The synthesis of a differentially protected oxygenated pyrroline nitrone and its application in routes to hyacinthacine family alkaloids

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Abstract

Glycosidases are involved in a range of important biological processes. The possibility of blocking or modifying these processes using glycosidase-inhibiting sugar mimics for therapeutic or biotechnological applications has attracted much interest. This may have significance in the treatment of cancer, viral infections, diabetes and obesity.

Iminosugars are analogues of mono- or disaccharides where the ring oxygen is replaced by a nitrogen atom. These compounds include polyhydroxylated derivatives of monocyclic structures such as piperidines and pyrrolizidines, and also of bicyclic structures such as indolizidines and pyrrolizidines.

Routes to nitrone 190 from L-xylose and D-arabinose were explored and the application of nitrone 190 towards the synthesis to hyacinthacine A₁ and hyacinthacine B₂ were investigated. The differential protection of the C-3 position of nitrone 190 meant that this position would be selectively deprotected after cycloaddition, liberating the C-1 hydroxyl group (hyacinthacine numbering) as a site for inversion. Inversion of the hydroxyl group would then provide the required stereochemical outcome for hyacinthacines A₁ and B₂, where the stereochemistry at C-1 and C-2 is cis.
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<tr>
<td>AIBN</td>
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<td>TPAP</td>
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1 INTRODUCTION
1.1 Iminosugars

Iminosugars have the potential to block or modify glycosidase enzymes which are involved in a wide range of biological processes, and they have therefore attracted a great deal of interest since they were first discovered. This interest has focused on the isolation of new iminosugars, their biological activity and indeed their synthesis. This thesis describes synthetic routes towards some of the recently isolated polyhydroxylated pyrrolizidines of the hyacinthacine family via a common nitrone precursor.

1.2 Structure of iminosugars

Iminosugars are potential glycosidase inhibitors and are analogues of mono- or disaccharides in which the ring oxygen has been replaced by nitrogen. The first iminosugar, nojirimycin (1, NJ) [figure 2], was discovered in 1966\(^1\) and numerous iminosugars have since been isolated from plants and microorganisms.\(^2\) Naturally occurring sugar mimics with a nitrogen in the ring are classified into five structural classes: polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines and nortropanes, and these can therefore be grouped as monocyclic or bicyclic structures as shown in Figure 1.

![Figure 1. General structure and numbering of iminosugar core structures; the numbering applied to the piperidine and pyrrolidine compounds is adapted from carbohydrate nomenclature.](image)
1.2.1 Monocyclic structures

There are two classes of monocyclic iminosugars, piperidines and pyrrolidines, with the general structures shown in Figure 1. Nojirimycin (or 5-amino-5-deoxy-D-glucopyranose) (NJ, 1) [figure 2] is the main representative of the piperidine class of monocyclic iminosugars. It is a polyhydroxylated piperidine corresponding to glucose in the pyranose form and was first described as an antibiotic produced in bacterial cultures of *Streptomyces roseochromogenes* R-468 and *Streptomyces nojiriensis* SF-426. As a hemiacetal, NJ is an inherently unstable structure and therefore its corresponding 1-deoxy analogue, 1-deoxynojirimycin (DNJ; 2) [figure 2], was synthesised by reduction with NaBH₄ by Paulsen and coworkers. DNJ was later isolated from the roots of mulberry leaves and was called moranoline. DNJ is also produced by many strains of the *Bacillus* and *Streptomyces* genera. DNJ is related to another natural product, 1-deoxymannojirimycin (DMJ, 3) [figure 2], where the hydroxyl group at C-2 has the opposite stereochemistry, mimicking the pyranose form of D-mannose. DMJ was found to be produced by *Streptomyces lavendulae* SF-425 and has recently been extracted from *Adenophora triphylla*. A number of other piperidine iminosugars have since been isolated from a range of plants and bacterial cultures and these are shown in Figure 2. Iminosugars with a hydroxyl group at C-1, nojirimycin B (manno-NJ, 4) and galactostatin (galaco-NJ, 5), were also isolated from species of *Streptomyces*. Fagomine, 1,2-dideoxynojirimycin (6) has been isolated from the seeds of Japanese buckwheat (*Fagopyrum esculentum*) as well as the Moreton Bay Chestnut and *Castanospermum australe*. The search for iminosugars continued and 3-epi-fagomine (7) and 3,4-di-epi-fagomine (8) were isolated from the leaves and roots of *X. zambesiaca*. Fagomine (6) and 6-deoxyfagomine (9) have been isolated from the roots of *Lycium Chinese* (Solanaceae) and 2S-carboxy-3R,4R,5S-trihydroxypiperidine (10) was isolated from the seeds of the legume *Baphhia racemosa*. The first naturally occurring DNJ derivative with a carbon substituent at C-1, α-homonojirimycin (α-HNJ, 11), was isolated from *O. diandra* and more recently has been isolated from the bulbs of *Hyacinthus orientalis* (Hyacinthaceae). The isomers and glycosides of α-HNJ such as β-homonojirimycin (β-HNJ, 12), α-homomannojirimycin (13), β-homomannojirimycin (14), α-homoallonojirimycin (15) and β-homoaltronojirimycin (16) are also produced by these plants. More recently, in 2002, piperidine iminosugars were isolated from the bulbs of *Scilla sibirica* including two new compounds, α-7-deoxyhomonojirimycin (17) and α-7-deoxyhomomannojirimycin (18).
Polyhydroxylated pyrrolidines resemble sugars in the furanose form and can be potent inhibitors of the corresponding glycosidases specific for carbohydrates with a matching pattern of hydroxyl substitution and stereochemistry. Some of the naturally occurring pyrrolidines are shown in Figure 3.

In 1976, 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP, 19), a naturally occurring analogue of β-D-fructofuranose was found in the leaves of the legume, *Derris*

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**Figure 2.** Naturally occurring piperidines
This compound is a very common metabolite as it has since been isolated from various other plants and microorganisms. A second example of a pyrrolidine analogue is 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1, 20), which resembles 19 but has only one hydroxymethyl group. D-AB1 was initially isolated from the fruits of *Anglyocalyx boutiqueanus* but, like DMDP, has since been isolated from different species of plants.  

**Figure 3.** Naturally occurring pyrrolidine iminosugars

The 6-deoxy derivative of DMDP (6-deoxy-DMDP, 2,5,-imino-1,2,3-trideoxy-D-mannitol, 21) was isolated from the seeds of *Anglyocalyx pynaertii*. The roots of *Morus* spp have been found to produce the 2-epimer of D-AB1, 1,4-dideoxy-1,4-imino-D-ribitol (22) and polyhydroxypyrrolidine nectrisine (23) was isolated from a broth culture of the fungus *Nectria Ludica*. The first naturally occurring 2,5-dideoxy-2,5-iminoheptitol, 2,5-dideoxy-2,5-imino-DL-glycero-D-manno-heptitol (homoDMDP, 24) was found in the leaves of bluebell (*Hyacinthoides non-scripta*). More recently, homoDMDP has been
found in the bulbs of hyacinths (*Hyacinthus orientalis*) along with 2,5-imino-2,5,6-trideoxy-\(\text{d-manno}\)-heptitol (6-deoxy-homoDMDP, 25) and 2,5-imino-2,5,6-trideoxy-\(\text{d-gulo}\)-heptitol (26). In 2002, homo-DMDP (24), 6-deoxy-homo-DMDP (25) and 2,5-imino-2,5,6-trIDEOXY-\(\text{d-gulo}\)-heptitol (26) were isolated from *Scilla sibirica* along with four new pyrrolidines (27-30). These new pyrrolidines were determined to be 7-deoxy-homoDMDP (2,5-imino-2,5,7-trIDEOXY-\(\text{glycero-d-manno}\)-heptitol 27), 2,5-dideoxy-2,5-imino-\(\text{glycero-d-galacto}\)-heptitol (28), 4-\(\text{O-\(\beta\)}\)-\(\text{d-mannopyranosyl}\)-6-deoxy-homoDMDP (29), and 4-\(\text{O-\(\beta\)}\)-[\(\text{d-mannopyranosyl-(1→4)}\]-\(\text{d-mannopyranosyl}\)]-6-deoxy-homoDMDP (30). Three further pyrrolidines (31-33) with longer side chains were isolated from the bulbs of *Scilla peruviana*.21 These \(\text{D-AB1}\) derivatives were found to possess, respectively, 2-hydroxypropyl, 1,2-dihydroxypropyl and 1,5,7,12,13-pentahydroxytridecyl side chains at the C-1\(\alpha\) position and were therefore identified as \(\alpha-1-\text{C-(1,5,7,12,13-pentahydroxytridecyl)-D-AB1}\) (31), \(\alpha-1-\text{C-(1,2-dihydroxypropyl)-D-AB1}\) (32) and \(\alpha-1-\text{C-(2-hydroxypropyl)-D-AB1}\) (33).
1.2.2 Bicyclic structures

There are three classes of natural bicyclic iminosugars: indolizidines, pyrrolizidines and nortropanes, which have the general structures as shown in Figure 1. The first bicyclic iminosugar, an indolizidine, was isolated from a legume called Swainsona canscens in 1979 and was named swainsonine (34) [figure 4]. It has since been isolated from locoweeds (Astragalus and Oxytropis species) which cause the disorder ‘locoism’ in animals. Another similar alkaloid, castanospermine (35) [figure 4], was later isolated from the seeds of the tree Castanospermum australe. It was found to share the same core indolizidine structure as 34 but differs in the substitution pattern of the hydroxyl groups. The seeds of Castanospermum australe were also found to produce 6-epi-castanospermine (36), 7-deoxy-6-epi-castanospermine (37) and 6,7-di-epi-castanospermine (38) the structures of which are shown in Figure 4. The leaves of Astragalus Lentiginous produce lentiginosine (39) and 2-epi-lentiginosine (40).

![Figure 4. Naturally occurring indolizidine iminosugars](image)

The first polyhydroxylated pyrrolizidines, alexine (41) [Figure 5] and australine (or 7a-epi-alexine, 42) [Figure 5] were isolated in 1988 from the legumes, Alexa leipetala and Castanospermum australe. These structures differ from the long known necine bases, pyrrolizidine alkaloids, with a hydroxymethyl side chain at the C-1 position. Castanospermum australe was subsequently found to be a valuable source of other pyrrolizidines bearing a carbon branch at the C-3 position, with 3,7-epi-alexine (43), 1,7a-di-epi-alexine (44), and 7,7a-di-epi-alexine (45), the structures of which are shown in Figure 5, all being isolated from this source. The pentahydroxylated indolizidine, casuarine (46) [Figure 5], which has been used for the treatment of cancer in Western Samoa, has been isolated from the bark of Casuarina equistifolia (Casuarinaceae) and
from the leaves of *Euginia jambolana* (Myrtaceae). The first naturally occurring polyhydroxylated pyrrolizidinecarboxylic acid, 7a-epi-alexaflorine (47) [Figure 5], which carries a carboxyl group at C-3, was found in the leaves of *Alexa grandiflora*.

A large family of hydroxylated pyrrolizidine alkaloids, that have a carbon branch at C-3 or both C-3 and C-5 have been isolated from plants belonging to the hyacinthaceae family (Figure 6); these are collectively known as hyacinthacines. Hyacinthacine B$_1$ (48), hyacinthacine B$_2$ (49) and hyacinthacine C$_1$ (50) were the first compounds to be discovered in this group and were isolated from *Hyacinthoides non-scripta* and *Scilla campanulata*. Four further hyacinthacines, A$_1$ (51), A$_2$ (52), A$_3$ (53), and B$_3$ (54), were subsequently isolated from the bulbs of *Muscari armeniacum* in 2000 along with the known hyacinthacine C$_1$ (50). In 2002, seven more hyacinthacines were isolated from the bulbs of *Scilla sibirica*—hyacinthacines A$_4$ (55), A$_5$ (56), A$_6$ (57), A$_7$ (58), B$_4$ (59), B$_5$ (60) and B$_6$ (61). Four new pyrrolizidines, with longer side chains at the C-5 position, were isolated from *Scilla peruviana* in 2004. These were, α-5-C-(3-hydroxybutyl)-7-epi-aurasline (62), α-5-C-(3-hydroxybutyl)hyacinthacine A$_1$ (63), α-5-C-(1,3-dihydroxybutyl)hyacinthacine A$_1$ (64), and α-5-C-(1,3,4-trihydroxybutyl)hyacinthacine A$_1$ (65). Finally, in 2007, an examination of the bulbs of *Scillia solcialis* resulted in the
isolation of eleven hyacinthacines, including six new compounds, hyacinthacine B\textsubscript{7} (66), C\textsubscript{2} (67), C\textsubscript{3} (68), C\textsubscript{4} (69), C\textsubscript{5} (70), and α-5-C-(3-hydroxybutyl)hyacinthacine A\textsubscript{2} (71).\textsuperscript{30} The known hyacinthacines A\textsubscript{3} (53), A\textsubscript{5} (56), B\textsubscript{3} (54), and B\textsubscript{4} (59) were also found in *Scillia solcialis*. 
Figure 6. Hyacinthacine family of polyhydroxylated pyrrolizidines arranged according to hyacinthacine letter grouping; A, B or C.

The name hyacinthacine is given to polyhydroxylated pyrrolizidines bearing a hydroxymethyl group at the C-3 and/or C-5 positions. These are assigned a letter and a number; the letter corresponds to the number of hydroxyl groups present and is related to
the mass of the compound. The hyacinthacine A group have 3 hydroxyl groups, the B class have 4 hydroxyl groups and the C have 5 hydroxyl groups. Numbers are then used to distinguish between compounds within the same class and are assigned in order of discovery.

Polyhydroxylated nortropanes were the final class of naturally occurring bicyclic iminosugars to be discovered when calystegines were found to be secondary metabolites in plants and were implicated in the maintenance of plant-bacterium relationships. A shown in Figure 7, Calystegines have a nortropane ring system, two to four hydroxyl functions of varying stereochemistries and position and a tertiary alcohol group at the bridgehead carbon C-1 of the bicyclic ring. The first calystegines, A₁ (72), B₁ (73) and B₂ (74), were extracted from root cultures of C. sepium and were classified into two groups, calystegines A and B, on the basis of their relative mobilities on paper electrophoresis. Examination of M. bombycis and M. alba both of the Moraceae family resulted in the discovery of calystegines B₂ and C₁ (75). Later, calystegines A₃, A₅ (76), B₁, B₂, and B₃ (77) were isolated from the roots of Physalis alkekengi var. francheti. Calystegines A₃, B₁, and B₂ have now been found in the Solanaceae plant family genera Atropa, Datura, Duboisia, Hyoscyamus and Scopolia. Low amounts of calystegines were detected in Datura wrightii leaves and D. stramonium roots and leaves. Calystegines A₅, A₆ (78), B₁, B₂, B₃, and N₁ (79) were subsequently isolated from Hyoscyamus niger. Calystegines A₃, A₅, B₁, B₂, B₃, B₄ (80), C₁ and calystegines B₁, B₂, B₄, C₁, and C₂ (81) were found in Scopolia japonica and in Duboisia leichhardtii respectively. More recently, new calystegines, A₈ (82) and B₆ (83), were isolated from H. niger and S. Japonica and it was discovered that Lycium chinense produced N-methyl-calystegines B₂ (84) and C₁ (85), and calystegines A₇ (86) and B₅ (87). Edible fruits and vegetables have also been found to be a source of calystegines; they have been detected in plants of the Convolvulaceae, Solanaceae and Moraceae families that include sweet peppers, chilli peppers, potatoes, eggplants, tomatoes, physalis fruits, sweet potatoes and mulberries.
Figure 7: Structures of polyhydroxylated nortropanes.
1.3 Biological activity of iminosugars

Iminosugars possess inhibitory activity against many of the glycosyltransferases and glycosidases that mediate, respectively, the biosynthesis and degradation of carbohydrate polysaccharides and glycoconjugates (glycoproteins, glycolipids, proteoglycans) in nearly all life forms. The first example involved the first isolated iminosugar NJ (1) which was reported to inhibit both α-glucosidases and β-glucosidases. The reduced form of NJ, DNJ (2), exhibited activity against glycosidases I and II, whereas DMJ (3) and its bicyclic counterpart Swainsonine (34) were reported to inhibit lysosomal α-mannosidases. Castanospermine (35) has been shown to be a powerful inhibitor of the α- and β-glucosidases in the mammalian gut. Australine was found to be a good inhibitor of the α-glucosidases, amylglucosidase and subsequently the glycoprotein enzyme glucosidase I.

Unsurprisingly, the inhibition of glycosidase and glycosyltransferase enzymes can affect the digestion and metabolism of polysaccharides as well as the maturation, transportation, and secretion of glycoconjugates. Glycoconjugates expressed at the cell surface – often possessing complex, branched extracellular carbohydrate structures – are involved in several fundamental biological functions such as cell-cell recognition, cell adhesion and signalling. The role of these biomacromolecules in cell differentiation, immune response, oncogenesis, tumor metastasis and viral infections has prompted considerable interest in iminosugars as a class of compounds for therapeutic intervention, with the emphasis of on their potential applications in the treatment of diabetes, cancer and viral disease.

1.3.1 Inhibition of digestive glycosidases

Digestive glycosidases, located in the small intestine, are enzymes that hydrolyze dietary carbohydrates to monosaccharides, which are then absorbed through the intestine wall. It was thought that treatment of non insulin dependent diabetes (type II diabetes) could be achieved by means of blocking these enzymes, thus regulating the absorption of carbohydrates through the intestine wall.

The original discovery of DNJ (2) resulted from investigations prompted by the knowledge that extracts of mulberry were able to suppress the rise in blood glucose that
follows eating, suggesting that this activity could be beneficial for diabetes treatment.\textsuperscript{15} DNJ (2) was subsequently found to have a good inhibitory effect on mammalian $\alpha$-glucosidase \textit{in vitro} and was thus considered to be a promising agent for treatment of diabetes.\textsuperscript{46} Unfortunately, the compound lacked efficacy \textit{in vivo} for this clinical indication but these studies stimulated the synthesis and evaluation of many derivatives of DNJ and $N$-alkylated analogues, such Migitol (88) (BAY m109), were subsequently found to be potent inhibitors of the glycogenolysis induced by glucagen in studies with hepatocytes.\textsuperscript{47} Migitol (GLYSET\textsuperscript{TM}) is now used in the treatment of type II diabetes and is available in the USA and Canada.

In type II diabetes, an increase in hepatic glucose production and blood glucose levels is observed. Inhibiting hepatic glycogen phosphorylase could prevent this from occurring. Isofagomine (89) [Figure 8] was recently found to be a good inhibitor of liver glycogen phosphorylase, blocking glycogen degradation in hepatocytes.\textsuperscript{48} The specificity of iminosugars is well illustrated by the fact that some $N$-alkylated derivatives of isofagomine (89) are active inhibitors at micromolar concentrations, whereas fagomine (6) and DNJ (2) lack activity (Figure 8).\textsuperscript{49}

![Figure 8: Structure showing specificity of inhibitory action on digestive glucosidase.](image)

1.3.2 Inhibition of lysosomal glycosidases

Disorders in the biosynthesis or catabolism of glycolipids in the cell have an impact on so called lysosomal storage diseases like type I Gaucher disease or Fabry disease.\textsuperscript{50} In normal cells there is a balance between the degradation of glycosphingolipids (GSLs) in the lysosome and their biosynthesis in the endoplasmic reticulum (ER)/Golgi system. The rates of influx of GSLs and efflux of metabolites are in equilibrium. In a lysosomal storage cell, enzyme activity in the lysosome is so low that GSLs accumulate. However,
although the catalytic activity of enzymes is reduced, it is not totally eliminated. Thus, drugs that could regulate the biosynthesis of GSLs to a concentration that fits well in the residual enzymic activity could prevent storage. Studies have thus been carried out with N-alkylated DNJs, which are inhibitors of ceramide-specific glycosyltransferases, and N-butyl DNJ (90) [Figure 9], marketed as Zavesca®, is now used in the treatment of type 1 Gaucher’s disease.

![N-butyl DNJ](image)

**Figure 9: N-butyl DNJ**

The discovery that swainsonine (34) can induce a reversible phenocopy of the genetic lysosomal storage disease, mannosidosis, in animals has led to the use of chemically induced deficiencies of lysosomal hydrolases as models for studying the pathogenesis of lysosomal storage disease. Mannosidosis is characterised by the accumulation in cells, and excretion in urine, of mannose rich oligosaccharides resulting from a deficiency of lysosomal α-mannosidase. Treatment with swainsonine (34) is particularly effective in mimicking lysosomal storage disease because of the compound’s potent inhibitory activity against lysosomal α-mannosidase and its lysosomotropic behaviour. It is a weak base with a $pK_a$ of 7.4, which ensures that it is taken up rapidly into cells by permeation and, once inside the lysosomes, becomes protonated and becomes concentrated. Swainsonine (34) has since been granted orphan drug status for the treatment of Gaucher disease.

Seeds containing castanospermine (35) have been reported to be toxic to animals and cause various symptoms, including gastrointestinal upset. Castanospermine (35) also disturbs lysosomal catabolism in animals, in this case glycogen and glucolipids by inhibition of lysosomal α-glucosidases or β-glucosidases. Its physiological effects therefore result in symptoms that resemble the genetic disorders of Pompe’s and Gaucher’s disease. The $N$-hydroxyethyl derivative of DNJ also induces the accumulation of glycogen, as demonstrated in normal human fibroblasts and the polarized HepG2 cells. It also delays the processing of the ER α-glucosidases I and II. Thus, lysosomal storage diseases can be induced and exacerbated by the effects of the inhibitory
action of iminosugar inhibitors on processing glycosidases. For this reason, attempts to harness iminosugar glycosidase inhibitors therapeutically to alter the glycosylation of proteins, or inhibit intestinal carbohydrate digestion carry the risk of inducing lysosomal storage as a chronic complication. There is some evidence, however, that the doses of inhibitor used effectively in experimental therapies may be too low to induce lysosomal storage.55

1.3.3 Processing glycosidases

The α-D-glucosidases and α-D-mannosidases involved in the post-translational processing of asparagine-linked glycans of glycoproteins can be selectively inhibited by various iminosugars.56 Using these inhibitors, the consequences of altering the glycosylation of a particular glycoprotein or the glyctype of cells can in a defined way be studied. Indeed, castanospermine (35), DMJ (3) and swainsonine (34), the structures of which are shown in Figure 10 have become the standard commercially available reagents used in biochemical studies for inhibition of α-glucosidases I, α-mannosidases I and α-mannosidases II. These compounds are not completely specific, however, and they do not inhibit processing unless they are present in relatively high concentrations, which in itself increases lack of specificity. Consequently, there is current interest in the discovery of more specific glycosidase inhibitors and this has prompted structure activity relationship studies. For example, N-methyl-DNJ (91) [Figure 10] has been found to be a poorer inhibitor of α-glucosidases I than DNJ (2) [Figure 10] and conversely for α-glucosidases II. Castanospermine also exhibited a similar inhibition of α-glucosidases I to N-methyl-DNJ (91) [Figure 10] suggesting that substitution of the piperidine nitrogen favours inhibition of α-glucosidases I.
Introduction

DMJ (3) was found to inhibit α-mannosidase I, which is particularly susceptible to iminosugar pyranose analogues. Although swainsonine (34) has been shown to be a potent inhibitor of α-mannosidase II, it does not inhibit other processing α-mannosidases in human cell cultures. The specificity of synthetic iminosugars for the various processing glycosidases has not yet been exhaustively evaluated, but the available information suggests that many are more specific \textit{in vitro} than their parent compounds. The effect of the common glycoprotein processing inhibitors on the biosynthesis, intracellular transport and function of most well characterised glycoproteins has been thoroughly evaluated however. Indeed, their effects on many cellular processes have been evaluated, but perhaps the two areas of most excitement and interest relate to anti-cancer and antiviral applications.

1.3.4 Anti-cancer activity and anti-viral activity

Both catabolic and processing glycosidases are involved in oncogenic transformation of cells as well as tumour cell invasion and migration behaviour that underpins metathesis. It has also been known for some time that levels of glycosidases are elevated in patients with various types of tumours. Significantly, castanospermine (35) and swainsonine (34) have been reported to inhibit metastasis, and this activity was attributed to their glycosidase-inhibitory activity.²

Host cell infection by the Human Immunodeficiency Virus (HIV) is initiated by interaction between gp-120, a glycoprotein peplomer on the HIV virion, and CD-4 on human lymphocytes. Gp-120 is generated in the Golgi apparatus together with a transmembrane glycoprotein, gp-41, by endoproteolytic cleavage of a precursor protein,
gp-160. Both gp-41 and gp-120 are highly N-glycosylated, with most of the glycans belonging to high mannose complex types. Indeed, gp-120 has 20 to 25 N-glycan linkable sites, and the total carbohydrate content comprises around 50% of the total molecular weight for the complete glycoprotein. Perturbation of viral glycoprotein processing could be an attractive target for chemotherapy to treat HIV, and some inhibitors of processing glycosidases I and II, such as castanospermine (35) and DNJ (3), are known to decrease the efficiency of HIV infection. Castanospermine (35), DMDP (19) and swainsonine (34) have also been reported to prevent the processing of the influenza viral glycoprotein (hemagglutinin) that is responsible for adsorption of the influenza virion to host cells. One of the most promising compounds in this class to date is N-butyl DNJ (Zavesca®, 90) which has shown good antiviral activity and low cell toxicity.

1.4 Enzyme mechanisms and structure activity relationships

Iminosugars are known to inhibit glycosidase enzymes and other carbohydrate-recognising proteins. It is generally accepted that pyranose hydrolysis catalysed by such carbohydrate processing enzymes (glycosidases and glycosyl transferases) proceeds through a half chair transition state with a substantial sp² character at the anomeric carbon. This reaction can occur with one of two stereochemical outcomes, inversion or retention of configuration, leading to two different mechanisms for the hydrolysis of a glycosidic bond (Figure 11). Both of these enzymic mechanisms involve a key oxonium intermediate or transition state (TS) with carbocation character that is generated by the action of acidic residues in the enzyme’s catalytic pocket. In the hydrolysis catalyzed by inverting glycosidases, one acidic residue acts as a general acid, facilitating the oxocarbenium-like intermediate formation, while a second (in the carboxylate state) acts as a general base and assists nucleophilic delivery of a water molecule to the anomeric carbon thus producing the glycoside with inversion of anomeric configuration (Fig. 11A). Retaining glycosidases convert the glycoside to glycoside with retention of the anomeric configuration. Instead of suffering direct nucleophilic attack by water, the oxocarbenium-like intermediate is stabilized by the carboxylate prior to its displacement from the anomeric centre by water (Fig. 11B). This double inversion at the anomeric centre leads to the retention of anomeric configuration.
Figure 11: Inverting (A) and retaining (B) glycosidase mechanisms.

The basic nitrogen atom of iminosugars may be protonated making these compounds analogues of the transition state oxonium ion.\(^{66}\) This is the key factor that underpins the capacity of iminosugars to bind to and to inhibit glycosidase and other carbohydrate processing enzymes.

Perhaps surprisingly the importance of shape, pattern of hydroxyl substitution and stereochemistry to an iminosugar’s selectivity is not always easily predictable.\(^{67}\) It was initially thought that inhibitors of glucosidases and mannosidases should have structures closely resembling glucose and mannose respectively. For example DNJ (2) has a structure similar to that of glucose and DMJ (3) has a structure similar to mannose, and indeed these compounds are known to inhibit \(\alpha\)-glucosidase and \(\alpha\)-mannosidase respectively, demonstrating that the differing stereochemical arrangement of the hydroxyl groups plays a key role in controlling inhibitory selectivity. This argument often holds for glucosidase inhibitors, which typically do have structures with hydroxyl substitution patterns similar to glucose.\(^{68}\) However, close mimicry between the structure of an inhibitory iminosugar and the enzyme’s substrate is not always seen. For example, 6-epi-castanospermine (36) has a structure closely resembling the D-mannosyl cation (92), but was found to inhibit \(\alpha\)-glucosidases and not the expected \(\alpha\)-mannosidase.\(^{64}\) With the exception of DMJ (3), \(\alpha\)-D-mannosidase inhibitors in reality generally do not have structures that closely resemble mannose. For example, swainsonine (34) exhibits potent inhibition of \(\alpha\)-mannosidases although its structural resemblance to \(\alpha\)-D-mannose is not
obvious. Winkler and Holan were able to offer an explanation for these inconsistencies based on the results of molecular orbital calculations and structure-activity relationship studies with $\alpha$-D-mannose analogues and mannosidase inhibitors. These calculations and studies showed that there are two optimized half chair geometries of the mannosyl cation, a “flap up” form ($93\,\text{D-}\text{mannosyl cation}$) and the “flap down” form ($94\,\text{D-}\text{mannosyl cation}$), the former being the lower in energy [Figure 12]. This study showed that the best inhibitors resembled the “flap up” half chair geometry of the mannosyl cation. This model was also able to rationalize why 6-$\text{epi}$-castanospermine (36), which superficially resembles mannose, is actually a poor mannosidase inhibitor, it has the incorrect ring configuration for good superimposition onto the mannosyl cation in the “flap up” half chair configuration. 

