that efficient removal of the MEM protection could not be achieved, an alternative strategy for the protecting group manipulation had to be established. The silyl family of protecting groups contains a series of similar ethers which increase in stability according to the bulkiness of the substituents attached to the silicon. These groups can generally be handled in a similar fashion since protection (silyl chloride and imidazole) and deprotection (TBAF) steps can be achieved using the same conditions for each. However, the massive variation in stability to certain reaction conditions, e.g. acid, means that they can also be treated as orthogonal protecting groups. Therefore it was thought that trimethylsilyl protection could be used in place of the MEM ether while manipulations were carried out to replace the acetate with the tert-butyldimethylsilyl ether. However, the trimethyl silyl ether was found to be intolerant to the basic deacetylation conditions. Considering this, and the failure of the tert-butyldimethylsilyl ether to improve the yield of the incorporation of the PMBOCH₂ moiety, it was decided that tert-butyldimethylsilyl protection could be used while the acetate was replaced with the highly stable tert-butyldiphenylsilyl ether.

The acetate 202 was synthesised as described previously and using the conditions described above, the alcohol was successfully transformed to the corresponding tert-butyldimethylsilyl ether 215 in 88% (scheme 2.11). In this case, the deacetylation with ammonia saturated methanol proceeded extremely satisfactorily with the alcohol 216 being obtained in quantitative yield. The tert-butyldiphenylsilyl protection was introduced

202 \[\overset{(a)}{\rightarrow}\] 215 \[\overset{(b)}{\rightarrow}\] 216

218 \[\overset{(d)}{\rightarrow}\] 217

Scheme 2.11

Reagents and Conditions: (a) TBDMSCI, imidazole, DCM; (b) ammonia saturated methanol; (c) TBDPSCI, imidazole, DCM; (d) acetic acid, THF, water 3:1:1.
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in the same way as the tert-butyldimethylsilyl protection, using tert-butyldiphenylsilyl chloride (scheme 2.11) to give the compound with 2 silyl protected alcohols 217, in up to 77% yield. The success of this strategy depended on being able to remove the tert-butyldimethylsilyl protection in the presence of the tert-butyldiphenylsilyl ether. This was achieved easily, by stirring the doubly protected species in a mixture of acetic acid, THF and water overnight (scheme 2.11). This gave the tert-butyldiphenylsilyl ether 218 after chromatography in 71% yield. Key aromatic signals at 7.6 and 7.3ppm showed that the tert-butyldiphenylsilyl protection remained intact, while no signal for the methyl protons of the tert-butyldimethylsilyl group could be seen. The yields given for this sequence are approximate and depend on whether the starting material at each stage had been purified or not. In fact the sequence could be performed with purification of only the final product, and in doing so, the tert-butyldiphenylsilyl ether 218 was obtained from the acetate 202 in 4 steps, with 56% yield. As such, the manipulations described here were considered a success.

Synthesis of ketone 221

Having synthesised the tert-butyldiphenylsilyl ether analogue of the chiral acetate, the original transformations could recommence. Accordingly, the alkene 218 was stirred vigorously with catalytic osmium tetroxide and NMO in a mixture of THF, water and

\[
\text{HO}_3\text{OTBDPS} \xrightarrow{(a)} \text{HO}_3\text{OTBDPS} \xrightarrow{(b)} \text{HO}_3\text{OTBDPS} \\
\text{218} \quad \text{84\%} \quad \text{219} \quad \text{80\%} \\
\text{219} \xrightarrow{(c)} \text{220} \quad \text{220} \quad \text{221} \quad \text{221} \\
\text{84\%} \quad \text{90\%} \quad \text{80\%}
\]

Scheme 2.12

Reagents and Conditions: (a) OsO\(_4\) (4% wt(aq) soln), NMO, THF, water, acetone; (b) DMP, pTSA, DCM; (c) Jones reagent (Cr\(_2\)O\(_7\), \(\text{H}_2\text{SO}_4\), \(\text{H}_2\text{O}\)), acetone, 0°C–RT.
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acetone overnight (scheme 2.12). After work up and purification by chromatography, the cis-diol 219 was obtained in 84% yield. It had been shown previously that this reaction proceeded with the dihydroxylation on the opposite side of the ring to the alcohol and acetate, and considering the steric bulk of the tert-butyldiphenylsilyl group when compared to the acetate, it was thought that the same would occur here.

Having found previously that the acetonide protection was effected more quickly and cleanly with dimethoxypropane than acetone, this technique was employed once more. As such, the diol was successfully protected to give acetonide 220 in a few minutes, in 90% yield, with no need for purification. The remaining free alcohol was oxidised by stirring 220 with Jones reagent in acetone for 3 hours (scheme 2.12). This reaction tended to go cleanly, and in contrast to the previous attempt, was high yielding (80%), giving the ketone 221 as a white solid, after purification by chromatography.

Key PMBOCH₂ insertion reaction and tert-butyldiphenylsilyl ether removal

Hence, once more, a candidate for the PMBOCH₂ insertion had been synthesised. This time the results for this transformation were significantly improved, with yields being consistently in the range 40–50%. However, yields were increased further after a new method for the synthesis of the tin moiety was employed (see later) and the concentration of n-butyllithium used was increased from 1.6M to 2.5M. In this instance, reaction of the ketone 221 with the lithiated Bu₃SnCH₂OPMB proceeded much more cleanly by TLC, with a single clearly visible spot appearing and minor by-products appearing only as faint traces. Accordingly after work up and purification, the desired product 222 was obtained in 76% yield (scheme 2.13). Successful incorporation of the PMBO moiety was confirmed by NMR spectroscopy. Aromatic doublets characteristic of an

![Scheme 2.13](image)

*Reagents and Conditions: Bu₃SnCH₂OPMB, BuLi, THF, -78°C.*
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AB strong coupling system for the PMB group could be seen at 7.3 and 6.9ppm. NMR spectroscopic evidence that the silyl protection remained intact included 2 aromatic multiplets at 7.6 and 7.4ppm and a large singlet at 1.0ppm for the tert-butyl group. This is a massive improvement even compared to the 20% which had previously been achieved for the acetate. In reality however, the unreliability of the acetate reaction lead to yields ranging from 0–20%, while yields in the range 65–76% were consistently observed for treatment of the tert-butyldiphenylsilyl protected species 221.

It can be said therefore that the hypothesis that a more robust protecting group would be more tolerant to the PMBOCH₂ insertion conditions was vindicated. To establish whether the synthetic route was viable still depended on whether or not the highly stable tert-butyldiphenylsilyl ether could be removed efficiently. In the original synthesis, the corresponding reaction would have been deacetylation, which has already been shown here to be straightforward and very high yielding.

<table>
<thead>
<tr>
<th>PMBO—, OH</th>
<th>PMBO—</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>a</td>
</tr>
<tr>
<td>222 94%</td>
<td></td>
</tr>
</tbody>
</table>

Scheme 2.14
Reagents and Conditions: TBAF, THF, 0°C.

In fact, it was possible to remove the tert-butyldiphenylsilyl group with similar effectiveness by stirring silyl ether 222 with TBAF in THF for 6 hours (scheme 2.14). This change could be clearly seen by NMR by the disappearance of the silyl aromatic and tert-butyl signals.

Fluoride reagents such as TBAF are particularly effective for desilylation as a result of the high affinity of fluoride ions for silicon. TBAF was chosen over HF because of the greater ease of handling. The alcohol 223 was obtained after purification by chromatography with yields as high as 83%, (94% taking into account reclaimed starting material). The resulting alcohol 223 was identical to that which had been synthesised by the original route and could be crystallised in ethyl acetate and petroleum ether. This allowed a crystal structure to be obtained, which allowed two points to be clarified. As mentioned previously, it was assumed that the osmium tetroxide mediated dihydroxylation would
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take place on the opposite side of the ring to the alcohol and silyl ether. Secondly, it was hoped that the PMBOCH₂ insertion would take place on the same side of the ring as the silyl ether, and the opposite side to the protected diol. This had been observed in the case of the acetate protected species, and was predicted to take place considering the cradle type structure which is adopted by 2 fused 5 membered rings like this. However the tert-butyl-diphenyl silyl group is considerably larger than the acetate group and it was conceivable that this could cause the PMBOCH₂ moiety to insert from the wrong side. The crystal data show however that both the dihydroxylation and the PMBOCH₂ insertion proceeded as expected. The crystal structure is shown in scheme 2.15 and the data are presented in appendix 1.

Scheme 2.15
Crystal structure of alcohol 223.

Final steps

The remaining steps were therefore identical to those in the original synthetic scheme. The first of these was the oxidation of the only remaining secondary alcohol. Jones reagent, or any such acidic reagent was rejected due to the acid lability of the tertiary hydroxyl group present. A mild oxidising agent which is suitable for such sensitive compounds is IBX which was readily synthesised in large quantities. Taking care to allow all the IBX to dissolve in DMSO, the alcohol was dissolved in the minimum THF and added to the IBX solution. The resulting transformation was complete, by TLC, in 5 hours (scheme 2.16).
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Scheme 2.16
Reagents and Conditions: IBX, DMSO, THF.

After work up and purification by flash chromatography, the ketone 224 was obtained in 92% yield.

The remaining two transformations were deprotection steps. Firstly, the PMB protection was removed by hydrogenation catalysed by palladium on charcoal (scheme 2.17). Initially however, this reaction was disappointing, with rather low yields (30–40%) of the diol 195 being observed. With vigorous stirring to ensure the catalyst was exposed to the hydrogen, the starting material was entirely consumed within 2 hours by TLC. Hence it was thought that the work up procedure of filtering through a pad of celite was responsible for the poor yield. With such a polar compound - two free hydroxyl groups and a ketone - it was thought that much of the product was adsorbed onto the celite and lost. This loss was prevented by removing the palladium on charcoal by passing the reaction mixture through a syringe filter. Accordingly yields as high as 89% after purification were achievable. While the syringe filters are only really suitable for small scale reactions, the loss on celite could be minimised by using the smallest amount of celite possible, and ensuring that the pad was washed with copious ethyl acetate.

Scheme 2.17
Reagents and Conditions: (a) H₂, Pd/C, THF; (b) TFA, DCM.
The final step was the removal of the acetonide protection. The fused 5-membered ring system is very stable and reasonably strong conditions are required to effect the deprotection. Previously, this had been carried out using TFA, although with poor yields. Even with very short reaction time, the labile tertiary hydroxyl group is still prone to elimination after protonation. Initially this approach was repeated and although TLC analysis showed some progress, no product was obtained. In an effort to improve the yield, alternative techniques were investigated. Treatment of the acetonide with acetic acid and PPTS yielded only starting material. pTSA, HCl, and triflic acid consumed the starting material, but with no significant production of the desired product. Further investigations into this deprotection step are required to establish suitable conditions. The complete synthesis of keto-tetrol (2R, 3S, 4S)-20 from cyclopentadiene 65 is presented in scheme 2.18.
RESULTS AND DISCUSSION: SYNTHESIS OF PUTATIVE INTERMEDIATES

Scheme 2.18
Reagents and Conditions: (a) Peracetic acid (36–40 wt% soln in aqueous acetic acid), sodium acetate, sodium carbonate, DCM, 0°C; (b) Pd(PPh₃)₄, acetic anhydride, THF, 0°C; (c) CAL-B Novozyme SP-435, phosphate buffer; (d) TBDMSCI, imidazole, DCM; (e) ammonia saturated methanol; (f) TBDPSCI, imidazole, DCM; (g) acetic acid, THF, water 3:1:1; (h) OsO₄ (4% wt(aq) soln), NMO, THF, water, acetone; (i) DMP, pTSA, DCM; (j) Jones reagent (CrO₃, H₂SO₄, H₂O), acetone, 0°C–RT; (k) Bu₃SnCH₂OPMB, BuLi, THF, -78°C; (l) TBAF, THF, 0°C; (m) IBX, DMSO, THF; (n) H₂, Pd/C, THF; (o) TFA, DCM.
2.1.2 Synthesis of Bu₃SnCH₂OPMB 230

The reagent for insertion of the PMBOCH₂ moiety, namely Bu₃SnCH₂OPMB 230, was not available commercially. Three different routes for the synthesis of this reagent were investigated, and these are described below.

Reaction of tributylstannyl methanol 227 with p-methoxybenzyl trichloroacetimidate 229

The first route (scheme 2.19), which was used in the original synthesis, involved 3 steps, beginning with the conversion of tributyltin hydride 225 to tributylstannyl methanol 227.

\[
\text{Bu}_3\text{SnH} \xrightarrow{(a)} [\text{Bu}_3\text{SnLi}] \xrightarrow{(b)} \text{Bu}_3\text{SnCH}_2\text{OH}
\]

Scheme 2.19
Reagents and Conditions: (a) diisopropylamine, BuLi, THF, 0°C; (b) paraformaldehyde, R.T.

The tributyltin hydride was lithiated with LDA which was formed in situ. The lithiated species was then reacted with paraformaldehyde to give the tributylstannyl methanol 227, in up to 67% yield.

Meanwhile, p-methoxybenzyl alcohol 228 was treated with sodium hydride, followed by trichloroacetonitrile, to give p-methoxybenzyl trichloroacetimidate 229 in yields as high as 95% (scheme 2.20).

\[
\text{MeO} \quad \text{OH} \quad \xrightarrow{} \quad \text{MeO} \quad \text{O} \quad \text{CCl}_3
\]

Scheme 2.20
Reagents and Conditions: NaH (60% dispersion in mineral oil), ether, CCl₃CN, 0°C.

These two products were then stirred with triflic acid in DCM to give Bu₃SnCH₂OPMB 230 in up to 32% yield (scheme 2.21).
RESULTS AND DISCUSSION: SYNTHESIS OF PUTATIVE INTERMEDIATES

[Chemical structure image]

Scheme 2.21
Reagents and Conditions: Triflic acid, DCM.

While the yields stated here are acceptable, these are the highest yields observed and the last step in particular was subject to large variation in the yield obtained. This meant that large quantities of alcohol 227 and trichloroacetimidate 229 were required, and it was very difficult to obtain significant quantities (~10g) of Bu₃SnCH₂OPMB 230. Furthermore, two of the three steps involve the handling and chromatography of organotin reagents, which are highly toxic. Hence, an alternative method was desirable.

*p*-Methoxybenzyl methyl thiomethyl ether 231

The second method was a three step linear sequence involving a thioether intermediate (scheme 2.22). *p*-Methoxybenzyl alcohol 228 was converted to *p*-methoxybenzylmethyl thiomethyl ether 231 in 56% yield by treatment with sodium hydride in dimethoxyethane followed by addition of chloromethyl methyl sulfide and sodium iodide. The thioether 231 was then converted to the chloromethyl ether 232 by exposing the thioether to sulfuryl chloride in DCM at -78°C. The crude product was obtained in 94% yield.

[Chemical structure image]

Scheme 2.22
Reagents and Conditions: (a) i. NaH, DME, 0°C ii. NaI, CH₂Cl₂, 0°C; (b) SO₂Cl₂, DCM, -78°C; (c) disopropylamine, n-BuLi then Bu₃SnH, THF.
and used without further purification. Finally, the chloromethyl ether was added to tributyltin hydride which had been lithiated with LDA and stirred at 0°C to give the desired tin compound 230 in up to 51% yield. Hence overall, there is a slight increase in yield, taking into account maximum yields for each reaction in both schemes (27% cf. 21%). Furthermore, the thioether route was less susceptible to large variation in yield for any of the synthetic steps. However, a more efficient route was still desirable, and the stench associated with the thioethers meant that handling was unpleasant.

Iodomethyl tributyl stannane 235

The third of these routes was a two step synthesis based around iodomethyl tributyl stannane\(^{105}\) 235 (scheme 2.23). This stannane was synthesised by treating zinc-diiodomethane 234 (Simmons-Smith reagent) with tributyltin chloride.\(^{106}\) After work up, the desired compound 235 was isolated by vacuum distillation in 48% yield.

\[
\begin{align*}
\text{CH}_2\text{I}_2 + \text{Zn}(s) & \xrightarrow{(a)} \text{[ICH}_2\text{ZnI]} \xrightarrow{(b)} \text{Bu}_3\text{SnCH}_2\text{I} \\
233 & \quad 234 & \quad 235
\end{align*}
\]

Scheme 2.23

Reagents and Conditions: (a) Cupric acetate, acetic acid, THF, 40°C; (b) Bu\(_3\)SnCl, THF, 40°C.

This was then added to a solution of \(p\)-methoxybenzyl alcohol 228 which had been pre-treated with potassium hydride (scheme 2.24). After work up and purification by chromatography, the Bu\(_3\)SnCH\(_2\)OPMB 230 was obtained in 38% yield.

\[
\begin{align*}
\text{MeO} & \quad \text{OH} \quad \text{Bu}_3\text{SnCH}_2\text{OPMB} \\
228 & \quad \text{MeO} \quad 230
\end{align*}
\]

Scheme 2.24

Reagents and Conditions: KH, 235, THF.
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As such, the overall yield of 18% was the lowest of all three approaches. However, this third route turned out to be the route of choice, since the synthesis was shorter and quicker. Secondly, the iodide 235 was easily purified by distillation, meaning that large quantities could be readily prepared. The distillation also reduced the amount of time that organo tin reagents were open to the atmosphere compared to chromatography, which lessened the problems associated with the handling of the organo tin compounds in comparison to the first route. Finally, of all three routes the third route had the most consistent yields, thereby making it the most reliable and reproducible.
2.2 Synthesis of keto-tetrol (2R, 3S, 4R)-20

The synthesis of the (2R, 3S, 4R)-diastereomer of the keto-tetrol 20 had been completed with reasonable success previously by Ian Archer and it was the intention to follow this procedure for this project. In fact the existing route was used as far as possible with only a few minor modifications.

Synthesis of ketone 237

Starting with L-ribose (+)-116 meant that chirality was already present in the molecule. The cis-hydroxyl groups were protected as the acetonide by stirring with sulfuric acid in acetone (scheme 2.25). Since formation of the furanose form 117 is the most thermodynamically stable product, only the 3 and 4 hydroxyl groups are protected in this procedure.

\[
\begin{align*}
\text{(-)-116} & \quad \rightarrow \quad \text{117 (92\%)} \\
\end{align*}
\]

Scheme 2.25

Reagents and Conditions: H₂SO₄, acetone.

Previously for the acetonide protection, work up had involved the addition of calcium hydroxide. This was also the procedure which had been used for this route originally, but here, this resulted in severe streaking of the products on TLC and the desired product was not obtainable by chromatography. It was found that addition of saturated sodium bicarbonate solution on completion of the reaction gave much more satisfactory results. It was therefore possible to obtain the acetonide protected species 117 satisfactorily pure without purification in 92% yield.

Manipulations were to be carried on the remaining hemiacetal alcohol. Therefore the primary alcohol was first protected as a silyl ether by stirring with 1.05 equivalents of tert-butyldimethylsilyl chloride in the presence of imidazole in DCM (scheme 2.26). Carrying out the reaction at 0°C ensured that formation of the doubly protected species was kept to a minimum. The mono silylated species 236 was obtained after chromatography in 64% yield. However, the product was often used in crude form.
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Scheme 2.26
Reagents and Conditions: (a) TBDMSCI, imidazole, DCM; (b) KMnO₄, acetone.

The remaining unprotected hydroxyl group was then oxidised to the corresponding ketone 237 using potassium permanganate in acetone (scheme 2.26). The product, obtained in 85% yield, was in a sufficiently pure form after work up.

Silyl ether removal and conversion to iodide 240

The silyl protecting group then had to be removed. The original procedure had used HF for the desilylation procedure having found TBAF to give unsatisfactory results. However, having already successfully carried out the similar procedure for the (2R,3S,4S)-diastereomer, with the less reactive tert-butyldiphenylsilyl ether, it was thought that the use of TBAF should be investigated further. The greater ease of handling made the TBAF mediated deprotection considerably more attractive. However, the first attempt at the deprotection with TBAF gave a yield of only 34%. The starting material was consumed entirely, and therefore the low yield suggested that side reactions were taking place. The reaction was then performed at -78°C and reducing the reaction time from thirty minutes to ten, but there was no formation of the desired product. Significant improvements were achieved by reducing the reaction time further to 5 minutes but cooling at only 0°C (scheme 2.27).

Scheme 2.27
Reagents and Conditions: TBAF, THF, 0°C.
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In this way, the alcohol 238 was obtained after purification by column chromatography in 76% yield. The yield was thought to be sufficiently high to justify the use of TBAF over HF which gave the alcohol in 78% yield.

The resulting alcohol 238 was then tosylated by stirring with tosyl chloride and pyridine in chloroform overnight (scheme 2.28). The reaction tended to proceed cleanly, although attempts to remove traces of starting material by continuing stirring for a second 24 hours had no effect. Thus after chromatography, the tosylate 239 was obtained in 75% yield.

The tosylate was then displaced by iodine by refluxing in acetone with sodium iodide at 65°C overnight (scheme 2.28). The formation of the iodide proceeded cleanly and after chromatography (primarily to remove traces of coloured by-product), the iodide 240 was obtained in 96% yield.

**Final steps**

DBU mediated elimination of HI gave the corresponding highly reactive enol lactone 241, which without purification was added to lithiated Bu₃SnCH₂OPMB. This Fujimoto-Belleau reaction should have resulted in ring opening at the furanose oxygen and reclosure to give the carbocyclic ring and a ketone 224 (scheme 2.29). This reaction was not completed successfully however.
RESULTS AND DISCUSSION: SYNTHESIS OF PUTATIVE INTERMEDIATES

Scheme 2.29
Reagents and Conditions: (a) DBU, THF; (b) Bu₃SnCH₂OPMB, n-BuLi, THF, -78°C.