Figure 12: Structural resemblance between selected iminosugars and carbohydrate cations

Nevertheless, in many cases, further exploration is required to establish a proper understanding of structure activity relationships for carbohydrate processing enzymes. Thus, for example, the basis for selective mannosidase-inhibitory activity of protonated
DNJ (95) remains unclear since this compound does not appear to closely mimic the structure of the “flap up” mannosyl cation (93). A possible explanation might be that binding in the mannosidase catalytic pocket is in this case dominated by ionic interactions between the protonated DNJ structure and the catalytic acidic residues lining the pocket.71

Given the promising enzyme inhibitory activity of iminosugars in general, considerable effort has been made over the last three decades to develop efficient synthetic routes to these compounds. This work has been prompted both by the need to provide material for biological assessment and clarification of structure activity relationships and also in the hope that synthesis of new structures might provide compounds with improved activity and selectivity.

1.5 Synthetic routes to hyacinthacines

There are many different methods for the synthesis of bicyclic polyhydroxylated alkaloids and indeed there are many examples to be found in the literature. General methods for building polyhydroxylated pyrrolizidines, indolizidines, quinolizidines and nortropanes have been reviewed by Lopez et al.72 This review classifies the general routes into three categories of cyclisation based on the key step in forming the bicyclic ring; these are: 1) standard cyclisation, 2) cycloaddition and 3) ring closing metathesis. In a review by Michael, the synthetic methods for producing polyhydroxylated indolizidines and quinolizidines have been discussed.73 El Nemr has also reviewed synthetic routes to polyhydroxylated indolizidines, with comprehensive coverage of synthetic approaches to swainsonine and its analogues.74 Given the vast number of reports concerning the synthesis of bicyclic iminosugars, only synthetic approaches to the more relevant pyrrolizidines will be discussed here.

A number of synthetic strategies have been devised by several groups for the preparation of hyacinthacines and these can be classified by the cyclisation method used. Routes using reductive cyclisation, ring closing metathesis (RCM), nucleophilic substitution, nitrone umpolung and nitrone 1,3-dipolar cycloaddition as the key reactions have all been applied (Figure 13), with starting materials derived from a carbohydrate-based chiral pool.
1.5.1 Reductive cyclisation

A series of hyacinthacines prepared by reductive cyclisation of pyrrolidine precursors have been reported by Izquierdo et al. In their first report, the Izquierdo group described the application of pyrrolidine derivative 96 for the synthesis of 7a-epi-hyacinthacine A₂ (97, Scheme 1) and 5,7a-diepi-hyacinthacine A₃ (98, Scheme 2).\(^7\)
Scheme 1: (a) TPAP, NMO, CH₂Cl₂, 4 Å MS, (95%); (b) Ph₃P=CHCHO, CH₂Cl₂, (44%); (c) H₂, 10% Pd/C, (45%); (d) TBAF, (59%); (e) H₂, 10% Pd/C, MeOH, HCl, (93%); (f) MeOH, Amberlite IRA-400 (OH⁻ form), (76%).

The pyrroloidine precursor (96) was prepared from D-fructose in 11 steps. Oxidation of the primary alcohol group catalysed by TPAP produced aldehyde 99. Wittig olefination of the aldehyde with (triphenylphosphoranylidene)acetalddehyde produced the homologated E-α,β-unsaturated aldehyde (100), which after hydrogenation gave saturated aldehyde 101. Intramolecular condensation to the bicyclic enamine (102), and further hydrogenation produced the pyrrolizidine (103). Removal of the TBDPS group to gave 104 and this was followed by further hydrogenation to give the hydrochloride salt of 7a-epi-hyacinthacine A₂ (105), which was converted into the free base (97) by treatment with a basic resin.

Pyrrolidine 96 was also used in similar chemistry to produce 5,7a-diepi-hyacinthacine A₃ (98) (Scheme 2). Again the route began with oxidation of the primary alcohol using TPAP to produce aldehyde 99. Wittig olefination of the aldehyde, this time with 1-triphenylphosphoranylidene-2-propanone instead of (triphenylphosphoranylidene)
acetaldehyde, produced ketone 106. The final steps in the route were identical to those applied in Scheme 1. Thus, after hydrogenation 106 furnished saturated ketone 107 and cyclisation delivered the bicyclic enamine (108). Further hydrogenation produced the pyrrolizidine (109) and the route was completed by the removal of the TBDPS group with TBAF, affording 110, followed by further hydrogenation under acidic conditions to obtain the target as a hydrochloride salt (110). The latter was again converted into the free base, 5,7a-diepi-hyacinthane A3 (98) by treatment with the basic resin. High stereoselectivity was observed in the key hydrogenation step that generated the pyrrolizidine core in intermediate 91 and this was attributed to delivery of hydrogen from the least hindered convex face of the bicyclic enamine 90.

Scheme 2: (a) TPAP, NMO, CH₂Cl₂, 4 Å MS (85%); (b) Ph₃P=CHCOCH₃, CH₂Cl₂ (57%); (c) H₂, 10% Pd/C (60% of 109 from 106); (d) TBAF; (85%) (e) H₂, 10% Pd/C, MeOH, HCl; (f) Amberlite IRA-400 (OH⁻ form) (83% of 98 from 110).

The Izquierdo group then applied the methodology developed in Schemes 1 and 2 to the synthesis of hyacinthane A₃ (53, Scheme 3)⁷⁷ and hyacinthane A₂ (52, Scheme 4).⁷⁸ Access to these members of the hyacinthane family required a modified pyrrolidine precursor however, and the group developed a suitably protected 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) derivative (112) from D-fructose for this purpose. The key
difference in the pyrrolidine precursor (112) used for synthesis of hyacinthacines A₃ and A₂ was the switch in the stereochemistry at the C-2 centre bearing the hydroxymethyl side arm. Additionally N-Boc protection rather than Cbz protection was applied in the route, but in other respects the steps were essentially the same as those applied previously in Schemes 1 and 2. Accordingly, the route to hyacinthacine A₃ started with the TPAP-catalysed oxidation of pyrrolidine 95, producing aldehyde 113. Wittig olefination of aldehyde 113 with 1-triphenylphosphoranylidene-2-propanone gave unsaturated ketone 114, and subsequent hydrogenation in the presence of hydrochloric acid produced the fully deprotected, saturated ketone (115) after treatment with a basic resin. This was then subjected to a further hydrogenation to achieve a reductive cyclisation via enamine 116. Hydrogenation from the less hindered β-face of 116 proceeded with very high steroselectivity to complete the first synthesis of hyacinthacine A₃ (53).

Scheme 3: (a) TPAP, NMO, CH₂Cl₂, 4 Å MS (90%); (b) Ph₃P=CHCOCH₃, toluene (100%); (c) H₂, 10% Pd/C, MeOH, HCl then Amberlite IRA-400 (OH⁻ form); (d) H₂, 10% Pd/C, MeOH, HCl then Amberlite IRA-400 (OH⁻ form), (68% of 53 from 114).

Shortly after disclosing the synthesis of hyacinthacine A₃, Izquierdo et al. described the application of the DMDP derivative 112 in the synthesis of hyacinthacine A₂ (52, Scheme 4).⁷⁸ A very similar strategy was used but aldehyde 113 was homologated by olefination with (triphenylphosphoranylidene)acetaldehyde to give 117. A first hydrogenation under neutral conditions, reduced the alkene side chain only. Deprotection of the O-TBDPS and N-Boc groups was achieved simultaneously with hydrochloric acid. A second hydrogenation, under acidic conditions, effected the reductive cyclisation and O-
debenzylation, with hyacinthacine A₂ (52) isolated in the free base form after treatment with basic resin.

Scheme 4: (a) TPAP, NMO, CH₂Cl₂, 4 Å MS; (b) Ph₃P=CHCHO, toluene (57%); (c) H₂, 10% Pd/C; (d) HCl, then Amberlite IRA-400 (OH⁻ form); (e) H₂, 10% Pd/C, MeOH, HCl, then Amberlite IRA-400 (OH⁻ form) (26% of 52 from 113).

This methodology – homologation of a suitable pyrrolidinecarbaldehyde followed by Wittig olefination and then reductive cyclisation – has subsequently been applied to the synthesis of many other pyrrolizidines. Thus, Izquierdo *et al.* have developed routes to 3-epi-hyacinthacine A₃ (120) and 3-epi-hyacinthacine A₂ (121),⁷⁹ and three dihydroxypyrrolizidines (122, 123 and 124) (Figure 14).⁸⁰ Synthesis of casuarine (46), 6,7-diepi-casuarine (125),⁸¹ 3-epi-hyacinthacine A₅ (126) and (+)-3-epi-hyacinthacine A₅ (127),⁸² (+)-5-epi-hyacinthacine A₅ (128) and ent-3-epi-hyacinthacine A₄ (129)⁸³ have also been described.
The earlier routes investigated by the Izquierdo group all targeted polyhydroxylated pyrrolizidines with *trans* relative stereochemistry across the C-1 and C-2 hydroxylated centres. A number of their later examples, as illustrated in Figure 14, possessed *cis*-1,2 relative stereochemistry. Access to these compounds was achieved by essentially the same pyrrolidinecarbaldehyde homologation and reductive cyclisation strategy as described previously but required a suitably protected pyrrolidinecarbaldehyde precursor embodying the desired *cis* stereochemistry. The approach is illustrated for the group’s recent synthesis of hyacinthacine A$_1$ (51) and hyacinthacine A$_6$ (57) (Scheme 5).$^{84}$ These two members of the alkaloid family were prepared from a common pyrrollidine derivative (130) possessing three orthogonal protecting groups synthesised in multiple steps from D-fructose.$^{84,85}$ Protection of the free hydroxymethyl side chain by benzylation followed by desilylation to remove the TBDPS protecting group exposed the required hydroxymethyl group for subsequent TPAP oxidation. This afforded the key pyrrolidinecarbaldehyde precursor (133). The latter was developed into hyacinthacine A$_6$ (57) by similar chemistry to that shown in Schemes 2 and 3 through homologation by Wittig olefination with 1-
triphenylphosphoranylidene-2-propanone followed by reductive cyclisation. In this case, an extra step was required to remove the benzoate protection and unmask the primary alcohol, and this was achieved by treatment with sodium methoxide in methanol following the reductive cyclisation and prior to hydrogenolysis of the benzyl groups.

The synthesis of hyacinthacine A₁ (51) (Scheme 5) was accomplished through a variation of the reductive cyclisation strategy previously discussed. Thus, pyrrolidincaraldehyde 133 was homologated to afford the α,β-unsaturated ester (134) rather than an aldehyde or ketone. Mild hydrogenation under neutral conditions selectively reduced the side chain alkene group as previously described to give saturated ester 136. N-Boc deprotection was achieved with trifluoroacetic acid, neutralising with sodium methoxide. The resulting intermediate was cyclised with concomitant O-debenzylation upon further treatment with sodium methoxide in boiling methanol, giving lactam 138. The latter was reduced with borane dimethylsulfide complex in THF and O-debenzylation, to unmask the alcohols at C-2 and C-3, was then carried out by hydrogenation under acidic conditions to complete the synthesis.
Scheme 5: (a) BzCl, CH₂Cl₂, TEA (78%); (b) TBAF, THF (88%); (c) NMO, TPAP, CH₂Cl₂, 4 Å MS (59%); (d) Ph₃P=CHCOMe, toluene, 80 °C (71%); (e) Ph₃P=CHCO₂Me, toluene, 80 °C (59%); (f) H₂, 10% Pd/C, MeOH; (g) i. TFA, DCM, ii. neutralization with MeONa in MeOH, iii. MeONa (cat.), MeOH, Δ to cyclise lactam (86% from 133); (h) H₂B.SMe₂, THF then MeOH (59%); (i) H₂, 10% Pd/C, MeOH, HCl then Amberlite IRA-400 (OH⁻ form) (93%).

In the preceding examples all of the pyrrolizidines were derived from pyrrolidine precursors that used the same protecting group for the alcohols that track through to the C-1 and C-2 positions of the target pyrrolizidine. This meant that the correct stereochemistry at these centres had to be included in the pyrrolidinecarbaldehyde on which a route was to be based. Greater synthetic versatility might be achieved if these alcohols carried distinct protecting groups, thereby allowing their separate manipulation.
after construction of the pyrrolizidine core. For example, deoxygenation or stereochemical inversion might be applied at one or other of the C-1 and C-2 positions in principle. With these considerations in mind, the Izquierdo group have recently evaluated a differentially protected analogue (139) of pyrrolidine 96 (Schemes 1 and 2) and utilized it to produce both hyacinthacine A_7 (58) and 1-epi-hyacinthacine A_7 (150) via a common pyrrolizidine intermediate (145, Scheme 6).^{86}

Scheme 6: (a) TPAP, NMO, CH_2Cl_2, 4 Å MS (89%); (b) Ph_3P=CHCOCH_3, toluene, 80 °C (78%); (c) H_2, 10% Pd/C, MeOH, 60 psi (44% of 145 and 40% of 144).

As before, TPAP-catalyzed oxidation of the free hydroxymethyl group in 139 was used to generate the key pyrrolidinecarbaldehyde precursor 140. Homologation via the Wittig olefination approach gave unsaturated ketone 141. A one-pot process was then used to achieve N-deprotection and reductive cyclisation under hydrogenation conditions. This process left the protecting groups on the more heavily substituted ring of the bicycle intact and furnished pyrrolizidine isomers 144 and 145 in 1:1 ratio. A key difference in the route described in Scheme 6 relative to those based on pyrrolidine 96 (Schemes 1 and 2) is the inverted stereochemistry at the C-2 position of the pyrrolidinecarbaldehyde precursor. This difference resulted in loss of stereocentre for the reductive cyclisation, though Izquierdo et al. did not comment on this feature in their paper.^{86}
Intermediate 145 was subsequently separated from 144 and debenzyolated to give alcohol 146 (Scheme 7). Further sequential deprotection of the remaining two masked hydroxyl groups led to 1-epi-hyacinthacine A7 (150). Implementation of an inversion strategy for the C-1 alcohol in 146 allowed access to hyacinthacine A7 (58) itself however. Unsuccessful initial attempts to invert the alcohol were made under Mitsunobu conditions (BzOH, DEAD, Ph3P, THF prior to hydrolysis of the benzoate). The failure of these conditions forced the group to undertake a Swern oxidation on 146 followed by borohydride reduction of the intermediate ketone. This yielded the inverted alcohol (147) and starting alcohol (146) as 1.5:1 mixture separable by chromatography. Deprotection of 147 then afforded hyacinthacine A7 (58).

Scheme 7: (a) MeONa (cat.),MeOH (86%); (b) i. oxalyl chloride, DMSO, TFA, DCM, -78°C ii. NaBH₄, MeOH (46% of 147 and 29% of 146); (c) TBAF, THF (65% for 148 and 85% for 149); (d) H₂, 10%, Pd/C, MeOH, HCl then Amberlite IRA-400 (OH⁻ form) (76% for 58 and 63% for 150); (e) i. BzOH, DEAD, Ph3P, THF, ii. MeONa (cat.), MeOH.

Although not reported, it can be envisaged that intermediate 144 could be treated in the same manner as above to produce hyacinthacine A₆ (57) and 1-epi-hyacinthacine A₆ (151) (Figure 15).
Figure 15. Potential hyacinthacine products from intermediate 134.
1.5.2 Ring closing metathesis

The first synthesis of hyacinthacine $A_2$ (52) was reported by Martin et al. in 2001, utilising ring-closing metathesis (RCM) as the key step in the route. (Scheme 8).^37

![Scheme 8](image_url)

Scheme 8: (a) (CH$_2$=CH)$_2$Zn (95%); (b) BzCl, n-Bu$_3$NI, CH$_2$Cl$_2$ / 1 N NaOH (1:1); (c) oxalyl chloride, DMSO, TFA, DCM, -78°C (63% from 153) ; (d) allylamine, AcOH, NaBH$_3$CN, 3 Å MS, MeOH (78%); (e) Grubbs I catalyst, toluene (30%); (f) H$_2$, Pd/C, MeOH, THF, 6N HCl (82%).

The synthesis started by addition of divinylzine to commercially available d-arabinose derivative 152 to give 153. Benzylation gave an inseparable 3:5:1 mixture of the desired product (154b) and side product 154a. The inseparable mixture was subjected to Swern oxidation and the required ketone (155) was isolated. Reductive amination of 155 with allylamine gave the amine intermediate, which underwent spontaneous intermolecular cyclisation by nucleophilic displacement of the allylic benzoate ester with the secondary amino group to provide RCM precursor 156 as a mixture of stereoisomers. RCM of the HCl salts of this epimeric mixture in the presence of Grubbs I catalyst resulted in the formation of 157 in an isolated yield of 30% based on the proportion of the appropriate
epimer in the precursor 156. Reduction of the double bond and debenzylation by hydrogenation gave hyacinthacine A₂ (52). The key RCM step in this synthesis was low-yielding and therefore this method has not been applied to the synthesis of many other hyacinthacines.

Recently another synthesis of hyacinthacine A₂ (52) starting from a d-arabinose derived nitrone (158) has been reported in which the RCM step has a much improved yield (Scheme 9).³⁸

Scheme 9: (a) vinlylmagnesium bromide, Et₂O (98%); (b) Zn, AcOH (100%); (c) allyl bromide, DMF, K₂CO₃, Bu₄NI (81%); (d) Grubbs second generation catalyst, toluene (87%); (e) H₂, 3 bar, Pd(OH)₂-C, MeOH-HCl, then Dowex (96%).

Nucleophilic addition of vinlylmagnesium bromide to nitrone 158 gave hydroxylamine 159 with nucleophilic attack occurring exclusively from the less hindered face of the nitrone. This step was followed by reductive scission of the hydroxylamine N-O bond and N-alkylation of intermediate 160 to produce the diallyl RCM precursor (156a). RCM was carried out using Grubbs second generation catalyst and bicycle 157 was obtained in 87% isolated yield. Finally, catalytic hydrogenation of 157 to reduce the double bond and remove the benzyl protection produced hyacinthacine A₂ (52).
1.5.3 Nucleophilic substitution

The method of using nucleophilic substitution as the key ring forming step has been applied to the synthesis of the pyrrolizidine, alexine (41) by Yoda et al. (Scheme 10).  

The synthesis started with conversion of D-arabinose tribenzyl ether 152 into the aminal (161) by reaction with 4-methylbenzyl amine (MPMMH₂). Nucleophilic attack of vinylmagnesium bromide on the aminal, followed by oxidation produced lactam 162. The terminal alkene in 162 then underwent oxidative cleavage to afford the aldehyde.
produced the primary alcohol, which upon treatment with TBDPSCI and then (Boc)Cl gave 165. Oxidative cleavage of the alkene group of 165 followed by reduction produced the primary alcohol, which upon treatment with TBDPSCI and then (Boc)_2O gave the N-Boc lactam (166). Addition ofvinylmagnesium bromide to 166 and subsequent reduction of the resulting enone, from the least hindered face, gave the allylic alcohol 167 with 5 contiguous stereocentres as the sole product. Mesylation and cyclisation under basic conditions produced pyrrolidine 168. Osmium tetroxide mediated oxidative cleavage of the vinyl group in the latter, with reduction of the resulting aldehyde and subsequent MOM protection of the alcohol formed, led to 169. The key cyclisation step was then achieved by removal of the silyl group and replacement with a tosyl moiety; cleavage of the N-Boc group with methanolic HCl triggered the nucleophilic substitution to afford bicycle 170. Finally catalytic hydrogenation unmasked the remaining protected hydroxyl groups to give alexine (41).

Yoda’s group then extended this strategy to achieve the first synthesis of a hyacinthacine series member, 7a-epi-hyacinthacine A2 (7-deoxyalexine, 97), Scheme 11.90

Scheme 11: (a) i. TBAF; ii. DMSO, DCM, -78 °C; (b) vinylmagnesium bromide, SmCl3; (c) i. TBDMSCl; ii. OsO4, NMO; iii. NaIO4; iv. NaBH4 (52 % from171); (d) i. TBAF; ii. TBDPSCI (87 %); (e) i. MsCl, TEA, CH2Cl2 (98 %); (f) i. BF3.OEt2; iii. KOH (73 %); (g) i. c. HCl; ii. H2, Pd/C (96 %).

Aminal 161, obtained from D-arabinose derivative 152 as before, was subjected to nucleophilic attack by butenylmagnesium bromide. This was followed by a similar
sequence of oxidation and protecting group manipulation to that described in Scheme 10, producing pyrrolidine 171 over 5 steps. After silyl deprotection, to unmask the terminal hydroxyl group of the side chain, followed by Swern oxidation, an aldehyde was obtained which was directly reacted with vinylmagnesium bromide in the presence of SmCl₃. The resulting allyl alcohol (not shown in Scheme 11) was silylated with TBDMSCl and the alkene cleaved by treatment with osmium tetroxide. Borohydride reduction of the resulting aldehyde then produced pyrrolidine 172. Removal of the secondary silyl protecting group and protection of the primary alcohol as the TBDPS ether gave compound 173. The free hydroxyl group of 173 was then derivatised as the mesylate to yield 174, which upon removal of the Boc group under mild acidic conditions underwent intramolecular nucleophilic substitution to afford the pyrrolizidine (175). Removal of the TBDPS group and the benzyl groups led to the desired 7z-epi-hyacinthacine A₂ (7z-deoxyalexine, 87).

The synthesis of both alexine (41) and 7z-epi-alexine (42) has recently been reported from L-xylose using similar methodology to that presented above. Syntheses of hyacinthacines B₁ (48) and B₂ (49) have also since been reported by a method employing intramolecular nucleophilic substitution commencing from the starting material (S)-(−)-2-pyrrolidone-5-carboxylic acid (176) (Schemes 12 and 13).
The route to hyacinthacines B₁ and B₂ involved construction of the key pyrrolidine (177) as a common intermediate. Thus, (S)-(−)-2-pyrrolidone-5-carboxylic acid (176) was converted into 177, which was then subjected to nucleophilic attack from a but-1-en-4-yl Grignard reagent. Reduction of the resulting ketone produced alcohol 178. Derivatisation of the free hydroxyl group as the mesylate followed by cyclisation produced a separable mixture of stereoisomeric pyrrolidines 179 and 180, with 180 being the major product. Desilylation of the latter was carried out to facilitate removal of the N-Boc group, assisted by hydride deprotonation of the resulting alcohol. This allowed installation of N-Cbz protection on the pyrrolidine and was followed by TBDMS silylation to reprotect the side chain alcohol in pyrrolidine 181. This pyrrolidine was applied to the synthesis of hyacinthacines B₁ (48) and B₂ (49) (Scheme 13).

Scheme 12: (a) CH₂=CH(CH₂)₂MgBr, THF, (83%); (b) NaBH₄, CeCl₃, EtOH (quantitative over 2 steps); (c) i. MsCl, Et₃N, CH₂Cl₂ (90%); ii. t-BuOK, THF (91% of 180 and 5% 179 from 178); (d) i. TBAF, THF (quantitative); ii. NaH, THF (quantitative); iii. CbzCl, NaHCO₃, DMF (97%).
**Scheme 13:** (a) AD-mix-α[(DHQ)_2PHAL ligand], t-BuOH/H_2O (1:1) (98%); (b) i. TBDMSCl, Et_3N, DCM (97%); ii. MsCl, Et_3N, DCM (92%); iii. H_2, Pd/C, EtOH (69% for 183 and 28% for 185); (c) i. TBAF, THF (97%); ii. TFA, H_2O (92%); (d) AD-mix-β[(DHQD)_2PHAL ligand], t-BuOH/H_2O (1:1) (98%).

Focusing first on the synthesis of hyacinthacine B_1 (48), catalytic Sharpless asymmetric dihydroxylation (AD) of 181 with AD-mix-α [(DHQ)_2PHAL ligand], produced diols 182 and 184 with moderate stereoselectivity (182:184, 71:29) as an inseparable mixture. After selective silylation of the primary alcohol, mesylation and cyclisation (triggered by hydrolytic cleavage of the N-Cbz group), the major pyrrolizidine isomer (183) could be separated by column chromatography. Silyl deprotection and treatment with TFA to remove the isopropyldiene group produced hyacinthacine B_1 (48). The asymmetric dihydroxylation of 181 with AD-mix-β [(DHQD)_2PHAL ligand] provided a mixture of 182 and 184 with reverse selectivity favouring isomer 184. This allowed access to
hyacinthacine $B_2$ (49) by application of the same series of transformations as described for hyacinthacine $B_1$.

A similar route, again using $(S)$-$(−)$-2-pyrrolidone-5-carboxylic acid (176) as the starting chiral building block, has very recently been applied to the synthesis of hyacinthacines $C_2$ (67), $C_3$ (68), and their C5 epimers.94
1.5.4 Nitrone umpolung

Desvergnes et al. have reported a synthesis of hyacinthacine $A_2$ (52) in which nitrone 158 served as the key intermediate for umpolung chemistry.\textsuperscript{71} This nitrone has, as discussed previously (\textit{vide supra} Section 1.4.2), also provided the basis for a ring closing metathesis approach to the same hyacinthacine family member, where the nitrone was prepared from D-arabinose. In the work described by Desvergnes et al., the nitrone was synthesised by a different route commencing from L-xylene; both methods for synthesis of this nitrone are discussed in detail later. The key reaction in the umpolung approach to hyacinthacine $A_2$ was the samarium diiodide induced reductive coupling of the nitrone with ethyl acrylate (Scheme 14).\textsuperscript{95}

\begin{equation*}
\text{Scheme 14: (a) ethyl acrylate, SmI}_2, H_2O (64\%); (b) SmI}_2, THF; (c) K}_2CO_3 (59\% from 186); (d) LiAlH}_4, THF (79\%); (e) H}_2, Pd/C, MeOH, HCl, DOWEX 1 x 8 (79\%).
\end{equation*}
Nitrone 158 was treated with samarium diiodide and ethyl acrylate in the presence of water, as a proton source, to afford hydroxylamine 186 in which the acrylate had selectively closed to the the least hindered lower face of the nitrone. In this reaction, the samarium diiodide reduced the C=N bond to generate an α-aza-nucleophilic species (radical or anion). The ethyl acrylate was activated in the reaction by coordination of the samarium to the carbonyl group and this allowed the 1,4-addition step to take place. The N-hydroxylamine (186) produced was reduced by the further addition of samarium diiodide to the reaction mixture, which resulted in formation of pyrrolidine 187. Partial cyclisation to lactam 188 was observed and complete conversion to 188 was achieved by treating the crude product with potassium carbonate. Reduction of the lactam to the tertiary amine (189) was followed by hydrogenation to produce hyacinthacine A₂ (52).
1.5.5 Nitrone 1,3-dipolar cycloaddition

A useful method for obtaining isoxazolidine ring systems, with highly selective regio- and stereocontrol, in a single step is from the nitrone-alkene 1,3-dipolar cycloaddition (Scheme 15).  

![Scheme 15: Nitrone-alkene 1,3-dipolar cycloaddition](image)

It is possible to generate up to 3 new stereocentres using this 1,3-dipolar cycloaddition, and the isoxazolidine N-O bond can be reductively cleaved to afford synthetically versatile amino alcohols. The nitrone 1,3-dipolar cycloaddition is best described as a concerted reaction between 2π and 4π electron addends. As with the Diels-Alder reaction, the thermally allowed 1,3-dipolar cycloaddition is a suprafacial process. Regio- and stereocontrol in nitrone 1,3-dipolar cycloadditions have been reviewed in detail in the literature and the scope of the following discussion will therefore be limited to 1,3-dipolar cycloadditions of cyclic nitrones with mono-substituted alkenes, as these are particularly relevant to this project.

1.5.5.1 1,3-Dipolar cycloaddition: regiochemistry and stereochemistry

There can be two regiochemical outcomes for the cycloaddition of a cyclic nitrone with a mono-substituted alkene. In principle, a C-4 or C-5 substituted isoxazolidine can be obtained (Figure 16). However, it has been shown that steric and electronic factors conspire to control the regioselectivity of the reaction which substantially favour a 5-substituted isoxazolidine product from alkenes bearing both electron donating and electron withdrawing substituents.
Figure 16. Regiochemical outcomes of 1,3-dipolar cycloaddition.

The electronic control of nitrone cycloaddition regiochemistry is typically rationalised by analysis of frontier molecular orbital interactions, where maximum orbital overlap contributing to the transition state involves interaction of the most energetically proximal HOMO-LUMO pair combination (Figure 17), and determines the reaction outcome. For nitrone-alkene cycloadditions, both sets of interactions favour a 5-substituted isoxazolidine product except in the case of alkenes carrying the most electron demanding of substituents (such as nitroethylene). Reactions of monosubstituted alkenes with 5-membered ring nitrones, such as 1-pyrrolidine-N-oxide, behave accordingly and 5-substituted isoxazolidines are predominantly formed.

Figure 17. Frontier molecular orbital interactions in 1,3-dipolar cycloadditions.

The 1,3-dipolar cycloaddition of a cyclic nitrone with a mono-substituted alkene can proceed with formation of two new stereogenic centres at C-3 and C-5, which can potentially yield four different diastereoisomers when 5-substituted isoxazolidines are formed. As cyclic nitrones do not have the capacity to exhibit E/Z isomerism, the stereocontrol in cycloadditions of these nitrones is typically higher than for their acyclic
counterparts. It is the dipolarophile orientation (end o or exo) and direction of approach (anti or syn) towards the 1,3-dipole that determines the product stereochemistry.

The endo/exo selectivity of cycloaddition reactions can be influenced by the interactions of secondary π orbitals (Figure 18).\textsuperscript{103} In the Diels-Alder reaction, the endo isomer is usually dominant with dienophiles carrying functionality that allows formation of favourable secondary orbital interactions in the transition state. In 1,3-dipolar cycloadditions of nitrone s, the interaction of the N-nitrone p\textsubscript{z} orbital with a vicinal p\textsubscript{z} orbital is small.\textsuperscript{104} Therefore, unlike Diels-Alder reactions, the endo/exo selectivity in nitrone cycloadditions is largely controlled by steric factors and hindrance between the nitrone and alkene substituents disfavours the endo transition state. The 1,3-dipolar cycloaddition of cyclic nitrone s generally occurs via an exo transition state therefore.

![Figure 18. Orbital interactions of exo and endo approach.](image)

The stereochemical outcome of the cycloaddition is further complicated for cyclic nitrone s bearing an existing stereogenic feature in the form of a substituent at one of the ring sp\textsuperscript{3} carbons. In this case, cycloaddition with a mono-substituted alkene may proceed to give a 5-substituted isoxazolidine product through four possible transition state combinations (Figure 19) and anti/syn selectivity, determined by the face from which the alkene preferentially attacks the cyclic nitrone, also has to be considered. This selectivity is usually determined by steric factors, with the dipolarophile attacking from the least sterically demanding anti face of the cyclic nitrone. The most favourable transition state for a 1,3-dipolar cycloaddition of this type is therefore generally exo-anti, and this has been observed by many groups.\textsuperscript{115,105,106,107} Minor products resulting from the endo-anti
and *exo-syn* are often seen, whereas the highly sterically demanding *endo-syn* product is rarely observed.

*Figure 19.* Possible approaches of dipolarophile in 1,3-dipolar cycloaddition.
1.5.5.2 Application to Hyacinthacine Synthesis

Ishibashi *et al.*\(^{108}\) and Goti *et al.*\(^{109}\) have both described the development of routes to hyacinthacine A\(_2\) (52) in which a 1,3-dipolar cycloaddition step is used (Scheme 16).

![Scheme 16](image-url)

**Scheme 16:** (a) 191 in CH\(_2\)Cl\(_2\), or 192 in C\(_6\)H\(_6\) (78% 193), (64% 194); (b), Zn, AcOH-H\(_2\)O (80% 195), (64% 196); (c), PhOCSCl, py, DMAP, CH\(_2\)Cl\(_2\) (80%); (d), Bu\(_3\)SnH, AIBN, C\(_6\)H\(_6\), Δ (58%); (e), 3 N HCl, MeOH (84%); (f), MsCl, TEA, CH\(_2\)Cl\(_2\), (100%); (g) LiAlH\(_4\), THF, Δ, (40%); (h), H\(_2\), Pd/C, MeOH (75%)

Ishibashi prepared nitrone 190 from L-xylose, as will be discussed later, and this nitrone was used in a 1,3-dipolar cycloaddition with tert-butyl acrylate (192) to produce the desired anti-exo cycloadduct (194) in 64% yield and the endo product in 31% yield. The Goti group used the bulkier \(N,N\)-dimethylamide (191) along with nitrone 158; this reaction proceeded with high stereoselectivity affording only the desired anti-exo adduct (193). Lactams 195 and 196 were then isolated following N-O bond cleavage of their respective parent isoxazolidines using zinc in acetic acid. Both lactams required removal of the hydroxy group from the lactam ring. Ishibashi achieved this using the using the
Barton-McCombie deoxygenation protocol. Thus, lactam 196 was transformed into the thionocarbonate 197 and subsequent treatment with tributyltin hydride and a catalytic amount of AIBN afforded the deoxygenated lactam (198) in 46% yield. Finally the MOM group was removed under acidic conditions to produce lactam 199. The authors state that lactam 199 could be used as an intermediate to hyacinthacines A1 and A2 but this was not reported. The route to hyacinthacine A2 was completed by Goti et al. however. This group undertook the deoxygenation of lactam 195 by mesylation of the hydroxyl group to give 200 followed by reduction with lithium aluminium hydride to give 201. Finally hydrogenation to remove the O-benzyl groups produced hyacinthacine A2 (52).

The synthesis of two new pentahydroxypyrrolizidines (202 and 203) involving a 1,3-dipolar cycloaddition of the previously mentioned nitrone (158) with 3-buten-1,2-diol derivatives 204 and 205 has been described very recently [Scheme 17 and 18]. The dipolarophiles were prepared by transesterification of 3-buten-1,2-diol (206) with vinyl acetate in the presence of Chirazyme L-2 in tert-butylmethyl ether at room temperature. This chemoenzmatic reaction produced a mixture of (R)-di-O-acetyl-3-butene-1,2-diol (204, 44%, 64% ee) and (S)-1-O-acetyl-3-butene-1,2-diol (205, 46%, 61% ee) (Scheme 17) which was separated by column chromatography.
Scheme 17: (a) vinyl acetate, Chirazyme® L-2, TBME (44%, 64% ee, 204), (46%, 61% ee, 205); (b) 205, CH₂Cl₂, 70 °C, 2.5 h, (70%); (c) MsCl, py, CH₂Cl₂ (74%); (d) Zn, AcOH/H₂O (9:1), 60 °C (60%); (e) Ambersep 900 (OH⁻), MeOH (83%); (f) i.Pd/C, H₂, c. HCl, ii. Dowex 50W X8 NH₄OH (85%).

Initially nitrone 158 was reacted with derivative 205, producing the anti-exo cycloaddition product, pyrroloisoxazolidine 207, as the major adduct. Cycloadduct 207 was converted into mesylate 208. Subsequent N-O bond cleavage in 208 using Zn/AcOH afforded pyrrolizidine 209. Deacetylation of 209 with Ambersep 900 (OH⁻) resin to diol 210 and subsequent hydrogenation afforded pyrrolizidine 202. Chiron 204 was then used in the synthesis of prrolizidine 203, the C-5 epimer of 202 (Scheme 18).
**Scheme 18:** (a) 204, DCM, 70 °C, 4, 74%; (b) Ambersep 900 (OH), MeOH (83%) (c) TBDPSCl, imidazole, DMF, (92%); (d) MsCl, py, DCM (81%); (e) Zn, AcOH/H2O (9:1), 60°C (76%); (f) TBAF, THF (61%); (g) i. Pd/C, H2, c.HCl, ii. Dowex 50W X8 NH4OH (85%).

Reaction of nitrone 158 and chiral alkene 204 produced isoxazolidine 211 as the major cycloaddition adduct. Deacetylation to give diol 212 and subsequent protection of the primary alcohol function as the silyl ether afforded 213. The hydroxyl function in 213 was then converted into a suitable leaving group, mesylate 214. This was followed by N-O bond cleavage and intramolecular N-alkylation to afford pyrrolizidine 215. Fluoride deprotection of 215 to 216 followed by hydrogenation produced pyrrolizidine 203.

### 1.6 Carbohydrate-derived cyclic nitrones

As discussed above, carbohydrate-derived nitrones have utility in synthetic routes to pyrrolizidine systems, both through exploitation of the principal chemical reactivity of nitrones, cycloaddition chemistry (Section 1.4.5), as well other aspects of nitrone chemistry – organometallic addition (Section 1.4.2) and formal ‘umpolung’ behaviour (Section 1.4.4). The use of nitrones 158 and 190 in particular has been discussed above and, as the work described in this thesis is focused on developing the use of carbohydrate-
derived nitrones for pyrrolizidine synthesis, the preparation of nitrones 158 and 190 is summarised below.

1.6.1 Routes to nitrone 158

Goti et al. employed two different approaches for the synthesis of nitrone 158 (Scheme 19) which gave comparable results in terms of overall yield.\(^{109}\) The first route, which commenced with L-xyllose as a chiral building block, has as its major drawback the expense of the starting carbohydrate. The second approach used D-arabinose as a building block and is preferred in terms of the lower cost involved.

![Scheme 19](image-url)

**Scheme 19:** (a) i. MeOH/H\(_2\)SO\(_4\), anhydr. Na\(_2\)SO\(_4\), rt, 21 h; ii. BnCl/KOH, Na\(_2\)SO\(_4\), reflux, 8 h; iii. 6 N HCl, CH\(_3\)COOH, 60-70 °C (50% over 3 steps); (b), NH\(_2\)OTHP, no solvent, r.t., 6 d, (100%); (c) MsCl, TEA, CH\(_2\)Cl\(_2\), r.t., 24 h (50-70%); (d) i. Dowex 50W X8, MeOH, r.t., 24 h (96%); ii. 0.1 M NaOH, dioxane, 0 °C, 2 h (55%); (e) i. NH\(_2\)OH.HCl, py, rt, 24 h; ii. TBDPSCl, py, rt, 18 h (100%); (f) I\(_2\), ImH, Ph\(_3\)P, toluene, reflux, 1 h (48%); (g) TBAF on silica gel, benzene, reflux, 1.5 h (91%).

Benzyl furanose derivatives 217 and 220 were produced from L-xyllose and D-arabinose respectively following the procedure reported by Fleet and Smith.\(^{112}\) Compound 217 was converted into tetrahydropyrylanoxime 218 which was subsequently mesylated to give 219. Removal of the THP protecting group by treatment with acidic resin in methanol, induced cyclisation to produce nitrone 158. A similar strategy was adopted for the D-arabinose derivative (220) in that reaction with hydroxylamine followed by TBDPSCl was used to generate an O-protected oxime intermediate (221). The alcohol function in
221 was then replaced by iodide with the double purpose of introducing a good leaving group and inverting the C-4 position, producing 222. Fluoride deprotection of the silyl group in 222 induced spontaneous cyclisation with displacement of the iodide to furnish nitron 158.

Py et al. have also synthesized nitron 158 from l-xylose (Scheme 20). This group first converted l-xylose (223) into the 2-O-methyl furanose derivative; benzyl protection of the remaining hydroxyl groups produced 224 in 90% yield over 2 steps. Cleavage of the acetal under acidic conditions to generate a hemiacetal (cf 217 in Scheme 19) was followed by reaction with TBDPSNH$_2$ to generate O-protected oxime 225. The free alcohol function in 225 was derivatised as the mesylate (226) and subsequent treatment with TBAF afforded oxime 227. Displacement of the mesylate in 227 by hydroxylamine and cyclisation efficiently produced nitron 158.

Scheme 20: (a) i. 0.5% MeOH, MeOH, r.t, 15 h; ii. NaH, BnBr, n-Bu$_4$N, DMF/THF, r.t, 48 h (90% over 2 steps); (b) i. AcOH/1 M H$_2$SO$_4$, 100 °C, 1 h; ii. TBDPSNH$_2$, cat. PPTS, MgSO$_4$, toluene, 110 °C, 30 min (79% over 2 steps); (c) MsCl, TEA, DCM, 0 °C, (93%); (d) TBAF, THF, 0 °C, 30 min (93%); (e) NH$_2$OH.HCl, NaHCO$_3$, MeOH/H$_2$O 4:1, 65 °C, 15h (92%).

1.6.2 Route to nitron 190

As mentioned earlier, Ishibashi et al. have used nitron 190 in the synthesis of a pyrrolizidine lactam precursor (199, Scheme 16) to hyacinthacine A$_2$. Nitron 190 possesses the same absolute stereochemistry as 158 but embodies a potentially more versatile combination of MOM and benzyl protecting groups of the C-3 and C-4 positions. We envisaged that this protecting group regime could be exploited to allow synthesis of a wider range of hyacinthacine family members through a common synthetic route with late stage divergency. Ishibashi et al. prepared nitron 190 in a route
commencing from L-xylose. As the preparation of nitrone 190 formed the starting point for this project, the route is discussed in detail in the Results and Discussion that follows.
2 RESULTS & DISCUSSION
2.1 Project objectives

A 1,3-dipolar cycloaddition reaction between a cyclic nitroson and a terminal alkene can be utilised for the synthesis of bicyclic pyrrolizidine alkaloids. Thus, the 1,3-dipolar cycloaddition of a suitable nitroson with a terminal alkene, which has a potential leaving group in the allylic position, will give a fused isoxazolidine. The N-O bond can be cleaved reductively allowing cyclisation to produce a pyrrolizidine (Scheme 21). If one isomer of the final product is to be achieved in high yield it is necessary to have control of stereochemistry in the cycloaddition. This strategy has been successfully utilised by several groups, including Ishibashi, Goti and in our group where cycloaddition reactions of functionalised cyclic nitrones have been used to prepare hydroxylated pyrrolizidines.

![Scheme 21: Route to pyrrolizidines via 1,3-dipolar cycloadditions between a cyclic nitroson and a terminal alkene bearing a leaving group in the allylic position.](image)

The aim of this PhD project was to investigate routes to hyacinthacine A1 and hyacinthacine B2 using a strategy related to that shown in Scheme 21. In principle, either nitrones of type 190 or 228 could be employed in the cycloaddition reaction (Scheme 22). The differential protection of the C-3 position of nitroson 190 means that this position can be selectively deprotected after cycloaddition, liberating the C-1 hydroxyl group (hyacinthacine numbering) as a site for inversion. Inversion of the hydroxyl group would then provide the required stereochemical outcome for hyacinthacines A1 and B2, where the stereochemistry at C-1 and C-2 is cis. A nitroson of type 228, on the other hand, has the protected hydroxyl groups at the C-3 and C-4 positions cis to each other prior to cycloaddition and this would remove the requirement for subsequent inversion in the pyrrolizidine ring system. However, it was considered likely that cycloaddition reactions of nitrones of this type would proceed with less stereochemical control. Nitroson 190 was therefore selected as the preferred cyclic nitroson for
our work as high stereo- and regioselectivity had already been established for cycloaddition reactions employing its use (Section 1.5.5.2).

**Scheme 22:** Potential routes to hyacinthacine $A_1$ and hyacinthacine $B_2$ via 1,3-dipolar cycloadditions of nitrone 190 or nitrone 228.

The initial scope of this work was to explore the literature for routes to nitrone 190 and compare these to alternative routes developed within our group. The application of nitrone 190 in synthetic routes to hyacinthacine $A_1$ and hyacinthacine $B_2$ was then to be examined.
2.2 Routes to nitrone 190

With nitrone 190 the key intermediate in the planned routes to hyacinthacines A1 and B2, efficient synthetic access was required. A synthesis starting from L-xylose has been discussed.\textsuperscript{113} An alternative synthetic route to nitrone 190 will be described here starting from L-xylose. In addition, a new route to 190 from D-arabinose, which has been developed by our group, will also be discussed.

2.2.1 Ishibashi route to nitrone 190 from L-xylose

\[ \text{Scheme 23: (a) 2,2,2-trichloroethanol, cat. TsOH, DCM, 39 ^\circ \text{C} (72\%); (b) NaH, MOMCl, THF-DMF, -20 ^\circ \text{C}; (c) Zn, NaOAc, AcOH, (80\%); (d) i. NH_2OTBDPS, cat. PPTS, MgSO}_4, benzene, reflux; ii. MsCl, Et_3N, toluene, 0 ^\circ \text{C}, (98\%); (e) TBAF (1.05 equiv.), 4 Å MS, THF, reflux, 15 min, (72\%).} \]

Ishibashi et al.\textsuperscript{113} have synthesised nitrone 190 from L-xylose (Scheme 23) via the known derivative (229).\textsuperscript{116} Lactol 229 was treated with trichloroethanol in the presence of a catalytic amount of TsOH to give 230 as a 3:1 mixture of α- and β-anomers in 72% yield. The hydroxyl group of 230 was then protected as its MOM ether in 231 and the trichloroethyl
Results and Discussion

Group reductively cleaved with zinc dust in acetic acid to produce lactol 232. Lactol 232 was then converted into oxime ether 233 by sequential treatment with NH$_2$OTBDPS and mesyl chloride. Treatment of 233 with TBAF in the presence of 4 Å MS in refluxing THF afforded the desired cyclic nitrone 190.

Our initial approach to the synthesis of nitrone 190 involved using the Ishibashi route described above and therefore commenced with the synthesis of lactol 229. Accordingly, L-xylose was initially protected as the isopropylidene xylofuranose derivative (234) [Scheme 24] as this masked both hydroxyl groups at C-1 and C-2 but left the C-3 hydroxyl group free, allowing for its subsequent differential protection by benzylaion. Installation of the isopropylidene group was achieved by acid-catalysed reaction of L-xylose (223) with acetone. The hydroxyl functions remaining at the C-3 and C-5 positions of 234 were then protected as dibenzyl ethers to give 235. Finally lactol 229 was obtained as a 3:1 mixture of α- and β-anomers after hydrolysis of the isopropylidene function using 50% aqueous acetic acid solution at 80 °C for 24 hours. Following the Ishibashi route, lactol 229 was next converted into trichloroethyl acetal 230. TLC analysis of this reaction indicated that the lactol had been completely consumed but produced a mixture of at least 3 components. Examination of the crude material by $^1$H NMR spectroscopy indicated predominant formation of the desired acetal, albeit as an inseparable 3:1 mixture of the α- and β-trichloroethyl isomers. The crude acetal 230 was then converted into MOM ether 231 by treatment with methoxymethyl chloride. TLC analysis showed complete consumption of 230 and the $^1$H NMR spectrum of the crude product indicated >90% formation of target 231. Without purification, selective cleavage of the trichloroethyl group was next attempted using zinc dust in acetic acid. Disappointingly this reaction cleaved the MOM ether as well as the trichloroethyl ether and returned lactol 229 (Scheme 24). As we were unable to reproduce Ishibashi’s route to nitrone 190, an alternative route from L-xylose was developed.
Results and Discussion

Scheme 24: (a) acetone, c. H$_2$SO$_4$, rt, 24 h (75%); (b) NaH, $n$-Bu$_4$NI, dry THF, BnBr, 0 °C to rt, 24 h, (84%); (c) acetic acid:water (1:1), 80 °C, 24 h, (86%); (d) 2,2,2-trichloroethanol, cat. TsOH, DCM, 39 °C, (72)%; (e) NaH, MOMCl, THF-DMF, -20 °C, (92%); (f) Zn, NaOAc, AcOH, (0 % for 232 and 80% for 229).

2.2.2 Modified route to nitrone 190 from L-xylose

Our failure to reproduce the Ishibashi route to nitrone 190 was caused by the acid sensitivity of the MOM group in intermediate 231. We were unable to selectively cleave the trichloroethyl group with zinc in acetic acid despite using conditions buffered with sodium acetate. We therefore explored a modified route from L-xylose-derived lactol 229 in which the hemiacetal group at the anomic position would be temporarily masked as a lactone [Scheme 25], allowing its regeneration under non-acidic conditions following installation of the MOM protecting group.
Scheme 25: (a) Ag₂CO₃/celite® (large excess), toluene, 80 °C, 1 h, (99%); (b) dimethoxymethane (large excess), chloroform dried over P₂O₅, 30 min, (92%); (c) DIBAL (8 equiv.), THF, -78 °C, 3 h, (78%); (d) NH₂OH.HCl, NaOMe, MeOH, r.t., 18 h, (82%); (e) TBDMS, dry py, 18 h, (73%); (f) H₂NØBDMS, MgSO₄, dry toluene, reflux, 30 min, (89%); (g) MsCl, Et₃N, DCM 0 °C, (93%); (h) TBAF or TBAT, THF or toluene (18-60%).

There is literature precedent for use of bromine and barium carbonate for the selective oxidation of the anomeric position of carbohydrates.¹¹⁸,¹¹⁹ Furthermore, Fleet et al. successfully used bromine with a barium carbonate buffer for preparation of hydroxy lactones 241, 242, 243 and 244 in yields ranging from 66% and 76% (Scheme 26).¹²⁰

Scheme 26: (a) aqueous CF₃CO₂H; (b) Br₂, BaCO₃ in aqueous dioxane, (66-76%).
When the bromine/barium carbonate oxidation procedure was applied to diol 229, however, lactone 236 was produced only with low conversion and diol 229 was returned largely unreacted. Moreover, upon $^1$H NMR analysis of the crude product signal corresponding to 4-bromobutan-1-ol were observed. This was possibly formed from the ring opening of the THF solvent by HBr. Unfortunately this compound could not be separated from the desired product as it had a very similar $R_f$ value. Formation of the bromobutanol side product was subsequently confirmed by comparison with of the NMR spectrum from an authentic sample.

In an attempt to increase the conversion of diol 229 and prevent the formation of the bromobutanol side product, the reaction was examined under a variety of different conditions where the solvent, molar ratio of Br$_2$ to starting material and bromine addition procedure were varied. All reactions were carried out at room temperature with a reaction time of 70 hours. The various conditions and isolated yields of 236 are summarised in Table 1.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Br$_2$ mol equiv.</th>
<th>Br$_2$ addition</th>
<th>Isolated Yield (%)</th>
<th>% bromobutanol in isolated product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioxane</td>
<td>1.5</td>
<td>Br$_2$ water</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>Dioxane</td>
<td>2</td>
<td>Br$_2$ water</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Dioxane</td>
<td>3</td>
<td>Br$_2$ water</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Dioxane</td>
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<tr>
<td>THF/water (1:3)</td>
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<td>Br$_2$ in THF</td>
<td>5</td>
<td>5</td>
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<td>Br$_2$ in THF</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>THF/water (1:3)</td>
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<td>Br$_2$ in THF</td>
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<td>6</td>
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<td>7</td>
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<tr>
<td>THF/water (1:3)</td>
<td>3</td>
<td>Br$_2$ in ether</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

**Table 1**: Summary of attempts at selective oxidation of lactol 229 using Br$_2$.

Unsurprisingly, it was found that removing THF from the system prevented the formation of the bromobutanol side product. The maximum yield of lactone 236 obtained was 51%, where
1,4-dioxane was used as a solvent and 1.5 equivalents of Br₂ were used. As the maximum yield observed for this step was relatively low this oxidation method was deemed unsuitable and an alternative method investigated.

It has long been known that silver carbonate is an effective reagent for oxidations of alcohols to aldehydes and ketones. In 1968 Fetizon et al. reported that freshly prepared silver carbonate, obtained by reaction of silver nitrate with sodium carbonate, is a suitable oxidising agent. However, the reagent generated in this way is difficult to filter, wash, and handle and therefore the reproducibility of oxidations using it can be poor. It was subsequently found that these problems could be eliminated by precipitating the reagent in the presence of a celite®. The resultant Ag₂CO₃/celite® reagent is a versatile and useful oxidising reagent and has been thoroughly investigated by Mikilop and Young. This reagent is commercially available but it was prepared in our laboratory using the procedure originally reported by Fetizon. Pleasingly, treatment of lactol with a large excess of the silver carbonate reagent in toluene produced lactone in quantitative yield as a white crystalline solid. X-ray crystallography confirmed the structure of lactone (Figure 20, data in Appendix A) with the stereochemistry as expected at this point of the synthesis. The structure was found to a monoclinic crystal system belonging to the P2₁ space group, with a zig-zag chain along the b axis. Pi stacking of the aromatic rings was not observed but intermolecular hydrogen bonding is present between the hydroxyl hydrogen and the lactone oxygen atom.

Figure 20: Crystal structure of lactone 236.
The free hydroxyl group in lactone 236 was then protected as the methoxymethyl ether using 10 equivalents of dimethoxymethane and P₂O₅ in chloroform, forming ether 237 cleanly as a colourless oil in a 98% yield without any need for purification. The presence of a singlet resonance of δ 3.37 in the ¹H NMR spectrum of 237, corresponding to the methoxy group, confirmed the successful installation of the MOM ether. The MOM methylene group was not easily observed in the ¹H NMR spectrum, but was more readily evident in the ¹³C NMR spectrum as a distinctive signal at δ 95.7.

A reduction using DIBAL was next carried out on 237 to afford lactol 232 in 86% yield as a 1.5:1 inseparable mixture of α- and β-isomers. The structure of 232 was confirmed by the disappearance of the lactone carbonyl signal in the ¹³C NMR spectrum of 237 at δ 172.6. Treatment of 232 with hydroxylamine hydrochloride then led to oxime 238 (E:Z ratio, 5:1 with the E-isomer assumed to be the major product) in an 82% yield. The oxime was then converted into its O-TBDMS derivative (239) in 73% yield by treatment with TBDMSCl. In principle, it should be possible to transform lactol 232 directly into compound 239 by treatment with H₂NOTBDMS, which can be prepared by a known procedure.¹²⁵ In order to explore this we therefore prepared H₂NOTBDMS by addition of tert-butyldimethylsilyl chloride to a mixture of hydroxylamine hydrochloride and ethylenediamine in dichloromethane. After stirring at room temperature for 24 hours, the silylated hydroxylamine was isolated in quantitative yield following work-up and distillation at reduced pressure. Ishibashi et al. had successfully transformed lactol 232 into the protected oxime 233 by treatment with H₂NOTBDPS, MgSO₄ and a catalytic amount of pyridinium p-toluenesulfonate in benzene (Scheme 23). The formation of O-TBDMS protected oxime 239 (Scheme 25) by similar treatment of 232 with H₂NOTBDMS was unsuccessful and produced oxime 238 quantitatively as the sole product. On consideration, this was not surprising as the catalyst employed is known to cleave TBDMS protecting groups.¹²⁶ A second attempt to achieve the direct transformation of 232 into 239 was therefore made under the same conditions but omitting the acid catalyst. Gratifyingly, this produced the TBDMS protected oxime (239) in 89% yield.
Oxime silyl ether 239 was then smoothly converted into mesylate 240 in 93% yield by treatment with MsCl and triethylamine in DCM. Finally, treatment of 240 with tetrabutylammonium fluoride (TBAF) in dry toluene produced the target differentially protected nitrone (190) successfully in 85% yield on the first attempt. The yield of the cyclisation step using TBAF was subsequently found to be variable, however, ranging typically from 28%-60%. Ishibashi et al. described optimum cyclisation conditions for their analogue (233) of 240 involving the use of tetrabutylammonium triphenylidifluorosilicate (TBAT) as an alternative desilylating agent to TBAF. They reported that these conditions led to greater yields of nitrones compared to those using TBAF. In our hands, however, treatment of 240 with TBAT afforded nitrone 190 in a disappointing yield of only 30% with no remaining starting material or identifiable side products isolable. With the variable yields observed in the silyl deprotection and cyclisation step leading to 190, it was decided to attempt the conversion of lactol 232 to nitrone 190 in a ‘one-pot’ process through initial reaction with hydroxylamine and then treatment with mesyl chloride. A similar ‘one-pot’ procedure for conversion of an analogous lactol (22a) into a nitrone has been reported by Goti et al. and this approach avoids the need for silyl protection of the intermediate oxime. Thus, under these conditions, the hemiacetal was to be converted into an oxime and the free hydroxyl group then derivatised as the mesylate [Scheme 27]. Under basic conditions, the oxime-mesylate was then expected to cyclise to the nitrone.