Initial attempts resulted in numerous compounds appearing by TLC, with no evidence of the desired product. Taking great care to maintain the anhydrous nature of the reaction, and careful titration of the n-butyllithium gave a cleaner reaction by TLC analysis. Furthermore, after initial purification attempts, there appeared to be some evidence of the desired product, but further purification caused the compound to decompose. Previous attempts within the group had demonstrated that the reaction could proceed as illustrated albeit with some difficulty. Scheme 2.30 shows the transformation in detail.

Scheme 2.30
Proposed mechanism of incorporation of PMBOCH₂ moiety.

Thus, attack by the PMBOCH₂ anion at the carbon results in ring opening at the lactone. Ring closure, this time as the carbocyclic species, produces the β-hydroxycyclopentanone 224 as a single diastereomer. In this instance, the PMBOCH₂ is incorporated on the same face as the acetonide ring. This direction of incorporation goes against steric arguments, which suggest that the opposite should be the case. Scheme 2.30 shows a possible rational for this. Chelation of the metal cation to the PMBO oxygen and the acetonide oxygen means that the hydroxymethyl side chain is directed to the more hindered face during the ring closing.
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Due to time constraints this reaction could not be investigated further. The final transformations would have been deprotection steps (scheme 2.31) to remove the PMB protection as described above, and the acetonide protection with acid to give keto-tetrol 20. The synthesis of the (2R, 3S, 4R)-diastereomer of the keto-tetrol 20 from L-ribose (+)-116 is shown in full in scheme 2.32.

![Scheme 2.31](image)

Scheme 2.31
Reagents and Conditions: (a) H₂, Pd/C, THF; (b) TFA, DCM.

2.3 Conclusions

The primary objective for this part of the project was to improve upon the synthesis of the (2R, 3S, 4S)-keto-tetrol to make it a viable synthetic route. Replacing the acetate protecting group in 197 with a tert-butyl-diphenyl silyl group to give the analogous compound 221 had the effect of increasing the yield for the key PMB insertion step from 20% to 76%. The required protecting group modifications (including removal of the tert-butyl-diphenyl silyl group) were high yielding. The result therefore, is a more reliable and replicable synthesis, and as such can be considered a success.

The synthesis of the both diastereomers was the secondary objective, with the intention that further work could be carried out on the feeding studies. However, this was not completed due to time constraints.
RESULTS AND DISCUSSION: SYNTHESIS OF PUTATIVE INTERMEDIATES

Scheme 2.32
Reagents and Conditions: (a) H$_2$SO$_4$, acetone; (b) TBDMSI, imidazole, DCM; (c) KMnO$_4$, acetone (d) TBAF, THF, 0°C (e) TsCl, pyridine, chloroform; (f) NaI, acetone, 65°C; (g) DBU, THF; (h) Bu$_3$SnCH$_2$OPMB, n-BuLi, THF, -78°C; (i) H$_2$, Pd/C, THF; (j) TFA, DCM.
2.4 Future work

The considerable improvement in the yield for the PMBOCH$_2$ insertion reaction using ketone 221 means that the synthetic route was made viable. However, work remains to optimise conditions for the acetonide deprotection.

The DBU mediated HI elimination and subsequent PMBOCH$_2$ insertion reaction for the iodide 240 requires attention, but it appears that with more time, this could have been made effective without any significant modification to the procedure.

In both cases, the incorporation of $^{13}$C labelling at the CH$_2$OH carbon could be achieved using the corresponding labelled PMBO$^{13}$CH$_2$SnBu$_3$ for the insertion reaction.

Thus with sufficient quantities of either unlabelled or labelled keto-tetros 20, the feeding experiments could undergo further study in order to establish the exact way in which the keto-tetrol 20 undergoes elimination to give the known enone 13.

Finally, on conclusion of the mechanistic studies into the biosynthetic pathway, it may be possible to feed modified carbocycles to mutants of *Streptomyces citricolor*. Those which are accepted by the organism would be expected to support the synthesis of novel carbocyclic nucleosides with possible therapeutic potential.
3. SYNTHESIS OF ABACAVIR

The importance of abacavir 5 in the treatment of HIV/AIDS means that the development of new synthetic routes to such compounds is of interest. Methods of incorporating the nucleoside are well documented4-12 and as such, attention is focused on the synthesis of enantiomerically pure precursors to the coupling reaction. In the case of abacavir, diol 243 would be a suitable precursor for a Mitsunobu type coupling. Work in these labs by Aitken107 had demonstrated the ability of Rhodococcus rhodochrous NCIMB 9703 to hydroxylate non-activated carbon centres in a range of compounds. As such, the protected alcohol 244 presented itself as an interesting synthetic target, to be examined as a substrate for this biohydroxylation.

Figure 3.1
Mitsunobu coupling precursor 243 and target substrate 244.

Retrosynthetic plan

An effective route to the biohydroxylation substrate 244 was required in order to allow repeated attempts and optimization of the biohydroxylation. Scheme 3.2 shows 2 possible approaches for the retrosynthesis of the target alcohol 245.

Scheme 3.2
Retrosynthesis of 3-benzoxymethylcyclopentene 245.
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

Approach B requires the potentially difficult introduction of the double bond by elimination, but otherwise requires only apparently straightforward manipulations. Approach A does not attempt to introduce the double bond, which must therefore be present in the starting material. The alcohol could be obtained by reduction of the corresponding carboxylic acid or ester. The approach was demonstrated by Branner-Jørgensen et al.\textsuperscript{108} starting from 3-chloro-cyclopentene which could be obtained from cyclopentadiene according to a procedure described by Alder et al.\textsuperscript{109}

3.1.1 Synthesis of benzyl ether 244 from cyclopentadiene

Chlorination of cyclopentadiene

Cyclopentadiene \( \text{65} \) was obtained by adding dicyclopentadiene dropwise to petroleum oil at \( \approx 200^\circ \text{C} \) in a flask fitted with a vigreaux column followed by a still head. The distillate was collected at a still head temperature of \( 40^\circ \text{C} \). This method resulted in rapid cracking of dicyclopentadiene and collection of cyclopentadiene, minimising the problem of polymerisation which is considerable when cracking neat dicyclopentadiene.

The chlorination was achieved by bubbling hydrogen chloride gas through cyclopentadiene\textsuperscript{10} (scheme 3.3).

![Scheme 3.3](image)

Reagents and Conditions: HCl(g).

This procedure results in an increase in volume of reaction mixture, and in the first attempt, the reaction was performed in a measuring cylinder, to observe this. The increasing volume stopped after around 2 hours and subsequent attempts used more conventional reaction vessels, and assumed that 2–3 hours reaction time was sufficient. Although purification was relatively easily achieved by distillation, the resulting clear colourless liquid was prone to discoloration (to purple) even when stored under argon at \(-20^\circ \text{C} \). It was therefore necessary to use the chloride 247 immediately after purification.
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

Carboxylic acid formation by Grignard reaction

The carboxylic acid 246 was obtained via reaction of the corresponding Grignard reagent 250 with carbon dioxide (scheme 3.4).

\[
\begin{align*}
\text{C}_7\text{H}_7\text{Cl} & \quad \xrightarrow{\text{(a)}} \quad \left[ \text{C}_7\text{H}_7\text{MgCl} \right] \quad \xrightarrow{\text{(b)}} \quad \text{C}_7\text{H}_7\text{CO}_2\text{H} \\
\text{247} & \quad \text{250} & \quad \text{246} \\
\end{align*}
\]

Scheme 3.4
Reagents and Conditions: (a) Mg, THF, -10°C; (b) CO$_2$, THF, -78°C.

At ambient temperature, there was a strong tendency for the Grignard reagent to dimerise, and it was necessary to keep the reaction mixture at -10°C or less. This in turn hindered formation of the Grignard reagent and high yields were unachievable. It was found to be most effective to add the reaction mixture to a slurry of solid carbon dioxide in THF. This gave a large concentration of carbon dioxide with which the Grignard reagent could react. Bubbling carbon dioxide through the reaction mixture had a tendency to evaporate the solvent. In this way the carboxylic acid 246 was obtained in around 13% yield. Significant improvements to this transformation may have been possible according to the literature. While some examples carried out the Grignard reaction using Rieke magnesium,$^{111,112}$ this was not an attractive proposition, due to the large quantities of potassium metal required. However, Jones et al.$^{113}$ suggested that a technique by Oppolzer$^{114}$ using a rotating solution reactor may be more effective. Jones went on to demonstrate a technique based on observations by Brown et al.$^{115}$ in which the magnesium is stirred under an atmosphere of argon for 2 days to give a black powder which when used in this reaction gave the desired acid in 84% yield. Had time allowed, these techniques may have been attempted.
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

Esterification and final steps

Conversion of the carboxylic acid 246 to the methyl ester 251 may have been possible using diazomethane according to the route described by Branner-Jørgensen, but in order to avoid using this hazardous material, alternative methods were sought. Formation of the acid chloride by refluxing with thionyl chloride, followed by reaction with methanol resulted in no formation of the desired product. If formation of the acid chloride was achieved it is possible that it was unstable and decomposed before reaction with methanol, possibly during reflux. Refluxing the acid in methanol with sulfuric acid also afforded no product. Finally, the use of a coupling agent in the presence of methanol gave the desired ester. Initial experiments using EDCI gave the desired ester 251 in 45% yield. The cheaper reagent EEDQ$^{16}$ was also tested for suitability and found to be just as effective (scheme 3.5).

![Scheme 3.5](https://example.com/scheme.png)

Conversion of carboxylic acid 246 to the methyl ester 251 using EEDQ in methanol.

However, once more, handling difficulties with the product may have caused the isolated yield to belie the effectiveness of the reaction. In one instance, the product isolated by rotary evaporation of column fractions was shown by NMR spectroscopy to contain DCM (product:solvent ~3:1). Taking this into account, along with the mass from the combined fractions, the yield would be in the region of 70%. After removing all traces of DCM, the yield of pure product was 30% suggesting that 40% had been lost during rotary evaporation. The methyl ester has a boiling point of 40-45°C at 9mmHg.
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

Therefore careful distillation of the column fractions would appear to be possible to give pure product. However, the product decomposed over time, even when stored at -18°C, and very rapidly with heat. Decomposition would likely be by isomerisation of the double bond to the more substituted site, and into conjugation with the carbonyl group. As such, this could result in loss of the ester moiety. Accordingly, a yield of 45% was the highest achieved.

Reduction of the resulting ester 251 using lithium aluminium hydride gave the more stable methyl alcohol 245 in a yield of 40% (scheme 3.6). This poor yield may have been due to impure starting material, or decomposition of the starting material during the reaction.

![Scheme 3.6](image)

**Scheme 3.6**

*Reagents and Conditions:* LiAlH₄, THF.

Benzylation of the resulting alcohol 245 using benzyl bromide in the presence of sodium hydride gave the protected species 244 in an excellent 83% yield. Analysis by NMR spectroscopy revealed a signal at 7.3ppm corresponding to the benzyl aromatic group, and multiplets at 5.8 and 5.7ppm characteristic of the two alkene protons. Hence, from cyclopentadiene, this route (summarised in scheme 3.7) gives the desired substrate

![Scheme 3.7](image)

**Scheme 3.7**

*Reagents and Conditions:* (a) HCl(g); (b) i. Mg, THF, -10°C; ii. CO₂, THF, -78°C; (c) EEDQ, MeOH; (d) LiAlH₄, THF; (e) BnBr, NaH, 'Bu₄NI, THF.
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

in less than 1% yield over only 5 steps. However, sufficient material had been obtained to begin preliminary biohydroxylation experiments.

Biohydroxylation of substrate 244 by *Rhodococcus rhodochrous*

Biohydroxylation reactions using *R. rhodochrous* tended to proceed to completion after incubation with the similar substrates overnight. Aitken found that this transformation however, was significantly slower, with consumption of starting material taking 5 days (scheme 3.8).

![Scheme 3.8](image)

*Reagents and Conditions: Rhodococcus rhodochrous NCIMB 9703, 18%.*

Organic extracts of the supernatant contained a number of products and after purification by silica column chromatography a single product was obtained in 20% yield. This hydroxylated product 252 was examined extensively by NMR in order to determine the regiochemistry and stereochemistry. Initial $^1$H NMR studies strongly suggested that the hydroxyl group had been added in a 1,4-relationship to the protected hydroxymethyl group. Furthermore, comparison with known NMR spectra for the *trans-* and *cis-* species appeared to suggest that the *trans-* isomer had formed. Aitken was able to confirm this by observing 'through space' proximity of relevant protons by a nOesy experiment. Figure 3.9 shows the key nOe crosspeaks.

![Figure 3.9](image)

*nOe crosspeaks for 252 indicated by arrows.*

At this stage however, the absolute configuration of the product was not known. A repeat of this biohydroxylation was attempted in order to obtain additional material for more extensive analysis. Under the same conditions, however, production of the
desired product appeared to peak after 2 days. After this time, other reactions became predominant, and the percentage of the desired product became negligible. Separation of the compounds produced failed to yield significant quantities of any of the components. An improved synthetic approach to the substrate for further testing was therefore required. Three alternative routes towards the synthesis of 3-benzyloxymethycyclopentene 244 were investigated by David Kwant for his final year project.

3.1.2 Alternative procedures for the synthesis of substrate 244

Wittig rearrangement

The first route investigated by Kwant was based around a [2,3]-sigmatropic Wittig rearrangement as described by Hildebrand et al.\textsuperscript{112} (scheme 3.10). The route described involves the vitamin-B$_{12}$ catalysed isomerisation of 1,2-epoxycyclopentane 253 to (R)-cyclopent-2-enol 254\textsuperscript{118} in 66% yield, with product e.e. 56%. This was treated with potassium hydride and (iodomethyl)tributyltin followed by butyllithium to effect the Wittig rearrangement and give the optically active 3-hydroxymethylcyclopentene 245 in 49% yield.

\[
\begin{align*}
\text{253} & \xrightarrow{(a)} \text{254} \quad (\text{66\%}) \\
\text{254} & \xrightarrow{(b)} \text{245} \quad (\text{49\%})
\end{align*}
\]

\textit{Scheme 3.10}

Reagents and Conditions: (a) Vitamin B$_{12}$, Zn, NH$_4$Cl, MeOH; (b) KH, then ICH$_2$SnBu$_3$, then BuLi, THF.

It was decided that given the prohibitive cost of vitamin B$_{12}$, and the rather poor e.e. of the product 254, the more straightforward reduction of 2-cyclopentenone 255 would be used to obtain the alcohol, albeit in racemic form. A non-racemic synthesis of the substrate is however desirable and the strategies attempted towards this will be discussed further. Having attempted the reduction of 2-cyclopentenone using lithium aluminium hydride with no success, Kwant carried out the reduction using sodium borohydride and cerium chloride in a procedure described by Luche\textsuperscript{119} particularly suited for this application due to a high selectivity for 1,2-addition over 1,4-addition. By this method,
the allylic alcohol 254 was obtained in up to 41% yield (scheme 3.11). Alcohol 254 was then treated with potassium hydride then (iodomethyl)tributyltin 235 (synthesis discussed elsewhere), and finally butyllithium, to effect the Wittig rearrangement and afford 3-hydroxymethylcyclopentene 245 in 38% yield. Benzylation of 245 as described previously was achieved by Kwant in 70% yield. Therefore, this sequence of reactions gave the desired substrate in around 11% yield (3 steps).

![Scheme 3.11](image)

Scheme 3.11
Reagents and Conditions: (a) NaBH₄, CeCl₃·7H₂O, MeOH, 41%; (b) KH, then ICH₂SnBu₃, R.T. then BuLi, THF, -78°C, 38%; (c) BnBr, NaH, Bu₄NI, THF, 70%.

The Wittig rearrangement proceeds entirely on one face of the cyclopentene ring. As observed for the vitamin B₁₂ case this means that optical activity is retained. An enantioselective reduction of 2-cyclopentenone 255 to give the alcohol R-254 would enable this route to provide a single enantiomer of the substrate 244. Although the route employed by Kwant is a marked improvement over the initial synthesis, the overall yield is still low, and synthesis of the required tin reagent 235 is relatively difficult. Furthermore, avoidance of organotin reagents would be ideal due to their high toxicity. It is worth noting that Hildebrand also investigated the 3-chlorocyclopentene route, but bypassed the esterification by direct reduction of the acid 246 in yield of 64%. Reduction of the acid was not attempted here, but may have improved the viability of the route.

Ene reaction

Work by Paulsen et al. and Snider et al. suggested that a Prins reaction may provide a good alternative. As such the dimethylaluminium chloride catalysed Prins reaction
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

Scheme 3.12
Reagents and Conditions: Paraformaldehyde, Me₂AlCl, DCM.

was investigated by Kwant (scheme 3.12). Snider had reported that the desired alcohol 245 was obtained in 7% yield, but the chloride 259, a precursor to alcohol 245 was produced in 39% yield. In fact, treatment of cyclopentene 257 with paraformaldehyde in the presence of dimethylaluminium chloride resulted in only 10% yield for the chloride and 4% yield for the alcohol 245. Therefore, Kwant abandoned this approach.

Introduction of double bond by elimination

Formation of the double bond by dehydoration of ethyl 2-hydroxycyclopentane-carboxylate 261 was reported by Scharf et al. This strategy is in keeping with retrosynthetic plan, path B (scheme 3.2). Starting from racemic ethyl cyclopentanone-2-carboxylate 260, the ketone was selectively reduced by PtO₂ catalysed hydrogenation (scheme 3.13). This gave the ester-alcohol 261 in trans-configuration. Treatment with phosphorus pentoxide gave a mixture of the desired double bond isomer 262 and the undesirable isomer 263 in a ratio of 9:1.

Scheme 3.13
Reagents and Conditions: (a) H₂/PtO₂, 3–4 atm.; (b) P₄O₁₀.

Presumably the tendency towards the product with double bond 2,3 to the substituent results from lack of a proton in an antiperiplanar arrangement relative to the OH at C–1 in the trans- isomer. Consequently, it was considered important that whatever reduction method was employed, it should give rise to the trans- isomer solely. However, this
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

appeared to be a facile route to ethyl 2-cyclopentenecarboxylate 262 which it was hoped would be more stable than the methyl ester 251 and have a higher boiling point (therefore avoiding loss during evaporation). Encouraging preliminary work by Kwant showed that the dehydration of alcohol 261 was possible although in low yield, and another project student Ross Stevenson continued this work.  

3.1.3 Synthesis of substrate 244 from ethyl cyclopentanone-2-carboxylate 260

Microbial reduction of keto-ester 260

The diastereo- and enantioselective microbial reduction of ethyl cyclopentanone-2-carboxylate 260 was reported by Buisson et al. The report indicates that ethyl 2-hydroxy-cyclopentanecarboxylate 261 is produced with the major product being of IS, 2S absolute configuration in >99% e.e. (scheme 3.14). The minor product is enantiomerically pure cis-hydroxycyclopentanecarboxylic acid ethyl ester (1S, 2R)-261 which can be removed by traditional flash chromatography.

![Scheme 3.14](attachment:image.png)

Reagents and Conditions: (a) Rhizopus arrhizus ATCC 11145, 500rpm, 25°C.

The reason that an enantiomerically pure compound is desired is that it may be an indication of whether or not the required diastereomer is produced in the biohydroxylation. The biohydroxylation process is enantioselective and enantiospecific. Therefore if the substrate is a racemic mixture, only one enantiomer is converted to the alcohol and a single diastereomer is produced. If the substrate is a single enantiomer, it will only be converted by the enzyme if it is the enantiomer which the enzyme is selective for. This in turn means that if the absolute configuration of the substrate is known, the absolute configuration of any product would be known and in the case
where there was no transformation, the absolute configuration of the product of the successful transformation would still be known by deduction. A possible example of this is shown in figure 3.15.

Figure 3.15
Hydroxylation by *Rhodococcus rhodochrous* NCIMB 9703

Stevenson revived a 4 year old sample of the fungus *Rhizopus arrhizus* ATCC 11145 and carried out the bioreduction. The literature indicated that the trans-(1S, 2S)-enantiomer was formed. The sample of 2-hydroxycyclopentanecarboxylic acid ethyl ester 261 obtained by the microbial transformation could therefore be used to identify the racemic trans-compound obtained by chemical means by comparison of the TLC. However, it was concluded much later that the major product from this biotransformation was in fact the cis-species, presumably as a result of contamination of the old sample of *R. arrhizus*.

Chemical reduction of 260 and dehydration

Initially the chemistry completed by Stevenson was repeated to verify results and obtain new material. The ester-alcohol 261 was initially obtained by selective reduction of ethylcyclopentanone-2-carboxylate 260 with sodium borohydride\(^\text{26}\) (scheme 3.16).

\[
\begin{align*}
\text{260} & \quad \text{OEt} \\
\text{trans-261} & \quad \text{OH} \\
\text{cis-261} & \quad \text{OH} \\
\text{38%} &
\end{align*}
\]

Scheme 3.16
*Reagents and Conditions: (a) NaBH₄, MeOH, 0°C.*
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The product thought to be of *trans*-configuration was identified by TLC comparison with the sample obtained by Stevenson. Thus after careful chromatography, the compound 261 obtained in this way was treated with phosphorus pentoxide in toluene (scheme 3.17). This resulted in low yields of alkene 262 even though TLC analysis appeared to show good consumption of starting material and formation of a new species.

![Scheme 3.17](image)

*Reagents and Conditions: P_2O_5, DCM.*

Considering the issues with boiling point encountered previously, it was noted that replacing toluene with a more volatile solvent could allow a greater quantity of product to be isolated. Therefore the reaction was run in THF, ether, DCM and toluene. TLC analysis indicated that the THF and ether reactions had not proceeded at all, while the DCM and toluene reactions appeared very similar by TLC. However, on removal of the solvent, similar yields were obtained. Taking great care with rotary evaporation failed to improve yields, since even with much solvent remaining (by NMR) yields were still poor.