When these conditions were applied to lactol 232, however, no nitrone 190 was formed and nitrile 245 was obtained cleanly in quantitative yield. The presence of a signal at δ 116.4 in the $^{13}$C NMR spectrum was attributed to the nitrile group of 245, and the structure was further confirmed by IR spectroscopy, where an absorption corresponding to a nitrile group was observed at 2232 cm$^{-1}$. A possible mechanistic explanation for this transformation is shown (Scheme 27).
Results and Discussion

Scheme 27: Attempted preparation of nitrone 190 and mechanistic formulation for the generation of nitrile 245.

Formation of nitrile 245 was a disappointing outcome but this probably explains the preference of Goti (Scheme 19), Py (Scheme 20) and Ishibashi (Scheme 23) who all elect to adopt a strategy that proceeds via a silyl protected oxime when undertaking a similar sequence of reactions. In an effort to further streamline the route, it was next decided to attempt an alternative one-pot procedure for transformation of lactol 229 into nitrone 190 [Scheme 28]. This involved altering the successful route shown in Scheme 25 and is summarised in Scheme 28. Thus, the solvent was removed after completion of each reaction stage (as determined by TLC); the appropriate solvent for the next step and reagents required were then added for each subsequent reaction without any purification. A variable yield of 18-43% of the desired nitrone 190 was achieved over the three steps.

Scheme 28: Telescop ing the lactol to nitrone transformation
This route was successful in the production of nitrone 190 and afforded reasonable yields at each step of the process. However, due to the modest overall yield and variability of the final step an alternative route to nitrone 190 from D-arabinose was also investigated.

2.2.3 New route to nitrone 190 from D-arabinose

The major drawbacks of the L-xylene-based routes described above are: (i) the high cost of the unnatural sugar employed as a starting material; (ii) the expense of preparing silver carbonate reagent employed as a selective oxidant and the large excess of this reagent used, and (iii) the variability in yield of the final step. Goti described routes to nitrone 158 starting from L-xylene or from the much cheaper D-arabinose 247 (Scheme 19).109 The route from D-arabinose involved the transformation of alcohol 221 into an iodide (222), inverting the stereochemistry prior to the cyclisation step leading to the nitrone. As the displacement of the iodide proceeds with inversion of configuration, nitrone 158 is formed by a route with an overall double inversion of stereochemistry. A new route to nitrone 190 from D-arabinose 247 was therefore planned involving a double inversion strategy commencing from alcohol 256 via iodide 257 (Scheme 29).
Results and Discussion

Scheme 29: (a) TBDPSCI, imidazole, DMF (55%); (b) acetone, CuSO₄, H₂O (38%); (c) TBAF, THF (75%); (d) Br₂, Ba₃(OMe)₂, THF-H₂O (85%); (e) CH₂(OH)₂, P₂O₅, CHCl₃ (92%); (f) DIBAL, THF, -78 °C (98%); (i) NH₂OTBDMS, toluene, MgSO₄, reflux, 5 min (72%); (j) PPh₃, I₂, imidazole, toluene (88%); (k) anhydrous TBAF, toluene, Δ, 5 min (83%).

D-Arabinose (247) was first protected at the 5-position as the TBDPS silyl ether 248 in 55% yield by the selective reaction of the primary alcohol function with TBDPSCI. The 1- and 2-positions of 248 were subsequently protected by isopropylidene derivatisation to 249. Deprotection of the silyl group by TBAF led to 250; this released the hydroxy groups at the 3- and 5-positions, allowing the benzyl groups in the target nitroxy to be put in place in compound 251. The isopropylidene protection was then removed under acidic conditions to produce lactol 252 in 89% yield as a 1.3:1 mixture of α- and β-anomers. Given the success of our previous strategy to install MOM protection in the xylose route to nitroxy 190 (Scheme 25), by oxidation of the lactol to the lactone prior to MOM protection and then reduction back to the lactol, we decided to use a similar strategy here. Accordingly, lactol 252 was selectively oxidised at the anomic position by treatment with bromine and barium carbonate in aqueous THF. Pleasingly, in contrast to the xylose route, where oxidation of lactol 229 with bromine had been problematic, the oxidation of arabinose-derived lactol 252 proceeded reliably and reproducibly to give lactone 253 in high yields. This obviated the
need for the expensive silver carbonate/celite reagent. In addition, the formation of the bromobutanol side product, which was a problem in the L-xylose route, was not observed here. With the 2-position free, lactone 253 was then differentially protected as its methoxymethyl ether 254 in 76% yield by reaction with dimethoxymethane. Reduction of lactone 254 by DIBAL produced lactol 255 in 97% yield as a mixture of anomers (α:β, 1.5:1). Lactol 255 was subsequently converted into TBDMS protected oxime 256 in a reasonable yield (60%) by reaction with H$_2$NOTBDMS in boiling toluene. The hydroxyl group was then replaced by iodide in 80% yield by treatment with triphenylphosphine and iodine, affording 257 with inversion at C-4. Finally, silyl deprotection with TBAF and S$_2$N$_2$ displacement of iodide furnished the desired nitrone (190) in a good yield (83%). The cyclisation step in this route was higher yielding and more consistent than cyclisation of 240 to 190 in the L-xylose route.
2.2.4 Summary of routes to nitrone 190

Three routes to differentially protected nitrone 190 have been described. The L-xylose route reported by Ishibashi et al.\textsuperscript{113} has successfully been implemented by that group. In our hands, however, attempts to install the MOM protection in lactol 232, by executing the Ishibari group’s three-step transformation from lactol 229 (Scheme 23), were unsuccessful. This necessitated the development of alternative strategies to access nitrone 190 efficiently. In the first approach, the Ishibashi synthesis was modified to avoid exposing the MOM-protected intermediate to acidic conditions. This was successfully accomplished [Scheme 25] via silver carbonate oxidation of lactol 229 to the corresponding lactone (236), protection of the 3-hydroxyl group and reduction of the lactone (237) back to the lactol (232). The subsequent steps then broadly followed the Ishibari route except that $O$-TBDMS protection of the oxime (239) was employed prior to the final steps—formation of the methanesulfonate (240) and cyclisation to the nitrone— which completed a nine-step route from L-xylose. Unfortunately the final cyclisation step was somewhat capricious and low-yielding. This problem was compounded by the requirement for the use of both the expensive starting material L-xylose and costly silver carbonate reagent which had to be used in large excess to achieve the oxidation of lactol 229 to lactone 236 in good yield. These issues led us to develop a second route to nitrone 190 [Scheme 29] commencing with the cheaper starting material, D-arabinose. We have successfully developed a route which avoided the use of silver carbonate reagent, albeit in a slightly longer eleven-step sequence. With nitrone 190 available, we were then able to proceed with the exploration of the 1,3-dipolar cycloaddition chemistry planned for construction of our bicyclic iminosugar targets.
2.3 1,3-dipolar cyclisation routes to hyacinthacines from nitrone 190

Routes to a differentially protected nitrone 190 have been described; such a nitrone can be useful in the synthesis of pyrrolizidine alkaloids with a hydroxymethyl group at C-3. The 1,3-dipolar cycloaddition of nitrone 190 with a terminal alkene would allow the development of routes to pyrrolizidines such as hyacinthacines A\textsubscript{1}, hyacinthacine B\textsubscript{2} etc, which have inverted stereochemistry at the C-1 position (hyacinthacine numbering system) [Scheme 22].

The differential protection at C-3 of the nitrone (destined to become the C-1 centre of the pyrrolizidines) was planned to enable its selective deprotection after construction of the bicycle in order to facilitate inversion or deoxygenation, thus allowing access to a range of hyacinthacines and polyhydroxylated pyrrolizidines from a single common synthetic precursor. This makes nitrone 190 a valuable synthetic intermediate and the application of nitrone 190 towards the synthesis of hyacinthacine A\textsubscript{1} and B\textsubscript{2} will be discussed in this thesis. The application of nitrone 190 in the first total synthesis of 1-epi-hyacinthacine B\textsubscript{2} will be demonstrated. To the best of our knowledge this constitutes the first synthesis of this non-natural hyacinthacine.
2.3.1 Routes to hyacinthacine A₁

As the simplest hyacinthacine with a hydroxymethyl group at the C-3 position and the hydroxyl groups at C-1 and C-2 cis to each other, hyacinthacine A₁ (51) was chosen as the first synthetic target (Figure 21).

Ishibashi’s group have previously exploited nitrone 190 previously in the preparation of a pyrrolizidine lactam (199, Scheme 16) that could be converted, in principle, into hyacinthacine A₁ through subsequent inversion of the stereochemistry at C-1 and reduction of the lactam.\(^\text{113}\) Surprisingly this sequence of reactions to conclude the synthesis has not been reported to date. The key step in the Ishibashi synthesis of pyrrolizidine lactam 199 was the 1,3-dipolar cycloaddition between nitrone 190 and tert-butyl acrylate (192), which proceeded with only modest selectivity to give the desired exo-anti cycloadduct (194) in 64% yield together with the endo-anti product (262) in 31% yield, [Scheme 30].
Scheme 30: Outcome of Ishibashi’s 1,3-dipolar cycloaddition between nitrone 190 and alkene 192.

The Goti group have investigated the 1,3-dipolar cycloaddition products formed upon reaction of a related nitrone (158) with maleic and acrylic acid derivatives 191, 263, and 264 (Scheme 31). With dimethyl maleate (263), nitrone 158 reacted cleanly to afford exclusively the exo-anti adduct (265). In contrast, methyl acrylate (264) gave a mixture of the anti adducts with poor selectivity (2.4:1, favouring the exo product over the endo). Approaches of dipolarophiles 263 and 264 occurred exclusively anti to both the benzyl group at C-3 and at C-5 of the nitrone, which are located on the same face of the ring. The benzyl group at C-4, which was expected to hamper formation of the endo cycloadducts was indeed effective with maleate 263, forming exclusively the exo cycloadduct 265, but not with acrylate 264, which gave a significant amount of endo adduct 266b. Goti et al. reasoned that by increasing the steric demand of the dipolarophile, the endo mode of approach should be disfavoured. This was confirmed in the reaction with the bulkier N,N-dimethylacrylamide dipolarophile (191), where the stereoselectivity of the cycloaddition was restored in favour of the exo-anti adduct 193 (Scheme 31).

Scheme 31: 1,3-dipolar cycloadditions of nitrone 158 with 263, 244 and 191 carried out by Goti et al.²⁰⁹
As nitrone 190 differs from nitrone 158 only in the protecting group at the C-3 position, it was thought that it should behave in a similar manner as nitrone 158 in 1,3-dipolar cycloaddition reactions. With this in mind and given the poor selectivity observed by Ishibashi’s group for the reaction of acrylate 192 with nitrone 190, we chose to use N,N-dimethylacrylamide (191) as the dipolarophile in our synthetic strategy (Scheme 32).

2.3.1.1 Attempted synthesis of hyacinthacine A₁: Route A

![Scheme 32: Planned route to hyacinthacine A₁ (51) – Route A.](image)

Based on the results of Goti et al. discussed above, then, the cycloaddition of nitrone 190 with acrylamide 191 was expected to furnish isoxazolidine 267, (Scheme 32). We anticipated that the route to hyacinthacine A₁ might then be completed by the following sequence of transformations. Firstly, after the cycloaddition, the N-O bond in 267 would be cleaved with subsequent cyclisation to form pyrrolizidinone 268. Mesylate derivatisation of the free hydroxyl group at C-6 would allow selective deprotection of the methoxymethyl ether, forming the key intermediate (270), where inversion at C-1 would subsequently put the oxygen functions at the 1- and 2-positions cis to each other. Hydrogenolytic cleavage of the remaining benzyl protecting groups would then lead to hyacinthacine A₁ (51). Clearly the
efficiency of this route would be strongly dependent on the outcome of the initial 1,3-dipolar cycloaddition, with four isomeric 5-isoxazolidine cycloadducts, (Figure 22), possible depending on the endo-exo and syn-anti selectivity achieved. In principle, both exo-anti and endo-anti products might be transformed into hyacinthacine A₁ by the route shown in Scheme 11, although it would be preferable to take a single isomer forward.

![Diagrams](image.png)

**Figure 22.** Possible cycloadducts from reaction between nitronitro 190 and acrylamide 191

Pleasingly, when nitronitro 190 was reacted with one equivalent of N,N-dimethylacrylamide (191) in dichloromethane at ambient temperature overnight only the exo-anti product was formed, and isoxazolidine 267 was obtained quantitatively as a pale brown oil following evaporation of the reaction mixture and column chromatography. The structure of the cycloadduct was confirmed by comprehensive 2-D NMR studies including NOESY. In particular a key set of cross-peaks in the NOESY spectrum confirmed that H-2 of the hexahydropyrrolo[1,2-b]isoxazole heterocycle was located on the face anti-to the 4- and 6-substituents were key NOESY interactions between CH-2 and CH-6, CH-2 and CH-3’’ and between CH-3’’ and CH-4 were observed (Figure 23). In addition, a NOESY interaction between CH-3a and CH-3’ was observed. The stereochemical outcome of the cycloaddition was further confirmed by the acquisition of a single crystal X-ray structure for a downstream intermediate in the route planned to hyacinthacine A₁ from isoxazolidine 267 (vide infra).
Figure 23: Key NOE interactions observed in the NOESY spectrum for isoxazolidine 267.

The remarkable selectivity in the reaction of nitrone 190 with acrylamide 191 can be explained by consideration of the steric interactions in the transition states for the cycloaddition modes leading to the four possible 5-substituted isoxazolidine products. Simple inspection of the structure of the nitrone and possible approaches of the alkene, shown for indicative purposes in Figure 24, clearly reveals that the \textit{exo-anti} mode of addition should incur the least steric penalty of all the four modes however. The exo approach is favoured due to the bulk of the alkene substituent and the alkene approaches form the more open lower ‘anti‘ face of the nitrone which is less hindered.
With the desired outcome from the 1,3-dipolar cycloaddition, the N-O bond of isoxazolidine 267 was then cleaved. There are various conditions commonly used to cleave the isoxazolidine N-O bond, including hydrogenation over Raney nickel, palladium or platinum catalysts,\textsuperscript{129} and reduction using zinc in acetic acid,\textsuperscript{130} or using lithium aluminium hydride.\textsuperscript{131} The conventional method of zinc dust and acetic acid was used in a first attempt with 267 and this afforded pyrrolizidine 268 directly in 57\% yield as white crystalline solid after work up and chromatographic isolation. As the yield was reasonable, no other methods of N-O bond cleavage were attempted and the structure of pyrrolizidine 268 was confirmed by X-ray crystallography (Figure 25, data in Appendix C). A triclinic crystal system was observed belonging to the P1 space group. Two molecules were found to be present in the asymmetric unit and are linked via OH...H hydrogen bonds into a discrete dimer.

\textbf{Figure 24:} Chem3D image showing the possible approaches of dipolarophile 191 to nitrone 190 in the cycloaddition reaction.

\textit{anti-endo approach} \hspace{1cm} \textit{anti-exo approach}

\textit{syn-endo approach} \hspace{1cm} \textit{syn-exo approach}
The hydroxyl function in compound 268 was then derivatised by treatment with methanesulfonyl chloride and triethylamine in dichloromethane, producing mesylate 269 in 98% yield. Deprotection of the MOM group by exposure to trifluoroacetic acid in dichloromethane at ambient temperature gave the key intermediate hydroxyzyrrolizidinone (270) in 87% yield. We anticipated that inversion of the hydroxyl group in pyrrolizidinone 270 might be achieved either by a redox procedure or by utilizing the Mitsunobu reaction. The redox procedure would involve oxidation to a ketone (271) [Scheme 32] followed by reduction back to the alcohol. Given the envelope-shaped structure of 271 we expected that the favoured trajectory for delivery of the hydride to ketone moiety of 271 would result in inversion of the C-1 stereocentre [Figure 26]. An attractive feature of this strategy was that simultaneous reductive deoxygenation of the mesylate group and lactam might then deliver the hyacinthacine A<sub>1</sub> precursor (260) directly in a one-pot transformation. Goti et al. had already successfully employed such a dual deoxygenation process with lactam-mesylate 200 in their route to hyacinthacine A<sub>2</sub> (52) [Scheme 16]. Moreover, Izquierdo and co-workers had also successfully applied the redox inversion procedure to an analogous pyrrolizidinone intermediate (146) [Scheme 7]. Thus, armed with separate precedent for the individual components of the proposed transformation of ketone 271 into pyrrolizidinone 260, we felt that the ambitious one-pot procedure should be feasible.
Common reagents for the oxidation of a secondary alcohol to a ketone are potassium dichromate, Dess-Martin periodinane\textsuperscript{133} and Swern conditions.\textsuperscript{134} Initially Dess-Martin oxidation of alcohol \textbf{270} to ketone \textbf{271} was attempted. The Dess-Martin periodinane (DMP), a hypervalent iodine compound, allows for the selective and very mild oxidation of secondary alcohols to ketones. Usually this reaction is performed at room temperature in DCM and is complete within 2 hours. When applied to alcohol \textbf{270}, TLC analysis after 2 hours indicated that no reaction had occurred and the alcohol remained unreacted. Further analysis at intervals over 24 hours also indicated no reaction. NMR analysis of the material returned following work up confirmed that no reaction had taken place, with no trace of a ketone-type carbonyl resonance present in the \textsuperscript{13}C NMR spectrum. The failure of this reaction is likely due to steric encumbrance of the secondary alcohol substrate. A Swern oxidation was then attempted on alcohol \textbf{270} using the standard oxalyl chloride/dimethylsulfoxide conditions but, as with the Dess-Martin procedure, TLC inspection and then NMR analysis of the crude material returned after work up indicated a complete failure in the reaction, with the substrate recovered unreacted. Both the Dess-Martin and the Swern oxidation procedures were attempted a further two times and as before
there was no reaction in any of these experiments. Finally, a PCC oxidation was attempted, but again without success, returning alcohol 270.

With the oxidation attempts unsuccessful, direct inversion of the alcohol under Mitsunobu conditions was tried. Accordingly, compound 270 was treated with a mixture of benzoic acid, triphenylphosphine and diethyl azodicarboxylate (DEAD) in dry THF in an attempt to obtain the inverted benzoate ester derivative (272) [Scheme 33]. However, this was also unsuccessful and the alcohol was returned.

**Scheme 33:** (a) BzOH, PPh₃, DEAD, THF (0%).

As a last resort it was decided to further modify the planned route to hyacinthacine A₁ (Scheme 32) by attempting to derivatise the free alcohol group in hydroxypyrrolizidinone 270 as a second mesylate, since this might then allow nucleophilic displacement with inversion by a suitable oxygen nucleophile such as an acetate anion, [Scheme 34]. Clearly the pre-existing mesylate at C-6 in the proposed dimesylate (273) might also suffer transformation under these conditions, but at this point we were keen to investigate the formation of the dimesylate to see if it was possible to secure any reaction at all on the C-1 alcohol. In the event, however, attempts to form dimesylate 273 from 270 were also unsuccessful.
Scheme 34: MsCl, TEA, DCM (0%).

The complete resistance of alcohol 270 to all of the reaction conditions described above came as a surprise. Although Izquierdo et al. had previously reported that the closely related pyrrolizidinol (146, Scheme 35) had been resistant to inversion of the 1-hydroxy group through a Mitsunobu strategy, they had successfully achieved the inversion to 147 by a redox procedure involving Swern oxidation followed by borohydride reduction. This yielded the inverted alcohol (147) and starting alcohol (146) as 1.5:1 mixture which was separable by chromatography.

Scheme 35: (a) Swern oxidation, (not isolated); (b) NaBH₄, MeOH; 147 (46%) and 146 (29%) (1.5:1); (c) i. BzOH, DEAD, Ph₃P, THF, ii. MeONa (cat.), MeOH, (no reaction).
2.3.1.2 Attempted synthesis of hyacinthacine A₁: Route B

Our route to pyrrolizidinol 270 is summarised in Scheme 36. Although we had no clear explanation for the lack of reactivity of this compound’s alcohol group, we were reluctant to abandon the route to hyacinthacine A₁ altogether. It was therefore decided to investigate the deoxygenation procedure on the MOM-protected mesylate lactam intermediate (269) first and return to the issue of C-1 inversion subsequently as a separate step. According to this modified route then, pyrrolizidine 269 was treated with lithium aluminium hydride in boiling THF for 1.5 h. Following an aqueous work up and chromatographic isolation, the deoxygenated pyrrolizidine 275 was successfully isolated in 89% yield. Selective deprotection of the MOM ether was then achieved by treatment with trifluoroacetic acid in dichloromethane to afford pyrrolizidinol 276 in 90% yield after purification by column chromatography. Although not investigated here, hydrogenolytic debenzylation of 276 would produce hyacinthacine A₂ (52). Rather than commit our material to this synthesis however, we focused attention on the inversion chemistry required to access hyacinthacine A₁ (51).
Scheme 36: (a) DCM, r.t., 2 h (100%); (b) Zn/AcOH, Δ, 3 h (57%); (c) MsCl, TEA, DCM, 0°C, 0.5 h (90%); (d) TFA, DCM, r.t., (87% for 270, 86% for 276); (e) Swern oxidation, (0%); (f) i. BzOH, DEAD, Ph₃P, THF, ii. MeONa (cat.), MeOH, (0%); (g) LiAlH₄, THF, Δ, 1.5 h (89%).

Surprisingly attempts at the Swern oxidation with 276 were also unsuccessful, returning 276 unreacted (notwithstanding the successful application of the Swern oxidation to closely related pyrrolizidinol 146 by Izquierdo et al.⁸⁶). Dess-Martin and PCC oxidations were then attempted on alcohol 276, but in both cases the alcohol remained unreacted. Mitsunobu inversion was then attempted (PPh₃/DEAD/benzoic acid) but again no reaction occurred and alcohol 276 was returned unreacted.
Scheme 37: Attempted mesylation of alcohol 253; MsCl, TEA, DCM, 0 °C, (0%).

A final attempt at inversion was made by derivatisation of alcohol 276 as mesylate 278 with a view to substituting the mesylate with a carboxylate nucleophile (Scheme 37). The mesylation of alcohol 276 was also unsuccessful, returning the unreacted alcohol.

2.3.1.3 Attempted synthesis of hyacinthacine A₁: Route C

Frustrated by the failure of the C-1 inversion strategies in Routes A and B above, a final approach to hyacinthacine A₁ was explored (Route C, Scheme 38), even though the route required extension of the synthetic sequence by a number of steps. Here the plan was to attempt the key stereocentre inversion on the hexahydropyrrolo[1,2-b]isoxazole cycloaduct (267) prior to cleavage of the isoxazolidine N-O bond and pyrrolizidine formation.
Scheme 38: Route C to Hyacinthacine A₁ (51); (a) TFA, DCM, r.t., (78%); (b) Swern oxidation, (no reaction); (c) i. BzOH, DEAD, Ph₃P, THF, (no reaction); ii. MeONa (cat.); (d) MsCl, DCM, TFA, (no reaction).

Cycloadduct 267 was selectively MOM-deprotected to compound 279 by TFA in 78% yield. This alcohol was then used as a substrate for the inversion attempts. As before, inversion was first attempted by a redox procedure. Attempts to oxidise alcohol 279 to ketone 280 by Swern oxidation, Dess-Martin oxidation and PCC oxidation were all unsuccessful, however, returning unreacted alcohol 279 in each instance. Alcohol 279 also failed to react when subjected to direct inversion under Mitsunobu conditions (PPh₃/DEAD/benzoic acid). Attempted conversion of alcohol 279 into mesylate 282 was also unsuccessful, and returned the unreacted alcohol.
2.3.1.4 Summary of attempted synthesis of hyacinthacine A₁ (51)

Three different routes have been explored towards the synthesis of hyacinthacine A₁ (51) where the inversion of the C-1 hydroxyl group at various intermediate stages was attempted (Figure 27). A redox procedure was investigated initially in each route as literature precedent suggested that Swern oxidation followed by reduction should be possible, where inversion by a Mitsunobu strategy might not proceed. However, when substrates 270, 276 and 279 were subjected to Swern oxidation conditions no reaction occurred and only the unreacted alcohol was returned in each case. These substrates were also found to be unreactive towards oxidations using Dess-Martin conditions and PCC. Unsurprisingly, these key intermediates were also resistant to inversion by the Mitsunobu reaction. Conversion of each substrate into the corresponding mesylate, with S₂N inversion and subsequent manipulation in mind, was similarly unsuccessful with each alcohol being returned unreacted. The failure of the inversion attempts on alcohols 270, 276 and 279 means that differentially protected nitrone 190 is not a suitable precursor in the synthesis of hyacinthacine A₁ (51), and it was therefore decided to explore the utility of the nitrone for synthesis of hyacinthacines bearing a carbon substituent at C-5. The apparent lack of reactivity found in the substrates tested could be that a hydrogen bond is forming between the bridgehead hydrogen and the oxygen of the free hydroxy group (Figure 26).

![Figure 27: Substrates investigated for inversion showing potential hydrogen bonding possibly explaining the lack of reactivity of these compounds.](image-url)
2.3.2 Routes to hyacinthacine B₂

Hyacinthacines B₁ (48) and B₂ (49) are identical to hyacinthacine A₁ (51) in one half of their structures, retaining the cis relationship between the C-1 and C-2 hydroxyl groups, but they possess an additional hydroxymethyl substituent at the 5-position of the pyrrolizidine core. At the outset of the project we anticipated that nitrone 190 might also be exploited for the construction of hyacinthacines B₁ and B₂ through cycloaddition with appropriate 4-carbon dipolarophiles. The strategy is illustrated in Figure 28 for hyacinthacine B₂, where a suitably protected but-3-ene-1,2-diol (287) would be used as the dipolarophile. An (R)-configured butenediol derivative would be required to establish the correct absolute stereochemistry at the C-5 centre in the case of hyacinthacine B₂ (49). Given our experience of the reaction between nitrone 190 and acrylamide 191, we could now be confident that a cycloaddition between this nitrone and the butenediol derivative would give predominantly an exo-anti adduct (288), although the intrinsic preferences of the chiral dipolarophile might, in principle, either reinforce or work against the selectivity afforded by the chiral nitrone structure. According to the plan in Figure 28, the free hydroxyl group in the isoxazolidine ring side-chain of 88 would be activated as a leaving group by derivatisation as the mesylate so that reductive cleavage of the N-O bond would proceed with concomitant intramolecular substitution to afford the pyrrolizidine core in 290. Deoxygenation and MOM-deprotection would then expose the C-1 hydroxyl group in 291 required for an inversion procedure that would deliver the hyacinthacine B₂ precursor (292). A final deprotection would afford the target pyrrolizidine (49), and we therefore anticipated using a benzyl protecting group in the butenediol dipolarophile (287) so that all three remaining protecting groups in intermediate 293 could be removed in a single hydrogenation step at the end. Given the problems encountered with the attempted inversion chemistry described in the preceding sections for hyacinthacine A₁, we were unsure at this stage whether the planned inversion step from 291 to 292 would be achievable, although we expected that deprotection of intermediate 291 would at least usefully furnish 1-epi-hyacinthacine B₂ (293), a polyhydroxylated pyrrolizidine that, to the best of our knowledge, has not previously been synthesised.
Figure 28: Potential route from nitrone 190 to hyacinthacine B₂.

Before commencing the route to hyacinthacine B₂, a suitable procedure for the synthesis of (R)-1-(benzyloxy)but-3-en-2-ol (294) was investigated. Mulzer et al.¹³⁵ have reported two routes to (R)-1-methoxybut-3-en-2-ol (295) starting from (R,R)-dimethyl tartrate (296) (Scheme 39) and (S)-oxiran-2-ylmethanol (297) (Scheme 40Scheme 39).
Scheme 39: (a) 2,2-dimethoxypropane, DCM, p-toluenesulfonic acid, Δ, 4 h, (85%); (b) LiAlH₄, Et₂O, -10 °C, 4 h, (70%); (c) NaH, Mel, DMF, r.t., 1 h, (65%); (d) TsCl, py, 0 °C, 1 h, (88%); (e) acetonitrile, NaI, Δ, 5 h, (93%); (f) Zn, EtOH, Δ, 5 h, (70%).

(R,R)-Dimethyl tartrate (296) was protected as the acetonide (298) which was subsequently reduced to diol 299. The monomethyl ether (300) was then formed by alkylation of 299 using methyl iodide and sodium hydride in DMF; only a small amount of the dimethyl ether were detected. The primary alcohol in 300 was then derivatised as the tosylate in 301 before being transformed into the iodide (302). Reductive elimination of the acetonide with activated Zn furnished allylic alcohol 295. Alternatively, a much shorter route was employed where (S)-glycidol (297) was O-methylated and then treated with trimethylsulfonylum ylide to give alcohol 295 directly (Scheme 40).
Scheme 40: (a) MeI, Ag₂O, DCM, Δ, 24 h, (60%); (b) Me₃S⁺I⁻, BuLi, THF, -15 °C, (76%).