### 3.1.4 Involvement of protecting groups

Still encouraged by the evidence from TLC, it was thought that the ester group may be hindering the effectiveness of the dehydration. With this in mind, the strategy was changed so that the dehydration could be carried out on a substrate not containing the carbonyl group. In fact, by introducing a benzyloxymethyl group in place of the ester group, the carbonyl would be removed, a compound of higher molecular weight would be obtained, and a successful dehydration would yield the desired substrate, which from the previous work, had seemed stable. Two strategies were envisaged for the introduction of the benzyloxymethyl group. The first used a protecting group approach, while the second relied on selectivity for primary alcohols over secondary alcohols.
Silyl ethers

For the first route, the free alcohol 261 was protected as the tert-butyldimethylsilyl ether using tert-butyldimethylsilyl chloride in the presence of imidazole, giving the desired product 264 in up to 95% after chromatography (scheme 3.18).

```
OEt
OH
261 (a) -> OEt
  (a) TBDMSCI, imidazole, DCM; (b) DIBAL, THF, -78°C; (c) BnBr, NaH, 'Bu 4 NI, THF; (d) TBAF, THF, 0°C–R.T.
```

Scheme 3.18

Initial attempts to reduce the ester to a hydroxymethyl group using lithium aluminium hydride in THF, which had been moderately successful in the case of the reduction of methyl 2-cyclopentenecarboxylate were not successful in this instance. While NMR spectroscopy suggested that the ester group was no longer present in the product, it appeared that the silyl ether was also removed under the reaction conditions.

An examination of the literature (e.g. Roush et al. and Corey et al.) revealed that DIBAL was known to be effective for reducing esters in the presence of TBDMS ethers. Hence the ester 264 was treated with DIBAL in DCM, giving the desired alcohol in 75% yield (scheme 3.18).

The alcohol was benzylated by treatment with sodium hydride, followed by benzyl bromide in the presence of tetrabutylammonium iodide. This reaction gave rise to not only the desired product 265 but also a number of by-products, of which one was of similar R 1 to the product. The desired benzylated alcohol 265 and by-product were of high R f, which meant that isolation of the product in pure form was extremely difficult. In fact, the chromatography proved too time consuming for this pathway to be useful. Therefore, the mixture containing these two compounds was used to continue the synthesis in the hope that the impurity could be removed more easily by chromatography after removal of the silyl protecting group. Tetrabutylammonium fluoride in THF was chosen to remove the tert-butyldimethylsilyl group (scheme 3.18).
Again, while the desired transformation was effected, it proved too difficult to separate the resulting alcohol 266 from a close running impurity. Thus, 2-benzylxoxymethyl-cyclopentanol 266 was obtained in semi-pure form in 57% yield. It may have been possible to proceed with the next step and find that conditions for chromatography were more favourable, but this was not attempted, as satisfactory conditions for the dehydration had not been found. Furthermore, it is possible that an alternative protecting group strategy could have resulted in simpler purification depending on the likely by-products.

Direct benzylation of primary alcohol

On the assumption that it would be possible to benzylate a primary alcohol in the presence of a secondary alcohol, ethyl 2-hydroxycyclopentanecarboxylate 261 was treated with lithium aluminium hydride in THF, to reduce the ester, giving 2-hydroxymethyl-cyclopentanol 267 in 83% yield (scheme 3.19). While it would be possible to reduce

\[
\text{Scheme 3.19}
\]

Reagents and Conditions: LiAlH₄, THF, 0°C.

ethyl cyclopentanone-2-carboxylate 260 to the diol in one step, this purely chemical scheme was still considered to be a pilot for the chemoenzymatic scheme, and therefore it was best practice to keep as many reactions as possible the same.

Using a new sample of \textit{Rhizopus arrhizus} ATCC 11145, the microbial reduction of ethyl cyclopentanone-2-carboxylate 260 was repeated. The fungal growth from the new spores appeared healthy and fitted the catalogue description well compared to the old sample. Perhaps unsurprisingly therefore rather different results were obtained to those reported by Stevenson, and \textit{trans-} and \textit{cis-} species 261 were obtained in a ratio (\textit{trans-}: \textit{cis-} 85:15) which agreed well with the literature ratio (87:13). These were separated by column chromatography and the \textit{trans-} species converted to the diol 267 in the same way as the \textit{cis-} (as can now be correctly assigned) species.
As suggested before, the synthesis of the racemic diol 267 could be achieved in a single step from ethyl cyclopentanone-2-carboxylate 260, and cis- and trans- isomers identified from authentic samples obtained previously. This was done according to a procedure described by Theil et al.,\textsuperscript{128} using a large excess of sodium borohydride (scheme 3.20). The cis- and trans- assignment of the products (by TLC) was in agreement that stated by Theil.

\[
\begin{align*}
\text{Scheme 3.20} \\
\text{Reagents and Conditions: NaBH}_4, \text{ EtOH, -20°C.}
\end{align*}
\]

Although the cis- and trans- isomers of the diol 267 were separated by meticulous chromatography, this is still a racemic route as the two trans-enantiomers are inseparable by chromatography. The following reactions were carried out on both enantiomers. Having obtained the diol 267 with relative ease, the question over the feasibility of selectively benzylating a primary alcohol could be addressed. By using only one equivalent of the benzylating reagents, it was deemed that it would be possible to introduce benzyl protection to the more reactive primary alcohol. Therefore the diol 267 was stirred in DMF at around -60°C with 1.5 equivalents of sodium hydride then 1 equivalent of benzyl bromide was added. After stirring overnight and allowing the reaction mixture to warm to room temperature, the reaction mixture was shown to contain starting material, the desired monoprotected product 266 and the diprotected species 268 (scheme 3.21). Consequently, although starting material could be recovered, yields were in the region of 40%.

\[
\begin{align*}
\text{Scheme 3.21} \\
\text{Reagents and Conditions: BnBr, NaH, THF, -60°C.}
\end{align*}
\]
Initially the reaction was quenched by addition of water, but NMR spectroscopy showed that the integral for the benzyl protons was too high, and comparison by TLC showed that benzyl alcohol co-eluted with the monoprotected species 266. Curiously, although the cis- and trans-isomers were of very similar $R_f$, this was only observed in the case of the cis-isomer. Fortunately the benzyl alcohol contamination could be avoided by quenching the reaction with dilute hydrochloric acid. Attempts to force the consumption of starting material by increasing the concentration or number of equivalents of benzyl bromide resulted in sole production of the doubly protected species 268, which had to be recovered by hydrogenation. The inefficiency of this reaction meant that an alternative was desirable.

**Benzyldiene acetal protection**

An intriguing possibility was the use of benzyldiene acetal protection. This is a protection strategy which is commonly used in carbohydrate chemistry, where a 1,3-diol is protected in this way, and after the required manipulations are made, the acetal ring can be opened selectively to give a benzyl protected primary alcohol and a deprotected secondary alcohol. The feasibility of applying this strategy to the diol in question 267 was therefore investigated. The presence of only two hydroxyl groups and lack of functionality elsewhere in the ring made this an attractive option. Only the possibility of bond strain, in particular for the trans-isomer was discouraging. However in a straightforward procedure, the diol 267 was dissolved in DMF and stirred with benzaldehyde dimethyl acetal and $p$TSA at 60°C overnight, successfully transforming both cis- and trans-isomers to the corresponding acetals 269 in around 80% yield (scheme 3.22). Conditions for this reaction were not optimised. The ring opening was

```
\begin{center}
\begin{tikzpicture}
  \node (267) at (0,0) {267};
  \node (269) at (2,1) {269};
  \node (266) at (4,0) {266};
  \draw[->] (267) -- node[above] {OH} (269);
  \draw[->] (269) -- node[above] {O} (266);
  \draw[->] (267) -- node[above] {OH} (269);
  \draw[->] (269) -- node[above] {Bn} (266);
  \node (trans) at (2,0.5) {trans- 81%, cis- 78%};
  \node (cis) at (4,0.5) {trans- 69%, cis- 63%};
\end{tikzpicture}
\end{center}
```

Scheme 3.22

*Reagents and Conditions*: (a) Benzaldehyde dimethyl acetal, $p$TSA, DMF, 60°C; (b) NaCNBH$_4$, HCl in ether, THF.
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effected by dissolving the acetal 269 in THF, with sodium cyanoborohydride and treating the resulting mixture with HCl solution in ether (scheme 3.22). Again, the reaction was successful for both diastereomers with yields in the range 63–69%. In the case of the cis-diastereomer, there appeared to be inseparable traces of a by-product in which the alternative ring opening had occurred giving the protection on the secondary alcohol instead of the primary alcohol. However, the desired trans-isomer 266 was obtained in 56% over the two steps. This represents a respectable improvement over the direct benzylation approach. Furthermore, while the direct benzylation approach was repeated many times in order to improve the effectiveness, due to time constraints, optimization of the acetal route was not possible. Hence by utilising the acetal protecting route, 2-benzyloxymethylcyclopentanol 266 was synthesised from ethyl cyclopentanone-2-carboxylate 260 in three steps to give the racemic product, or one enzymatic step followed by three chemical steps to give the product as a single enantiomer.

3.1.5 Double bond formation by dehydration/elimination

Strategies for formation of the alkene 244 via elimination could therefore continue to be investigated. As such, the benzyloxymethyl alcohol 266 was treated with phosphorus pentoxide in DCM (scheme 3.23), and as observed by TLC for the dehydration of ethyl 2-hydroxycyclopentanecarboxylate 261, the reaction went cleanly with starting material being consumed over twenty minutes. However, once more yields were low (20%) and around 60% of the isolated product was the 1,2-double bond isomer 270 (as determined by NMR spectroscopy), which was inseparable from the desired product 244 by chromatography. The alkene protons in the benzyl ether 244 give rise to a pair

![Scheme 3.23](image_url)

Reagents and Conditions: P2O5, DCM.
of multiplets at 5.8ppm, with pronounced "roofing" effect. In place of these signals, for the undesired isomer 270, a multiplet at 5.7ppm corresponding to the single olefinic proton can be seen. The C-6 protons are at higher shift for alkene 270 relative to the desired alkene 244 in accordance with the increase in electron density resulting from the proximity of the double bond to the benzyloxyethyl substituent in alkene 270.

Many of these reactions were done prior to the correct assignment of cis- and trans-, and as such only the compound now known to be of cis- configuration was tested. Abandoning the direct dehydration approach, it was thought that by transforming the hydroxy group to a better leaving group it may be possible to effect a base mediated elimination. Such an approach would still be constrained in the direction of elimination by the availability of an antiperiplanar configuration for the acidic proton and leaving group.

**Sulfonation**

The first leaving group to be studied was the tosylate. However, treatment of the 2-benzyloxyethyl-cyclopentanol 266 with toluene sulfonyl chloride or toluene sulfonic anhydride in pyridine failed to effect any reaction even with heating (scheme 3.24). This may be due to steric reasons.

A similar approach with triflic anhydride appeared, by TLC analysis, to consume the starting material. Attempts to purify the product by column chromatography were unsuccessful as the product decomposed on the silica. It is likely that the triflate 272 eliminated on the acidic silica to give the wrong double bond isomer 270 (scheme 3.25).

![Scheme 3.24](image)

*Reagents and Conditions: pTsCl or pTs₂O, pyridine, R.T. or 50°C.*

Treatment of the crude triflate with a strong hindered base could have given the desired product, but this was not attempted due to time constraints.
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

\[ \text{Scheme 3.25} \]
Reagents and Conditions: Tf\(_2\)O, pyridine.

Finally, conversion of the hydroxyl group to the mesylate was investigated.\(^{30}\) Mesyl chloride and triethylamine were added to a solution of the alcohol 266 in DCM in an ice bath and the starting material was entirely consumed in 15 minutes (scheme 3.26).

\[ \text{Scheme 3.26} \]
Reagents and Conditions: MsCl, triethylamine, DCM, 0\(^\circ\)C.

This time however, after work up, the product could be purified by column chromatography giving the mesylate 273 in 66% \(\text{(trans-)}\) and 85% \(\text{(cis-)}\) yield. Confirmation of the transformation was obtained in the form of an NMR singlet at 2.9ppm, corresponding to the mesyl CH\(_3\) protons, and by the disappearance of the OH signal present in the IR spectrum for alcohol 266.

Hence the alcohol was successfully converted to a better leaving group. It now remained to identify a suitable method for elimination of the mesylate to give the correct double bond isomer 244.

**Base mediated elimination**

Stirring the mesylate 273 \(\text{(cis- and trans-)}\) in THF with DBU at R.T. failed to cause any change to the starting material. LDA was added to the reaction mixture, and although by TLC analysis, the starting material was consumed, a complex mixture of products was formed which didn’t appear to contain the desired product.

The DBU mediated elimination was repeated for the \(\text{cis-}\) isomer in DMF, with microwave radiation. After work up and purification, NMR revealed that the product contained the
wrong double bond isomer 270 in a ratio of 2:1 with the desired isomer 244. Repeating
the reaction with conventional heating produced predominantly the wrong alkene, and
in a significantly lower yield (18% cf. 90%). It was thought that the conditions that
were required to force the reaction were causing the E1 type elimination to proceed
much more quickly than the desired E2 elimination. Therefore, it was concluded that at
stronger, but still highly hindered base may be able to effect the reaction at moderate
temperatures. As such, the cis- mesylate 273 was stirred with potassium tert-butoxide
in DCM. After stirring for 70 hours, little change was observed by TLC, and the small
amount of product obtained after work up was shown by NMR spectroscopy to be a
mixture of isomers.

Alumina mediated elimination

Papers by Posner et al. and Kobayashi et al. described the elimination of sulfonate
esters, using alumina. The cis- mesylate 273 was stirred in DCM with a large excess of
activated acidic alumina (for chromatography) and after stirring overnight, TLC showed
that very little starting material remained. After work up, NMR spectroscopy showed
that the product was mainly the wrong double bond isomer 270. However, the literature
stated that super I type alumina was suitable. As, activated, neutral brockmann I
(standard) alumina was available this was used and the reaction repeated for both
isomers. An initial small scale reaction revealed by NMR spectroscopy that the cis-
species had eliminated to give the undesired 1,2-alkene 270, while the trans- species
had eliminated to give the desired isomer 244 as the major product (6.33:1 major:
minor). Scheme 3.27 shows possible elimination mechanisms for the trans isomer.
The anti elimination process is generally more favourable, but requires that the
mesylate and H, are in an antiperiplanar configuration. Experience with base mediated
elimination, which failed to provide the desired double bond isomer 244, suggests
that this may not be the mechanism of elimination. This is in agreement with the
perspective drawings in scheme 3.27 which show the mesylate 273 in the most likely
conformation, with the large benzylxoyymethyl group in a pseudo equatorial position.
As such, no protons are antiperiplanar to the mesylate group. This implies therefore that
syn elimination, in which loss of the mesylate group is assisted by coordination to the
aluminium, is the most likely mechanism or formation of alkene 244. Syn elimination
to give the wrong double bond isomer may be restricted by the relative inaccessibility of
H, to the alumina. In the case of the cis- mesylate an antiperiplaner configuration exists
RESULTS AND DISCUSSION: SYNTHESIS OF ABACAVIR

Scheme 3.27
Anti and Syn elimination mechanisms for mesylate trans-273.

between the mesylate group and $H_1$, and consequently, anti elimination occurs readily to give the wrong double bond isomer 270.

Scale up using the trans- mesylate showed that the product could be isolated in up to 76% yield. The 2 double bond isomers elute at the same Rf and therefore could not be separated by chromatography. Optimization of this reaction to improve product ratio and yield was not investigated due to time constraints. Furthermore, the work by Kwant on the Prins reactions had shown that it was possible to separate the double bond isomers for the equivalent alcohols 245 and 258 and therefore if the deprotection and reprotection could be performed efficiently, the correct alkene 244 could be obtained in pure form. However, it was thought that the small amount of contamination could be tolerated for the biohydroxylation.
3.2 Conclusions

The final route gives the required substrate 244 in 12% yield for the racemic chemical route in 5 steps, or 6% yield for the non racemic chemoenzymatic synthesis in 6 steps. Both routes are summarised in scheme 3.28. Therefore, the chemical route compares favourably with the previous successful attempts at the synthesis of the substrate. But of greatest importance is the effectiveness of the route to the optically active substrate (3R)-244, which could provide valuable information on the biohydroxylation and potential an attractive route to alcohol 252. In many cases, the yield of the cis-isomer is significantly greater than the trans-isomer. While this may be inherent in the structural differences between the two, the reactions were generally repeated many times for the cis-isomer, while the same reactions for the trans-isomer were often carried out only once. Conceivably therefore overall yields of 16% and 14% for the chemical and chemoenzymatic reactions respectively (taking highest yields for cis- or trans- for each step) seem feasible.

Scheme 3.28
Reagents and Conditions: (a) Rhizopus arrhizus ATCC 11145, 500rpm, 25°C; (b) NaBH₄, EtOH, -20°C; (c) LiAlH₄, THF, 0°C; (d) Benzaldehyde dimethyl acetal, pTSA, DMF, 60°C; (e) NaCNBH₄, HCl in ether, THF; (f) MsCl, triethylamine, DCM, 0°C; (g) Al₂O₃, activated, neutral, Brockmann I (standard) ca 150 mesh, DCM.

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3.3 Future work

The synthesis of the desired substrate 244 is reliable and reasonably efficient. Further optimization of reaction conditions, particularly the elimination of the mesylate could improve the overall yield and selectivity for the desired double bond isomer. Thiel et al. showed that the diol 267 could be resolved by transesterification using *Pseudomonas cepacia* lipase, giving the acetylated species in up to 48% yield and >99% e.e. This is attractive because the procedure uses equipment and techniques normal found in a synthetic chemistry laboratory. However, Only 25% of the material can be used in this way, since the cis-diol is discarded, as well as the wrong trans-diastereomer.

A third attempt the biohydroxylation procedure using *Rhodococcus rhodochrous* 9703 failed to produce the expected hydroxylated species 252. This reaction therefore requires significant optimization efforts. If conditions could be found for the synthesis of significant quantities of alcohol 252 final steps could be taken to demonstrate the transformation to carbocyclic nucleosides such as abacavir using a base coupling technique with inversion.
4. EXPERIMENTAL

4.1 General experimental

4.1.1 Instrumentation

$^1$H and $^{13}$C NMR spectra were recorded on either a Varian Gemini 200, Bruker AC 250 or Bruker WH 360 instrument. Chemical shifts ($\delta_{H}$, $\delta_{C}$) are reported in parts per million (ppm) and coupling constants (J) are reported in Hertz (Hz) and quoted to 1dp.

Electron Impact (EI) mass spectrometry was carried out on a Finnegan 4500 or a Finnegan 4600 instrument and Fast Atom Bombardment (FAB) mass spectrometry was performed using a Kratos MS50TC instrument.

Infrared spectra were recorded on a Biorad FTS-7, a Jasco FT/IR-460 plus FT-IR spectrometer or a Perkin Elmer Paragon 1000 FT-IR spectrometer with the frequencies ($\nu$) being measured in wavenumbers (cm$^{-1}$). Liquid samples were recorded as thin films using sodium chloride plates. Solids samples were recorded as nujol mulls or as a potassium chloride disc.

Optical rotations were measured on an Optical Activity AA-1000 polarimeter or Optical Activity PolAAr 2, AA Series, Automatic Polarimeter with a cell path length 1 dm and concentrations quoted in g/100 mL (sodium line 589 nm detection). [$\alpha$]$^\circ_{D}$ values are given in $10^{-1}$ deg cm$^{2}$ g$^{-1}$.

Elemental Analysis was performed using a Perkin-Elmer 2400 CHN Elemental Analyser.

Melting points were obtained on a Gallenkamp melting point apparatus and are given (in °C) uncorrected.

GCMS was run on a Hewlet Packard HP6890 Series GC System (HP-5 5% Phenyl Methyl Siloxane capillary column (30m x 320$\mu$m x 0.25$\mu$m) coupled to a HP 5937 Mass Selective Detector.

4.1.2 Chromatography

Analytical thin layer chromatography (tlc) was carried out on Merk aluminium backed plates coated with silica gel 60 F$_{25}$ and visualised using $p$-anisaldehyde, ammonium molybdate and potassium permanganate dips.

Flash column chromatography was carried out using the appropriately sized parallel-
sided glass column filled with silica gel 60 (Merk 9385, particle size 0.04-0.063mm) or appropriately sized Biotage Flash System, with Biotage cartridge.

4.1.3 Solvents and reagents

All solvents and reagents were used as supplied from commercial sources unless otherwise stated. Anhydrous solvents were either purchased as anhydrous grade or distilled prior to use. Dichloromethane was distilled from calcium hydride and tetrahydrofuran was distilled from sodium benzophenone ketyl. Reactions performed under anhydrous conditions were carried out in an atmosphere or nitrogen or argon using standard techniques.

Petroleum ether refers to the fraction bp 40–60°C.

Novozyme® was received as a gift from Novo-Nordisk.

n-BuLi was titrated against diphenylacetic acid before use.

Jones reagent (1.34M) was prepared by dissolving chromium trioxide (13.4g, 0.13mol) in concentrated sulfuric acid (12mL) and then diluting to 100mL with distilled water.
4.2 Experimental Procedures

4.2.1 Synthesis of putative intermediates

Cyclopentadiene monoepoxide 66

Freshly cracked cyclopentadiene (90.00g, 1.36mol, 1.00eq) and sodium carbonate (483g) were added to DCM (818mL) in a 3 neck round bottom flask. This mixture was mechanically stirred, and cooled in an ice bath. Peracetic acid (36–40 wt% solution in aqueous acetic acid, 196mL, 1.05–1.16mmol, 0.77–0.86eq) which had been pretreated with sodium acetate (12g) was then added to the mixture via dropping funnel over a period of 1h. Stirring at R.T. was continued overnight. The solids were removed by suction filtration and washed thoroughly with DCM. The solution was dried (MgSO$_4$) and the solvent removed under slight (600mbar) vacuum. The resulting residue was purified by distillation at reduced pressure, giving a clear colourless liquid 66 (12.56g 15%);

$\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3050 (C=C–H), 2909 (C–H); $\delta_H$ (200 MHz; CDCl$_3$) 6.16–6.11 (m, 1H, H–2), 6.10–5.94 (m, 1H, H–1), 3.92–3.87 (m, 1H, H–3), 3.83–3.78 (m, 1H, H–4), 2.59 (ddd, 1H, $J$=19.0, 2.2, 4.0Hz, H–5), 2.44–2.31.. (m, 1H, CH–5); $\delta_C$ (63 MHz; CDCl$_3$) 137.68 (C–2), 131.09 (C–1), 59.02 (C–3), 56.64 (C–4), 35.40 (C–5); m/z (EI) 82 (M+).
EXPERIMENTAL

cis-1,4-Diacetoxycyclopent-2-ene\textsuperscript{a} 200

\[
\begin{array}{c}
\text{AcO} \\
\text{1} \\
\text{OAc}
\end{array}
\]

A solution of palladium tetrakis (triphenylphosphine) (1.06g, 0.92mmol, 0.5mol\%) and acetic anhydride (19.40g, 17.9ml, 0.19mol, 1.04eq) in THF (150mL) was cooled in an ice bath. To this, a solution of cyclopentadiene monoepoxide 66 (15.00g, 0.18mol 1.00eq) in THF (30mL) was added dropwise over 10 minutes. Stirring was continued for a further 15 minutes, after which TLC (eluting with hexane/ethyl acetate 1:1, permanganate stain, product $R_f = 0.59$) indicated that the reaction had gone to completion.

The reaction mixture was filtered through a silica pad which was washed with ether. The solvent was removed by rotary evaporation to give a light brown oil. This was purified by flash column chromatography (eluting with hexane/ethyl acetate 85:15). Appropriate fractions were combined and the solvent removed to give an orange oil 200 (18.84g, 56%).

$\nu_{\max}$ (neat)/cm$^{-1}$ 2950 (C—H), 1737 (C=O); $\delta_{H}$ (200 MHz; CDCl$_3$) 6.05 (s, 2H, H-3 & H-2), 5.53–5.47 (m, 2H, H-1 & H-4), 2.91–2.76 (m, 1H, H-5$\beta$), 2.02 (s, 3H, CH$_3$COO), 1.75–1.64 (m, 1H, H-5$\alpha$); $\delta_{C}$ (63 MHz; CDCl$_3$) 170.50 (OC=O), 134.40 (C-2&3), 76.41 (C-1&4), 36.91 (C-5), 20.91 (CH$_3$); $m/z$ (FAB +ve) 184 (M+H).
A suspension of Novozyme SP-435 (1.33g) in phosphate buffer (400ml, pH 8, 50mM) was prepared, and to this, the diacetate 200 (7.98g, 43.6mmol, 1.00eq) was added. The suspension was stirred at R.T. for 72h. TLC (eluting with ethyl acetate/hexane 1:1, anisaldehyde stain, product $R_f = 0.23$) indicated that only a slight trace of starting material remained.

The solids were removed by filtration and washed with ethyl acetate. The aqueous layer was saturated with NaCl and NaHCO$_3$, and extracted with ethyl acetate. The organic extracts were combined, dried (MgSO$_4$) and the solvent removed in vacuo to give an orange solid. This was purified by flash column chromatography (eluting with ethyl acetate:hexane 1:1) to give a pale cream coloured crystalline solid 202 (3.53g, 58%).

$\nu_{\text{max}}$ (nujol)/cm$^{-1}$ 3333 (OH), 1723 (C=O); $\delta_{\text{H}}$ (200 MHz; CDCl$_3$) 6.09–6.05 (m, 1H, H–3), 5.95–5.91 (m, 1H, H–2), 5.48–5.42 (m, 1H, H–4), 4.70–4.65 (m, 1H, H–1), 2.84–2.70 (m, 1H, H–5), 2.33 (s, 1H, OH (disappears on shaking with D$_2$O)), 2.01 (s, 3H, CH$_3$COO), 1.60 (dt, 1H, $J$=14.5, 3.9Hz, H–5$_{\text{a}}$); $\delta_{\text{C}}$ (63 MHz; CDCl$_3$) 170.72 (OC=O), 138.37 (C–3), 132.29 (C–2), 76.94 (C–4), 74.56 (C–1), 40.28 (C–5), 21.03 (CH$_3$); [$\alpha$]$_{D}^2$ (c 1.00 CHCl$_3$) +71.2$^\circ$ (Lit 66.3$^\circ$ (c 1.53 CHCl$_3$)$^{134}$, 68.0$^\circ$ (c 1.02 CHCl$_3$)$^{135}$); $m/z$ (FAB +ve) 143; Anal. calcd for C$_7$H$_{10}$O$_3$ (142.15): C, 59.15; H, 7.04; Found: C, 58.84; H, 6.99; mpt 46–49°C (Lit 46–48.5°C$^{134}$ 49–50°C$^{135}$).
(1R, 4S)-4-(2-Methoxyethoxymethoxy)-1-acetoxycyclopentene 203

MEMO

The alcohol 202 (2.00g, 14.1mmol, 1.00eq) was dissolved in DCM (26mL) along with diisopropylethylamine (2.73g, 3.68mL, 21.1mmol, 1.50eq) and methoxyethoxymethyl chloride (2.63g, 2.39mL, 21.13mmol, 1.50eq). The mixture was stirred overnight. TLC (eluting with hexane/ethyl acetate 1:1, anisaldehyde or molybdate stain, product Rf = 0.45) indicated that the reaction had gone to completion.

Water (30mL) was added and stirring continued for 10 minutes. The organic layer was removed, and the aqueous layer extracted with DCM (3 x 30mL). The organic extracts were combined, dried (MgSO4) and the solvent removed by rotary evaporation, to give a clear yellow oil 203 (3.88g, 100%);
EXPERIMENTAL

(1S, 4R)-4-(2-Methoxyethoxymethoxy)-cyclopent-2-enol 204

MEMO

The acetate 203 (1.00 g, 4.35 mmol, 1.00 eq) was dissolved in ammonia saturated methanol (120 mL) and this solution was stirred at R.T. overnight. TLC (eluting with ethyl acetate, anisaldehyde stain, product \( R_f = 0.32 \)) indicated that the starting material had been consumed.

The solvent was removed by rotary evaporation giving a yellow oil. This oil was purified by flash column chromatography (eluting with ethyl acetate). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 204 (0.73 g, 89%);

\[ \nu_{\text{max}} \text{(neat)/cm}^{-1} = 3430 \text{ (OH)} \]
\[ \delta_{\text{H}} (250 \text{ MHz; CDCl}_3) = 6.04-5.96 \text{ (m, 2H, H-3 & H-2), 4.79 & 4.74 (d, 2H, J=11.3, CH-6), 4.62-4.52 (m, 2H, H-1 & H-4), 3.75-3.61 (m, 2H, CH}_2-7), 3.56-3.52 (m, 2H, CH}_2-8), 3.37 (s, 3H, CH}_3-9), 2.72-2.60 (m, 1H, H-5), 2.20 (s, 1H, OH), 1.61 (dt, 1H, J=14.2, 4.0Hz, H-5 \_a); \delta_{\text{C}}(63 \text{ MHz; CDCl}_3) = 137.2 \text{ (C-2), 134.0 (C-3), 94.4 (C-6), 80.09 (C-1), 74.55 (C-4), 71.49 (C-7), 66.68 (C-8), 58.80 (C-9), 41.17 (C-5); } \lbrack \alpha \rbrack_{D}^{22}(c 1.00 \text{ CHCl}_3) +8.6^\circ \text{; } m/\text{z(FAB +ve)} = 189 \text{ (M+H), HRMS(FAB +ve) } 189.11281 \text{ (calculated for C}_9\text{H}_{17}\text{O}_4 189.11268, \text{ (Dev 0.69 ppm)).} \]
(1R, 4S)-(tert-Butyldimethylsilyloxy)-4-(2-methoxyethoxymethoxy)cyclopent-2-ene 204

The alcohol 203 (1.86g, 9.90mmol, 1.00eq) was dissolved in dry DCM (27mL) and tert-butyldimethylsilyl chloride (1.57g, 10.4mmol, 1.05eq) followed by imidazole (1.01g, 14.9mmol, 1.50eq) were added. After 3 hours TLC (eluting with ethyl acetate, anisaldehyde stain, product Rf = 0.68) indicated that the reaction had gone to completion. The solids were removed by filtration and the resulting solution was concentrated by rotary evaporation to give 2 oils. The lower layer was removed using a pasteur pipette, leaving a colourless oil 204 (2.65g, 89%);

\[ \Delta \text{max (neat)/cm}^{-1} 2953-2856 (\text{CH}_3); \delta H (250 \text{ MHz; CDCl}_3) 5.93-5.84 (\text{m, 2H, H-3 & H-2}), 4.77 (s, 2H, \text{CH}_2-6), 4.67-4.63 (\text{m, 1H, H-1}), 4.61-4.46 (\text{m, 1H, H-4}), 3.76-3.63 (\text{m, 2H, CH}_2-7), 3.57-3.53 (\text{m, 2H, CH}_2-8), 3.38 (s, 3H, \text{CH}_3-9), 2.70 (dt, 1H, J=13.3, 7.2Hz, H-5), 1.56 (dt, 1H, J=13.3, 5.6Hz, H-5'), 0.87 (s, 9H, 'Bu), 0.57 (dd, 6H, J=1.5, 0.3Hz, Si(CH}_3)_2); \delta c (63 \text{ MHz; CDCl}_3) 137.25 (C-3), 134.52 (C-2), 94.69 (C-6), 80.21 & 74.66 (C-1 & 4), 71.59 (C-8), 66.64 (C-7), 58.86 (C-9), 42.03 (C-5), 25.74 (C(CH}_3)_2), 25.51 (C(CH}_3)_2), 4.73 (Si(CH}_3)_3); |\alpha|_D^{23}(c 1.00 \text{ CHCl}_3) +15.2^\circ; m/z(\text{FAB +ve}) 303 (M+H), \text{HRMS(\text{FAB +ve}) 303.19941 (calculated for C}_{15}H_{31}O_4Si, 303.19916, (Dev 0.80ppm));
EXPERIMENTAL

(1S, 2R, 3R, 5S)-3-(tert-Butyldimethylsilyloxy)-5-(2-methoxyethoxymethoxy)cyclopentane-1,2-diol 210

Alkene 205 (1.00g, 3.30mmol, 1.00eq) was dissolved in THF (5mL), acetone (2mL), and water (0.75mL). Osmium tetroxide (4% wt(aq) soln 1.68mg, 40µl, 6.6µmol, 0.2mol%) was added, followed by N-methylmorpholine N-oxide (0.85g, 6.30mmol, 1.90eq). The mixture was stirred at R.T. for 72h. TLC (eluting with ethyl acetate, molybdate stain, product R<sub>f</sub> = 0.4) indicated that the starting material had been consumed. The solvents were removed by rotary evaporation. Saturated sodium bisulfite solution (10mL) was added, and this aqueous layer was extracted with ethyl acetate (3 x 10mL). The combined organic extracts were dried (MgSO<sub>4</sub>) and the solvent removed by rotary evaporation to give a yellow oil 210 (0.89g, 80%);

\[ \nu_{\text{max}} \text{(neat)/cm}^{-1} \text{ 3425 (OH), 2949–2822 (C–H)}; \delta_{\text{H}} \text{(250 MHz; CDCl}_3\text{) 4.77 (d, 1H, J=7.1Hz, H–1), 4.70 (d, 1H, J=7.1, H–2), 4.08–4.03 (m, 8H, H–3, H–5, CH}_2\text{–6, CH}_2\text{–7 & CH}_2\text{–8), 3.39 (s, 3H, CH}_3\text{–9), 2.57–2.45 (m, 1H, H–4), 1.57–1.50 (m, 1H, H–4'), 0.86 (s, 9H, 'Bu), 0.08–0.03 (m, 6H, Si(CH}_3\text{)_2); \delta_{\text{C}}(63 \text{ MHz; CDCl}_3\text{) 95.74 (C–6), 84.59 (C–2), 78.04 (C–1), 76.15 (C–5), 74.31 (C–3), 71.40 (C–7), 66.65 (C–8), 58.81 (C–9), 37.77 (C–4), 25.62 (C(CH}_3\text{)_2), 17.85 (C(CH}_3\text{)_2), -5.01 (Si(CH}_3\text{)_2); [\alpha]^{25}_D(c 1.00 \text{ CHCl}_3) +35.8°; m/z(FAB +ve) 337 (M+H), HRMS(FAB +ve) 337.20421 (calculated C\text{_{15}H\text{_{33}O\text{_{6}Si}}, 337.20464, (Dev 1.27ppm))}; \]

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(1R, 2S, 3R, 4S)-(tert-Butyldimethylsilyloxy)-4-(2-methoxyethoxyethoxy)-
2,3-(isopropylidenedioxy)cyclopentane 211

MEMO

The diol 210 (0.80g, 2.56mmol, 1.00eq) and p-toluene sulfonic acid (40mg, 0.23mmol, 9.1mol%) were dissolved in acetone (50mL) and the solution stirred at R.T. overnight. TLC (eluting with ethyl acetate, molydate stain, product R_f = 0.80) showed that the reaction had gone to completion. Calcium hydroxide was added until the solution was basic, and stirring was continued for 10 minutes. The solids were removed by filtration and the resulting solution was concentrated by rotary evaporation, to give a clear colourless oil 211 (0.86g, 96%);

ν_max (neat)/cm⁻¹ 2929–2817 (CH); δ_H (250 MHz; CDCl₃) 4.75 (d, 1H, J=7.0Hz, H-3), 4.71 (d, 1H, J=7.0Hz, H-2), 4.61–4.57 (m, 1H, H-4), 4.43–4.40 (m, 1H, H-1), 4.14–4.05 (m, 2H, CH₂-6), 3.71–3.66 (m, 2H, CH₂-7), 3.56–3.52 (m, 2H, CH₂-8), 3.37 (s, 3H, CH₃-9), 2.22–2.12 (m, 1H, H-5), 1.83–1.75 (m, 1H, H-5), 1.40 & 1.26 (s, 6H, C(CH₃)₂), 0.86 (s, 9H, 'Bu), 0.06 & 0.04 (s, 6H, Si(CH₃)₂); δ_C (63 MHz; CDCl₃) 110.76 (C(CH₃)₂), 93.85 (C-6), 86.83 (C-4), 84.92 (C-1), 80.88 (C-3), 77.41 (C-2), 71.56 (C-7), 66.78 (C-8), 58.86 (C-9), 37.39 (C-5), 26.55 & 24.23 (C(CH₃)₂), 25.63 (C(CH₃)₂), 17.90 (C(CH₃)₂), -4.94 & -4.98 (Si(CH₃)₂); [α]_D^2 = [c 1.00 CHCl₃] +12.4°; m/z(FAB +ve) 377 (M+H), HRMS(FAB +ve) 377.23600 (calculated for C₁₈H₃₇O₆Si, 377.23594, (Dev 0.15ppm));
EXPERIMENTAL

(1S, 2R, 3S, 4R)-4-(tert-Butyldimethylsilyloxy)-2,3-(isopropylidenedioxy)cyclopentanol 212

The MEM species 211 (2.0 g, 5.30 mmol, 1.00 eq) was dissolved in toluene (40 mL) and finely powdered zinc bromide (7.0 g, 6.00 eq) was added. Stirring was continued for 16 hours after which time TLC (eluting with hexane/ethyl acetate 5:1, molybdate stain, product Rf = 0.39) indicated that some starting material remained. The solvent was decanted and fresh ZnBr2 (4.0 g) added. After stirring for three days, TLC indicated that the starting material had been entirely consumed.

The organic layer was washed with water (40 mL), saturated sodium bicarbonate (40 mL) and saturated brine (40 mL). The combined aqueous layers were extracted with ether (3 x 40 mL). The combined organic extracts were dried (MgSO4), and the solvent removed by rotary evaporation, to give an orange oil. This was purified by flash column chromatography (eluting with petroleum ether:ethyl acetate 19:1). Appropriate fractions were combined and the solvent removed to give a clear colourless oil 212 (0.60 g, 39%);

νmax (neat)/cm−1 3526 (OH), 2950–2858 (C–H);

1H (250 MHz; CDCl3) 4.63 (d, 1H, J=5.4 Hz, H–3), 4.48 (d, 1H, J=5.4 Hz, H–2), 4.23 (d, 1H, J=3.5 Hz, H–4), 4.06 (d, 1H, J=5.4 Hz H–1), 3.10 (d, 1H, J=10.5 Hz, OH), 2.06 (ddd, 1H, J=14.3, 4.6, 3.5 Hz, H–5), 1.77–1.70 (m, 1H, H–5), 1.36 (d, 3H, J=0.5 Hz, C(CH3)), 1.26 (d, 3H, J=0.5 Hz, C(CH3)), 0.86 (s, 9H, ‘Bu), 0.11–0.04 (m, 6H, Si(CH3)2);

13C (63 MHz; CDCl3) 109.91 (C(CH3)2) 86.28 (C–3), 85.67 (C–2), 78.26 (C–4), 77.41 (C–1), 37.17 (C–5), 25.98 & 23.66 (C(CH3)2), 25.56 (C(CH3)2), 17.75 (C(CH3)2), -5.11 & -5.23 (Si(CH3)2); [α]22D (c 1.00 CHCl3) +4.2°; m/z (FAB +ve) 289, HRMS (FAB +ve) 289.18352 (calculated for C14H25O4Si, 289.18351, (Dev 0.02 ppm));
EXPERIMENTAL

(2S, 3S, 4R)-4-(tert-Butyldimethylsilyloxy)-2,3-(isopropyldenedioxy)cyclopentanone 213

The alcohol 212 (61.7 mg, 0.21 mmol, 1.00 eq.), was dissolved in acetone (2.5 mL) and the solution cooled to 0°C (ice bath). Jones reagent (0.12 mL, 0.17 mmol, 0.77 eq.) was added dropwise, and stirring at 0°C was continued for around 15 minutes. The ice bath was removed and stirring at R.T. was continued for 3 hours. TLC (hexane/ethyl acetate 5:1 molybdate stain, product Rf = 0.49) indicated that the starting material had been entirely consumed.

Sodium bicarbonate (0.1 g) was added and stirring continued for around 10 minutes. The mixture was filtered and the solvent removed by rotary evaporation. The resulting residue was purified by flash column chromatography (eluting with hexane/ethyl acetate 4:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear oil 213 (18.7 mg, 30%);

νmax (neat)/cm⁻¹: 2986–2858 (C–H), 1764 (C=O); δH (250 MHz; CDCl3) 4.54–4.51 (m, 1H, H–2), 4.41–4.38 (m, 1H, H–3), 4.30–4.27 (m, 1H, H–4), 2.86–2.76 (m, 1H, H–5α), 2.20–2.11 (m, 1H, H–5β), 1.41 & 1.31 (d, 6H, J=0.5 Hz, C(CH₃)₂), 0.86 (s, 9H, tBu), 0.12–0.02 (m, 6H, Si(CH₃)₂); δC (63 MHz; CDCl3) 212.09 (C=O), 112.6 (C(CH₃)₂), 82.65 (C–2), 77.92 (C–3), 69.09 (C–4), 43.07 (C–5), 26.65 & 24.71 (C(CH₃)₂), 25.50 (C(CH₃)₃), 17.84 (C(CH₃)₃), -5.02 (Si(CH₃)₂); [α]D²⁰ (c 1.00 CHCl₃) +100.9°;
EXPERIMENTAL

(1S, 2S, 3S, 4R)-4-(tert-Butyldimethylsilyloxy)-1-(p-methoxybenzyloxymethyl)-2,3-(isopropylidenedioxy)cyclopentanol 214

Bu$_3$SnCH$_2$OPMB (0.51g, 1.16mmol, 1.05eq) was dissolved in THF (11mL) and the solution cooled to -78°C (dry ice/acetone). BuLi (1.6M soln in hexanes, 0.69mL, 1.16mmol,1.05eq) was added over 2 minutes and stirring at -78°C continued for 5 minutes. The ketone 213 (0.32g, 1.10mmol, 1.00eq) in THF (6mL) was then transferred to the reaction mixture via canula. Stirring was continued till TLC (eluting with petroleum ether/ethyl acetate, molybdate stain, product R$_f$ = 0.18) suggested that the reaction had proceeded as far as possible (30 minutes).