For convenience, we decided to synthesise (R)-1-(benzyloxy)but-3-en-2-ol (294) by the shorter route from the commercially available (R)-2-(benzyloxymethyl)oxirane (304). Pleasingly, the ring opening of the enantiopure oxirane by the sulphur ylide produced the desired allylic alcohol (294) in an excellent yield (91%), [Scheme 41]. With the chiral alkene in hand, we next investigated the key 1,3-dipolar cycloaddition with nitrone 190 in order to commence the route to hyacinthacine B₂ (Scheme 41).
The cycloaddition between nitrone 190 and alkene 294 was initially attempted in dichloromethane at room temperature as the previous reaction with acrylamide 191 had been found to proceed effectively without heating. Alkene 294 proved to be substantially less reactive as a dipolarophile, however, and under these conditions the reaction did not occur to any significant extent over 24 h. Reaction between the addends required reflux in toluene to proceed and afforded an inseparable two-component mixture in a 3:1 ratio and quantitative yield. Unfortunately, as the product mixture was inseparable, the identity of the structures of the products could not initially be confirmed on the basis of 2D NMR experiments. The major product was therefore assumed to the desired exo-anti adduct (304a) and the minor product the less favoured endo-anti product (304b), (Figure 29). In principle, the formation of a mixture of exo and endo products (though inconvenient) would not be detrimental to the

**Scheme 41:** (a) Me$_3$S$^+$I, BuLi, THF, -15 °C, (91%); (b) 190, toluene, Δ, 24 h, (100% as inseparable mixture); (c) MsCl, TEA, DCM, 0 °C, 24 h (0%).
synthesis, so long as anti-selectivity was preserved. This is because the isoxazolidine ring C-5 stereocentre would be removed in the later deoxygenation step of the planned route to hyacinthacine B₂ (Figure 28). It was therefore decided to progress with the synthetic route as shown in Scheme 41, and the product mixture from the cycloaddition step was taken directly forward to the next stage. This involved treatment with methanesulfonyl chloride and triethylamine in dichloromethane. Surprisingly, however, this attempted conversion of the hydroxyl group into the mesylate only appeared to progress for the minor component within the cycloadduct mixture. This suggested that the minor adduct may in fact have been the exo-anti product (304a) and the major adduct the endo-anti isomer (304b); the lack of reactivity of the latter can possibly be explained by formation of a stabilising intramolecular hydrogen bond interaction between the hydroxyl group and the oxygen of the isoxazolidine ring (Figure 29). The identity of the minor adduct as the exo-anti cycloaddition product (304a) was indeed confirmed subsequently by independent synthesis through a modified route (vide infra).

![Structure of assumed cycloadducts 304a and 304b](image)

**Figure 29:** Structure of assumed cycloadducts 304a and 304b provided from the reaction of nitrone 190 with alkene 294; an intramolecular hydrogen bond in the major adduct is tentatively proposed to account for the failure of the compound’s conversion into its mesylate derivative.

The lack of reactivity of the major adduct within the product mixture derived from nitrone 190 and alkene 294, tentatively identified as the endo-anti isomer (304b), necessitated modification to the route to increase the cycloaddition selectivity in favour of an adduct that could successfully be developed into the pyrrolizidine. With this in mind, the hydroxyl group of allylic alcohol 294 was derivatised as the bulky silyl ether in compound 309, (Scheme 42), by treatment with TBDPSCl and imidazole. The hope was that this radical change in the dipolarophile’s structure would lead to a more selective cycloaddition outcome, though it was difficult to predict at the outset whether the favoured product would have the appropriate
stereochemistry required for successful conversion through to the mesylate needed in the synthesis of hyacinthacine B₂.

![Scheme 42](image)

**Scheme 42**: (a) TBDPSCl, imidazole, THF, r.t., 24 h, (96%); (b) toluene, reflux, 24 h, (92%).

The presence of the bulky TBDPS group in the dipolarophile did indeed increase the selectivity of the 1,3-dipolar cycloaddition reaction. Thus, after boiling alkene 309 and nitrone 190 in toluene overnight a single cycloadduct (310) (Scheme 42) was obtained in 92% yield after evaporation of the solvent and isolation by column chromatography. Adduct 310 was identified as the desired *exo-anti* product on the basis of comprehensive 2D NMR experiments and the observation of key cross-peaks in the NOESY spectrum that confirmed the orientation of the isoxazolidine ring CH-5 atom on the opposite face of the bicycle to the methoxymethyl group. In addition, NOESY interactions between CH-3’ and CH-2 and between CH3’ and CH-4 were observed (Figure 30, spectra in Appendix D).

![Figure 30](image)

**Figure 30**: Key NOESY interactions in cycloadduct 310.

It was anticipated that removal of the TBDPS protecting group from cycloadduct 310 would furnish alcohol 304, thus allowing a return to the planned synthetic route for hyacinthacine B₂ (Scheme 41). The silyl ether was successfully cleaved under standard conditions with
Results and Discussion

TBAF in THF at ambient temperature over 1 h, producing alcohol 304 in 85% yield, (Scheme 43). Comparison of the $^1$H NMR spectrum of the alcohol (304) thus obtained with that of the product mixture previously formed in the reaction of nitrone 190 with allyl alcohol 294 confirmed the identity of the minor isomer from that reaction as the exo-anti product (304). Mesylation of alcohol 304 now proceeded smoothly, forming mesylate 305 in quantitative yield, (Scheme 43). Previously only the minor adduct provided from the cycloaddition of allyl alcohol 304 had successfully formed the mesylate. This finding was therefore also consistent with the identity of that minor adduct as the exo-anti product (304). Cleavage of the isoxazolidine N-O bond in 305 was next accomplished under standard conditions by treatment with Zinc dust in hot acetic acid. Pleasingly this reaction proceeded efficiently with concomitant substitution of the mesylate to form pyrrolizidinol 306 in 87% yield after work up and chromatographic isolation.

Two methods for the deoxygenation of alcohol 306 to 307 were then attempted— first, by derivatisation of the alcohol as the mesylate (311) with subsequent reduction by hydride, and secondly, by a Barton-McCombie procedure. The mesylation-reduction route was attempted initially in order to avoid the toxic conditions used in the Barton-McCombie deoxygenation. Mesylate 311 was formed quantitatively from alcohol 306 under standard conditions with methanesulfonyl chloride and triethylamine in dichloromethane. Attempts at reducing mesylate 311 with lithium aluminium hydride in boiling THF over 12 h produced target pyrrolizidine 308 in only very low yield (ca. 2%), however, with mesylate 311 returned as the principal component. The use of Super-Hydride® increased the yield of 308 modestly to ca. 10% but again returned the mesylate. The Barton-McCombie deoxygenation was therefore investigated. Initial attempts at converting alcohol 306 into xanthate 312 using sodium hydride, carbon disulfide and methyl iodide in THF were largely unsuccessful, providing 312 in only 5% yield, this was despite taking rigorous precautions to ensure operation under anhydrous conditions. Changing the base to potassium hydride, however, allowed successful conversion of the alcohol into xanthate 312 in good yield (70%). Xanthate 312 was then subjected to treatment with AIBN and tributyl tin hydride in toluene at reflux to produce the deoxygenated pyrrolizidine (307) in a reasonable yield of 59%. Deprotection of the methoxymethyl ether in 307 was achieved with trifluoroacetic acid in dichloromethane and furnished alcohol 308 as the substrate for the key inversion step. As in the attempted
synthesis of hyacinthacine A₁, the alcohol inversion was tried, in the first instance, by a redox procedure. Swern oxidation of alcohol 308 to ketone 313, attempted under standard conditions (oxalyl chloride/dimethylsulfoxide), was unsuccessful and returned the alcohol unreacted. Disappointingly the application of Dess-Martin and PCC reagents also failed to effect the necessary oxidation, once again returning alcohol 308. Inversion by a Mitsunobu strategy was then attempted (benzoic acid/PPh₃/DEAD), but again no reaction took place, and the configurationally inverted carboxylate intermediate was not formed. As with the attempted synthesis of hyacinthacine A₁, all inversion attempts of C-1 on alcohol 308 were unsuccessful.

Scheme 43: Synthetic route to hyacinthacine B₂; (a) 309 and 190, toluene, reflux, 18 h, (92%); (b) TBAF, THF, r.t., 1 h, (85%); (c) MsCl, TEA, DCM, r.t., (99%); (d) Zn dust, 1/1 acetic acid/water, 85 °C, (87%); (e) MsCl, TEA, DCM, r.t., (99%); (f) Super-Hydride®, THF,
r.t., 24 h, (10%); (g) KH, CS₂, MeI, THF, r.t., 20 min, (70%); h) AIBN, Bu₃SnH, toluene, reflux, 1 h, (59%); (i) TFA, DCM, r.t., 48 h, (81%).

2.3.3 Synthesis of 1-epi-hyacinthacine B₂.

Given the remarkable resistance of alcohol 308 to derivatisation and the complete failure of the planned inversion step, final efforts were committed to the debenzylation of 308 in order to furnish the previously unknown pyrrolizidine, 1-epi-hyacinthacine B₂ (293) (Scheme 44). Initially the hydrogenation was attempted using a ThalesNano H-cube™, whereby a methanolic solution of 308 was pumped through a 10% Pd/C catalyst cartridge at a flow rate of 0.5 mL/min at 30 bar. This failed to effect the debenzylation to 293, however, and the experiment was therefore repeated with the addition of a little acetic acid to the solvent. As before no debenzylation was observed and only the starting material 308 was recovered. Successful deprotection was finally achieved by hydrogenation under acidic conditions using the conventional balloon technique. Thus, an acidic solution of compound 308 in methanol was hydrogenated over a 10% Pd/C catalyst for 48 hours at ambient temperature and pressure. After removal of the catalyst by filtration and evaporation of the solvent, the target pyrrolizidine, 1-epi-hyacinthacine B₂ (293), was obtained in 88% yield following ion exchange chromatography over a basic resin.

![Scheme 44](image)

Scheme 44: (a) i. H₂/Pd/C, MeOH, HCl, r.t., 48 h; ii. Amberlite resin IR-400 (OH⁻ form) (88%).
2.4 Conclusions

The synthesis of differentially protected nitrone 190 has been successfully achieved and reported in this thesis. A route to nitrone 190 from L-xylose over nine synthetic steps has been described (Scheme 25). An alternative route involving eleven synthetic steps starting from D-arabinose has also been presented (Scheme 29). The L-xylose route has the advantage of being the shorter one, but D-arabinose is a less expensive starting material. The D-arabinose route has the added advantage of avoiding an expensive silver carbonate oxidation that was necessary in the L-xylose route. Overall, the D-arabinose route to nitrone 190 would be the recommended one for reliable preparation of the nitrone for further development of the synthetic routes to biologically active polyhydroxylated pyrrolizidines.

Nitrone 190 was found to be remarkably selective in its 1,3-dipolar cycloaddition reactions with N,N-dimethylacrylamide (191) and (R)-(1-(benzyloxy)but-3-en-2-yloxy)(tert-butyl)diphenylsilane (309), forming exclusively the desired exo-anti cycloadducts 267 and 310 respectively.

![Figure 31](image)

The differential protection built into nitrone 190 allowed these cycloadducts to be successfully manipulated and transformed into pyrrolizidinols 276 and 308, (Figure 32), possessing structures suitable for the preparation of hyacinthacines A1 and B2 respectively. The key feature of the synthetic design here was to allow access to compounds with an unmasked alcohol at the pyrrolizidine C-1 position in order to perform a configurational inversion at the penultimate stage in the route; thereby establishing the correct
(1S,2R,3R,7aR)-absolute stereochemistry in the right-hand ring of these hyacinthacine alkaloids.

![Chemical structures of hyacinthacine A and B](image)

**Figure 32.** Substrates on which inversion was attempted.

To our considerable surprise, however, the hydroxy group in pyrrolizidinols 276 and 308 proved completely resistant to derivatisation and attempts to facilitate the inversion. With the work directed towards synthesis of hyacinthacine A₁, we also examined the suitability of other intermediates (270 and 279, Figure 32) as substrates for the inversion chemistry. However, in each case the secondary alcohol resisted derivatisation and we were ultimately unable to complete the routes to either of the natural compounds, hyacinthacines A₁ and B₂. The apparent lack of reactivity found in the substrates tested could be due to the presence of a hydrogen bond between the bridgehead hydrogen and the oxygen of the free hydroxy group.

Pyrrolizidinol intermediate 278 was, however, successfully transformed into 1-epi-hyacinthacine B₂ (293, Figure 33) by hydrogenolytic cleavage of the benzyl ether protecting groups. This, to the best of our knowledge, completed the first total synthesis of 1-epi-hyacinthacine B₂, which has yet to be isolated from a natural source and would therefore be an interesting candidate for biological testing.

![Chemical structures of 1-epi-hyacinthacine B₂](image)

**Figure 33:** Formation of 1-epi-hyacinthacine B₂.
2.5 Future Work

The apparent lack reactivity of the free hydroxyl group in the substrates investigated is likely due to hydrogen bonding between the bridge head hydrogen and the oxygen of the free hydroxyl group (figure 34).

![Figure 34: Potential Hydrogen bonding of inversion substrates.](image-url)

Since the oxidation (Swern, and Dess-Martin) and inversion strategies (Mitsunobu and via a mesylate) involved reactions at low temperature (ambient to -78 °C) their lack of reactivity is perhaps not surprising. Therefore, higher temperature oxidations (e.g. Oppenauer oxidation) and inversions (e.g. via tosyl group) could be attempted in the hope that the hydrogen bond interaction could be overcome. Conversion to a tosylate could provide routes not only to the desired inverted alcohol, but also opens up the substrate to nucleophilic attack allowing a wide variety of functional changes at the C-1 position (e.g alkyl, halide, sulfide and alkyl sulfide) (Figure 35). Additionally, deoxygenation of these substrates would provide novel compounds (Figure 35).
Figure 35: Further reactivity of inversion substrates to be explored leading to desired and alternative structures.
3 EXPERIMENTAL
Instrumentation

NMR spectra were recorded on a Bruker AC200 at 200 MHz (\(^1\text{H}\)) and 50 MHz (\(^{13}\text{C}\)), or a Bruker DPX400 at 400 MHz (\(^1\text{H}\)) and 100 MHz (\(^{13}\text{C}\)). Coupling constants (\(J\)) are quoted in Hz. Accurate mass measurements (electrospray) were performed using Finnigan MAT 900 XLT and Finnigan MAT 95 XP instruments at the EPSRC National Mass Spectrometry Service Centre, University of Wales, Swansea. Elemental analyses were conducted using an Exeter CE-440 elemental analyser. Infrared spectra were recorded using a Perkin-Elmer RX Fourier Transform spectrometer as a thin film on NaCl plates. Specific rotations were measured at room temperature using a Bendix-NPL 143D automatic polarimeter (path length 1 cm) with solvent as stated; units for \([\alpha]_D\) values are 10\(^{-1}\) deg cm\(^2\) g\(^{-1}\).

Chromatography

Analytical t.l.c. was performed on Merck aluminium-backed Kieselgel 60 silica plates (0.25mm), visualised with UV light (254nm) and developed with 5% aqueous ammonium molybdate solution to which concentrated sulfuric acid had been added. Flash chromatography was carried out with the indicated solvents using 230-400 mesh Kieselgel 60 silica as the stationary phase.

General Details

All reagents used were purchased from Aldrich, Avocado or Lancaster chemical companies unless otherwise stated. Solvents were routinely distilled before use and, where indicated, dried by standard procedures. Light petroleum refers to material of boiling range 40-60 °C. All air and moisture sensitive reactions were carried out using usual inert atmosphere techniques under an argon atmosphere. All glassware was flame-dried, and then cooled under argon prior to use. Reactions with H\(_2\) were carried out at 1 atm. pressure, using a balloon unless otherwise stated.
1,2-\textit{O}-Isopropylidene-\textit{\textalpha}L-\textit{xylofuranose} (234)

L-\textit{xylose} (5.00 g, 33.32 mmol) was added to acetone (130 mL) containing conc. H\textsubscript{2}SO\textsubscript{4} (5 mL) and the mixture was stirred at room temperature for 30 minutes. A solution of Na\textsubscript{2}CO\textsubscript{3} (6.50 g) in water (50 ml) was added with external cooling and the mixture was left to stir for a further 5 h. The mixture was then neutralised with Na\textsubscript{2}CO\textsubscript{3}. Filtration and concentration produced an oil, which was chromatographed on silica gel, eluting with DCM containing a MeOH gradient (5-10 %), to generate the title compound 234 (4.95 g, 75 %) as a white solid.

\( R_f \) 0.33 (DCM/methanol, 95:5); m.p 46-47 °C (lit. m.p 42-43 °C\textsuperscript{136}; \( \delta \text{H} \) (200 MHz, CDCl\textsubscript{3}): 1.23 (3H, s, CMe\textsubscript{2}), 1.42 (3H, s, CMe\textsubscript{2}), 2.83 (1H, b, s, OH-3), 3.93-4.19 (4H, m, C4-H, C5-H\textsubscript{2}, C5-OH), 4.28 (1H, d, \( J = 2.5 \), C3-H), 4.48 (1H, d, \( J = 3.7 \), C2-H), 5.93 (1H, d, \( J = 3.7 \), C1-H); \( \delta \text{C} \) (50 MHz): 26.4 (CH\textsubscript{3}), 27.0 (CH\textsubscript{3}), 61.2 (CH\textsubscript{2}), 77.0 (C-3), 79.1 (C-4), 85.8 (C-2), 105.1 (C-1), 112.1 (C); IR \( \nu_{\text{max}} \) 3401.4 (br OH), 2988.7, 1377.4, 1217.6, 1165.4, 1074.3, 1016.8 cm\textsuperscript{-1}; LRMS (ES): 208.2, 191.1, 173.1, 150.1, 133.0, 115.1, 76.3; HRMS (ES): 208.1177 [(M+NH\textsubscript{4})\textsuperscript{+}; requires 208.1179]; Anal. Calcd for C\textsubscript{8}H\textsubscript{14}O\textsubscript{5}: C, 50.52; H, 7.54. Found: C, 50.72; H, 7.53.

3,5-\textit{Di-O}-benzyl-1,2-\textit{iso-O}-propyldene-\textit{\textalpha}L-\textit{xylofuranose} (235)

Compound 234 (4.10 g, 23.67 mmol) in THF (10 mL) was added to a suspension of NaH (60% dispersion in oil, 3.00 g, 25.60 mmol) and \( n \)-Bu\textsubscript{4}NI (6.80 g, 18.41 mmol) in anhydrous THF (100 mL) at 0 °C. The mixture was stirred at this temperature for 20 min then allowed
to warm up to room temperature and left to stir for 30 minutes. Subsequently, the mixture 
was cooled to 0 °C and treated with a solution of BnBr (5.70 mL, 47.99 mmol) in THF (5 
ml). The mixture was then warmed up to ambient temperature and left to stir for 5 h. The 
mixture was quenched with water (50 ml) and extracted with ethyl acetate (2 x 60ml). The 
combined organic layers were washed with brine (30 ml), dried over MgSO₄, filtered and 
evaporated to produce an orange oil. The crude material was purified by flash 
chromatography using hexanes containing ethyl acetate gradient (10-20 %) to produce 235 
(6.70 g, 84 %) as an orange oil.

\[ R_f \text{0.36 (light petrol/ethyl acetate, 5:1); } \delta_H (200 \text{ MHz, CDCl}_3): \]
\[ 1.29 (3\text{H, s, CCH}_3), 1.46 (3\text{H, s, CCH}_3), 3.75 (2\text{H, m, C5-H}_2), 3.96 (1\text{H, d, }J = 3.2, \text{C3-H}), 4.27 (1\text{H, dt, }J = 6.1, 3.2, \text{C4-H}), 4.56 (5\text{H, m, 2 x OCH}_2\text{Ph, C2-H}), 6.18 (1\text{H, d, }J = 3.05, \text{C1-H}), 7.47-7.51 (10\text{H, m, Ph}); \]
\[ \delta_c (50 \text{ MHz): 26.5 (CH}_3), 28.00 (CH}_3), 69.8 (OCH}_2), 73.4 (OCH}_2), 75.3 (OCH}_2), 78.6 (C-4), 80.9 (C-3), 82.2 (C-3), 106.5 (C-1), 110.4 (C), 127.7, 127.6, 127.8, 128. 3, & 128.4 (CH}_ar), 137.5 & 137.9 (C); IR v_{max} 3065.8, 3029.7, 2932.3, 2336.6, 1497.1, 1451.1, 1374.1, 1309.4, 1075.4, 1018.5, 888.8, 858.5, 736.3, 697.5 \text{ cm}^{-1}. \text{Anal. Calcd for C}_{22}\text{H}_{26}\text{O}_5: C, 71.33; H, 7.07. \]
Found: C, 71.02; H, 7.01.

3, 5-Di-O-benzyl-L-xylofuranose (229)\textsuperscript{113}

\[ \text{A stirred solution of 3,5-di-O-benzyl-1,2-iso-O-propylidene-α-L-xylose 235 (4.01 g, 10.81 \text{ mmol) in acetic acid-water (1:1, 30 mL) was heated to 80 °C and stirred for 24 h. The mixture was cooled, diluted with water (30 ml), and extracted with DCM (60 ml). The organic layer was washed with saturated sodium bicarbonate (20 ml), brine (20 ml), dried over Na}_2\text{SO}_4, \text{filtered and concentrated to give the diol 229 (3.09 g, 86 %) as a white wax.} \]

\[ R_f \text{0.11 (light petrol/ethyl acetate 6:4); m.p 67-70 °C (no lit. value); } \delta_H (200 \text{ MHz, CDCl}_3): \]
\[ 3.38-3.65 (2\text{H, m, C5-H}_2), 3.83-4.11 (4\text{H, m, C2-H, C3-H, 2x OH}), 4.30-4.60 (1\text{H, m, C4-H)}, \]
Experimental

4.33-4.61 (4H, m, 2 x O\text{CH}_2\text{Ph}), 5.12 (\alpha\text{-anomer, s, C1-H}), 5.39 (\beta\text{-anomer, d, } J = 3.3, \text{C1-H}), 7.13-7.33 (10H, m, Ph); \delta_c (50 MHz): 68.5 & 68.7 (\alpha & \beta \text{CH}_2), 71.6 & 72.5 (\alpha & \beta \text{CH}_2), 73.2 & 73.4 (\alpha & \beta \text{CH}_2), 77.24 & 79.1 (\alpha & \beta \text{CH}), 82.5 & 83.2 (\alpha & \delta \text{CH}), 95.7 (\alpha \text{C1-H}), 103.2 (\delta \text{CH}), 127.3, 127.4, 127.5, 127.6, 127.8, 128.1 & 128.3 (\text{CH}_\text{ar}), 137.5 & 137.6 (\text{c}); IR \nu_{\text{max}} 3365.8 (\text{br. OH}), 3061.6, 2893.3, 1497.4, 1453.9, 1356.0, 1279.3, 1096.6, 1045.3, 977.0, 913.6, 734.1, 697.0 cm\textsuperscript{-1}. LRMS (ES): 348.3, 330.3, 240.3, 198.2, 168.2, 108.2, 91.2; HRMS (ES): 348.1805 [(\text{M+NH}_4)^+]; requires 348.108. Anal. Calcd for C\textsubscript{19}H\textsubscript{22}O\textsubscript{5}: C, 69.07; H, 6.71. Found: C, 68.88; H, 6.66.

3,5-Di-O-benzyl-1-xylano-1,4-lactone (236)

![Diagram](image)

Method 1:

Barium carbonate (490 mg, 2.48 mmol) and bromine (100 \mu L, 1.94 mmol) were added to a stirred ice cold solution of 229 (0.50 g, 1.51 mmol) in THF-water (1:3, 25 mL). The mixture was allowed to warm to ambient temperature and was left to stir overnight. The mixture was then filtered through Celite\textsuperscript{®} and the filtrate concentrated under reduced pressure. The remaining aqueous solution was removed by extraction with DCM (2 x 30ml) and the combined organic layers were concentrated to give the crude material. This was purified by chromatography, eluting with light petroleum with an ethyl acetate gradient (10- 40 %) to produce the lactone 236 (0.089 g, 18%) as a white solid.

Method 2:

A mixture of diol 229 (3.96g, 11.99 mmol) and Ag\textsubscript{2}CO\textsubscript{3}/Celite\textsuperscript{®} (7.50g, 12.50 mmol) were heated at 80 °C in toluene (200 mL) for 1 h. The mixture was filtered and washed with DCM (100 ml). The filtrate was then evaporated and the residue was purified by flash
Experimental

chromatography, using light petroleum/ethyl acetate (7:3) as the eluent, to give the pure lactone **236** (1.75 g, 45%) as a white solid.

Method 3:

A mixture of diol **229** (1.32 g, 3.99 mmol) and Ag$_2$CO$_3$/Celite® (7.50 g, 12.50 mmol) were heated at 80 °C in toluene (200 mL) for 1 h. The mixture was filtered and washed with DCM (100 ml). The filtrate was then evaporated and the residue was purified by flash chromatography, using light petrol/ethyl acetate (7:3) as the eluent, to give the pure lactone **236** (1.29 g, 99%) as a white solid. A small sample of the white solid was recrystallised from a 1:1 mixture of heptane/ethyl acetate providing crystals which were analysed by x-ray crystallography.

$R_f$ 0.2 (light petroleum/ethyl acetate 7:3); m.p 84-87 °C; $\delta_H$ (200 MHz, CDCl$_3$): 2.68 (1H, d, $J =3.1$, C2-H), 3.51 (2H, 2 dd, $J = 10.9$, 2.3, 5a-H$_2$), 3.53 (2H, 2 dd, $J = 10.9$, 2.7, C5b-H), 4.15-4.72 (6H, m, 2 OCH$_2$Ph, C3-H, C4-H), 7.02-7.25 (10H, m, Ph); $\delta_c$ (50 MHz): 66.4 (C-5), 71.1 (C-3), 72.1 (OCH$_2$), 73.0 (OCH$_2$), 77.1 (C-2), 79.8 (C-4), 126.9, 127.2, 127.5, 127.7, 127.9 & 127.9 (CH$_{ar}$), 136.7 (C), 136.9 (C), 174.7 (CO); IR $\nu_{max}$ 3450.7 (br OH), 3034.5, 2866.3, 1777.8, 1654.2, 1318.9, 1124.2, 1020.3, 763.2 cm$^{-1}$. LRMS (ES): 346.4, 330.4, 256.3, 222.3, 168.2, 106.3; HRMS (ES): 346.1655 [(M+NH$_4$)$_+$. C$_{19}$H$_{23}$NO$_5$ requires 346.1649]. Anal. Calcd for C$_{19}$H$_{20}$O$_5$: C, 69.50; H, 6.14. Found: C, 69.39; H, 6.14.

**Preparation of Ag$_2$CO$_3$/Celite® reagent**

Celite® (30 g) was added to a stirred solution of silver (I) nitrate (30 g) in distilled water (200 mL). To this mixture was added slowly a solution of sodium carbonated decahydrate (30 g) in distilled water (300 mL). The precipitate was collected by filtration and washed well with distilled water (500 ml). The solid was then dried on the rotary evaporator for 4 h in the dark. It has been estimated that 0.6 g of this material contains roughly 1 mmol Ag$_2$CO$_3$.  

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To a stirred solution of 236 (0.23g, 0.97 mmol) in chloroform (22 mL) at ambient temperature was added dimethoxymethane (4.40 mL, 48.41 mmol) and phosphorous pentoxide (20.24 g, 5.72 mol). The mixture was stirred for 30 min, the solids decanted and the filtrate poured in to an ice cold solution of saturated sodium bicarbonate (17 mL). The two phases were separated and the aqueous phase was extracted with chloroform (2 x 20 ml) and the combined organic layers were washed with brine (20 ml), dried over Na₂SO₄, and evaporated in vacuo to give the ether 237 (354 mg, 98 %) as a yellow oil.