The reaction was quenched by addition of saturated ammonium chloride solution (5mL). Once the resulting mixture had warmed to room temperature the aqueous layer was extracted with ethyl acetate (80mL, then 50mL). The combined organic extracts were washed with water (100mL), brine (85mL) and dried (MgSO$_4$). The solvent was removed by rotary evaporation to give a brown oil. This was purified by column chromatography (Biotage flash system petroleum ether/ethyl acetate 99:1 up to 9:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 214 (70mg, 16%);

$\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 3513 (OH), 2933–2858 (C=H); $\delta_{\text{H}}$ (250 MHz; CDCl$_3$) 7.25 (dt, 2H, J=8.8, 2.5Hz, Ar), 6.85 (dt, 2H, J=8.8, 2.5Hz, Ar), 4.58 (d, 1H, J=11.9Hz, H–7), 4.46 (d, 1H, J=6.2Hz, H–3), 4.43 (d, 1H, J=11.9Hz, H–7), 4.32 (d, 1H, J=6.2Hz, H–2), 4.14–4.08 (m, 1H, H–4), 3.79 (s, 3H, OCH$_3$), 3.50 (d, 1H, J=9.9Hz, H–6), 3.41 (d, 1H, J=9.9Hz, H–6), 3.00 (s, 1H, OH), 2.04 (dd, J=13.9, 5.6Hz, H–5$_e$), 1.74 (dd, 1H, J=13.9, 3.5Hz, H–5$_p$), 1.48 & 1.32 (s, 6H, C(CH$_3$)$_3$), 0.8 (s, 9H, 'Bu), 0.03 & 0.01 (s, 6H, Si(CH$_3$)$_2$);
(1R, 4S)-4-(tert-Butyldimethylsilyloxy)-cyclopent-2-enyl acetate 215

The alcohol 202 (5.00g, 35.0mmol, 1.00eq) was dissolved in DCM (100mL). Imidazole (3.60g, 53.0mmol, 1.50eq) and tert-butyldimethylsilyl chloride (5.54g, 37.0mmol, 1.05eq) were added and stirring continued for 3 hours. TLC (petroleum ether/ethyl acetate 2:1, molybdate stain, product Rf = 0.79) indicated that the reaction had gone to completion.

The solids were removed by filtration and the filtrate was concentrated by rotary evaporation to give a yellow oil and a lower brown oil layer. The lower layer was removed using a pasteur pipette and the resulting yellow oil could be used without purification. Purification was possible by column chromatography (Biotage flash system, eluting with petroleum ether/ethyl acetate 19:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 215 (88%).

$\nu_{\text{max}}$ (neat)/cm$^{-1}$: 2954–2858 (C–H), 1739 (C=O), 1472 (C=C); $\delta_H$ (250 MHz; CDCl$_3$) 6.00 (ddd, 1H, $J$=5.6, 1.7, 1.5Hz H–2), 5.90 (ddd, 1H, $J$=5.6, 1.7, 1.4Hz, H–3), 5.50–5.44 (m, 1H, H–1), 4.76–4.70 (m, 1H, H–4), 2.82 (dt, 1H, $J$=13.8, 6.9Hz, H–5$\beta$), 2.06 (s, 3H, C=OCH$_3$), 1.62 (dt, 1H, $J$=13.8, 5.1Hz, H–5$\alpha$), 0.91 (s, 9H, 'Bu), 0.10 & 0.09 (s, 6H, Si(CH$_3$)$_2$); $\delta_C$ (63 MHz; CDCl$_3$) 171.27 (C=O), 139.29 (C–2), 131.56 (C–3), 77.33, (C–1), 75.26, (C–4), 41.56 (C–5), 26.26 (C(CH$_3$)$_3$), 21.57 (C=OCH$_3$), 18.56 (C(CH$_3$)$_3$), -4.24 & -4.28 (Si(CH$_3$)$_2$); $[\alpha]^2_D$ (c 1.00 CHCl$_3$) +3.1°; m/z(FAB +ve) 257 (M+H); HRMS(FAB +ve) 257.15731 (calculated for C$_{13}$H$_{25}$O$_3$Si, 257.15730, (Dev 0.03ppm)).
(1R, 4S)-4-(tert-Butyldimethylsilyloxy)cyclopent-2-enol 216

The acetate 215 (8.96g, 35.0mmol, 1.00eq) was dissolved in ammonia saturated methanol (300mL), and the solution was stirred at R.T. overnight. TLC (petroleum ether/ethyl acetate 2:1, molybdate stain, product Rf = 0.15) indicated that the reaction had gone to completion.

The solvent was removed by rotary evaporation to give a brown oil which could be used without purification 216 (8.52g crude).

\( \nu_{\text{max}} \) (neat)/cm\(^{-1} \) 3346 (OH), 2955–2857 (C–H), 1472 (C=C); \( \delta_{\text{H}} \) (250 MHz; CDCl\(_3\)) 5.92 (ddd, 1H, \( J=5.6, 1.9, 1.2 \)Hz, H–3), 5.85 (ddd, 1H, \( J=5.6, 1.9, 1.2 \)Hz, H–2), 4.66–4.60 (m, 1H, H–1), 4.59–4.53 (m, 1H, H–4), 2.65 (dt, 1H, \( J=14.0, 6.9 \)Hz, H–5\( \_p \)), 2.64 (s, 1H, OH), 1.49 (dt, 1H, \( J=14.0, 4.5 \)Hz, H–5\( \_o \)), 0.87 (s, 9H, 'Bu), 0.63 (s, 6H, Si(CH\(_3\))\(_2\)); \( \delta_{\text{C}} \) (63 MHz; CDCl\(_3\)) 137.13 (C–3), 136.11 (C–2), 75.59 (C–4), 75.44 (C–1), 44.95 (C–5), 26.29 (C(CH\(_3\))\(_2\)), 18.58 (C(CH\(_3\))\(_2\)), -4.26 (Si(CH\(_3\))\(_2\)); \([\alpha]^{20}_{\text{D}}\)(c 1.00 CHCl\(_3\)) -19.7°; m/z(FAB +ve) 215 (M+H), 197 (M-OH).
The alcohol 216 (7.49g, 35.0mmol, 1.00eq) was dissolved in DCM (150mL). Imidazole (3.57g, 53.0mmol, 1.50eq) and tert-butyldiphenylsilyl chloride (10.1g, 9.56ml, 37.0mmol, 1.05eq) were added and stirring continued overnight. TLC (petroleum ether/ethyl acetate 4:1, molybdate stain, product Rp = 0.81) indicated that the reaction had gone to completion.

The solids were removed by filtration and the filtrate was concentrated by rotary evaporation to give two oils. The lower layer was removed using a pasteur pipette leaving the crude product (18.36g). This product could be used in crude form or purified by column chromatography (Biotage flash system, eluting with petroleum ether/ethyl acetate 99:1). Appropriately combined fractions were concentrated by rotary evaporation to give a clear colourless oil 217 (60%).

\[ \nu_{\text{max}} \text{ (neat)/cm}^{-1} 2955-2857 \text{ (C–H)}, 1472 \text{ (C=C)}; \delta_{\text{H}} \text{ (250 MHz; CDCl}_3) 7.74-7.66 \text{ (m, 4H, Ar), 7.47-7.36 \text{ (m, 6H, Ar), 5.77 \text{ (s, 2H, H–2 & 3), 4.56 \text{ (dd, 1H, J=6.1, 7.0Hz, H–4), 4.54 \text{ (dd, 1H, J=6.1, 7.0Hz, H–1), 2.54 \text{ (dt, 1H, J=12.9, 7.0Hz, H–5)})}}, 1.70 \text{ (dt, 1H, J=12.9, 6.1Hz, H–5), 1.08 \text{ (s, 9H, ‘Bu), 0.9 \text{ (s, 9H, ‘Bu), 0.09 & 0.08 \text{ (s, 6H, Si(CH}_3}_2)}, \delta_{\text{C}} \text{ (63 MHz; CDCl}_3) 135.67 & 135.65 \text{ (C–2&3), 134.16 & 134.07 (CH Ar), 129.46 \text{ (Ar C), 127.46 (Ar C), 75.64 & 74.72 (C–1&4), 44.90 (C–5), 26.76 (SiMe}_2C(CH}_3}_2)}, 18.98 \text{ (SiMe}_2C(CH}_3}_2), 10.09 \text{ (SiPh}_2C(CH}_3)_2), -4.64 \text{ (SiCH}_3)_2, -4.73 \text{ (SiCH}_3); [\alpha]^{22}_D \text{ (c 1.00 CHCl}_3) -20.2^\circ; m/z(\text{FAB +ve}) 197 (M-OTBDPS).} \]
EXPERIMENTAL

(1S, 4R)-4-((tert-Butyldiphenylsilyloxy)cyclopent-2-enol 218

\[
\begin{align*}
&\text{HO} \\
&\text{OTBDPS}
\end{align*}
\]

The silyl ether 217 (~18.36g, ~35.0mmol - crude) was dissolved in acetic acid (150mL), THF (50mL) and water (50mL). After stirring for 3 days at R.T., TLC (eluting with petroleum ether/ethyl acetate 9:1, permanganate stain, product Rf = 0.28) showed that the reaction had gone to completion.

Water (150mL) was added and the resulting mixture extracted with ethyl acetate (3 × 150mL). The combined organic layers were washed with brine (~100mL), dried (MgSO\(_4\)), and the solvent removed by rotary evaporation to give a brown oil. This oil was purified by column chromatography (biotage flash system (in 3 portions) eluting with petroleum ether/ethyl acetate 9:1). Appropriate fractions were combined and solvent removed by rotary evaporation, to give a white solid 218 (6.64g, 56% from acetate (4 steps).

\[\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}: 3332 (\text{OH}), 1589 (\text{C=C}); \delta_{\text{H}} (250 \text{ MHz; CDCl}_3) 7.73-7.69 (m, 4H, Ar), 7.47-7.41 (m, 6H, Ar), 5.89 (dt, 1H, J=5.4, 1.4Hz, H-2), 5.83 (dt, 1H, J=5.4, 1.5Hz, H-3), 4.67-4.62 (m, 1H, H-1), 4.69-4.64 (m, 1H, H-4), 2.55 (dt, 1H, J=13.7, 7.0Hz, H-5), 1.94 (s, 1H, OH), 1.64 (dt, 1H, J=13.7, 4.6Hz, H-5), 1.97 (s, 9H, 'Bu); \delta_{\text{C}} (63 \text{ MHz; CDCl}_3) 136.69 (C-2), 135.60 (CH Ar), 135.45 (C-3), 133.91 (C Ar), 133.76 (C Ar), 129.57 (CH Ar), 127.52 (CH Ar), 75.83 (C-1), 74.85 (C-4), 44.35 (C-5), 26.75 (SiPh\(_2\)C(CH\(_3\))\(_2\)), 18.91 (SiPh\(_2\)C(CH\(_3\))\(_2\)); [\alpha]^{20}_{D} (c 1.00 \text{ CHCl}_3) +4.7^\circ ; m/z(\text{FAB +ve}) 339 (M+H), 321 (M- OH);\]
EXPERIMENTAL

(1S, 2R, 3S, 4R)-4-(tert-Butyldiphenylsilyloxy)cyclopentane-1,2,3-triol 219

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

The alkene 218 (2.00g, 5.90mmol, 1.00eq) was dissolved in THF (10mL), acetone (4mL) and water (1.4mL). N-methylmorpholine N-oxide (1.50g, 11.1mmol, 1.90eq) was added and the mixture stirred until all the NMO had dissolved. Osmium tetroxide (4% wt(aq) soln, 2.8mg, 70μL, 11μmol, 0.2mol%) was added and the solution stirred vigorously overnight. TLC (eluting with ethyl acetate, molybdate stain, product \( R_f = 0.47 \)) indicated that the reaction had gone to completion.

The solvents were removed by rotary evaporation. Saturated sodium bisulfite solution (20mL) was added and the resulting solution was extracted with ethyl acetate (4 × 20mL). The combined organic extracts were dried (MgSO₄) and the solvent removed by rotary evaporation. The resulting residue was purified by flash column chromatography (biotage flash system, eluting with petroleum ether/ethyl acetate 3:2). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 219 (1.84g, 84%).

\[ v_{\text{max}} \text{ (neat)/cm}^{-1} \ 3375 \text{ (OH)}, \ 2931-2858 \text{ (C-H)}; \delta_{\text{H}} \text{ (250 MHz; CDCl}_3) \ 7.78-7.65 \text{ (m, 4H, Ar), 7.46-7.40 \text{ (m, 6H, Ar), 4.08-3.88 \text{ (m, 4H, H-2,3,1&4), 3.66 \text{ (s, 1H, OH), 3.26 \text{ (s, 1H, OH), 2.67 \text{ (s, 1H, OH), 2.24 \text{ (dt, 1H, J=13.8, 7.0Hz, H-5)}, 1.56 \text{ (dt, 1H, J=13.8, 6.0Hz, H-5)}, 1.10 \text{ (s, 9H, 'Bu); \delta_{\text{C}}\text{ (63 MHz; CDCl}_3) \ 136.16 \text{ (CH Ar), 136.14 \text{ (CH Ar), 134.04 \text{ (C Ar), 133.95 \text{ (C Ar), 130.34 \text{ (CH Ar), 130.30 \text{ (CH Ar), 128.23 \text{ (CH Ar), 128.18 \text{ (CH Ar), 78.48 \text{ (C-2), 78.19 \text{ (C-3), 77.47 \text{ (C-1), 76.23 \text{ (C-4), 39.19 \text{ (C-5), 27.34 \text{ (SiPh}_2\text{C(CH}_3)_3), 19.47 \text{ (SiPh}_2\text{C(CH}_3)_3); [\alpha]_D^{20} \text{(c 1.00 CHCl}_3) -6.0^\circ; m/z\text{(FAB +ve) 373 (M+H), HRMS(FAB +ve) 373.18386 (calculated C}_{21}\text{H}_{29}\text{O}_4\text{Si, 373.18351, (Dev 0.94ppm)).}}}}}}}\]
EXPERIMENTAL

(1S, 2R, 3S, 4R)-4-(tert-Butyldiphenylsilyloxy)-2,3-(isopropylidenedioxy)cyclopentanol 220

The triol 219 (1.84g, 4.95mmol, 1.00eq) was dissolved in DCM (100mL) and dimethoxy propane (1.10g, 1.30mL, 10.6mmol, 2.10eq) was added. With stirring, a microspatula full of pTSA (a few mg’s) was added. After stirring for 5 minutes, TLC (eluting with ethyl acetate, molybdate stain, product Rf = 0.83) showed that the reaction had gone to completion.
Calcium hydroxide was added and stirring continued for approximately 10 minutes. The solids were removed by filtration and the filtrate concentrated by rotary evaporation to give a clear colourless oil 220 (1.84g, yield 90%).

$\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3532 (OH), 2933–2859 (C–H); $\delta_{\text{H}}$ (250 MHz; CDCl$_3$) 7.68–7.61 (m, 4H, Ar), 7.47–7.36 (m, 6H, Ar), 4.70 (dd, 1H, $J$=5.5, 1.7Hz, H–2), 4.58 (dd, 1H, $J$=5.5, 1.7Hz, H–3), 4.31–4.29 (m, 1H, H–4), 4.13–4.06 (m, 1H, H–1), 3.30 (d, 1H, $J$=11.2, OH), 1.98 (dt, 1H, $J$=14.5, 4.0Hz, H–5$^p$), 1.75 (d, 1H, $J$=14.5Hz, H–5$^o$), 1.30 &1.22 (s, 6H, C(CH)$_3$)$_3$, 1.07 (s, 9H, 'Bu); $\delta_{\text{C}}$(63 MHz; CDCl$_3$) 136.18 (CH Ar), 136.11 (CH Ar), 133.01 (C Ar), 132.91 (C Ar), 130.56 (CH Ar), 128.36 (CH Ar), 110.44 (C(CH$_2$)$_2$), 86.88 (C–2), 86.01 (C–3), 79.90 (C–1), 78.09 (C–4), 37.65 (C–5), 27.38 (SiPh$_2$C(CH$_3$)$_3$), 26.50 (C(CH$_3$)), 24.20 (C(CH$_3$)), 19.39 (SiPh$_2$C(CH$_3$)$_3$); [$\alpha$]$^D$$_\text{D}$ (c 1.00 CHCl$_3$) +13$^\circ$; $m/z$ (FAB +ve) 413 (M+H), HRMS(FAB +ve) 413.21482 (calculated for C$_{24}$H$_{33}$O$_4$Si, 413.21481, (Dev 0.00ppm)).
(2S, 3S, 4R)-4-(tert-Butyldiphenylsilyloxy)-2,3-(isopropylidenedioxy)cyclopentanone 221

The alcohol 220 (1.96g, 4.70mmol, 1.00eq) was dissolved in acetone (60mL) and the resulting solution was cooled in an ice bath. Jones reagent (2.76mL, 3.70mmol, 0.79eq) was added dropwise and stirring at 0°C was continued for 15 minutes. The ice bath was removed, and stirring continued for 3 hours. TLC (eluting with petroleum ether/ethyl acetate 19:1, molybdate stain, product Rf = 0.24) revealed that the reaction had gone to completion.

Sodium bicarbonate (2.0g) was added and stirring continued for a further 10 minutes. The solids were removed by filtration and the filtrate was concentrated by rotary evaporation. The resulting oil was purified by flash column chromatography (biotage flash system, eluting with petroleum ether/ethyl acetate 19:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give an oil, which upon standing became a white solid 221 (1.57g, 80%).

$\nu_{max}$ (KBr)/cm$^{-1}$ 3025-2857 (C–H), 1755 (C=O); $\delta_{H}$ (360 MHz; CDCl$_3$) 7.64–7.56 (m, 4H, Ar), 7.46–7.34 (m, 6H, Ar), 4.66 (dd, 1H, J=5.1, 1.2Hz, H–3), 4.49 (d, 1H, J=5.1Hz, H–2), 4.42 (dd, 1H, J=5.0Hz, H–4), 2.90 (dd, 1H, J=17.8, 5.0Hz, H–5), 2.20 (d, 1H, J=17.8Hz, H–5$^a$), 1.39 & 1.34 (s, 6H, C(CH$_3$)$_2$), 1.09 (s, 9H, 'Bu); $\delta_C$(63 MHz; CDCl$_3$) 212.55 (C=O), 136.06 (CH Ar), 136.03 (CH Ar), 133.29 (C Ar), 130.54 (CH Ar), 128.36 (CH Ar), 128.34 (CH Ar), 113.23 (C(CH$_3$)$_2$), 82.81 (C–2), 78.53 (CH–3), 70.55 (C–4), 43.38 (C–5), 27.24 (SiPh$_2$C(CH$_3$)$_2$), 27.24 (C(CH$_3$)$_2$), 25.22 (C(CH$_3$)$_2$), 19.50 (SiPh$_2$C(CH$_3$)$_3$); $[\alpha]_D^2$ (c 1.00 CHCl$_3$) +112°; m/z(FAB +ve) 411 (M+H), HRMS(FAB +ve) 411.19923(calculated for C$_{24}$H$_{31}$O$_4$Si, 411.19916, (Dev 0.17ppm)); mpt 87.0–88.5°C.
EXPERIMENTAL

(1S, 2S, 3S, 4R)-1-\((p\text{-Methoxybenzyloxymethyl})\)-4-\((\text{tert-butyldiphenylsilyloxy})\)-2,3-(isopropylidenedioxy)cyclopentanol 222

Bu₂SnCH₂OPMB (2.20g, 5.00mmol, 1.20eq) was dissolved in THF (100mL) and the solution cooled to -78°C (dry ice/acetone). BuLi (2.5M soln in hexanes, 2.11mL, 5.00mmol, 1.20eq) was added and stirring at -78°C continued for 5 minutes. A solution of the ketone 221 (1.68g, 4.10mmol, 1.00eq) in THF (40mL) was added slowly via canula and stirring at -78°C continued for 15 minutes. TLC (eluting with petroleum ether/ethyl acetate 9:1, molybdate stain, product R_f = 0.10) indicated that negligible starting material remained.

The reaction was quenched by addition of ammonium chloride (100mL). The mixture was allowed to warm to room temperature and was extracted with ethyl acetate (3 x 100mL). The combined organic extracts were washed with brine (100mL) and dried (MgSO₄). The solvent was removed by rotary evaporation to give a pale yellow oil. This was purified by flash column chromatography (Biotage flash system eluting with petroleum ether/ethyl acetate 19:1, up to 9:1). Appropriate fractions were combined and the solvent removed by rotary evaporation, to give a clear colourless oil 222 (1.76g, 76%).

\( \nu_{\text{max}} \) (neat)/cm⁻¹ 3519 (OH), 2932–2858 (C–H); \( \delta_\nu \) (250 MHz; CDCl₃) 7.64–7.57 (m, 4H, Si–Ar), 7.43–7.32, (m, 6H, Si–Ar), 7.27 (d, 2H, J=8.4Hz, Ar(PMB)), 6.87 (d, 2H, J=8.4Hz, Ar(PMB)), 4.61 (d, 1H, J=11.8Hz, H–2), 4.49, (s, 2H, CH₂–7), 4.47 (d, 1H, J=11.8Hz, H–3), 4.22–4.21 (m, 1H, H–4), 3.79 (s, 3H, OMe), 3.61 & 3.49 (d, 2H, J=8.4Hz, CH–6), 2.96 (s, 1H, OH), 1.95 (dd, 1H, J=14.1, 5.5Hz, H–5), 1.84 (dd, 1H, J=14.1, 3.4Hz, H–5'), 1.41 & 1.28 (s, 6H, C(CH₃)₂), 1.03 (s, 9H, 'Bu); \( \delta_\nu \) (63 MHz; CDCl₃) 159.61 (C Ar(PMB)), 136.17 (CH Ar–Si), 133.86, (C Ar–Si), 133.75 (C Ar–Si), 130.72 (C Ar(PMB)), 130.23 (CH Ar–Si), 130.19 (CH Ar–Si), 129.76 (CH Ar(PMB)), 128.11 (CH Ar–Si), 114.21 (CH Ar(PMB)), 112.49 (C(CH₃)₂), 87.49 (C–2), 80.63 (C–3), 79.09 (C–1), 75.69 (C–4), 74.86 (C–7), 73.68 (C–6), 55.68 (OMe), 42.91 (C–5), 27.35 (SiPh₂C(CH₃)₂), 26.66 (C(CH₃)₂), 24.98 (C(CH₃)₂), 19.49 (SiPh₂C(CH₃)₂); [α]²² D (c 1.00 CHCl₃) +1.0°; m/z (FAB +ve) 585 (M+Na), HRMS (FAB +ve) 585.26470(calculated for C₃₅H₄₂O₆SiNa, 585.26487, (Dev-0.28ppm)).
EXPERIMENTAL

(1S, 2S, 3S, 4R)-1-(p-Methoxybenzylxoyxmethyl)-4-hydroxy-2,3-(isopropylidenedioxy)cyclopentanol 223

The silyl ether 222 (2.50g, 4.45mmol, 1.00eq), was dissolved in THF (55mL), and the solution cooled to 0°C (ice bath). To this, TBAF (1.0M soin in THF, 4.35mL, 4.35mmol, 1.00eq) was added. Stirring was continued for around 6 hours and TLC (eluting with petroleum ether/ethyl acetate 1:1, molybdate stain, product Rf = 0.16), showed that the amount of starting material remaining was negligible.

Water (50mL) was added and the resulting mixture extracted with ethyl acetate (3 x 50mL), adding sodium chloride as required to aid separation. The combined organic extracts were dried (MgSO4) and the solvent removed by rotary evaporation to give a dark brown oil. This oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 1:4 then 3:7). Appropriate fractions were combined and the solvent removed by rotary evaporation to give starting material (0.31 g) and the product as a pale yellow oil 223 (1.19g, 94%) which could be crystallised from ethyl acetate and petroleum ether;

\[
\begin{align*}
\text{v}_{\text{max}} \text{(KBr)/cm}^{-1} & \quad 3538 \text{ (OH)}, 3423 \text{ (OH)}, 2971–2841, \text{ (C–H)}; \\
\delta_{\text{H}} \text{(250 MHz; CDCl}_3) & \quad 7.09 \text{ (d, 2H, J=8.7Hz, Ar)}, 6.72 \text{ (d, 2H, J=8.7Hz, Ar)}, 4.37 \text{ (s, 2H, CH}_2-7), 4.30 \text{ (d, 1H, J=5.8Hz, H–2)}, 4.21 \text{ (d, 1H, J=5.8Hz, H–3)}, 3.90 \text{ (d, 1H, J=6.3Hz, H–4)}, 3.65 \text{ (s, 3H, OMe)}, 3.38 & 3.30 \text{ (d, 2H, J=8.8Hz, CH–6)}, 2.06 \text{ (dd, 1H, J=14.8, 6.3Hz, H–5)}, 1.80 \text{ (d, 1H, J=14.8Hz H–5)}, 1.33 & 1.18 \text{ (s, 6H, C(CH}_3)_2); \\
\delta_{\text{C}} \text{(63 MHz; CDCl}_3) & \quad 160.02 \text{ (C Ar)}, 130.24 \text{ (CH Ar)}, 129.16 \text{ (C Ar)}, 114.42 \text{ (CH Ar)}, 112.26 \text{ (C(CH}_3)_2), 88.16 \text{ (C–2)}, 82.16 \text{ (C–3)}, 78.48 \text{ (C–1)}, 75.60 \text{ (C–7)}, 74.04 \text{ (C–6)}, 73.62 \text{ (C–4)}, 55.69 \text{ (OMe)}, 44.95 \text{ (C–5)}, 26.70 \text{ (C(CH}_3)_2), 24.85 \text{ (C(CH}_3)_2); [\alpha]_{D}^{25} \text{(c 1.00 CHCl}_3) +25.7^\circ; m/z \text{(EI+) 324 (M+)}; \text{Anal. Calcd for C}_{15}H_{26}O_6 (324.37): C, 62.90; H, 7.34, Found C, 62.76; H, 7.20; mpt 67.0–68.0°C.}
\end{align*}
\]
EXPERIMENTAL

(1R, 2S, 4S)-4-(p-Methoxybenzyl)oxymethy-4-hydroxy-2,3-(isopropylidenedioxy)cyclopentanone \(^{18}\) 224

IBX (1.80 g, 6.45 mmol, 2.60 eq), was stirred in DMSO (15 mL), until it had completely dissolved (25 minutes). The alcohol 223 (0.80 g, 2.47 mmol, 1.00 eq) was dissolved in the minimum THF and the resulting solution transferred to the DMSO. Stirring was continued for 5 hours and TLC (eluting with petroleum ether/ethyl acetate 1:1, molybdate stain, product R<sub>f</sub> = 0.3) showed that the reaction had gone to completion. Water (25 mL) was added and stirring continued for 5 minutes. The resulting white precipitate was filtered off and washed with ether. The filtrate was extracted with ether (4 x 50 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub> ) and the solvent removed by rotary evaporation to give a pale yellow/brown oil. This oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 65:35). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 224 (0.73 g, 92%).

\[ \nu_{\text{max}} \text{(neat)/cm}^{-1} \] 3490 (OH), 2988–2839 (C–H), 1759 (C=O); \( \delta \_4 \) (250 MHz; CDCl<sub>3</sub>) 7.17 (d, 2H, \( J=8.8 \text{Hz, Ar} \)), 6.90 (d, 2H, \( J=8.8 \text{Hz, Ar} \)), 4.55 (d, 1H, \( J=5.7 \text{Hz, H–2} \)) 4.44 (s, 2H, CH<sub>2</sub>–7), 4.37 (d, 1H, \( J=5.7 \text{Hz, H–3} \)), 3.82 (s, 3H, OMe), 3.53 & 3.30 (d, 2H, \( J=8.5, 1 \text{H, } \)), 2.68 (s, 1H, OH), 2.72 (d, 1H, \( J=17.9 \text{Hz, H–5} \)), 2.50 (d, 1H, \( J=17.9 \text{Hz, H–5} \)), 1.50 & 1.38 (s, 6H, C(CH<sub>3</sub>)<sub>2</sub>); \( \delta \_c \) (63 MHz; CDCl<sub>3</sub>) 208.53 (C=O), 159.53 (C Ar), 129.52 (CH Ar), 129.15 (C Ar), 114.00 (CH Ar), 113.05 (C(CH<sub>3</sub>)<sub>2</sub>), 81.91 (C–2), 81.03 (C–3), 74.30 (C–7), 73.52 (C–6), 55.37 (OMe), 46.33 (C–5), 26.72 ((C(CH<sub>3</sub>)<sub>2</sub>), 24.98 (C(CH<sub>3</sub>)<sub>2</sub>); [\( \alpha \] ]<sub>20</sub> <sup>D</sup> (c 1.00 CHCl<sub>3</sub>) +115.0°; \( m/z \) (EI+) 324 (M+).
EXPERIMENTAL

(1R, 2S, 4S)-4-Hydroxymethyl-4-hydroxy-2,3-(isopropylidenedioxy)cyclopentanone\(^\text{18}\) \textbf{195}

The PMB ether \textbf{224} (200mg, 0.62mmol, 1.00eq), was dissolved in THF (6mL), and palladium on charcoal (10\%, 0.31g, 0.29mmol, 0.47eq) was added. The stirrer was placed on its side to ensure the solvent surface was broken. While stirring, hydrogen was introduced into the reaction vessel and vented repeatedly till the vessel had been purged. Stirring was continued for 2 hours and TLC (eluting with chloroform/methanol 7:3, anisaldehyde stain, product R\(_f\) = 0.56) showed that the reaction had gone to completion.

The palladium/charcoal was removed by passing the reaction mixture through a syringe filter and the solvent was removed by rotary evaporation. The resulting residue was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 3:7). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a white solid \textbf{195} (0.11g, 89\%);

\[ \nu_{\text{max}} \text{(KBr)/cm}^{-1} 3521 \text{ (OH), 3481 (OH), 2999–2896 (C–H), 1763 (C=O);} \quad \delta_{\text{H}} \text{(250 MHz; CDCl}_3) 4.62 & 4.35 \text{ (d, 2H, J 5.8, H–6), 3.64 \text{ (d, 1H, J=10.7Hz, CH–2), 3.51 \text{ (d, 1H, J=10.7Hz, CH–3), 2.99 \text{ (s, 1H, OH), 2.68 \text{ (d, 1H, J=18.0 Hz, H–5)}, 2.41 \text{ (d, 1H, J=18.0Hz, H–5)}, 1.45 & 1.34 \text{ (s, 6H, C(CH}_3)_2); \delta_{\text{C}} \text{(63 MHz; CDCl}_3) 208.91 \text{ (C=O), 113.77 \text{ (C(CH}_3)_2), 80.56 \text{ (C–2), 80.44 \text{ (C–3), 74.29 \text{ (C–1), 67.46 \text{ (C–6), 45.98 \text{ (C–5), 26.64 \text{ (C(CH}_3)_2), 24.99 \text{ (C(CH}_3)_2); m/z(FAB+) 203 (M+H).}}}}} \]
The alcohol 117 (100mg, 0.52mmol, 1.00eq) was dissolved in DCM (2mL). With stirring, imidazole (53mg, 0.78mmol, 1.50eq) and tert-butyldimethylsilyl chloride (83mg, 0.55mmol, 1.05eq) were added. Stirring was continued overnight and TLC (eluting with ethyl acetate, permanganate stain, product Rf = 0.69), showed that the reaction had gone to completion.

The solids were removed by filtration and the solvent removed by rotary evaporation. The resulting oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 9:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a white solid 236 (0.10g, 64%).

\[
\begin{align*}
\nu_{\text{max}} \text{(KBr)/cm}^{-1} & \quad 3357 \text{ (OH)}, \quad 2992-2860 \text{ (C-H)}; \quad \delta_{\text{H}} \text{ (250 MHz; CDCl}_3) \quad 5.28 \text{ (d, 1H, J=11.8Hz, CH-1)}, \quad 4.76 \text{ (d, 1H, J=11.8Hz, OH)}, \quad 4.68 \text{ (d, 1H, J=5.9Hz, CH-2)}, \quad 4.50 \text{ (d, 1H, J=5.9Hz, CH-3)}, \quad 4.35 \text{ (t, 1H, J=2.1Hz, CH-4)} \quad 4.35 \text{ (t, 2H, J=2.1Hz, CH-5)}, \quad 1.48 \quad \text{&} \quad 1.32 \text{ (s, 6H, C(CH)_2)}, \quad 0.92 \text{ (s, 9H, 'Bu)}, \quad 0.14 \quad \text{&} \quad 0.13 \text{ (s, 6H, Si(CH)_2)}; \quad \delta_{\text{C}} \text{(63 MHz; CDCl}_3) \quad 111.91 \text{ (C(CH)_2)}, \quad 103.35 \text{ (C-1)}, \quad 87.51 \text{ (C-2)}, \quad 86.86 \text{ (C-3)}, \quad 79.31 \text{ (C-4)}, \quad 64.70 \text{ (C-5)}, \quad 26.35 \quad \text{&} \quad 24.81 \text{ (C(CH)_2)}, \quad 25.65 \text{ (C(CH)_3)}, \quad 18.13 \text{ (C(CH)_3)}, \quad -5.80 \text{ (Si(CH)_2)}; \\
[\alpha]_D^{20} \text{(c 0.12 CHCl}_3) & \quad +8.6^\circ; \quad \text{mpt} \quad 50.8-51.8^\circ C.
\end{align*}
\]
5-O-( tert-Butyldimethylsilyloxymethyl)-2,3-O-isopropylidene-L-ribonolactone*2

The alcohol 236 (77 mg, 0.25 mmol, 1.00 eq), was dissolved in acetone (2 mL) and the solution immersed in a water bath at R.T. Potassium permanganate (60 mg, 0.38 mmol, 1.52 eq) was added and stirring continued overnight. TLC (eluting with petroleum ether/ethyl acetate 9:1, permanganate stain, product Rr = 0.38) showed that negligible starting material remained.

The solvent was removed by rotary evaporation and the resulting residue resuspended in ethyl acetate. The resulting mixture was passed through a pad of celite. The clear colourless solution was concentrated by rotary evaporation to give a white solid 237 (65 mg, yield 85%);

νmax (KBr)/cm⁻¹ 2987–2859 (C–H) 1774 (C=O); δH (250 MHz; CDCl₃) 4.73 (d, 1H, J=5.6 Hz, CH–2), 4.70 (d, 1H, J=5.6 Hz, CH–3), 4.60 (dd, 1H, J=2.1, 1.5 Hz, CH–4), 3.89 (dd, 1H, J=11.3, 2.1 Hz, CH–5), 3.79 (dd, 1H, J=11.3, 1.5 Hz, CH–5), 1.47 & 1.39 (s, 6H, C(CH₃)₂), 0.87 (s, 9H, 'Bu), 0.08 & 0.07 (s, 6H, Si(CH₃)₃); δC (63 MHz; CDCl₃) 174.04 (C=O), 112.86 (C(CH₃)₂), 82.16 (C–2), 78.34 (C–3), 75.65 (C–4), 62.84 (C–5), 26.67 & 25.46 (C(CH₃)₂), 25.64 (C(CH₃)₃), 18.08 (C(CH₃)₃), -5.75 & -5.90 (SiCH₃); [α]D²⁰ (c 1.00 CHCl₃) +41°; mpt 62.0–65.0°C.
EXPERIMENTAL

2,3-O-Isopropylidene-L-ribonolactone² 238

The silyl ether 237 (2.00g, 6.62mmol, 1.00eq). was dissolved in THF (150mL), and the resulting solution cooled to 0°C (ice bath). TBAF (1.0M solution in THF, 6.80mL, 6.80mmol, 1.00eq) was added dropwise with stirring. After stirring for 5 minutes saturated ammonium chloride solution (100mL) was added. The resulting mixture was extracted ethyl acetate (3 × 150mL). The combined organic extracts were dried (MgSO₄) and the solvent removed by rotary evaporation to give a clear orange oil. TLC (eluting with petroleum ether/ethyl acetate 1:1, molybdate stain, product R₉ = 0.18) showed that the reaction had gone to completion.

The orange residue was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 1:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give white solid 238 (0.95g, 76%);

ν<sub>max</sub> (KBr)/cm<sup>-1</sup> 3468 (OH), 1775 (C=O); δ<sub>H</sub> (250 MHz; CDCl₃) 4.85 (d, 1H, J=5.6Hz, CH-2), 4.79 (d, 1H, J=5.6Hz, CH-3), 4.65-4.63 (m, 1H, CH-4), 4.00 & 3.80 (d, 2H, J=12.4Hz, CH₂-5), 3.08 (s, 1H, OH), 1.47 & 1.38 (s, 6H, C(CH₃)₂); δ<sub>C</sub> (63 MHz; CDCl₃) 175.19 (C=O), 112.98 (C(CH₃)₂), 82.83 (C-2), 78.15 (C-3), 75.52 (C-4), 61.66 (C-5), 26.55 & 25.26 (C(CH₃)₂); [α]<sup>20</sup> (e 1.03 CHCl₃) +63.0°, mpt 127.5-130.0°C.
The alcohol 238 (0.86g, 4.55mmol, 1.00eq) and tosyl chloride (1.28g, 6.72mmol, 1.47eq), were dissolved in chloroform (12mL), and pyridine (0.72g, 0.74mL, 9.15mmol, 2.00eq) added. Stirring was continued overnight. TLC (eluting with petroleum ether/ethyl acetate 1:1, molybdate stain, product $R_f = 0.47$) showed that only a small amount of starting material remained.

Water (12mL) and additional chloroform were added as required. After shaking thoroughly, the aqueous layer was removed and the organic layer washed with citric acid (2 × 12mL), dried (MgSO$_4$), and the solvent removed by rotary evaporation to give a yellow oil. This oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 1:4). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a white solid 239 (1.16g, 75%).

$\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 2993–2948 (C–H) 1785 (C=O); $\delta_{\text{H}}$ (250 MHz; CDCl$_3$) 7.79 (d, 2H, $J=8.2$Hz, Ar), 7.76 (d, 2H, $J=8.2$Hz, Ar), 4.80 (d, 1H, $J=5.7$Hz, CH–2), 4.74 (d, 1H, $J=5.7$Hz, CH–3), 4.70 (t, 1H, $J=2.3$Hz, CH–4), 4.36 (dd, 1H, $J=11.2$, 2.3Hz, CH–5), 4.20 (dd, 1H, $J=11.2$, 2.3Hz, CH–5), 2.49 (s, 3H, CH$_3$), 1.48 & 1.40 (s, 3H, C(CH)$_2$); $\delta_{\text{C}}$ (63 MHz; CDCl$_3$) 172.96 (C=O), 145.76 (OC Ar), 131.38 (CH$_3$C Ar), 130.10 (CH Ar), 127.79 (CH Ar), 113.70 (C(CH$_2$)$_2$), 78.89 (C–2), 77.20 (C–3), 74.84 (C–4), 68.12 (C–5), 26.45 & 25.34 (C(CH$_2$)$_2$), 21.57 (CH$_3$); $[\alpha]_D^0$ (c 0.50 MeOH) -36.0°; mpt 104.0–108.0°C.
EXPERIMENTAL

5-Iodo-5-deoxy-2,3-O-isopropylidene-L-ribonolactone\textsuperscript{a} 240

The lactone 239 (0.75g, 2.20mmol, 1.00eq) was dissolved in acetone (9mL) and sodium iodide (0.65g, 4.34mmol, 2.00eq) was added. The resulting solution was refluxed gently (oil bath temperature 65°C) with stirring overnight. TLC (eluting with petroleum ether/ethyl acetate 3:1, molybdate stain, product \( R_f = 0.35 \)) showed that the reaction had gone to completion.

The solids were removed by filtration and the filtrate concentrated by rotary evaporation. The resulting orange oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 9:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a yellow powder 240 (0.29g, yield 96%);

\( \nu_{\text{max}} (\text{KBr})/\text{cm}^{-1} \) 3034–2941 (C–H), 1777 (C=O); \( \delta_{\text{H}} \) (250 MHz; CDCl\(_3\)) 4.99 (d, 1H, \( J=6.1\text{Hz} \), CH–2), 4.65–4.60 (m, 1H, CH–4), 4.60 (d, 1H, \( J=6.1\text{Hz} \), CH–3), 3.47–3.35 (m, 2H, CH\(_2\)–5), 1.47 & 1.40 (s, 6H, C(CH\(_3\))\(_2\)); \( \delta_{\text{C}} \) (63 MHz; CDCl\(_3\)) 172.88 (C=O), 113.89 (C(CH\(_3\))\(_2\)), 80.69 (C–2), 80.20 (C–3), 75.09 (C–4), 26.36 & 25.28 (C(CH\(_3\))\(_2\)), 5.48 (C–5); [\( \alpha \)]\(_{D}^{20}\) (c1.01 CHCl\(_3\)) +30.7°; mpt 89.5–92.0°C.
Tributylstannyl methanol** 227

Diisopropylamine (4.15g, 5.75mL, 40.7mol, 1.10eq) was dissolved in THF (50mL) and the resulting solution cooled to 0°C (salt ice bath). n-BuLi (1.6M solution in hexanes, 23.13mL, 37.0mmol, 1.00eq) was added. Tributyltin hydride 225 (1.08g, 10mL, 37.0mmol, 1.00eq) was then added to the resulting mixture. The ice bath was removed and the solution was allowed to warm to R.T. Paraformaldehyde (1.11g, 37.0mmol, 1.00eq) was added and the mixture stirred for 3 hours. Hexane (150mL) was added and the resulting mixture washed with water (3 x 100mL), dried (Na₂SO₄) and the solvent removed by rotary evaporation to give a clear colourless oil. This oil was purified by flash column chromatography (Biotage flash system, eluting with petroleum ether/ethylacetate 29:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 227 (6.54g, 55%);

v_{max} (neat)/cm^{-1} 3333 (OH), 2954–2849 (C–H); \delta_{H} (200 MHz; CDCl₃) 4.01 (s, 2H, CH₂OH), 1.70–1.20 & 1.00–0.80 (m, 27H, (CH₂)₃Sn); \delta_{C}(63 MHz; CDCl₃) 53.52 (CH₂OH), 29.03 (CH₃CH₂CH₂Sn), 27.24 (CH₃CH₂CH₂Sn), 13.48 (CH₃(CH₂)₂Sn), 8.79 (CH₃(CH₂)₂CH₂Sn).
Sodium hydride (60% dispersion in mineral oil, 0.41g, 10.1mmol, 12mol%) was transferred to a round bottom flask and washed with petroleum ether, which was removed by filter canula. Anhydrous ether (15mL) was added, followed by a solution of freshly distilled p-methoxybenzyl alcohol 228 (11.98g, 86.5mmol, 1.00eq) in ether (15mL) via canula. The pale yellow mixture was then cooled to 0°C (salt ice bath) and trichloroacetonitrile (12.49g, 8.7mL, 86.5mmol, 1.00eq) added dropwise via syringe. This mixture was stirred at 0°C for 1 hour 20 minutes, after which time, the ice bath was removed and the solution stirred for a further 2 hours 20 minutes. The solvent was removed by rotary evaporation and anhydrous methanol (3.5mL) was added, followed by pentane (35mL). The solution was shaken vigorously then stirred for 10 minutes, then filtered through a pad of celite. The filtrate was then concentrated by rotary evaporation to give a clear oil 229 (17.33g, 70%);

\[ \delta_H (250 \text{ MHz}; \text{CDCl}_3) 8.36 (s, \text{NH}), 7.37 (d, 2H, J=8.8 Hz, \text{Ar-4&8}), 6.90 (d, 2H, J=8.8 Hz, \text{Ar-5&7}), 5.27 (s, 2H, \text{CH}_2), 3.81 (s, 3H, \text{CH}_3); \delta_C (63 \text{ MHz}; \text{CDCl}_3) 162.46 (\text{C Ar}), 159.55 (\text{C=NH}), 129.57 (\text{CH Ar}), 127.35 (\text{C Ar}), 113.76 (\text{CH Ar}), 91.33 (\text{CCl}_3), 70.54 (\text{CH}_2), 55.13 (\text{CH}_3). \]
Cupric acetate (0.08g) was dissolved in glacial acetic acid (8mL) by stirring at 95°C (oil bath temperature). Zinc (5.2g) was added to the resulting solution and stirred for 5 minutes. The acetic acid was decanted off and the couple was washed with further glacial acetic acid (8mL) at 90°C. The couple was then washed with ether (3 x 16mL) and allowed dry under a flow of nitrogen. The flask was fitted with a reflux condenser, dropping funnel and thermometer. THF (14mL) was added to the couple and a soluton of diiodomethane 233 (21.5g, 6.46mL, 80.2mmol, 1.86eq) in THF (14mL) was placed in the dropping funnel. The reaction mixture was warmed to around 35°C with a heat gun. A few drops of the diiodomethane solution were added and the mixture heated gently with the heatgun. The diiodomethane solution was then added dropwise at a rate which maintained a temperature of 40°C. Stirring at 40°C was then continued for 2 hours. The flask was cooled in an ice bath and the solution transferred to another 3N round bottom flask via filter canula. A solution of tributytin chloride (14.06g, 11.72mL, 43.2mmol, 1.00eq) in THF (20mL) was added dropwise via dropping funnel at 40°C. The reaction was then stirred for an additional 2.5 hours and left to stand overnight. Toluene (50mL) was added to the resulting mixture and this solution was washed with 5% HCl (4 x 100mL). The organic phase was dried (MgSO₄) and purified by vacuum distillation (still head temperature 98°C, 0.1mbar), giving a clear colourless oil 235 (16.79g, 48%);
EXPERIMENTAL

[(p-Methoxybenzyloxy)methyl]tri-\textit{n}-butylstannane$^{84}$ 230

\[
\begin{array}{c}
\text{O} \\
\text{SnBu_3} \\
\text{O} \\
\end{array}
\]

Tributylstannyl methanol 227 (0.84g, 2.60mmol, 1.00eq) and PMBtrichloroacetimidate (1.5g, 5.30mmol, 2.04eq) were dissolved in DCM (4mL). Triflic acid (51.6mg, 0.03mL, 0.34mmol, 13mol\%) was added dropwise (causing the reaction mixture to warm up). TLC (eluting with petroleum ether/ethyl acetate 19:1, molybdate stain, product $RF = 0.71$) showed that the reaction had gone to completion in 15 minutes.