*Rf 0.76 (light petroleum/ethyl acetate 1:1); δ<sub>H</sub> (200 MHz, CDCl₃): 3.37 (3H, s, OMe), 3.59 (1H, dd, J = 10.93, 3.12, C5a-H), 3.61 (1H, dd, J = 10.93, 3.25, C5b-H), 4.21-4.69 (8H, m, 2 x OCH<sub>2</sub>Ph, OCH<sub>2</sub>O, C3-H, C4-H), 4.93 (1H, d, J = 6.65, C2-H), 7.01-7.29 (10H, m, Ph); δ<sub>c</sub> (50 MHz): 55.81 (OCH₃), 66.66 (C-5), 71.92 (OCH₂), 72.59 (OCH₂), 73.22 (C-3), 76.98 (C-2), 79.14 (C-4), 95.74 (OCH₂O), 127.22, 127.38, 127.44, 127.67, 127.82, 128.06, 128.16 & 128.24 (CH<sub>ar</sub>) 136.78 (C), 137.20 (C), 172.595 (C=O); IR ν<sub>max</sub> 2898.3, 1789.9, 1496.5, 1453.8, 1244.8, 1165.8, 1123.7, 1023.3, 736.8 cm<sup>-1</sup>. LRMS (ES): 372.3, 341.4, 327.5, 299.2, 281.8, 265.3, 249.6, 235.3, 228.1, 220.8, 108.6, 91.7; HRMS (ES): 390.1908 [(M+NH₄)<sup>+</sup>; C₂₁H₂₈NO₆ requires 390.1911]. Anal. Calcd for C₂₁H₂₄O₆: C, 67.73; H, 6.50. Found: C, 67.91; H, 6.78.

3,5-Di-O-benzyl-2-O-methoxymethyl-L-xylofuranose (232)<sup>113</sup>
Lactone 237 (1.00 g, 2.69 mmol) was dissolved in THF (80 mL) and cooled to -78 °C. A solution of DIBAL in toluene (1.5M, 3.60 mL, 5.40 mmol) was added dropwise over 30 min and the mixture allowed to stir for 1.5 h. A further portion of the DIBAL solution (5.00 mL, 7.50 mmol) was then added dropwise over 30 min. The mixture was then stirred until TLC analysis showed no remaining starting material. The reaction was then quenched with saturated ammonium chloride (22 mL), filtered through celite® and the residue was washed well with DCM (200 mL). The filtrate was then concentrated in vacuo to produce hemiacetal 232 (0.78g, 78%) as a pale yellow/green oil.

\[ R_f 0.48 \text{ (light petroleum/ethylacetate 1:1); } \delta_H (400 MHz, CDCl}_3): 3.22 (3H, 2 s, OMe α- and β-anomers), 3.51-3.72 (2H, m, C5-H), 3.88-4.07 (3H, m, C2-H, C3-H & C4-H), 4.21-4.62 (6H, m, 2 x OCH}_2Ph & OCH}_2O), 5.09-5.11 (1H, 2 x s, C1-H, α and β), 5.38 (1H, br OH), 7.08-7.29 (10H, m, Ph); \delta_c (100 MHz): 55.9 & 56.2 (α & β OMe), 68.9 & 69.1 (ά & β CH}_2), 72.6 & 72.9 (α & β CH}_2), 73.7 & 73.9 (α & β CH}_2), 77.6 (β-anomer C-2), 80.0 & 80.3 (α & β C-3), 81.5 & 81.6 (α & β C-4), 84.2 (α-anomer C-2), 96.2 & 96.9 (α & β OCH}_2O), 96.6 (β-anomer C-1), 102.3 (α-anomer C-1), 127.9, 128.0, 128.1, 128.2, 128.4, 128.6, 128.7 & 128.8 (CH}_ar), 137.6 (C), 138.1 (C); IR \nu_max 3430.0 (br. OH), 3063.5, 2931.4, 1718.3, 1496.7, 1454.3, 1367.9, 1271.8, 1209.9, 1156.9, 1103.4, 1041.7, 918.1, 738.2, 698.5 cm\textsuperscript{-1}.

3,5,-Di-O-benzyl-2-O-methoxymethyl-L-xylose oxime (238)

\[ \text{A solution of Lactol 232 (158 mg, 0.422 mmol), hydroxylamine hydrochloride (553 mg, 7.91 mmol), and sodium methoxide (442 mg, 7.92 mmol) were stirred in methanol (15 mL) for 16 h at ambient temperature. The reaction mixture was then concentrated in vacuo and the residue was dissolved in DCM (20ml). The resulting solution was washed with water (2 x 20ml) and the combined organic layers were dried over Na}_2SO}_4 and filtered. The filtrate was concentrated in vacuo to give 238 (134mg, 82 %) as a dark yellow oil.} \]
Experimental

R\text{f} 0.27 (light petroleum/diethyl ether 1:1); \(\delta_{\text{H}}\) (200 MHz, CDCl\textsubscript{3}): 3.21 (3H, s, OMe), 3.25 (1H, d, \(J = 5.73\), E-isomer, 4-OH), 3.29 (3H, m, C5-H & Z-isomer, 4-OH), 3.8-3.74 (6H, m, 2 x OCH\textsubscript{2}Ph, OCH\textsubscript{2}O), 6.25 (1H, d, \(J = 6.22\), C1-H, Z-isomer), 7.12-7.28 (10H, m, Ph), 7.34 (1H, d, \(J = 7.71\), C1-H, E-isomer), 9.02 (1H, br. N-OH); \(\delta_{\text{c}}\) (50 MHz): 55.6 (E-isomer, OCH\textsubscript{3}), 55.9 (Z-isomer, OCH\textsubscript{3}), 68.9 (CH), 69.6 (CH), 70.6 (Z-isomer, CH\textsubscript{2}), 71.0 (E-isomer, OCH\textsubscript{2}), 73.2 (OCH\textsubscript{2}), 74.4 (E-isomer, CH\textsubscript{2}), 74.8 (Z-isomer, CH\textsubscript{2}), 78.5 (CH), 94.9 (E-isomer, OCH\textsubscript{2}O), 95.8 (Z-isomer, OCH\textsubscript{2}O), 127.6, 127.8, 128.2 & 128.2 (CHar), 137.6 (E-isomer, C), 137.6 (Z-isomer, C); IR \(\nu_{\text{max}}\) 3327.7 (br. OH), 3080.4, 3024.8, 2865.8, 1458.8, 1093.1, 1067.4, 732.6, 695.7 cm\textsuperscript{-1}.

3,5-Di-O-benzyl-2-O-methoxymethyl-\textalpha;-xylose-O-(\textit{tert}-butyldimethylsilyl)oxime (239)

Method 1:

Oxime 238 (118 mg, 304 mmol) was dissolved in dry pyridine (35 mL) and \textit{tert}-butyldimethylsilyl chloridc (46 mg, 304 mmol) was added. The mixture was allowed to stir for 12 h at ambient temperature. Concentration left a residue which was dissolved in DCM (50 ml) and washed with water (2 x 20 ml). The organic layers were washed with 1M HCl (5 ml), Sodium Bicarbonate (10ml) and brine (20 ml) before being dried over MgSO\textsubscript{4}, filtered and evaporated \textit{in vacuo} to give 239 (0.262 g, 34 %) (E:Z, 5:1) as a white/green oil.

Method 2:
To a stirred solution of lactol 232 (577 mg, 1.54 mmol) in dry toluene (6 mL) was added MgSO₄ (670 mg). The suspension was brought to reflux for 5 min then tert-butyldimethylsilylhydroxyamine (340 mg, 1.54 mmol) was added. The reaction mixture was left at reflux for a further 30 min before being allowed to cool. The filtrate was washed with saturated sodium bicarbonate (5ml), brine (10ml) then dried over MgSO₄ and filtered. The filtrate was concentrated to give 239 (0.114 g, 89%) (E:Z, 5:1) as a yellow/green oil.

Rᵋ 0.68 (light petroleum/diethyl ether 1:1); δ_H (200 MHz, CDCl₃): 0.00 (6H, s, SiMe₂), 0.78 (9H, s, SiCMe₃), 2.34 (1H, br s, OH), 3.13 (3H, s, OMe), 3.23-3.41 (2H, m, C5-H), 3.55-3.89 (3H, m, C2-H, C3-H, C4-H), 4.28-4.65 (6H, m, 2 x OCH₂Ph & OCH₂O), 6.89 (1H, d, J =5.11, Z-isomer C1-H), 7.08-7.21 (10H, m, Ph), 7.40 (1H, d, J =7.7, E-isomer C1-H); δ_c (50 MHz): (E-isomer) -8.1 (2x CH₃), 17.9 (CMe₃), 25.8 (3 x CH₃), 55.6 (OCH₃), 69.1 (CH₄), 69.9 (CH₂), 73.1 (CH₂), 73.8 (CH-3), 74.1 (CH₂), 77.5 (CH-2), 94.7 (OCH₂O), 127.5, 127.6, 127.7, 128.0 & 128.2 (CH₆), 137.5 & 137.7 (C), 152.0 (CH-1); IR ν_max 3352.8 (br. OH), 3069.2, 3030.6, 2935.3, 2854.5, 1428.9, 1113.7, 1069.4, 920.5, 732.4, 701.1 cm⁻¹, HRMS (ES): 504.2760 [(M+H)⁺], requires 504.2776.

**Preparation of tert-butyldimethylsilylhydroxyamine**

Ethylenediamine (30.22 g, 0.50 mol) was dissolved in DCM (150 mL) and hydroxylamine hydrochloride (35.32 g, 0.50 mol) was added. This mixture was stirred at ambient temperature for 6 h. A condenser with water cooling was fitted and tert-butyldimethylsilylhydroxychloride (75.32 g, 0.50 mol) was added portionwise down the condenser over a 1 h period. The mixture was allowed to cool, the condenser was removed, the flask was stoppered and the mixture allowed to stir for 24 h. The reaction mixture was filtered and the filtrate was distilled under vacuum, product distilled at 80-90 °C to produce the product as a white solid (24 g, 83 %).

δ_H (200 MHz, CDCl₃): 0.00 (6H, s, SiMe₂), 0.79 (9H, s, CMe₃), 4.98 (2H, s, br. NH₂).
3,5-Di-O-benzyl-4-O-methanesulfonyl-2-O-methoxymethyl-1-xylose-O-(tert-butyldimethylsilyl) oxime (240)\textsuperscript{113}

![Compound 239 structure](image1)

Compound 239 (0.26 g, 0.52 mmol) was dissolved in DCM (1.5 mL), cooled to 0 °C and stirred under an argon atmosphere. Triethylamine (113 µL, 0.81 mmol), and methanesulfonyl chloride (49 µL, 0.62 mmol) were added successively. The mixture was stirred for 30 min at 0 °C and then water (0.5 mL) was added. The layers were separated and the aqueous layer was extracted with DCM (2x 2 ml). The combined organic layers were washed with brine (5 ml), dried over MgSO\textsubscript{4}, filtered and evaporated to give 240 (0.270 g, 93%) (E:Z, 5:1) as a yellow oil.

R\textsubscript{f} 0.72 (light petroleum/diethyl ether 1:1); δ\textsubscript{H} (200 MHz, CDCl\textsubscript{3}): 0.00 (6H, s, SiMe\textsubscript{2}), 0.79 (9H, s, CMe\textsubscript{3}), 2.78 (3H, s, SO\textsubscript{2}Me), 3.11 (3H, s, OMe), 3.58-3.66 (2H, m, C5-H), 3.75-3.83 (1H, m, C3-H), 4.16-4.49 (7H, 2 x OCH\textsubscript{2}Ph, OCH\textsubscript{2}O, C4-H), 4.72-4.89 (1H, m, C2-H), 6.92 (1H, d, J =5.11, Z-isomer C1-H), 7.08-7.22 (10H, m, Ph), 7.32 (1H, d, J =7.36, E-isomer H-1); δ\textsubscript{C} (50 MHz): (E-isomer) -7.9 (2 x CH\textsubscript{3}), 18.5 (CMe\textsubscript{3}), 26.4 (3 x CH\textsubscript{3}), 38.7 (SCH\textsubscript{3}), 56.6 (OCH\textsubscript{3}), 68.9 (C-5), 73.6 (CH), 73.80 (OCH\textsubscript{2}), 75.5 (OCH\textsubscript{2}), 79.1 (CH), 81.2 (CH) 94.7 (OCH\textsubscript{2}O), 128.3, 128.3, 128.5 128.7 & 128.9 (CH\textsubscript{ar}), 137.6 & 137.7 (C), 152.62 (CH-1); IR ν\textsubscript{max} 3031.8, 2930.5, 2888.3, 1497.2, 1471.8, 1454.8, 1361.1, 1252.2, 1176.7, 1099.9, 1028.4, 924.4, 838.2, 818.5, 794.2 cm\textsuperscript{-1}.

5-O-tert-Butyldiphenylsilyl-d-arabinofuranose (248)\textsuperscript{137}

![D-Arabinose structure](image2)

D-Arabinose
D-Arabinose (7.67 g, 51.06 mmol) was added to a solution of tert-butylidiphenylsilyl chloride (13.65 g, 49.65 mmol) and imidazole (6.78 g, 99.59 mmol) in dry DMF (105 mL). The suspension was heated to 60 °C and stirred for 2 h. The reaction mixture was then cooled to ambient temperature then poured into aqueous HCl solution (1M, 225 mL) and extracted with DCM (150 mL). The organic phase was washed with water (75 mL), saturated sodium bicarbonate (75 mL) and brine (75 mL). The organic layer was dried over MgSO₄, filtered and evaporated to give a yellow oil. The crude oil was purified by flash chromatography (3:2 light petroleum:EtOAc) to give the silylated product 248 (10.90 g, 55%) (3:1, α:β) as a viscous pale yellow oil.

Rf 0.10 (3:2 Light petroleum:EtOAc); δH (200 MHz): 1.03 (9H, s, α-anomer CMe₃), 1.05 (9H, s, β-anomer, CMe₃), 3.66-4.35 (2x 7H, m, C2-H, C3-H, C4-H, C5-H and 2x -OH), 5.34 (1H, dd, J = 8.7, 4.2, β-anomer, C1-H), 5.43 (1H, pseudo d, J = 4.6, α-anomer C1-H), 7.32-7.46 (2x 6H, m, CHar), 7.62-7.74 (2x 4H, m, CHar); δC (50 MHz): 18.9 (α-anomer CSi), 19.0 (β-anomer CSi), 26.6 (α-anomer CMe₃), 26.7 (β-anomer CMe₃), 64.0 (α-anomer C-5), 64.5 (β-anomer C-5), 78.0 (CH), 78.8 (CH), 82.9 (β-anomer C-2), 86.9 (α-anomer C-2), 96.8 (β-anomer C-1), 103.4 (α-anomer C-1), 127.8, 130.0 & 130.1 (CHar), 131.6 & 131.8 (α-anomer C), 132.0 & 132.2(β-anomer C), 135.5 & 135.5 (CHar); IR νmax 3400.3 (br. OH), 3072.3, 2931.7, 2858.5, 1659.9, 1428, 1113.2, 1069.4, 702.7 cm⁻¹.

5-O-(tert-Butylidiphenylsilyl)-1,2-O-isopropylidene-β-D-arabino-furanose (249)

Anhydrous copper (II) sulfate (7.97 g, 49.93 mmol) and sulfuric acid (sp. gr. 1.84, 0.36 mL, 6.40 mmol) were added to a solution of 248 (7.02 g, 18.07 mmol) in anhydrous acetone (80 mL). After stirring for 17 h at ambient temperature, the blue reaction mixture was filtered and neutralised with ammonia gas. The precipitated ammonium sulfate was filtered off and the filtrate was concentrated to dryness. The product was purified by flash chromatography (4:1 light petroleum:EtOAc) to give the acetal 249 (2.93 g, 38%) as a yellow oil.
R<sub>f</sub> 0.32 (4:1 Light petroleum:EtOAc); δ<sub>H</sub> (200 MHz): 1.06 (9H, s, CMe<sub>3</sub>), 1.28 & 1.32 (2x 3H, 2s, CMe<sub>2</sub>), 2.04 (1H, s, OH), 3.81 (2H, d, J = 6.6, C5-H), 4.01 (1H, dd, J = 6.6, 2.5, C4-H), 4.40-4.45 (1H, m, C3-H), 4.53 (1H, d, J = 4.1, C2-H), 5.87 (1H, d, J = 4.1, C1-H), 7.32-7.45 (6H, m, CH<sub>ar</sub>); δ<sub>C</sub> (50 MHz): 19.1 (C-Si), 26.0 and 26.7 (CMe<sub>2</sub>), 63.5 (CH<sub>2</sub>), 76.2 (C-4), 86.9 (C-3), 87.3 (C-2), 105.5 (C-1), 112.4 (CMe<sub>2</sub>), 127.7 (CH), 129.7 (CH), 133.0 (C), 135.5 (CH); IR ν<sub>max</sub> 3452.2 (br. OH), 3071.9, 2932.8, 2858.3, 1428.1, 1113.6, 1066, 1018.5, 702.7 cm<sup>-1</sup>.

1,2-O-Isopropylidene-β-D-arabinofuranose (250)<sup>136</sup>

To a solution of 249 (2.88 g, 6.72 mmol) in dry THF (120 mL) was added tetra-n-butylammonium fluoride in THF (1M, 13.44 mL, 13.44 mmol). The reaction mixture was stirred for 1 h at ambient temperature, then evaporated to give a brown oil. The crude residue was purified by flash chromatography (100% EtOAc) to give the diol 250 (0.94 g, 75%) as an off-white crystalline solid.

R<sub>f</sub> 0.34 (100% EtOAc); m.p.: 113-116 °C (no lit. m.p given); δ<sub>H</sub> (200 MHz, CD<sub>3</sub>OD): 1.30 & 1.48 (2x 3H, 2s, CMe<sub>2</sub>), 3.68 (2H, d, J = 6.8, C5-H), 3.97 (1H, dt, J<sub>4</sub> = 6.6, 2.5, C4-H), 4.13 (1H, d, J = 2.1, C3-H), 4.50 (1H, d, J = 4.2, C2-H), 5.87 (1H, d, J = 4.2, C1-H); δ<sub>C</sub> (50 MHz, CD<sub>3</sub>OD): 26.3 (CH<sub>3</sub>), 27.2 (CH<sub>3</sub>), 63.5 (C-5), 76.6 (CH), 88.4 (CH), 89.9 (CH), 107.2 (C-1), 113.5 (C); IR ν<sub>max</sub> 3391.7 (br. OH), 2940.2, 1378.4, 1213.6, 1163.3, 1066.1, 1015.1 cm<sup>-1</sup>.

3,5-Di-O-benzyl-1,2-O-isopropylidene-β-D-arabinofuranose (251)<sup>136</sup>
Experimental

To an ice cold solution of 250 (5.52 g, 31.52 mmol) in DMF (100 mL) was added NaH in mineral oil (60% w/w, 3.64 g, 91.0 mmol). The mixture was stirred for 15 min, allowed to warm to ambient temperature and stirred for a further 15 min. Tetrabutylammonium iodide (11.2 g, 30.32 mmol) was added, followed by benzyl bromide (10 mL, 84.18 mmol) and the mixture was stirred for 4 h. The reaction was quenched with MeOH (4 mL), reduced *in vacuo* and the resultant residue was dissolved in EtOAc (20 mL). The organic solution was washed with brine (20 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash chromatography (8:1 light petroleum:EtOAc) to give the dibenzyl ether 251 (7.2 g, 62%) as a colourless oil.

R_f 0.32 (8:1 Light petroleum:EtOAc); δ_H (200 MHz): 1.33 & 1.43 (2x 3H, 2s, CMe₂), 3.64 (2H, d, J= 6.2, C5zH), 4.03 (1H, d, J= 3.3, C4zH), 4.27 (1H, dt, J= 6.2, 2.9, C3zH), 4.54 (1H, d, J= 12.0, OCH₂Ph), 4.56 (1H, d, J= 11.6, OCH₂Ph), 4.60 (1H, d, J= 12.0, OCH₂Ph), 4.63 (1H, d, J= 11.6, OCH₂Ph), 4.63 (1H, d, J= 3.7, C2zH), 4.63 (1H, d, J= 3.7, C1zH), 5.90 (1H, d, J= 3.7, C1-H), 7.22-7.37 (10H, m, Ph); δ_C (50 MHz): 26.2 (CH₃), 26.9 (CH₃), 69.9 (C₅), 71.5 (CH₂), 73.2 (CH₂), 82.9 (CH), 83.5 (CH), 85.1 (CH), 105.6 (C-1), 112.6 (O-C-O), 127.6, 127.6, 127.8, 128.3 & 128.4 (CH₆), 137.2 & 137.9 (C); IR ν_max 3063.9, 3031.1, 2937.3, 2865.3, 1454.3, 1374.1, 1211.5, 1098.5, 1072.6, 1027.2, 737.9, 698.3 cm⁻¹.

3,5-Di-O-benzyl-D-arabinofuranose (252)¹³⁶

A solution of the isopropylidene derivative 251 (6.60 g, 17.83 mmol) in acetic acid-water (1:1, 50 mL) was heated at 60 °C for 5 h. The reaction mixture was cooled to ambient temperature, diluted with water (125 mL), and extracted with DCM (2 x 125 mL). The combined organic layers were washed with saturated sodium bicarbonate (125 mL) and brine (125 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo* to give the diol 252 (5.38 g, 92%) (1:1, α:β) as a colourless syrup.
Rf 0.56 (1:1 Light petroleum:EtOAc); δH (200 MHz): 3.38-3.65 (2H, m, C5-H), 3.83-4.11 (4H, m, C2-H, C3-H, 2x OH), 4.30-4.60 (1H, m, C4-H), 4.37-4.65 (4H, m, OCH2Ph), 5.24 (α-anomer, s, C1-H), 5.30 (β-anomer, d, J = 3.3, C1-H), 7.15-7.33 (10H, m, Ph); δc (50 MHz): 69.1 & 69.9 (α and β CH2), 71.4 & 71.8 (α and β CH2), 73.5 (CH2), 75.4 & 76.2 (α and β CH), 80.7, 82.9, 83.2 & 84.2 (α and β 2x CH), 97.3 (β-1anomer Cz1), 103.6 (α-1anomer Cz1), 127.3, 127.5, 127.8, 128.0 & 128.2 (CHar), 136.1, 136.1, 136.3 & 137.1 (C); IR vmax 3401.6 (br. OH), 3063.7, 2925.8, 2867.9, 1454.3, 1364.1, 1272.3, 1208.8, 1072.4, 1027.7, 738, 698.2 cm⁻¹.

3,5-Di-O-benzyl-D-arabinono-1,4-lactone (253)¹³⁶

![Diagram of molecules 252 and 253]

To an ice cold solution of 252 (3.90 mg, 12 mmol) in THF-water (1:3, 200 mL), was added barium carbonate (39 g, 19 mmol) and bromine (8 mL, 16 mmol). The reaction mixture was allowed to warm to ambient temperature and was left stirring overnight. The mixture was filtered through Celite® and the filtrate was reduced under pressure. The residue was purified via flash chromatography (7:3 light petroleum:EtOAc) to give the lactone 253 (3.34 g, 85%) as a white solid.

Rf 0.36 (7:3 Light petroleum:EtOAc); m.p.: 83-85 °C; δH (200 MHz): 3.28 (1H, br. s, OH), 3.53 (1H, dd, J = 11.6, 3.7, H-5a), 3.69 (1H, dd, J =11.6, 2.5, C5-Hb), 4.16 (1H, t, J3,2 = 6.6, C3-H), 4.34 (1H, ddd, J= 6.6, 3.7, 2.5, C4-H), 4.43 (1H, d, J =12.0, OCH2Ph), 4.44 (1H, dd, J = 6.6, C2-H) 4.50 (1H, d, J = 12.0, OCH2Ph), 4.52 (1H, J = 11.6, OCH2Ph), 4.72 (1H, J = 11.6, OCH2Ph), 7.17-7.32 (10H, m, Ph); δc (50 MHz): 67.9 (C-5), 72.5 (CH2), 73.7 (CH2), 74.3 (C-4), 79.8 (C-3), 80.2 (C-2), 127.9, 128.1, 128.2 & 128.6 (CHar), 137.1 (C), 137.2 (C), 174.6 (C=O); IR vmax 3446.4 (br. OH), 3031, 2866.4, 1771.3, 1653.9, 1453.9, 1318.2, 1127.6, 1026.6, 762.6, 737, 698.1 cm⁻¹.
3,5-Di-O-benzyl-2-O-methoxymethyl-D-arabinono-1,4-lactone (254)\textsuperscript{136}

![Chemical Structure](image)

To a solution of 253 (3.00 g, 8.8 mmol) in dry chloroform (200 mL) at ambient temperature was added dimethoxymethane (40 mL, 440 mmol) and phosphorus pentoxide (184 g, 52.8 mmol). After stirring for 30 min, the mixture was decanted and the filtrate poured into an iced-cold saturated sodium bicarbonate solution (160 mL). The mixture was extracted with chloroform (2 x 30 mL), and the combined organic layers were washed with brine (50 mL), dried over Na\textsubscript{2}SO\textsubscript{4}, and evaporated to give the product 254 (3.20 g, 92\%) as a colourless oil.

$R_f$ 0.69 (1:1 Light petroleum:EtOAc); $[\alpha]_D^{20}$ +23.88 (c 0.49 CHCl\textsubscript{3}); $\delta_H$ (200 MHz): 3.47 (3H, s, OMe), 3.56 (1H, dd, $J_{CH} = 11.2, 3.7$, C5-H\textsubscript{a}), 3.71 (1H, dd, $J_{CH} = 11.2, 2.9$, C5-H\textsubscript{b}), 4.29-4.37 (2H, m, C2-H & C3-H), 4.49-4.77 (5H, m, C4-H & OCH\textsubscript{2}Ph), 4.75 (1H, d, $J_{CH} = 6.6$, OCH\textsubscript{2}O), 5.09 (1H, d, $J_{CH} = 6.6$, OCH\textsubscript{2}O), 7.18-7.37 (10H, m, Ph); $\delta_C$ (50 MHz): 55.9 (OCH\textsubscript{3}), 67.6 (C-5), 72.6 (CH\textsubscript{2}), 73.2 (CH\textsubscript{2}), 76.1 (CH), 78.7 (CH), 79.1 (CH), 95.7 (OCH\textsubscript{2}O), 127.4, 127.6, 127.9, 128.2 & 128.2 (CH\textsubscript{ar}), 126.9 (C), 137.1 (C), 171.8 (C=O); IR $\nu_{max}$ 2899.3, 1791.8, 1454.9, 1211.6, 1151.4, 1123.7, 1026.4, 739.4, 689.6 cm\textsuperscript{-1}.

3,5-Di-O-benzyl-2-O-methoxymethyl-D-arabinofuranose (255)\textsuperscript{136}

![Chemical Structure](image)

Lactone 254 (2.52 g, 9 mmol) was dissolved in anhydrous THF (120 mL) and cooled to -78 °C. DIBAL (1M solution in toluene, 10.2 mL, 10.2 mmol) was slowly added and the reaction mixture was stirred for 20 min. The reaction was then quenched by the careful addition of ammonium chloride (60 mL). The mixture was filtered through Celite\textsuperscript{®}, washed well with
DCM and then reduced under vacuum to give 255 as a 1:1 mixture of α:β anomers (2.57 g, 98%).

R\text{f} 0.43 (1:1 Light petroleum:EtOAc); \(\delta_H (200 \text{ MHz})\): 3.26 & 3.35 (3H, 2 s, OMe), 3.38-3.57 (2H, m, C5-H), 3.66-3.85 (1H, m, CH), 3.97-4.12 (2H, m, 2x CH), 4.35-4.68 (6H, m, 2 x OCH\text{Ph} \& OCH\text{H}_2O), 5.17-5.32 (1H, m, C1-H); \(\delta_C (50 \text{ MHz})\): 55.7 & 55.9 (α and β OCH\text{3}), 70.0 & 70.2 (α and β C-5), 71.9, 72.2, 73.3 & 73.5 (α and β 2x CH\text{2}), 80.2, 81.5, 82.2, 82.6, 82.8 & 83.9 (α and β 3x CH), 95.8 (β-anomer C-1), 96.4 & 96.5 (α and β OCH\text{2}O), 101.6 (α-anomer C-1), 127.7, 127.9, 128.3, 128.5 & 128.5 (CH\text{ar}), 137.3 & 137.8 (C); IR \(\nu_{\text{max}}\) 3413.9 (br. OH), 2926.7, 1453.8, 1366, 1151.9, 1104.7, 1035.2, 738.3, 698.2 cm\text{⁻¹}.