The reaction mixture was filtered through a pad of celite, and the pad washed repeatedly with pentane. The filtrate was concentrated giving an oily solid. This was purified by flash column chromatography (biorage flash system, petroleum ether/ethyl acetate 99:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 230 (0.37g, yield 32%).

Potassium hydride (30% dispersion in mineral oil, 0.97g, 7.28mmol, 1.05eq) and THF (10mL) were transferred to a round bottom flask and cooled to 0°C (ice bath). p-Methoxybenzyl alcohol 228 (1.00g, 0.90mL, 7.25mmol, 1.04eq) was added dropwise via syringe and stirring at 0°C was continued for 45 minutes. Tributyl-iodomethyl-stannane 235 (3.00g, 6.96mmol, 1.00eq) was added and the resulting mixture stirred at R.T. for a further 6h. TLC (as previous) suggested that the reaction would go no further.

The solids were removed by filtration, but this was a tedious process and conveniently, the solids could be more easily removed by centrifugation (5 minutes at 4000rpm). The filtrate was concentrated by rotary evaporation and the resulting oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 97.5:2.5). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 230 (1.15g, 38%).

\[ \nu_{\text{max}} \text{(neat)/cm}^{-1} 2955-2852 \text{ (C–H); } \delta_{\text{H}} \text{ (250 MHz; CDCl}_3\text{) 7.34 (d, 2H, } J=8.8\text{Hz, Ar–4&8), 6.97 (d, 2H, } J=8.8\text{, Ar–5&7), 4.47 (s, 2H, OCH}_2\text{Ar), 3.92 (s, 3H, CH}_3\text{), 3.82 (s, 2H, SnCH}_2\text{O), 1.82–1.30 & 1.12-0.90 (m, 27H, (CH}_2\text{)}^\text{3}\text{CH}_2\text{); } \delta_{\text{C}} \text{(63 MHz; CDCl}_3\text{) 158.83 (Car), 130.87 (CAr), 128.99 (CHAr), 113.44 (CHAr), 76.64 (OCH}_2\text{Ar), 60.94 (SnCH}_2\text{O), 55.07 (OCH}_3\text), 29.02 (CH}_3\text{Sn), 27.21 (CH}_2\text{CH}_2\text{Sn), 13.61 (CH}_3\text{(CH}_2\text{)}^\text{3}\text{Sn), 8.86 (CH}_2\text{(CH}_2\text{)}^\text{2}\text{Sn).} \]
4.2.2 Abacavir

3-Chlorocyclopentene\textsuperscript{109,110} 247

HCl(g) was bubbled through freshly cracked cyclopentadiene 65 (34.5g, 0.52mol, 1.00eq) for around 2 hours. The light pink liquid was stored at -20°C overnight. This crude product was purified by distillation at reduced pressure (collecting fraction corresponding to still head temperature of ~ 26°C at a pressure of 23mmHg). This gave a clear colourless liquid 247 (~16g, ~30%);

ν\textsubscript{max} (neat)/cm\textsuperscript{-1} 3060.7 (C=\textit{C}-\textit{H}), 2849–2973 (C-H); δ\textsubscript{H} (250 MHz; CDCl\textsubscript{3}) 6.05–6.03 (m, 1H, H-2), 5.90–5.86 (m, 1H, H-1), 5.05–5.00 (m, 1H, H-3), 2.60–2.11 (m, 4H, CH\textsubscript{2}-5 & CH\textsubscript{2}-4); δ\textsubscript{C}(63 MHz; CDCl\textsubscript{3}) 136.03 (C-2), 132.00 (C-1), 65.53 (C-3), 34.37 (C-4), 30.93 (C-5); m/z (EI) 102 (M+).
EXPERIMENTAL

2-Cyclopentene carboxylic acid<sub>108, 113</sub> 246

![Structure of 2-Cyclopentene carboxylic acid](image)

A solution of the chloride 247 (10.00g, 97.6mmol, 1.00eq) in THF (30mL) was added slowly to a stirred mixture of magnesium (3.9g, 0.16mol, 1.64eq) in THF (30mL), over a period of half an hour. The temperature was kept below -10°C by adjusting external cooling (dry ice/acetone bath). After addition was complete, stirring was continued while maintaining a temperature of -10°C. The reaction mixture was transferred to a THF (10mL)/CO<sub>2</sub> slurry using a pasteur pipette and left for around 1 hour.

Saturated ammonium chloride (80mL) was added and the mixture stirred until effervescence had ceased. The organic layer was removed, and the aqueous layer was acidified with 2M HCl. The aqueous layer was extracted with ether (3 x 100mL). The combined organic extracts were dried (MgSO<sub>4</sub>) and the solvent removed by rotary evaporation to give a brown oil. This oil was purified by flash column chromatography (eluting with dichloromethane). Appropriate fractions were combined, and the solvent removed by rotary evaporation to give a clear colourless oil 246 (1.38g, 13%);

ν<sub>max</sub> (neat)/cm<sup>-1</sup> 2952 (OH), 2500–2750 (C–H), 1706 (C=O); δ<sub>h</sub> (200 MHz; CDCl<sub>3</sub>) 5.95–5.90 (m, 1H, H-2), 5.77–5.72 (m, 1H, H-3), 3.62–3.55 (m, 1H, H-1), 2.48–2.21 (m, 2H, CH<sub>2</sub>-4), 2.17–2.10 (m, 2H, CH<sub>2</sub>-5); δ<sub>c</sub> (63 MHz; CDCl<sub>3</sub>) 181.28 (C–6), 134.25 (CH–2), 127.25 (CH–3), 50.29 (CH–1), 32.06 (CH<sub>2</sub>-4), 26.25 (CH<sub>2</sub>-5), m/z (EI) 112 (M+).
The acid 246 (0.60g, 8.48mmol, 1.00eq) and EEDQ (2.10g, 8.48mmol, 1.00eq) were dissolved in methanol (10mL). The flask was fitted with a bubbler and the reaction mixture stirred for approximately 8 hours. Bubbling ceased and TLC (hexane/ethyl acetate 3:1, anisaldehyde stain, product $R_f = 0.45$) indicated that the reaction had gone to completion.

The methanol was removed by rotary evaporation, and the resulting residue was purified by flash column chromatography (eluting with dichloromethane). Appropriate fractions were combined and the solvent removed by rotary evaporation to give 251 (0.28g, 42%);

$\nu_{\text{max}}$ (neat)/cm$^{-1}$: 3056 (C=C—H), 2949–2850 (C—H), 1731 (C=O); $\delta_{\text{H}}$ (200 MHz; CDCl$_3$) 5.92–5.86 (m, 1H, H–2), 5.74–5.68 (m, 1H, H–3), 3.68 (s, 3H, CO$_2$CH$_3$), 3.60–3.50 (m, 1H, H–1), 2.60–2.40 (m, 2H, CH$_2$–4), 2.18–2.10 (m, 2H, CH$_2$–5); $\delta_{\text{C}}$ (63 MHz; CDCl$_3$) 175.24 (C–6), 133.77 (C–2), 128.32 (C–3), 51.68 (C–7), 50.34 (C–1), 32.04 (C–4), 26.36 (C–5); $m/z$ (EI) 127 (M$^+$), 67 ($C_5H_7$).
Lithium aluminium hydride (0.13 g, 3.42 mmol, 0.58 eq) was stirred in THF (4 mL), and to this, the ester 251 (0.75 g, 5.91 mmol, 1.00 eq) in THF (4 mL) was added slowly over 5–10 minutes. Once all the ester solution had been added, stirring was continued for a further 20 minutes. Any excess LiAlH₄ was hydrolysed by addition of the minimum amount of water. The reaction mixture was then exposed to the atmosphere and stirred for around 2 hours. The solids were removed by filtration, and washed with THF. The THF was removed by rotary evaporation to give a clear brown oil. This oil was purified by flash column chromatography (eluting with dichloromethane:methanol 95:5). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear pale brown oil 245 (0.24 g, 40%);
Ethyl 2-hydroxycyclopentanecarboxylate

Microbial Transformation

The mould (Rhizopus Arrhizus ATC 11145) was obtained as a freeze dried sample of spores and protein. This was grown on potato dextrose agar plates at 30°C. The liquid medium was prepared by dissolving glucose (30.00g), KH₂PO₄ (1.00g), K₂HPO₄ (2.00g), corn steep liquor (10.00g), MgSO₄·7H₂O (0.50g), NaNO₃ (2.00g), FeSO₄·7H₂O (0.02g) and KCl (0.50g) in 1L of deionised water. The resulting solution was distributed in 500mL portions to 2 x 2L flasks and these were autoclaved. 1 flask was inoculated with around half a plate of non-sporing mould. Both flasks were then shaken at 500rpm at 25°C for 3 days. Sufficient growth was judged to have taken place in the inoculated flask, and none in the control flask. A solution of the keto-ester (0.50g, 3.21mmol) in ethanol (5mL) was added to the medium containing the mould. Shaking at the same speed and temperature was continued for 3 days. TLC (of a sample shaken with ethyl acetate, eluting with petroleum ether/ethyl acetate 3:1, permanganate stain, product minor Rᵣ = 0.22, product major Rᵣ = 0.14) revealed that the reaction had gone to completion.

The suspension was filtered through celite and the filtrate extracted with ethyl acetate (3 x 500mL). The combined organic extracts were dried (MgSO₄) and the solvent removed by rotary evaporation to give a brown oil. This oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 6:1 up to 3:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give 2 pale brown oils (trans- 240mg, 47% & cis- 40mg, 8.3%).
Sodium borohydride reduction

The ester 260 (8.38g, 53.7mmol, 1.00eq) was dissolved in methanol (134mL), and the resulting solution cooled to 0°C (ice bath). Sodium borohydride (2.26g, 59.5mmol, 1.10eq) was added portionwise with stirring, allowing the reaction to subside between additions. After 5-10 minutes, water (80mL) and 5% HCl (20mL) were added. Stirring was continued for 5-10 minutes. The resulting mixture was extracted with ethyl acetate (3 × 100mL). Salt was added to saturate the aqueous layer and extra water/ethyl acetate added until adequate separation had occurred. The combined organic extracts were washed with brine, dried (MgSO₄) and the solvent removed by rotary evaporation. The resulting residue was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 3:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 261 (cis- 3.28g, 38%).

(1S, 2S)-Ethyl 2-hydroxycyclopentanecarboxylate (1S, 2S)-261

\( \text{trans- } \nu_{\text{max}} \) (neat)/cm\(^{-1}\) 3438 (OH), 2964–2876 (C–H), 1732 (C=O); \( \delta_{\text{H}} \) (250 MHz; CDCl₃) 4.42 (td, 1H, \( J=6.5, 6.5 \text{Hz}, CH-2 \)), 4.20 (q, \( J=7.1 \text{Hz}, \text{OCH}_2\text{CH}_3 \)), 2.69 (td, 1H, \( J=8.7, 6.5 \text{Hz}, CH-1 \)), 2.11–2.04 (m, 2H, CH₂-3), 1.85–1.69 (m, 4H, CH₂-5 & 4), 1.31 (t, 3H, \( J=7.1 \text{Hz}, \text{OCH}_2\text{CH}_3 \)); \( \delta_{\text{C}} \) (63 MHz; CDCl₃) 175.51 (C=O), 76.60 (C-2), 60.93 (OCH₂CH₃), 53.00 (C-1), 34.46 (C-3), 27.61 (C-5), 22.41 (C-4), 14.52 (OCH₂CH₃); \([\alpha]_{\text{D}}^{20} \) (c 1.28 MeOH) +64.0°;

(1S, 2R)-Ethyl 2-hydroxycyclopentanecarboxylate (1S, 2R)-261

\( \text{cis- } \nu_{\text{max}} \) (neat)/cm\(^{-1}\) 3441 (OH), 2959–2876 (C–H), 1730 (C=O); \( \delta_{\text{H}} \) (250 MHz; CDCl₃) 4.45 (dd, 1H, \( J=4.3, 3.5 \text{Hz}, CH-2 \)), 4.22 (q, 2H, \( J=7.1 \text{Hz}, \text{OCH}_2\text{CH}_3 \)), 2.68 (ddd, 1H, \( J=4.9, 4.9, 4.3 \text{Hz}, CH-1 \)), 2.08–1.53 (m, 6H, CH₂-3, 5 & 4), 1.29 (t, 3H, \( J=7.1 \text{Hz}, \text{OCH}_2\text{CH}_3 \)); \( \delta_{\text{C}} \) (63 MHz; CDCl₃) 174.80 (C=O), 73.59 (C-2), 60.48 (OCH₂CH₃), 49.32 (C-1), 33.78(C-3), 26.20 (C-5), 21.84 (C-4), 14.02 (OCH₂CH₃); \([\alpha]_{\text{D}}^{20} \) (c 0.94 MeOH) +22.0°.
EXPERIMENTAL

**Ethyl 2-cyclopentenecarboxylate 262**

![Ethyl 2-cyclopentenecarboxylate](image)

The alcohol 261 (100mg, 0.63mmol, 1.00eq) was dissolved in DCM (1mL) and phosphorus pentoxide (100mg, 0.70mmol, 1.12eq) added. The mixture was stirred for 30 minutes and TLC indicated that the reaction had gone to completion. Water (5mL) was added and the resulting mixture extracted with DCM (3 x 5mL). The combined organic extracts were washed with brine (10mL), dried (MgSO₄) and the solvent removed by rotary evaporation to give a colourless oil. Insufficient material was obtained for analysis.

**Ethyl 2-(tert-butyldimethylsilyloxy)cyclopentanecarboxylate 264**

![Ethyl 2-(tert-butyldimethylsilyloxy)cyclopentanecarboxylate](image)

The alcohol 261 (3.00g, 19.0mmol, 1.00eq) was dissolved in DMF (6mL). Imidazole (3.24g, 47.6mmol, 2.50eq) and tert-butyldimethylsilyl chloride (3.42g, 22.7mmol, 1.19eq) were added and the resulting mixture stirred overnight. TLC (eluting with petroleum ether/ethyl acetate 3:1, molybdate stain, product Rf = 0.63) showed that the reaction had gone to completion. Water (10mL) was added and the resulting mixture extracted with ethyl acetate (4 x 10mL). The combined organic extracts were dried (MgSO₄) and the solvent removed by rotary evaporation. The resulting oil was purified by column chromatography (eluting with petroleum ether/ethyl acetate 9:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 264 (4.93g, 95%);

\[
\begin{align*}
\nu_{\text{max}} \, (\text{neat})/\text{cm}^{-1} & \quad 2956-2857 \, (C-H), 1743 \, (C=O); \\
\delta_{\text{H}} \, (250 \, \text{MHz; CDCl}_3) & \quad 4.48 \, (dd, 1H, J=4.7, 4.0Hz, H-2), 4.11 & 4.01 \, (dq, 2H, J=10.8, 7.2Hz, OCHCH_3), 2.71 \, (dt, 1H, J=4.7, 8.8Hz, H-1), 2.25-2.09 & 1.92-1.49 \, (m, 6H, CH_2-3, 5 & 4), 1.26 \, (t, 3H, J=7.2Hz, OCH_2CH_3), 0.84 \, (s, 9H, 'Bu), 0.04 & 0.03 \, (s, 6H, SiMe); \\
\delta_{\text{C}} \, (63 \, \text{MHz; CDCl}_3) & \quad 172.61 \, (C=O), 75.29 \, (C-2), 59.98 \, (OCH_2CH_3), 51.21 \, (C-1), 35.24 \, (C-3), 25.51 \, (CH(CH)_3), 24.66 \, (C-5), 21.85 \, (C-4), 17.76 \, (C(CH)_3), 14.08 \, (OCH_2CH_3), -4.74 & -5.33 \, (Si(CH)_3);
\end{align*}
\]
Hydroxymethyl-2-(tert-butyldimethylsilyloxy)cyclopentane 274

![Structural formula]

The ester 264 (1.00g, 3.77mmol, 1.00eq) was dissolved in DCM (25mL) and the resulting solution cooled to -78°C (dry ice/acetone). DIBAL (20% weight solution in toluene, 7.25mL, 8.83mmol, 2.34eq) was added dropwise. Stirring was continued for 3h and TLC (eluting with petroleum ether/ethyl acetate 5:1, molybdate stain, product \( R_f = 0.27 \) ) showed that the reaction had gone to completion.

The reaction was quenched by addition of saturated potassium sodium tartrate solution (Rochelle’s salt) (25mL). The resulting mixture was extracted with ethyl acetate (3x25mL). The combined organic extracts were dried (MgSO\(_4\)) and the solvent removed by rotary evaporation. The resulting oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 9:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 274 (0.64g, 75%);
The alcohol 274 (200mg, 0.87mmol, 1.00eq) was dissolved in DMF (5mL). To this solution, sodium hydride (60% dispersion in mineral oil, 54mg, 1.30mmol, 1.50eq) was added and the resulting mixture stirred for 30 minutes. Benzyl bromide (164mg, 0.11mL, 0.96mmol, 1.10eq) was added, followed by a microspatula of TBAI. After stirring overnight, TLC (eluting with petroleum ether/ethyl acetate 5:1, molybdate stain, product Rf = 0.61) showed that negligible starting material remained. Water (5mL) was added and the resulting mixture extracted with ethyl acetate (3 × 10mL). The combined organic extracts were dried (MgSO4) and the solvent removed by rotary evaporation. Purification was attempted, by flash column chromatography (eluting with petroleum ether up to petroleum ether/ethyl acetate 19:1) but a slightly lower running compound contaminated the majority of the fractions and only small portions of pure material could be isolated. Appropriate fractions were combined and the solvent removed by rotary evaporation to give the semi-purified oil 265 (0.36g, 65%);

\[
\begin{align*}
\text{v} & (\text{neat})/\text{cm}^{-1} \quad 2955-2856 \text{ (C–H)}; \\
\delta & (250 \text{ MHz; } \text{CDCl}_3) \quad 7.42-7.30 \text{ (m, 5H, Ar),} \\
4.59 & \text{ & } 4.47 \text{ (d, 2H, } J=11.8\text{Hz, CH}_2-7\text{),} \\
4.29 & \text{ (dd, 1H, } J=6.1, 4.0\text{Hz, CH–1),} \\
3.66 & \text{ (dd, 1H, } J=8.6, 7.7\text{Hz, CH–6),} \\
3.45 & \text{ (dd, 1H, } J=8.6, 6.3\text{Hz, CH–6),} \\
2.08 & \text{ (m, 1H, H–2),} \\
1.77–1.51 & \text{ (m, 6H, CH}_2–3\text{, 5 & 4),} \\
0.92 & \text{ (s, 9H, } \text{‘Bu),} \\
0.07 & \text{ (s, 6H, SiMe}_2\text{); } \\
\delta & (63 \text{ MHz; } \text{CDCl}_3) \quad 138.13 \text{ (C Ar),} \\
127.57 & \text{ (CH Ar),} \\
127.14 & \text{ (CH Ar),} \\
126.53 & \text{ (CH Ar),} \\
73.18 & \text{ (C–1),} \\
72.58 & \text{ (C–7),} \\
70.17 & \text{ (C–6),} \\
45.37 & \text{ (C–2),} \\
34.76 & \text{ (C–5),} \\
25.53 & \text{ (C–3),} \\
25.21 & \text{ (C(CH}_3)_3\text{),} \\
21.02 & \text{ (C–4),} \\
17.47 & \text{ (C(CH}_3)_3\text{),} \\
-5.17 & \text{ & } -5.90 \text{ (SiCH}_3\text{).}
\end{align*}
\]
2-Hydroxymethylcyclopentanol\textsuperscript{28} \textit{267}

1 Step Sodium Borohydride

Ethanol (240mL) was cooled to -30°C (dry ice/acetone) and sodium borohydride (25.00g, 0.66mol, 5.10eq) added. A solution of the keto-ester \textit{260} (20.00g, 0.13mol, 1.00eq) in ethanol (60mL), was added at a rate to maintain the temperature in the range -20 to -30°C. This temperature was maintained for a further 1h, then stirring was continued at R.T. for 64h. TLC (eluting with ethyl acetate, permanganate stain, \textit{trans-} product \(R_f = 0.15\), \textit{cis-} product \(R_f = 0.19\)) revealed that the reaction had gone to completion.

A little ethanol was added to mobilise the now solid reaction mixture and glacial acetic acid (100mL) was added slowly. The solvents were removed by rotary evaporation using toluene as required to aid removal of acetic acid. To the resulting solid, brine (200mL) was added. The resulting mixture was extracted with ethyl acetate (3 x 200mL). The combined organic extracts were dried (MgSO\textsubscript{4}), and the solvent removed by rotary evaporation to give a clear colourless oil. This oil was purified by flash column chromatography (eluting with petroleum ether, then slowly up to ethyl acetate). Mixed fractions were subjected to repurification in the same way, thus giving 2 clear colourless oils \textit{267} (\textit{trans-} 6.57g, 44\% & \textit{cis-} 4.05g, 27\%).

**Ethyl 2-hydroxycyclopentanecarboxylate reduction with LiAlH\textsubscript{4}**

Lithium aluminium hydride (50mg, 1.32mmol, 2.10eq) was suspended in THF (1mL) and resulting mixture was cooled to 0°C. A solution of the ester \textit{261} (\textit{cis-} and \textit{trans-} in separate experiments, 100mg, 0.63mmol, 1.00eq) in THF (1mL) was added dropwise. After 20 minutes TLC analysis (as previous) showed that the reaction had gone to completion.

Water was added dropwise until no further effervescence occurred, taking care to add only what was necessary. The solids were removed by filtration and the resulting solution concentrated by rotary evaporation to give a clear colourless oil \textit{267} (\textit{trans-} 32mg, 44\% & \textit{cis-} 61mg, 83\%).
(1S, 2R), trans- $\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3348 (OH), 2955–2872 (C–H); $\delta_{H}$ (250 MHz; CDCl$_3$) 4.06 (td, 1H, $J=$6.6, 6.6Hz, H–1), 3.79 (dd, 1H, $J=$10.3, 5.3Hz, H–6), 3.57 (dd, $J=$10.3, 8.9Hz, H–6), 2.70 (s, 1H, OH), 2.03–1.60 (m, 6H, CH$_2$–3 & 5, H–2 & 4), 1.25–1.21 (m, 1H, H–4); $\delta_c$ (63 MHz; CDCl$_3$) 77.08 (C–1), 65.46 (C–6), 49.48 (C–2), 34.21 (C–5), 26.29 (C–3), 21.68 (C–4); m/z (Ei+) 98 (M–H$_2$O), 80 (M–2H$_2$O).

cis- $\nu_{\text{max}}$ (neat)/cm$^{-1}$ 3443 (OH), 2951–2875 (C–H); $\delta_{H}$ (250 MHz; CDCl$_3$) 4.49–4.46 (m, 1H, H–1), 4.22–4.16 & 3.98–3.93 (m, 2H, H–6), 2.17–2.12 (m, 1H, H–2), 1.79 (s, 1H, OH), 1.80–1.68 (m, 6H, CH$_2$–3, 5 & 4); $\delta_c$ (63 MHz; CDCl$_3$) 77.92 (C–1), 63.24 (C–6), 40.22 (C–2), 34.77 (C–5), 25.10 (C–3), 22.31 (C–4); MS(FAB+) 81 (M+H–2H$_2$O).
EXPERIMENTAL

2-Hydroxymethylcyclopentanol benzylidene acetal 269

The diol 267 (0.50g, 4.31mmol, 1.00eq) was dissolved in DMF (10mL) and with stirring, benzaldehyde dimethyl acetal (1.48mL, 1.51g, 9.90mmol, 2.3eq, was added, followed by p-toluenesulfonic acid (0.16g, 0.86mmol, 0.20eq). Stirring was continued overnight at 60°C after which time TLC (eluting with ethyl acetate, anisaldehyde stain, trans- product Rf = 0.73, cis- product Rf = 0.72) showed that very little starting material remained.

Rotary evaporation in a warm water bath to remove the solvent was commenced immediately. The resulting residue was suspended in saturated NaHCO₃ solution and washed with DCM (2×10mL). The combined organic layers were dried and the solvent removed by rotary evaporation to give a yellow oil. This was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 9:1). Appropriate fraction were combined and the solvent removed by rotary evaporation to give clear colourless oils 269 (trans- 0.71g, 81% & cis- 0.68g, 78%).

\[ \text{trans-} \nu_{\text{max}} \text{(neat)/cm}^{-1} 2962-2873 (C=H); \delta_{H} (250 \text{ MHz; CDCl}_3) 7.59-7.38 (m, 5H, Ar), 5.57 (s, 1H, H-7), 4.48 (dd, 1H, J=10.6, 4.1Hz, H-6), 3.78 (d, 1H, J=10.6Hz, H-6), 3.57 (ddd, 1H, J=10.0, 10.0, 7.3Hz, H-1), 2.05-2.15 (m, 1H, H-2), 1.88-1.69 (m, 5H, CH₂-3, 5 & H-4), 1.10-1.24 (m, 1H, H-4); \delta_{C} (63 \text{ MHz; CDCl}_3) 138.20 (C Ar), 129.57 (CH Ar), 128.82 (CH Ar), 126.11 (CH Ar), 101.62 (C-7), 83.65 (C-1), 72.99 (C-6), 41.60 (C-2), 28.14 (C-5), 22.29 (C-3), 18.46 (CH₂-4); m/z (EI+) 204 (M+); HRMS(EI+) 204.11461(calculated for C_{13}H_{16}O₂, 204.11503, (Dev -2.07ppm)).

\[ \text{cis-} \nu_{\text{max}} \text{(neat)/cm}^{-1} 2959-2871 (C=H); \delta_{H} (250 \text{ MHz; CDCl}_3) 7.90-7.50 & 7.39-7.36 (m, 5H, Ar), 5.48 (s, 1H, H-7), 4.36-4.33 (m, 1H, H-1), 4.26-4.21 & 4.18-4.13 (m, 2H, H-6), 2.13-1.64 (m, H7, H-2, CH₂-3, 5 &4); \delta_{C} (63 \text{ MHz; CDCl}_3) 138.85 (C Ar), 128.57 (CH Ar), 128.10 (CH Ar), 125.97 (CH Ar), 100.22 (C-7), 80.02 (C-1), 67.46 (C-6), 39.31 (C-2), 33.02 (C-5), 25.43 (C-3), 22.61 (C-4); m/z (EI+) 204 (M+). \]
Direct benzylation of diol

The diol \(267\) (2.77g, 23.9mmol, 1.00eq), was dissolved in DMF (60mL) and the resulting solution cooled to -78°C (dry ice/acetone). Sodium hydride (0.96g, 40.0mmol, 1.67eq) was added and the resulting mixture stirred for 10 minutes. Benzyl bromide (4.10g, 2.84mL, 24.0mmol, 1.00eq) was added and stirring continued for 4 hours. TLC (eluting with ethyl acetate, permanganate stain, \(\text{trans- product } R_f = 0.57, \text{cis- product } R_f = 0.58\)) showed that a new species was now present. Although starting material remained, work up was commenced, by quenching the reaction by the addition of water. For the cis- species, (separate reaction, with diol (0.56g)), the reaction was quenched by addition of HCl (2M, 15mL). The resulting mixture was extracted with ethyl acetate. The combined organic extracts were dried and the solvent removed by rotary evaporation to give a clear colourless oil. This was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 9:1 up to 1:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil \(266\) (trans- 2.35g, 46% & cis- 0.38g, 38%).

Ring opening of benzylidene acetal

The benzylidene acetal \(269\) (0.57g, 2.80mmol, 1.00eq) was dissolved in THF (20mL) and sodium cyanoborohydride (1.58g, 25.0mmol, 8.93eq) was added. To the resulting solution, HCl (2.0M in Et₂O) was added portionwise until the evolution of gas had ceased and the solution remained acidic. During addition, a white precipitate formed. TLC (eluting with ethyl acetate, anisaldehyde stain, \(\text{trans- product } R_f = 0.56, \text{cis- product } R_f = 0.58\)) showed that the reaction had gone to completion. The reaction mixture was concentrated to approximately 5mL and DCM (20mL) was added. The resulting mixture as neutralised by addition of saturated NaHCO₃ and extracted with DCM 3 x 20mL). The combined organic extracts were washed with water (20mL), dried (MgSO₄), and the solvent removed by rotary evaporation. The
resulting oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 9:1, up to ethyl acetate). Appropriate fractions were combined and the solvent removed to give a clear colourless oil \(266\) (trans-0.40g, 69% & cis-0.37g, 63%).

**TBDMS removal with TBAF**

The silyl ether \(265\) (340mg, 1.06mmol, 1.00eq) was dissolved in THF (25mL) and the resulting solution cooled to 0°C (ice bath). TBAF (1.0M soln in THF, 1.17mL, 1.17mmol, 1.10eq) was added and stirring continued overnight. TLC (eluting with petroleum ether/ethyl acetate 5:1, molybdate stain, product \(R_f = 0.15\)) showed that new species had formed, and although starting material remained, work up was commenced.

Saturated ammonium chloride solution (25mL) was added and the resulting mixture shaken thoroughly. The layers were separated and the aqueous layer extracted with ethyl acetate (3 × 25mL). The combined organic extracts were dried (MgSO\(_4\)), and the solvent removed by rotary evaporation. The resulting oil was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 9:1 up to 4:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil \(266\) (cis-0.13g, yield 57%). This contained inseparable impurities probably arising from the impure starting material however, and was not deemed useful.
**EXPERIMENTAL**

*trans*- ν<sub>max</sub> (neat)/cm<sup>-1</sup> 3388 (OH), 2955–2869 (C–H); δ<sub>H</sub> (250 MHz; CDCl<sub>3</sub>) 7.44–7.34 (m, 5H, Ar), 4.59 (s, 2H, CH<sub>2</sub>–7), 4.05 (dt, 1H, J=6.8, 6.8 Hz, H–1), 3.66 (dd, 1H, J=9.0, 5.4 Hz, H–6), 3.42 (dd, 1H, J=9.0, 9.0 Hz, H–6), 2.30–1.60 (m, 6H, CH–2 & 4, CH<sub>2</sub>–3 & 5), 1.27–1.16 (m, 1H, H–4); δ<sub>C</sub> (63 MHz; CDCl<sub>3</sub>) 137.55 (C Ar), 127.76 (CH Ar), 126.94 (CH Ar), 126.87 (CH Ar), 77.46 (C–1), 73.26 (C–7), 72.59 (C–6), 46.82 (C–2), 33.23 (C–5), 25.82 (C–3), 21.12 (C–4); m/z (El+) 206 (M+), 188 (M–H<sub>2</sub>O); HRMS(EI+) 206.13037 (calculated for C<sub>13</sub>H<sub>18</sub>O<sub>2</sub>, 206.13068, (Dev -1.50 ppm)).

*cis*- ν<sub>max</sub> (neat)/cm<sup>-1</sup> 3375 (OH), 2956–2871 (C–H); δ<sub>H</sub> (250 MHz; CDCl<sub>3</sub>) 7.45–7.33 (m, 5H, Ar), 4.76–4.48 (m, 2H, CH<sub>2</sub>–7), 4.40 (m, 1H, H–1), 3.72–3.69 (m, 2H, CH<sub>2</sub>–6), 2.18 (s, 1H, OH), 1.89–1.84 (m, 1H, CH–2), 1.76–1.52 (m, 6H, CH<sub>2</sub>–3, 5 & 4); δ<sub>C</sub> (63 MHz; CDCl<sub>3</sub>) 137.93 (C Ar), 128.32 (CH Ar), 127.59 (CH Ar), 127.33 (CH Ar), 74.28 (C–1), 73.09 (C–7), 70.23 (C–6), 44.19 (C–2), 34.65 (C–5), 25.91 (C–3), 22.12 (C–4); m/z (El+) 206 (M+), 188 (M–H<sub>2</sub>O).
2-Benzylxoxymethylcyclopentyl mesylate 273

The alcohol 266 (0.31g, 1.51mmol, 1.00eq), was dissolved in DCM (30mL) and the resulting solution cooled to 0°C (ice bath). Mesyl chloride (0.27g, 0.18mL, 2.32mmol, 1.54eq) and triethylamine (0.23g, 0.31mL, 2.23mmol, 1.48eq) were added and the resulting solution stirred at 0°C for 15 minutes. TLC (eluting with petroleum ether/ethyl acetate 1:1, molybdate stain, trans- product Rf = 0.58, cis- product Rf = 0.55) showed that the reaction had gone to completion.

HCl (2M, 30mL) was added and the layers mixed thoroughly. The layers were separated and the aqueous layer extracted with DCM (3 x 30mL). The combined organic extracts were dried (MgSO4) and the solvent removed by rotary evaporation to give a clear colourless oil. This was purified by flash column chromatography (eluting with petroleum ether/ethyl acetate 9:1). Appropriate fractions were collected and the solvent removed by rotary evaporation to give a clear colourless oil 273 (trans- 0.28g, 66% & cis- 0.36g, 85%).

trans- νmax (neat)/cm⁻¹ 2960–2871 (C–H); δH (250 MHz; CDCl3) 7.43–7.27 (m, 5H, Ar), 4.98 (dt, 1H, J=4.5, 4.5Hz, H–1), 4.33 (s, 2H, CH2–7), 3.53 (dd, 1H, J=9.4, 5.4Hz, H–6), 3.35 (dd, 1H, J=7.7, 9.4Hz, H–6), 2.94 (s, 3H, CH3), 2.46–2.43 (m, 1H, H–2), 2.01–1.69 (m, 5H, CH2–3, 5 & H–4), 1.39–1.31 (m, 1H, H–4); δC (63 MHz; CDCl3) 137.92 (C Ar), 128.30 (CH Ar), 127.58 (CH Ar), 86.56 (C–1), 73.10 (C–7), 70.86 (C–6), 46.07 (C–2), 37.93 (CH3), 33.34 (C–5), 26.95 (C–3), 22.94 (C–4); m/z (EI+) 188 (M–C4H9SO3).

cis- νmax (neat)/cm⁻¹ 2958–2873 (C–H); δH (250 MHz; CDCl3) 7.42–7.32 (m, 5H, Ar), 5.19–5.16 (m, 1H, H–1), 4.57 & 4.52 (d, 2H, J=11.85Hz, CH–7), 3.67–3.54 (m, 2H, CH2–6), 2.95 (s, 3H, CH3), 2.17–1.29 (m, 7H, H–2, CH2–3, 5 & 4); δC (63 MHz; CDCl3) 137.87 (C Ar), 128.28 (CH Ar), 127.58 (CH Ar), 85.50 (C–1), 73.09 (C–7), 68.73 (C–6), 44.97 (C–2), 37.68 (CH3), 33.34 (C–5), 25.57 (C–3), 21.15 (C–4); m/z (EI+) 284 (M+), 188 (M=C4H9SO3).
3-Benzylxomethylcyclopentene 244

Benzylation of 3-hydroxymethylcyclopentene

The alcohol 245 (0.20g, 2.02mmol, 1.00eq) was dissolved in THF (7mL) and benzyl bromide (0.38g, 0.26ml, 2.22mmol, 1.10eq), followed by sodium hydride (60% dispersion in mineral oil, 0.12g, 3.00mmol, 1.49eq) and TBAI (trace). The reaction vessel was immersed in an ice bath and the reaction mixture stirred overnight. TLC (eluting with dichloromethane/hexane 1:1, anisaldehyde stain, product R_f = 0.42) indicated that starting material remained. Although extra benzyl bromide (0.02m1, 0.03mmol, 0.10eq), and sodium hydride (0.02g, 0.60mmol, 0.30eq) were added, further time (3 hours) resulted in no apparent further consumption of starting material.

Ether (5mL) was added to dilute and the resulting solution was washed with brine (5mL), saturated aqueous sodium bicarbonate (5mL) and water (5mL). The organic layer was dried (MgSO_4_) and the solvent removed by rotary evaporation. The resulting residue was purified by flash column chromatography (eluting with dichloromethane/hexane 1:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 244 (0.32g, 83%).
Elimination of mesylate

The mesylate 273 (0.91 g, 3.20 mmol, 1.00 eq) was dissolved in DCM (100 mL) and while stirring, alumina (Al₂O₃, activated, neutral, Brockmann I (standard) ca 150 mesh, 30 g) was added. Stirring was continued for 2h and TLC (eluting with dichloromethane, anisaldehyde stain, product Rₓ = 0.64) showed that the reaction had gone to completion. The solids were removed by filtration and the solvent by rotary evaporation. The resulting oil was purified by column chromatography (eluting with dichloromethane/hexane 1:1). Appropriate fractions were combined and the solvent removed by rotary evaporation to give a clear colourless oil 244 (0.46 g, 76%).

ν_max (neat)/cm⁻¹ 3047 (C=C–H), 2933 & 2857 (CH); δ_H (250 MHz; CDCl₃) 7.40–7.25 (m, 5 H, Ar), 5.83–5.78 (m, 1 H, H–2), 5.75–5.70 (m, 1 H, H–1), 4.54 (s, 2 H, CH₂–7), 3.43–3.31 (m, 2 H, CH₂–6), 3.04–2.97 (m, 1 H, H–3), 2.40–2.29 (m, 2 H, CH₂–5), 2.10–1.96 (m, 1 H, H–4), 1.64–1.27 (m, 1 H, H–4); δ_C (63 MHz; CDCl₃) 138.51 (C, Ar), 131.92 (C–2), 128.21 (C–1), 127.47 (CH Ar), 127.35 (CH Ar), 74.28 (C–7), 72.94 (C–6), 46.01 (C–3), 31.74 (C–5), 26.57 (C–4); m/z (El) 188; HRMS (FAB +ve) 188.11961 (calculated for C₁₃H₁₆O 188.12012, (Dev 2.68ppm)).
4-Benzylxymethylcyclopent-2-enol<sup>107</sup> 252

The resting cell transformation was carried out by resuspending the cell mass in one tenth growth volume of phosphate buffer (50mM, pH 7). A solution of 244 (100mg) in ethanol (1mL) was added to the resting cell culture (200mL) of *Rhodococcus rhodochrous* NCIMB 9703.

After 5 days incubation as above, the cells were removed from the cell suspension by centrifugation and the supernatant extracted into ethyl acetate. Purification by flash column chromatography gave a clear colourless oil 252 (20mg, 18% Aitken<sup>107</sup>)

Subsequent attempts failed to yield sufficient product for analysis;

δ<sub>H</sub> (360 MHz; CDCl<sub>3</sub>) 7.41–7.30 (m, 5H, Ar), 6.04 (ddd, 1H, J=0.8, 2.0, 5.6, CH–2), 5.95–5.92 (m, 1H, CH–3), 4.94–4.91 (m, 1H, CH–1), 4.55 (s, 2H, CH<sub>2</sub>–7), 3.44–3.35 (m, 2H, CH<sub>2</sub>–6), 3.27–3.19 (m, 1H, CH–4), 2.00 (ddd, 1H, J=5.0, 7.2, 14.1, CH–5), 1.91 (ddd, 1H, J=3.2, 7.7, 14.1, CH–5); δ<sub>C</sub>(90 MHz; CDCl<sub>3</sub>) 139.0 (C Ar), 137.41 (CH–2), 134.77 (CH–3), 128.80 & 128.00 (CH Ar), 77.50 (CH–1), 74.39 (CH<sub>2</sub>–7), 73.53 (CH<sub>2</sub>–6), 45.34 (CH–4), 37.89 (CH<sub>2</sub>–5); MS (EI) 204 (M+).
5. REFERENCES

REFERENCES

REFERENCES

58. H. J. Bestmann and D. Roth, Synlett, 1990, 12, 751.
REFERENCES


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REFERENCES


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REFERENCES

125. R. Stevenson, Final Year Honours Project, University of Edinburgh, 2002.
6. APPENDICES

6.1 Appendix 1

X-ray crystal structure for (1S, 2S, 3S, 4R)-1-(p-methoxy-benzyloxymethyl)-4-hydroxy-2,3-(isopropylidenedioxy)cyclopentanol 223
Crystal data and structure refinement for (1S, 2S, 3S, 4R)-1-(p-methoxybenzyl)oxymethyl)-4-hydroxy-2,3-(isopropylidenedioxy)cyclopentanol 223

Crystal data

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Data collection

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**Solution and refinement**

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Table 1. Atomic coordinates (x $10^4$) and equivalent isotropic displacement parameters (A$^2$ x $10^3$) for 223. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

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### Table 2. Bond angles for 223

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Table 2 cont. Bond angles for 223

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Table 3. Anisotropic displacement parameters ($A^2 \times 10^3$) for 223.

The anisotropic displacement factor exponent takes the form:

$$-2\pi^2 [h^2 a^* U_{11} + \ldots + 2h \times \times b^* U_{12}]$$

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6.2 Appendix 2

2D $^1$H--$^1$H nOesy 4-Benzoyloxymethylcyclopent-2-enol$^{107}$ 252