3,5-Di-\text{O}-benzyl-2-\text{O}-methoxymethyl-\text{d}-arabinose-\text{O}-(\text{tert}-\text{butyldimethylsilyl})oxime (256)\textsuperscript{136}

To a solution of Lactol 255 (2.5 g, 6.68 mmol) in dry toluene (35 mL) was added MgSO\textsubscript{4} (1g). The suspension was stirred at reflux for 5 minutes then tert-butyldimethylsilyl hydroxylamine (1.84 g, 12.51 mmol) was added. The reaction mixture was allowed to heat at reflux for 30 minutes then cooled to ambient temperature and filtered. The filtrate was washed with saturated sodium bicarbonate (25 mL) and brine (30 mL), dried over MgSO\textsubscript{4} then filtered. The filtrate was concentrated to give an oily residue. Purification by column chromatography (light petroleum:EtOAc 7:3) yielded silyl oxime 256 (2.41 g, 72%) (E:Z, 2:1) as a yellow/green oil.

R\text{f} 0.42 (light petroleum:EtOAc 1:1); \(\delta_H (200 \text{ MHz, CDCl}_3)\): \(\delta_H (200 \text{ MHz})\): 0.11 (6H, SiMe\textsubscript{3}, E-isomer), 0.13 (6H, SiMe\textsubscript{3}, E-isomer), 0.81 (9H, s, Z-isomer, 3x CH\text{3}), 0.85 (9H, s, E-isomer, 3x CH\text{3}), 2.05 (1H, d, \(J = 6.6\), Z-isomer, OH), 2.13 (1H, d, \(J = 5.8\), E-isomer, OH), 3.27 (3H, s, E-isomer, OMe), 3.29 (3H, s, Z-isomer, OMe), 3.56-3.67 (2x 2H, m, C5-H), 3.68 (1H, dd, \(J = 6.6, 3.3\), E-isomer, C3-H), 3.89-3.95 (2x 1H, m, C4-H), 4.29-4.42 (2x 1H, m, Z-.
isomer C3-H and E-isomer C2-H), 4.43-4.63 (2x 4H, m, OCH₂Ph), 5.15 (1H, dd, J = 5.8, 2.5, Z-isomer, C2-H), 7.11 (1H, d, Z-isomer, H-1), 7.14-7.29 (2x 10H, m, Ph), 7.45 (1H, d, E-isomer, H-1); δC (50 MHz): 25.4 (CMe₂, Z-isomer), 25.5 (CMe₃, Z-isomer), 55.5 (OCH₃) 68.9 (C-4), 69.9, 70.8, 73.3 & 74.0 (CH₂), 75.9 (CH), 79.9 (CH), 94.6 & 96.1 (2 x OCH₂O E- and Z-isomers), 125-140 (CH₃), 154.1 (C-1); IR νmax 3352.8 (br. OH), 3069.2, 3030.6, 2935.3, 2854.5, 1428.9, 1113.7 1069.4, 920.5, 732.4, 701.1 cm⁻¹.

(2R,3S,4S,E)-3,5-Bis(benzyloxy)-4-iodo-2-(methoxymethoxy)pentanal O-tert-butyldimethylsilyl oxime (257)\textsuperscript{136}

![Reaction Scheme](image)

A suspension of alcohol 256 (2.20 g, 4.35 mmol), triphenylphosphine (4.57 g, 19.85 mmol), imidazole (1.18 g, 17.41 mmol) and iodine (3.32 g, 13.06 mmol) in toluene (175 mL) was heated at reflux for 1.5 h. The dark brown reaction mixture was then cooled to 0 °C. Sodium bicarbonate (175 mL) was added and the mixture was stirred for 10 min until the organic layer became yellow. Excess iodine was added in small portions until the colour of the organic layer became burgundy. This excess iodine was then removed via the addition of saturated sodium thiosulfate until the organic layer retained its original yellow colour. The two phases were then separated and the organic layer was washed with water (50mL). The combined organic layers were evaporated and the resultant oil was dissolved in diethyl ether. The crystalline triphenylphosphine oxide was filtered off and the filtrate was concentrated in vacuo to give an oil. The crude residue was purified by flash chromatography (light petroleum:EtOAc 8:2) to afford product 257 (2.94 g, 88%) (E:Z, 7:3) as a pale yellow oil. With careful chromatography the two isomers were separated as pale oils, with the E-isomer eluting first.

\textit{E-isomer}

Rₜ 0.49 E-isomer (8:1 Light petroleum:EtOAc); δH (200 MHz): 0.3 (6H, s, SiMe₂), 1.18 (9H, s, SiMe₃), 3.41 (OCH₃), 3.61 (1H, dd, J = 7.5, 2.9, C3-H), 3.79-3.91 (2H, m, C5-2H), 4.22-
Experimental

4.28 (2H, m, C4-H and C2-H), 4.45 (2H, s, OCH2Ph), 4.51-4.71 (2H, m, OCH2Ph), 5.45 (2H, s, OCH2O), 7.19-7.46 (10H, m, Ph), 7.63 (1H, d, J = 8.2, C1-H); \( \delta \) (50 MHz): 18.5 (SiC), 26.5 (SiMe3), 31.7 (CH), 56.1 (OCH3), 72.9 (CH2), 73.1 (CH2), 75.1 (CH2), 78.1 (CH), 78.8 (CH), 95.5 (OCH2O), 125.2, 127.6, 127.7, 127.9, 128.2, 128.4, 128.9 & 129.7 (CHar), 133.0 (C), 133.2 (C), 135.5 (CHar), 137.4, 137.6 & 138.3 (C), 151.9 (C-1); IR \( \nu_{\text{max}} \) 3069.8, 3030.6, 2930.8, 2857.8, 1428, 1115, 1071.7, 922, 739.3, 699.1 cm\(^{-1}\).

Z-isomer

Rf 0.44 Z-isomer (8:1 Light petroleum:EtOAc); \( \delta \)H (200 MHz): 0.13 (6H, s, SiMe2), 0.88 (9H, s, SiMe3), 3.21 (OCH3), 3.45 (1H, dd, J = 7.5, 2.9, C3-H), 3.62-3.71 (2H, m, C5-H2), 3.87 (1H, t, J = 5.0, C3-H), 4.20-4.30 (1H, m, C4-H), 4.39-4.61 (2H, m, OCH2Ph), 4.71-4.75 (2H, m, OCH2Ph), 5.26 (1H, m, C2-H), 7.07 (1H, d, J = 6.2, C1-H), 7.36-7.44 (10H, m, Ph); \( \delta \)C (50 MHz): 18.1 (SiC), 26.1 (SiMe3), 31.4 (C4-H), 56.1 (OCH3), 72.6 (CH2), 74.7 (CH2), 74.8 (CH2), 78.4 (CH), 78.5 (CH), 95.5 (OCH2O), 125.2, 127.6, 127.7, 127.9, 128.2, 128.9 & 129.7 (CHar), 133.0 (C), 133.2 (C), 135.5 (CHar), 137.4, 137.6 & 138.3 (C), 154.1 (C-1); IR \( \nu_{\text{max}} \) 3069.8, 3030.6, 2930.8, 2857.8, 1428, 1115, 1071.7, 922, 739.3, 699.1 cm\(^{-1}\).

**(3R,4R,5R)-3-Methoxymethyl-4-benzyloxy-5-benzyloxymethyl-\( \Delta \)-pyrroline-N-Oxide (179)**

Method 1:

\[
\text{BrO} \quad \text{OMe} \quad \text{OTBDMS} \quad \text{BnO} \quad \text{OMOM} \quad \text{N} \quad \text{OMOM} \quad \text{BnO} \\
240 \quad \text{179}
\]

To a solution of 240 (0.25 g, 0.43 mmol) in dry toluene (8 mL) was added tetrabutylammonium fluoride solution in THF (1M, 0.65 mL, 0.65 mmol). The mixture was heated at reflux and held for 30 min then cooled to ambient temperature. Concentration in vacuo produced a brown residue (300 mg) which was purified by flash chromatography (EtOAc) to give nitrone 179 as a brown oil (0.135 g, 84%).
Method 2:

To a solution of 240 (715 mg, 1.23 mmol) in dry THF (50 mL) was added tertbutylammonium triphenyldifluorosilicate (TBAT) (682 mg, 1.23 mmol). The mixture was heated and held at reflux for 7 min then concentrated in vacuo to give a brown residue. The residue was purified by flash chromatography (EtOAc) to give nitrone 179 as a brown oil (137 mg, 30 \%).

Method 3:

To a solution of 257 (528 mg, 0.86 mmol) in dry THF (25 mL) was added tetrabutylammonium fluoride 1M solution in THF (1.3 mL, 1.3 mmol). The mixture was refluxed for 10 minutes, cooled to ambient temperature then concentrated in vacuo to give a brown residue. The residue was purified by flash chromatography (EtOAc) to give nitrone 179 as a brown oil (83 \%).

R_f 0.15 (light petroleum/ethyl acetate 1:1); \( \delta_H \) (200 MHz, CDCl_3): 3.28 (3H, s, OMe), 3.65 (1H, dd, \( J = 2.22, 9.71 \)), 3.94 (1H, m, C5'-H_a), 4.23-4.54 (7H, m, 2 x PhCH_2O, C3-H, C4-H& C5-H), 6.91 (d, \( J = 1.92 \), C2-H), 7.19-7.31 (10H, m, Ph); \( \delta_C \) (50 MHz): 55.3 (OCH_3), 65.3 (CH_2), 71.5 (CH_2), 72.9 (CH_2), 76.7 (CH), 80.3 (CH), 81.4 (CH), 95.9 (OCH_2O), 127.2, 127.3, 127.4, 127.6, 127.9 & 128.1 (CH_ar), 133.34 (CH-1), 136.68 & 137.23 (C); LRMS (ES): 372.2, 242.2, 102.0; HRMS (ES):372.1806 [(M+H)^+; C_{21}H_{26}NO_5 requires 372.1805].

(2R,3aR,4R,5R,6R)-5-(benzyloxy)-6-(benzyloxyethyl)-4-(methoxymethoxy)-N,N-dimethylhexahydropyrrolo[1,2-b]isoxazole-2-carboxamide (267)
To a solution of nitrone 179 (1.50 g, 4.04 mmol) in DCM (29 mL) was added \textit{N}, \textit{N}-dimethyacrylamide (0.43 mL, 4.04 mmol). The mixture was stirred at ambient temperature overnight then concentrated \textit{in vacuo} producing a brown residue. Purification by flash chromatography (EtOAc) yielded an isoxazole 267 as a brown oil (2.21 g, 100%).

Rf 0.33 (ethyl acetate); δH (400 MHz, CDCl3): 2.33 (1H, m, C3\textsubscript{z}H), 2.92 (3H, s, NCH\textsubscript{3}), 2.93-3.02 (1H, m, C3\textsuperscript{3-}H), 3.03 (3H, s, NCH\textsubscript{3}), 3.30-3.33 (1H, m, C6\textsubscript{z}H), 3.34 (3H, s, OCH\textsubscript{3}), 3.58-3.63 and 3.68-3.73 (2 x 1H, m, C7\textsubscript{z}H), 3.75-3.80 (1H, m, 2 x OCH\textsubscript{2}Ph), 4.62 (2H, s, OCH\textsubscript{2}O), 4.82 (1H, t, J = 7.1, C2-H), 7.19-7.31 (10H, m, Ph); δc (100 MHz): 35.8 (NCH\textsubscript{3}), 35.9 (CH\textsubscript{2}-3), 36.9 (NCH\textsubscript{3}), 55.6 (OCH\textsubscript{3}), 68.8 (CH-3a), 69.98 (CH-6CH\textsubscript{2}), 70.0 (CH-6), 72.3 (OCH\textsubscript{2}Ph), 73.3 (OCH\textsubscript{2}Ph), 74.5 (CH-2), 83.9 (CH-5), 84.7 (CH-4), 95.8 (OCH\textsubscript{2}O), 127.5 (CH-Ar), 127.7 (CH-Ar), 127.7 (CH-Ar), 128.2 (CH-Ar), 128.2 (CH-Ar), 128.3 (CH-Ar), 137.8 (CH-Ar), 137.8 (C-Ar), 138.2 (C-Ar), 168.3 (C=O); IR ν\textsubscript{max} 3362.3, 3030.8, 2931.6, 2867.8, 1645.7 (C=O), 1496.7, 1453.9, 1403.0, 1361.5, 1260.2, 1102.1, 1028.4, 912.1, 800.1, 738.9, 699.2 cm\textsuperscript{-1}; LRMS (ES): 439.1, 425.4, 398.2, 371.5, 354.3, 349.2, 327.8, 310.3; HRMS (ES): 471.2484 [(M+H)+]; C\textsubscript{26}H\textsubscript{35}N\textsubscript{2}O\textsubscript{6} requires 471.2490.

\textbf{(1R,2R,3R,6R,7aR)-2-(Benzyloxy)-3-(benzyloxymethyl)-6-hydroxy-1-(methoxymethoxy)-hexahydropyrrolizin-5-one (268)}

To compound 267 (1.8 g, 3.83 mmol) was added 10M AcOH (12 mL, 120 mmol) and Zinc dust (6.00 g, 91.22 mmol). The mixture was stirred at 80 °C for 3 hrs and then the pH was adjusted to 14 using aqueous NaOH. The product was extracted into DCM (2x 10ml) and the combined organic layers were dried over MgSO\textsubscript{4}, filtered and concentrated. Purification by column chromatography (EtOAc) yielded 268 a white solid (0.793 g, 57%). The solid was
recrystallised form heptanes and ethyl acetate to provide crystals for x-ray crystallography analysis.

Rf 0.46 (ethyl acetate); m.p 135-138 °C; δH (400 MHz, CDCl3): 1.91 (1H, ddd, J 12.5, 10.3, 8.5, C7-H), 2.85 (1H, ddd, J 12.5, 7.8, 6.1, C7'-H), 3.19 (1H, br s, OH), 3.37 (3H, s, OCH3), 3.53, (1H, dd, J = 9.9, 3.9, C3-CH2-Ha’), 3.66 (1H, ddd, J = 12.5, 7.8, 6.1, C7a-H), 3.82-3.87 (1H, m, C7a-H), 3.83 (1H, dd, J = 7.1, 5.5, C1-H), 4.09 (1H, dd, J = 9.1, 4.7, C3-H), 4.34 (1H, t, J = 4.9, C2-H), 4.54-4.59 (3H, m, OCH2Ph, C6-H), 4.62-4.65 (2H, m, C5-CH2OCH2Ph), 4.67 (2H, m, OCH2O), 7.28-7.42 (10H, m, Ph); δc (100 MHz): 36.9 (CH2-7), 55.6 (OCH3), 58.2 (CH-3), 59.6 (CH-7a), 68.7 (C3-CH2), 71.8 (CH-6), 72.5 (OCH2Ph), 72.6 (OCH2Ph), 85.6 (CH-2), 87.7 (CH-1), 96.6 (OCH2O), 127.6, 127.6, 127.7, 127.8, 128.4 & 128.4 (CHAr), 137.8 & 137.9 (CAr), 174.2 (C=O); IR νmax 3350.5, 3030.6, 2892.3, 1675.9, 1458.6, 1360.5, 1213.1, 1128.8, 1041.0, 911.8, 733.8, 696.7, 604.0 cm⁻¹; LRMS (ES): 428.4, 412.4, 338.3, 91.2; HRMS (ES) 428.2070 [(M+H)+]; C24H30NO6 requires 428.2068; Anal. Calcd for C24H29NO6: C, 67.43; H, 6.84; N, 3.28. Found: C, 67.38; H, 6.82; N, 3.21.

(1R,2R,3R,6R,7aR)-2-(Benzyloxy)-3-(benzyloxymethyl)-1-(methoxymethoxy)-5-oxohexahydro-7H-pyrrolizin-6-yl methanesulfonate (269)

268 (0.76 g, 1.77 mmol) was dissolved in DCM (10 mL) and stirred at 0 °C under an argon atmosphere. Triethylamine (0.44 mL, 3.88 mmol), and methanesulfonyl chloride (2 mL, 3.00 mmol) were added successively. The mixture was stirred for 30 min and water (5 mL) was added and the layers separated. The aqueous layer was extracted with DCM (2 x 10ml) and the combined organic layers were washed with brine (10ml), dried over MgSO4, filtered and evaporated to give mesylate 269 (0.270 g, 100 %).
Rf 0.33 (ethyl acetate); δH (400 MHz, CDCl3): 2.12 (1H, m, C7-H), 2.97 (1H, ddd, J = 13.0, 8.3, 6.2, C7’-H), 3.31 (3H, s, CH3SO3), 3.37 (3H, s, OCH3), 3.52, (1H, dd, J = 10.0, 3.7, C3-CH2-Ha’), 3.67, (1H, dd, J = 9.9, 4.6, CH-3CH2-Hb’), 3.72 (1H, dd, J = 7.7, 6.3, C7a-H), 3.81 (1H, dd, J = 7.4, 5.6, C1-H), 4.07 (1H, dd, J = 8.6, 4.4, C3-H), 4.36 (1H, t, J = 4.9, C2-H), 4.54-4.62 (2H, m, OCH2Ph), 4.64-4.66 (2H, m, CH2OCH2Ph), 4.67-71 (2H, m, OCH3), 5.40 (1H, d, J = 9.8, 8.6), 7.28-7.42 (10H, m, Ph); δc (100 MHz): 34.6 (CH2-7), 39.8 (SO2CH3), 55.6 (OCH3), 58.4 (CH-3), 59.4 (CH-7a), 68.4 (C3-CH2), 72.6 (OCH2Ph), 73.2 (OCH2Ph), 78.5 (CH-6), 85.4 (CH-2), 87.9 (CH-1), 96.7 (OCH2O), 127.5, 127.6, 127.7, 127.71, 127.8, 128.84 & 128.9 (CHAr), 137.6 & 137.7 (Car), 167.9 (C=O); IR vmax 3030.0, 2936.6, 1713.9, 1496.1, 1358.9, 1271.4, 1176.1, 1046.0, 844.9, 737.0, 699.2, cm⁻¹; LRMS (ES): 506.4, 412.4, 322.3, 134.1, 108.2; HRMS (ES): 506.1848 [(M+H)⁺; C25H32NO8S requires 506.1843]. Anal. Caled for C25H31NO8S: C, 59.39; H, 6.18; N, 2.77. Found: C, 59.31; H, 6.15; N, 2.68.

(1R,2R,3R,6R,7aR)-2-(Benzyloxy)-3-(benzyloxymethyl)-1-hydroxy-5-oxo-hexahydro-7H-pyrrolizin-6-yl methanesulfonate (270)

To a solution of 269 (100 mg, 0.198 mmol) in DCM (2.5 mL) was added TFA (0.2 mL). The reaction mixture was stirred at ambient temperature for 48 hours. The mixture was washed with sodium bicarbonate (2 mL) and extracted with DCM (3 x 3 mL). The Organic phase was dried over MgSO4, filtered and the filtrate concentrated. Purification by column chromatography (petrol/EtOAc 1:1) yielded 270 (78 mg, 86 %) as a yellow oil.

Rf 0.20 (heptane:ethyl acetate, 1:1); δH (400 MHz, CDCl3): 2.07 (1H, m, C7-H), 2.95 (1H, m, C7’-H), 3.26 (3H, s, SO2CH3), 3.64 (1H, dd, t, J = 10.0, 2.8, C3CH2-Ha), 3.68 (1H, dd, J = 5.6, 3.3, C7a-H), 3.72 (1H, dd, t, J = 10.0, 3.5, C3CH2-Hb), 3.97 (1H, t, J = 3.7, C1-H), 4.10 (1H, t, J = 3.1, C2-H), 4.23 (1H, q, J = 3.0, C3-H), 4.54-4.58 (2H, m, OCH2Ph), 4.59-4.63 (2H, d, J = 4.9, OCH2Ph), 5.42 (1H, dd, J = 10.4, 8.0, C6-H), 7.32 (10H, m, Ph); δc (100 MHz): 34.9
Experimental

(\(\text{CH}_2-7\)), 39.8 (\(\text{SO}_2\text{CH}_3\)), 59.7 (CH-3), 62.8 (CH-7a), 70.0 (C3-CH2), 71.9 (OCHPh), 73.8 (OCH2Ph), 78.2 (CH-6), 80.9 (CH-1), 87.6 (CH-2), 127.7, 127.8, 127.9, 128.0, 128.3, 128.5, 128.6 & 128.6 (CHAr), 136.7 & 137.3 (CAr), 169.46 (C=O); HRMS (ES): 462.1584 [(M+H)\(^+\); \(\text{C}_{23}\text{H}_{28}\text{NO}_7\text{S}\) requires 462.1581]. Anal. Calcd for \(\text{C}_{23}\text{H}_{28}\text{NO}_7\text{S}\): C, 59.85; H, 5.90; N, 3.03. Found: C, 59.31; H, 5.79; N, 2.97.

**Attempted preparation of \((2R,3R,6R,7aR)-2-(\text{Benzyloxy})-3-(\text{benzyloxymethyl})-1,5\text{-dioxo-hexahydro-7H-pyrrolizin-6-yl methanesulfonate (271)}\)**

Method 1: Dess-Martin Periodinane Oxidation

270 (50 mg, 0.11 mmol) and Dess – Martin periodinane in DCM (15 % w/w, 70 mg, .25 mmol) were stirred in DCM (2 mL) at ambient temperature. The reaction progress was monitored by TLC (petrol/EtOAC 1:1) and on completion was diluted with ether (3 mL). A mixture of Na2S2O3 and Sodium bicarbonate was added and the mixture stirred until homogeneous and the products isolated by extraction with DCM (2 x 5 mL). TLC and Crude NMR showed no reaction had taken place after 18 hours. (0 mg, 0 %).

Method 2: Swern Oxidation

To a stirred solution of 2M oxalyl chloride (33 µL, 0.38 mmol) in anhydrous DCM (3 mL) was added DMSO (52 µL, 0.74 mmol) dropwise under a nitrogen atmosphere and the mixture stirred for 30 mins. A solution of 270 (1.5 mg, 0.32 mmol) in DCM (2 mL) was added and the reaction stirred at -78 °C for 1 h. TEA (134 µL, 0.96 mmol) was added and the reaction stirred until it reached ambient temperature then water (5 mL) was added. The product was extracted with DCM (5 mL), washed with brine (5 mL) and concentrated. TLC and Crude NMR showed no reaction had taken place after 18 hours. (0 mg, 0 %).
**Experimental**

**Attempted preparation of (1S,2R,3R,6R,7aR)-2-(Benzyloxy)-3-(benzyloxymethyl)-6-(methylsulfonyloxy)-5-oxohexahydro-1H-pyrrolizin-1-yl benzoate (272)**

A solution of alcohol 271 (50 mg, 0.11 mmol) in PPh₃ (83 mg, 0.32 mmol) and benzoic acid (15 mg, 0.13 mmol), in dry THF (4 mL) was stirred at 0 °C under an atmosphere of nitrogen. DEAD (50 μL, 0.32 mmol) was added dropwise and the mixture stirred at 0 °C for 1 h, allowed to warm to ambient temperature then stirred overnight. The solvents were evaporated under reduced pressure to give an orange oil. No reaction was observed from NMR and TLC analysis of the crude product.

**(1R,2R,3R,7aR)-2-(Benzyloxy)-3-(benzyloxymethyl)-1-(methoxymethoxy)hexahydro-1H-pyrrolizine (275)**

To a solution of amide 269 (450 mg, 0.89 mmol) in anhydrous THF (5 mL) was added LiAlH₄ (118 mg, 3.12 mmol) and mixture heated at reflux for 1.5 hr. The reaction was quenched by addition of NaOH, the solids were solids filtered off and the filtrate partitioned between EtOAc (5 mL) and water (5 mL). The organic phase was separated and aqueous layer extracted with EtOAc (5 ml). The combined organic phases were concentrated to give a dark yellow oil. Purification by column (EtOAc) yielded 275 (297 mg, 84 %) as a yellow oil.

Rₜ 0.15 (ethyl acetate); δH (400 MHz, CDCl₃): 1.83-1.91 (3H, m, C6-H₂, C7-H), 2.03-2.08 (1H, m, C7’-H), 2.81-2.84 (1H, m, C5-H), 2.98 (1H, ddd, J = 7.5, 7.0, 3.7, C3-H), 3.10-3.13 (1H, m, C5’-H), 3.40 (3H, s, OCH₃), 3.46-3.49 (1H, m, CH-7a), 3.51-3.58 (2H, m, C3-H₂),
Experimental

3.92 (1H, t, J = 6.1, H-1), 4.05 (1H, dd, J = 7.5, 6.2, H-2), (1H, d, J = 3.4), 4.55-4.59 (3H, m, CH$_2$OCH$_3$Ph, OCH$_2$Ph-H$_a$), 4.61-4.72 (3H, m, OCH$_2$Ph-H$_b$, OCH$_2$O7.28-7.42 (10H, m, Ph); $\delta_c$ (100 MHz): 25.4 (CH$_2$-6), 31.0 (CH$_2$-7), 55.2 (CH$_2$-5), 55.5 (OCH$_3$), 67.3 (CH-7a), 68.0 (CH-3), 71.8 (C5=CH$_2$), 72.7 (OCH$_2$Ph), 73.2 (C5-CH$_2$OCH$_2$Ph), 85.5 (CH-2), 86.6 (CH-1), 91.9 (OCH$_2$O), 127.4, 127.5, 127.6, 127.6, 127.65, 127.68, 127.7, 128.2, 128.2, 128.3, 128.3, & 128.4 (CH$_2$), 138.42 & 138.5 (C$_{ar}$); IR $\nu_{\text{max}}$ 3030.4, 2889.6, 1721.8, 1496.4, 1452.9, 1361.7, 1309.3, 1260.7, 1207.7, 1107.3, 1035.1, 844.9, 917.8, 736.7, 698.2, cm$^{-1}$; LRMS (ES): 398.2; HRMS (ES): 398.2318 [(M+H)$^+$]; C$_{24}$H$_{31}$NO$_4$ requires 398.2326. Anal. Calcd for C$_{24}$H$_{31}$NO$_4$: C, 72.52; H, 7.86; N, 3.52. Found: C, 72.49; H, 7.77; N, 3.48.

(1R,2R,3R,7aR)-2-(Benzyloxy)-3-(benzyloxyethyl)hexahydro-1H-pyrrolizin-1-ol (276)

![Chemical Structure](image)

To a solution of 275 (297 mg, 0.75 mmol) in DCM (18 mL) was added TFA (1.75 mL, 22.74 mmol). The reaction mixture was stirred at ambient temperature for 48 h. The mixture was washed with sodium bicarbonate (2 mL) and extracted with DCM (3 x 3 mL). The combined organics were dried over MgSO$_4$, filtered and the filtrate concentrated. Purification by column chromatography (EtOAc) yielded the alcohol 276 (237 mg, 90%) as a yellow oil.

$R_f$ 0.03 (ethyl acetate); $\delta_H$ (200 MHz, CDCl$_3$): 2.16-2.23 (4H, m, C6-H2, C7-H2), 2.83 (1H, dt, J = 10.7, 6.6, C5-H$_b$), 2.98 (1H, ddd, J = 7.5, 7.0, 3.7, C3-H), 3.10 (1H, dt, J = 12.1, 6.1, C5-H$_a$), 3.46 (1H, m, C7a-H), 3.59-3.64 (2H, m, C3-H$_2$), 3.92 (1H, t, J = 6.1, C1-H), 4.05 (1H, dd, J = 7.5, 6.2, C2-H), 4.55-4.59 (2H, m, CH$_2$OCH$_2$Ph), 4.59-4.62 (1H, m, OCH$_2$Ph-H$_a$), 4.70-4.73 (1H, m, OCH$_2$Ph-H$_b$), 7.28-7.42 (10H, m, Ph); $\delta_c$ (50 MHz): 23.8 (CH$_2$-6), 27.9 (CH$_2$-7), 55.2 (CH$_2$-5), 67.3 (CH-7a), 68.03 (CH-3), 71.8 (C5-CH$_2$), 72.7 (OCH$_3$Ph), 73.2 (C5-CH$_2$OCH$_2$Ph), 85.5 (CH-2), 86.6 (CH-1), 91.9 (OCH$_2$O), 127.4, 127.5, 127.59, 127.61, 127.63, 127.65, 127.68, 127.7, 128.22, 128.24, 128.27, 128.30, & 128.35 (CH$_2$), 138.34 & 138.40 (C$_{ar}$); IR $\nu_{\text{max}}$ 3413.9 (br. OH), 3030.8, 2888.9, 1721.7, 1496.2, 1453.3, 1361.7, 1309.5, 1260.1, 1208.2, 1107.1, 1035.2, 845.3, 917.2, 736.5, 698.2, cm$^{-1}$; LRMS
(ES): 353.2, 328.1, 292.2, 276.2, 248.1, 232.1, 91.0; HRMS (ES): \text{354.2062 [(M+H)$^+$; C$_{22}$H$_{28}$NO$_3$ requires 354.2069]; Anal. Calcd for C$_{22}$H$_{28}$NO$_3$: C, 74.55; H, 7.96; N, 3.95. Found: C, 74.50; H, 7.98; N, 3.87.}

**Attempted preparation of (1R,2R,3R,7aR)-2-(Benzyloxy)-3-(benzyloxymethyl) hexahydro-1H-pyrrolizin-1-one (277)**

![Chemical Structure](image)

**Method 1: Dess–Martin Periodinane Oxidation**

276 (50 mg, 0.11 mmol) and Dess – Martin periodinane in DCM (15 % w/w, 70 mg, .25 mmol) were stirred in DCM at ambient temperature. The reaction was monitored by TLC (petrol/EtOAC 1:1) and on completion was diluted with ether. A mixture of Na$_2$S$_2$O$_3$ and Sodium bicarbonate was added and the mixture stirred until homogeneous and the products isolated by extraction. TLC and Crude NMR showed no reaction had taken place after 18 h. (0 mg, 0 %).

**Method 2: Swern Oxidation**

To a stirred solution of 2M oxalyl chloride (33 µL, 0.38 mmol) in anhydrous DCM (3 mL) was added DMSO (52 µL, 0.74 mmol) dropwise under a nitrogen atmosphere and the mixture stirred for 30 mins. A solution of 276 (1.5 mg, 0.32 mmol) in DCM (2 mL) was added and the reaction stirred at -78 °C for 1 h. TEA (134 µL, 0.958 mmol) was added and reaction stirred until it reached ambient temperature then water (5 mL) was added. Product was extracted with DCM, washed with brine and concentrated. TLC and Crude NMR showed no reaction had taken place after 18 hours. (0 mg, 0 %).
**Experimental**

**Attempted preparation of (1S,2R,3R,7aR)-2-(Benzyloxy)-3-(benzyloxymethyl) hexahydro-1H-pyrrolizin-1-ol (260)**

![Chemical structure of 260](image)

A solution of alcohol 276 (50 mg, 0.11 mmol), PPh₃ (83 mg, 0.32 mmol) and benzoic acid (15 mg, 0.13 mmol), in dry THF (4 mL) was stirred at 0 °C under an atmosphere of nitrogen. DEAD (50 µL, 0.32 mmol) was added dropwise and mixture stirred at 0 °C for 1 h then at ambient temperature overnight. The solvents were evaporated under reduced pressure to give an orange oil. No reacton was observed from NMR and TLC analysis of the crude product.

**(2R,3aR,4R,5R,6R)-5-(Benzyloxy)-6-(benzyloxymethyl)-4-hydroxy-N,N-dimethylhexahydropyrrolo[1,2-b]isoxazole-2-carboxamide (279)**

![Chemical structure of 279](image)

To a solution of 267 (150 mg, 0.32 mmol) in DCM (5 mL) was added TFA (0.5 mL). The reaction mixture was stirred at ambient temperature for 48 h. The mixture was washed with sodium bicarbonate (2 mL) and extracted with DCM (3 x 3 mL). The combined organic layers were dried over MgSO₄, filtered and the filtrate concentrated. Purification by column chromatography (petrol/EtOAc 1:1) yielded alcohol 279 (78 mg, 86 %) as a yellow oil.

Rᵣ 0.33 (ethyl acetate); δH (400 MHz, CDCl₃): 2.38 (1H, ddd, J = 12.7, 7.5, 5.5, C3-Hₐ), 2.86 (1H, ddd, J = 12.4, 9.2, 7.3, C3-H₈), 2.94 (3H, s, NCH₃), 2.99 (3H, s, NCH₃), 3.38-3.43 (1H, m, C6-H), 3.69 (1H, dd, J = 9.6, 3.7, C7-Hₐ), 3.73 (1H, dd, J = 9.6, 3.7, C7-Hₗ), 3.86 (1H, m, CH-3a), 3.92 (1H, dd, J = 3.7, 2.2, C5-H), 4.21 (1H, t, J = 3.7, 2.2, C4-H), 4.55-4.62 (2H, m, OCH₂Ph), 4.66-4.73 (2H, m, OCH₂Ph), 4.83 (1H, t, J = 7.4, C2-H), 7.19-7.31 (10H, m,
Experimental

\[ \delta_c (100 \text{ MHz}): 35.8 \text{ (NCH}_3\text{)}, 36.3 \text{ (CH}_2\text{-3)}, 36.9 \text{ (NCH}_3\text{)}, 70.8 \text{ (CH-6CH}_2\text{)}, 72.1 \text{ (0CH}_2\text{Ph), 72.55 (CH-7a)}, 72.6 \text{ (CH-6)}, 73.7 \text{ (OCH}_2\text{Ph)}, 74.8 \text{ (CH-2)}, 79.3 \text{ (CH-5)}, 86.7 \text{ (CH-4)}, 127.75 \text{ (CH-Ar), 127.88 (CH-Ar)}, 128.5 \text{ (CH-Ar)}, 137.8 \text{ (CH-Ar)}, 137.4 \text{ (C-Ar), 137.6 (C-Ar), 168.3 (C=O)}; \text{ IR } \nu_{\text{max}} 3380.2 \text{ (br. OH), 3362.7, 3030.7, 2931.1, 2866.7, 1646.0 } \text{ (C=O), 1496.9, 1453.9, 1403.3, 1361.3, 1260.3, 1101.8, 1028.1, 912.3, 800.2, 738.7, 699.0 cm}^{-1}; \text{ HRMS (ES) 427.2222 [(M+NH}_4\text{)]}; \text{ C}_{26}\text{H}_{35}\text{N}_2\text{O}_6 \text{ requires 427.2227}; \text{ Anal. Calcd for } \text{C}_{26}\text{H}_{35}\text{N}_2\text{O}_6: C, 66.22; H, 7.48; N, 5.94. \text{ Found: C, 66.17; H, 7.39; N, 5.87.}

**Attempted preparation of (2R,3aR,5R,6R)-5-(Benzyloxy)-6-(benzyloxymethyl)-N,N-dimethyl-4-oxohexahydropyrrolo[1,2-b]isoxazole-2-carboxamide (280)**

Method 1: Dess-Martin Periodinane Oxidation

279 (50 mg, 0.108 mmol) and Dess – Martin periodinane in DCM (15 % w/w, 70 mg, 0.25 mmol) were stirred in DCM at ambient temperature. The reaction was monitored by TLC (petrol/EtOAC 1:1) and on completion was diluted with ether. A mixture of Na$_2$S$_2$O$_3$ and Sodium bicarbonate was added and the mixture stirred until homogeneous and the products isolated by extraction. TLC and Crude NMR showed no reaction had taken place after 18 h. (0 mg, 0 %).

Method 2: Swern Oxidation

To a stirred solution of 2M oxalyl chloride (33 µL, 0.38 mmol) in anhydrous DCM (3 mL) was added DMSO (52 µL, 0.74 mmol) dropwise under a nitrogen atmosphere and the mixture stirred for 30 min. A solution of 279 (1.5 mg, 0.32 mmol) in DCM (2 mL) was added and the reaction stirred at -78 °C for 1 h. TEA (134 µL, 0.96 mmol) was added and reaction stirred until it reached ambient temperature then water (5 mL) was added. Product was extracted with DCM, washed with brine and concentrated. TLC and Crude NMR showed no reaction had taken place after 18 hours. (0 mg, 0 %).
**Experimental**

**Attempted preparation of (2R,3aR,4S,5R,6R)-5-(benzylxoy)-6-(benzyloxymethyl)-4-hydroxy-N,N-dimethylhexahydropyrrolo[1,2-b]isoxazole-2-carboxamide (281)**

A solution of alcohol 279 (50 mg, 0.11 mmol) in PPh₃ (83 mg, 0.32 mmol) and benzoic acid (15 mg, 0.13 mmol), in dry THF (4 mL) was stirred at 0 °C under an atmosphere of nitrogen. DEAD (50 µL, 0.32 mmol) was added dropwise and mixture stirred at 0 °C for 1 h then at ambient temperature overnight. The solvents were evaporated under reduced pressure to give an orange oil. No reaction was observed from NMR and TLC analysis of the crude product.

**(R)-1-(Benzyloxy)but-3-en-2-ol (294)**

Trimethylsulfonium iodide (4.95 g, 24.23 mmol) was suspended in toluene (30 mL) and heated at reflux for 30 min on a Dean-Stark trap. Toluene was removed and residue dried under high vacuum. After flushing with nitrogen, THF (50 mL) was added and solution cooled to -15 °C in an ice/salt bath and butyllithium (2.5 M, 8.7 mL, 21.75 mmol) was added over 30 min. After stirring at -15 °C for 30 min (R)-Glycidol-benzyl ether (1 g, 0.93 mL, 6.09 mmol) in THF (10 mL) was added within 15 min. The mixture was stirred at -10 °C for 1 h then at ambient temperature overnight. The reaction was quenched with water (50 mL) and extracted with diethyl ether (2 x 50 mL). Concentration and purification by column chromatography (petroleum ether:EtOAc 8:1) yielded the product 294 (1.01 g, 91 %) as a clear oil.
Rf 0.17 (petroleum ether: EtOAc 8:1); δH (400 MHz, CDCl3): 2.59 (1H, br. s, OH), 3.42 (1H, dd, J = 9.6, 7.9, C1-Ha), 3.58 (1H, dd, J = 9.6, 3.4, C1-Hb), 4.39 (1H, m, C2-H), 4.61 (2H, s, OCH2Ph), 5.24 (1H, dt, J = 10.6, 1.5 Hz, C4-Ha), 5.40 (1H, dt, J = 17.3, 1.5 Hz, C4-Hb), 5.88 (1H, ddd, J = 17.3, 10.6, 5.6 Hz, C3-H), 7.39 (10H, m, Ph); δc (100 MHz): 71.5 (CH-2), 73.3 (OCH2Ph), 73.9 (CH2-1), 116.4 (CH2-4), 127.72, 127.73, 127.79, 128.38, 128.43, 128.46, & 128.48 (CHAr), 136.5 (CH-3), 137.8 (C8); IR νmax 3346 (br. OH), 3049.3, 2931.0, 2857.4, 1589.6, 1487.6, 1472.1, 1427.8, 1390.5, 1361.9, 1259.8, 1112.9, 1028.2, 998.2, 822.0, 739.4, 700.8, cm⁻¹; m/z (ES) 178.0992 [(M+H)+; C11H14NO2 requires 178.0994]. Anal. Calcd for C11H14O2: C, 74.13; H, 7.92. Found: C, 74.32; H, 7.99.

(R)-1-(Benzyloxy)but-3-en-2-yloxy)(tert-butyl)diphenylsilane (309)

To a solution of alcohol 294 (0.9 g, 5.05 mmol) in anhydrous DMF (15 mL) and imidazole (1.02 g, 15 mmol) was added TBDPSCl (2.08 mL, 7.58 mmol) and mixture attired at room temperature overnight. The reaction was quenched with 1M HCl (20 mL), extracted with ethyl acetate (2 x 15 ml), washed with water (20 ml), sodium bicarbonate (20 ml) and brine (10 ml) then dried over MgSO4, filtered and concentrated. Purification by flash chromatography (light petroleum ether: EtOAc 9:1) yielded 309 (2.03 g, 96 %) as a green/yellow oil.

Rf 0.58 (light petroleum ether: EtOAc 9:1); δH (200 MHz, CDCl3): 0.99 (9H,s, 3 x CH3), 3.27 (1H, dd, J = 9.7, 7.9, C1-Ha), 3.36 (1H, dd, J = 9.6, 3.4, C1-Hb), 4.26-4.31 (1H, m, C2-H), 4.29 (2H, s, OCH2Ph), 4.99 (1H, dt, J = 10.8, 1.6 Hz, C4-Ha), 5.10 (1H, dt, J = 17.4, 1.6 Hz, C4-Hb), 5.81 (1H, ddd, J = 17.4, 10.8, 5.4 Hz, C3-H) 7.36-7.42 (10H, m, Ph), 7.69-7.82 (5H, m, Ph); δc (50 MHz): 19.1 (CSi), 26.7 (CH3), 72.8 (CH-2), 73.0 (OCH2Ph), 74.3 (CH2-1), 115.4 (CH2-4), 127.7, 127.73, 127.8, 128.4, 128.43, 128.46, & 128.48 (CHAr), 135.6 (CH-3), 133.9, 134.5 & 138.0 (C8); IR νmax 3049.3, 2931.0, 2857.4, 1589.6, 1487.6, 1472.1, 1427.8, 1390.5, 1361.9, 1259.8, 1112.9, 1028.2, 998.2, 822.0, 739.4, 700.8, cm⁻¹.
To a solution of nitrone 179 (800 mg, 2.15 mmol) in toluene (50 mL) was added alkene 309 (740 mg, 1.78 mmol) and the mixture was heated at reflux overnight. The mixture was cooled and concentrated in vacuo and purification by column chromatography (hexane: Et₂O, 7:3) yielded 310 (1.287 g, 92%) as a brown oil.

Rf 0.28 (heptane: EtOAc, 7:3); δH (400 MHz, CDCl₃): 1.09 (9H, s, SiMe₃), 2.28 (1H, ddd, J = 12.5, 7.3, 5.4, C3-H), 2.50 (1H, ddd, J = 12.3, 9.2, 7.8, C3’-H), 3.27 (1H, dd, J = 10.8, 6.0, C6-H), 3.37 (3H, s, OCH3), 3.39, (2H, m, C8-CH₂O), 3.62-3.69 (2H, m, C7-Ha, C3a-H), 3.72-3.76, (1H, m, C7-Hb), 3.97-4.03 (2H, m, C5-H, C8-H), 4.08 (1H, t, J = 4.1, C4-H), 4.21-4.24 (2H, m, OCH₂O), 4.38 (1H, td, J = 7.5, 4.6, C2-H), 4.59-4.69 (6H, m, 3 x OCH₂Ph), 7.12-7.77 (35H, m, Ph); δc (100 MHz): 19.4 (SiC), 26.9 (CMe₃), 35.4 (CH₂-3), 55.5 (OCH₂), 68.2 (CH-7a), 68.9 (CH-6), 69.76(CH-7CH₂), 69.77 (CH-6CH₂), 71.9 (CH₂), 72.3 (CH-5), 72.34 (OCH₂Ph), 72.9 (OCH₂Ph), 73.3 (OCH₂Ph), 83.5 (CH-2CH), 84.9 (CH-4), 95.9 (OCH₂O), 127.3 (CH₃), 127.4 (CH₃), 127.42 (CH₃), 127.51 (CH₃), 127.58 (CH₃), 127.6 (CH₃), 127.68 (CH₃), 127.74 (CH₃), 127.83 (CH₃), 128.1 (CH₃), 128.2 (CH₃), 128.3 (CH₃), 128.5 (CH₃), 129.6 (CH₃), 133.2 (C₆), 134.4 (C₆), 135.8 (C₆), 136.2 (CH₃), 138.1 (C₆), 138.2 (C₆), 138.3 (C₆); IR νmax 3066.3, 2930.9, 1417.9, 1453.8, 1427.8, 1361.8, 1149.9, 1111.6, 1042.1, 916.0, 822.3, 737.5, 699.6, 611.0 cm⁻¹; LRMS (ES): 788.2, 730.3, 242.2; HRMS (ES): 788.3979 [(M+H)⁺]; C₄₈H₇₈NO₇Si requires 788.3977.
(S)-2-(Benzyloxy)-1-((2R,3aR,4R,5R,6R)-5-(benzyloxy)-6-(benzyloxymethyl)-4-(methoxymethoxy)hexahydropyrrolo[1,2-b]isoxazol-2-yl)ethanol (304)

![Chemical Structure](image)

To a solution of silyl ether (0.48 g, 0.61 mmol) in dry THF (10 mL) was added TBAF (1M, 1.20 mL, 1.20 mmol). The reaction mixture was stirred at ambient temperature for 1 h then evaporated to give a brown residue. Purification by column chromatography (light petroleum:EtOAc, 3:7) yielded 304 as an orange oil (0.283 g, 85 %).

Rf 0.42 (heptane: EtOAc, 3:7); δH (200 MHz, CDCl3): 2.28 (1H, ddd, J = 12.4, 7.2, 5.0, C3-H*), 2.44 (1H, ddd, J = 12.6, 8.9, 7.5, C3-H), 2.68 (1H, d, J = 2.9, OH), 3.18-3.22 (1H, m, C6-H), 3.31 (3H, s, CH2OCH3), 3.40-3.73 (5H, m, 2 x CH2, C3a-H) 3.86-4.26 (4H, m, C5-H, C2-H, C8-H, C4-H), 4.44-4.64 (8H, m, OCH2O, 3x OCH2Ph), 7.12-7.77 (35H, m, Ph); δc (100 MHz): 34.4 (CH2-b), 55.4 (OCH3), 68.2 (CH-7a), 69.1 (CH-6), 69.3 (CH-6CH2), 70.2 (CH-6), 70.9 (OCH2Ph), 72.0 (OCH2Ph), 73.1 (OCH2Ph), 73.2 (OCH2Ph), 76.8 (CH-5), 83.2 (CH-2CH), 85.2 (CH-4), 95.7 (OCH2O), 127.3 (CHAr), 127.5 (CHAr), 127.51 (CHAr), 128.0 (CHAr), 128.1 (CHAr), 128.2 (CHAr), 137.6 (CAr), 137.8 (CAr), 138.0 (CAr); IR νmax 3413.9 (br. OH), 3069.3, 2930.6, 1417.1, 1454.1, 1427.6, 1360.9, 1150.3, 1111.3, 1041.8, 916.4, 822.7, 737.2, 699.6, 610.6 cm⁻¹; LRMS (ES): 549.2, 458.1, 428.1, 398.1, 276.1, 91.0; HRMS (ES) 550.216 [(M+H)⁺; C32H40NO7 requires 550.280]. Anal. Calcd for C32H39NO7: C, 69.92; H, 7.32; N, 2.55. Found: C, 69.88; H, 7.35; N, 2.59.

(S)-2-(Benzyloxy)-1-((2R,3aR,4R,5R,6R)-5-(benzyloxy)-6-(benzyloxymethyl)-4-(methoxymethoxy)hexahydropyrrolo[1,2-b]isoxazol-2-yl)ethyl methanesulfonate (305)

![Chemical Structure](image)
Alcohol 304 (200 mg, 0.36 mmol) in DCM (20 mL) was stirred at 0 °C under an atmosphere of argon. Et₃N (0.10 mL, 0.90 mmol) and MsCl (0.04 mL, 0.70 mmol) were successively added. The mixture was stirred at 0 °C for 30 min then the reaction was quenched by the addition of water (10 ml). The phases were separated and the aqueous layer extracted with DCM (2 x 15 ml), washed with brine (10 ml), dried over MgSO₄, filtered and concentrated. Purification by flash chromatography (light petroleum:EtOAc, 1:1) yielded mesylate 305 (221 mg, 97 %) as a yellow oil.

Rf 0.47 (heptane:EtOAc, 4:6); δH (200 MHz, CDCl₃): 2.23-2.56 (2H, m, C₃-H₂), 3.01 (3H, s, SMe), 3.24-3.38 (1H, m, C₆-H), 3.29 (3H, s, CH₂OCH₃), 3.45-3.70 (5H, m, CH₂, CH₃, C₃a-H), 3.91 (1H, m, C₅-H), 4.04-4.18 (1H, m, C₂-H), 4.29-4.37 (1H, m, C₄-H), 4.41-4.59 (8H, m, OCH₂O, 3x OCH₂Ph), 4.79 (1H, q, J = 5.2, C₂CH), 7.12-7.77 (35H, m, Ph); δc (100 MHz): 34.6 (CH₂-C₃), 38.2 (SO₂Me), 55.3 (OCH₂), 68.3 (CH₇a), 69.2 (CH₂), 69.3 (CH₂), 69.6 (CH-6), 71.9 (CH₂), 72.9 (CH₂), 73.1 (CH₂), 75.31 (CH₆), 80.02 (CH₅), 83.40 (C₂CH), 84.31 (CH₄), 95.57 (OCH₂O), 127.25 (CH₇a), 127.35 (CH₆), 127.41 (CH₅), 127.59 (CH₆), 127.97 (CH₇a), 128.03 (CH₆), 128.15 (CH₅), 136.93 (C₆), 137.49 (C₅), 137.80 (C₇a); IR νmax 3030.6, 2935.9, 2892.3, 1496.4, 1454.0, 1358.3, 1208.6, 1174.8, 1104.4, 1038.0, 971.1, 919.2, 808.2, 739.9, 698.9 cm⁻¹; LRMS (ES): 628.2, 506.2, 413.2, 354.1, 344.1, 108.0, 91.0, 79.0; HRMS (ES) 628.2575 [(M+H)⁺]; C₃₃H₄₂NO₉S requires 628.2575.

(1R,2R,3R,5R,6R,7aR)-2-(Benzyloxy)-3,5-bis(benzyloxymethyl)-1-(methoxymethoxy) hexahydro-7a-H-pyrrolizin-6-ol (306)

To isoxazole 306 (950 mg, 1.51 mmol) was added AcOH (10 M, 2 mL, 20.00 mmol) and Zn dust (1 g, 30.42 mmol). The mixture was stirred at 85 °C for 2h then the pH was adjusted to 14 by addition of saturated NaOH solution. The layers were separated and the mixture extracted with DCM (2 x 13 ml). The combined organic layers were dried over MgSO₄, filtered and concentrated. Purification by column chromatography (EtOAc) yielded alcohol 306 (668 mg, 87 %) as a pale green oil.
Rf 0.20 (EtOAc); δH (400 MHz, CDCl3): 1.89 (1H, ddd, J = 14.1, 5.6, 3.0, C7-H), 2.32 (1H, ddd, J = 14.0, 9.7, 6.3, C7-H), 3.32 (1H, m, C5-H), 3.33 (3H, s, OCH3), 3.49 (2H, m, C3-CH2), 3.59 (1H, m, C7a-H), 3.79 (1H, ddd, J = 14.1, 5.6, 3.0, C3-H), 3.92 (2H, ddd, J = 14.1, 5.6, 3.0, C5-CH2), 3.98 (1H, t, J = 2.9, C2-H), 4.20 (1H, t, J = 2.9, C1-H), 4.27 (1H, s, C6-H) 4.49-4.67 (8H, m, 4 x OCH2), 7.28-7.35 (15H, m, 3 x Ph); δc (100 MHz): 39.4 (CH2-7), 55.4 (0Me), 62.9 (CH-3), 65.9 (CH-5), 67.2 (C5-CH2), 67.8 (CH-7a), 71.9 (OCH2), 72.6 (OCH2), 72.9 (OCH2), 73.4 (OCH2), 74.3 (CH-6), 85.5 (CH-1), 86.4 (CH-2), 95.5 (OCH2O), 127.4 (CHar), 127.6 (CHar), 127.61 (CHar), 127.8 (CHar), 127.88 (CHar), 127.89 (CHar), 128.2 (CHar), 128.22 (CHar), 128.3 (CHar), 128.36 (CHar) 128.4 (CHar), 137.9 (Car), 138.4 (Car); IR νmax 3413.9 (br. OH), 3030.7, 2889.7, 1496.6, 1453.8, 1174.9, 1103.3, 1037.3, 971.8, 919.5, 739.1, 698.9 cm⁻¹; LRMS (ES): 533.3, 502.2, 488.2, 472.2, 442.2, 426.2, 413.2, 91.0; HRMS (ES): 533.2778 [M⁺; C32H39NO6 requires 533.2772]. Anal. Calcd for C32H39NO6: C, 72.02; H, 7.37; N, 2.63. Found: C, 72.12; H, 7.42; N, 2.68.

(1R,2R,3R,5R,6R,7aR)-2-(Benzyloxy)-3,5-bis(benzyloxymethyl)-1-(methoxymethoxy) hexahydro-1H-pyrrolizin-6-yl methanesulfonate (311)

Alcohol 306 (400 mg, 1.12 mmol) in DCM (20 mL) was stirred at 0 °C under an atmosphere of argon. Et3N (0.35 mL, 3.14 mmol) and MsCl (0.14 mL, 2.46 mmol) were successively added. The mixture was stirred at 0 °C for 30 min then the reaction was quenched by the addition of water (10 ml). The layers were separated and the aqueous layer extracted with DCM (2 x 10 ml), washed with brine (10ml), dried over MgSO4, filtered and concentrated. Purification by flash chromatography (light petroleum:EtOAc, 3:7) yielded mesylate 311 (458 mg, 100 %) as a pale green oil.

Rf 0.34 (heptanes:EtOAc, 3:7); δH (200 MHz, CDCl3): 2.35-2.41 (2H, m, C7-H2), 2.83 (3H, s, SO2CH2), 3.31 (3H, s, OCH3), 3.38-3.57 (5H,m, C3-CH2, C7a-H, C3-H, C5-H), 3.78 (2H, d, J = 5.5, C5-CH2), 3.98 (1H, t, J = 5.1, C2-H), 4.08 (1H, t, J = 5.1, C1-H), 4.49-4.55 (4H, m,
2 × OCH₂), 4.59-4.63 (4H, m, 2 × OCH₂), 5.21 (1H, q, J 5.1 Hz, H-6), 7.31-7.41 (15H, m, 3 × Ph); δc (100 MHz): 35.8 (CH₂-7), 38.2 (SCH₃), 55.4 (OCH₃), 61.6 (CH-3), 64.1 (CH-5), 65.7 (C₅-CH₂), 66.6 (CH-7a), 72.2 (OCH₂), 72.4 (OCH₂), 73.3 (OCH₂), 73.5 (OCH₂), 82.8 (CH-6), 85.1 (CH-1), 86.6 (CH-2), 95.9 (OCH₃O), 127.6 (CH₃), 127.7 (CH₃), 127.9 (CH₃), 128.3 (CH₃), 137.8 (C₆), 138.3 (C₆), 138.4 (C₆); IR νmax 3063.0, 3030.2, 2888.5, 1496.3, 1453.8, 1358.5, 1175.0, 1103.5, 1037.8, 970.0, 918.3, 861.6, 738.6, 698.9 cm⁻¹; LRMS (ES): 611.2, 490.2, 424.2, 396.2, 352.2, 163.0, 107.0, 79.0; HRMS (ES): 611.2547 [M⁺; C₃₃H₄₁NO₈S requires 611.2547]; Anal. Calcd for C₃₃H₄₁NO₈S: C, 63.35; H, 7.03; N, 2.38. Found: C, 63.47; H, 7.12; N, 2.51.

(1R,2R,3R,5R,6R,7aR)-2-(Benzyloxy)-3,5-bis(benzyloxymethyl)-1-(methoxymethoxy) hexahydror-1H-pyrrolizin-6-yl S-methyl carbonodithioate (312)

Method 1:

Alcohol 306 (800 mg, 1.50 mmol) was added to a suspension of NaH (30 % in mineral oil, 158 mg, 3.95 mmol) in dry THF (30 mL) at 0 °C and then the mixture was stirred at rt for 20 min. The suspension was then cooled to 0 °C and CS₂ (0.25 mL, 303 mg, 3.95 mmol) was added and the reaction mixture allowed to stir at 0 °C for 10 min. Iodomethane (0.25 mL, 565 mg, 3.95 mmol) was then added and mixture stirred at ambient temperature for 20 min. The reaction was then quenched by careful addition of water until effervescence had stopped. The layers were separated and the organic layer was extracted with diethyl ether (10 mL), dried over MgSO₄, filtered and concentrated to give a yellow residue. Purification by column chromatography (light petroleum:EtOAc 7:3) produced the xanthate 312 (47 mg, 5 %) as a yellow oil.

Method 2:

Alcohol 306 (800 mg, 1.50 mmol) was added to a suspension of KH (30 % in mineral oil, 533 mg, 3.98 mmol) in dry THF (30 mL) at 0 °C and then the mixture stirred at RT for 20
min. The suspension was then cooled to 0 °C and CS₂ (0.25 mL, 303 mg, 3.95 mmol) was added and the reaction mixture allowed to stir at 0 °C for 10 min. Iodomethane (0.25 mL, 565 mg, 3.95 mmol) was then added and mixture stirred at ambient temperature for 20 min. The reaction was then quenched by careful addition of water until effervescence had stopped. The organic layer was extracted with diethyl ether (3 mL), dried over MgSO₄, filtered and concentrated to give a yellow residue. Purification by column chromatography (light petroleum:EtOAc 7:3) produced the xanthate 312 (649 mg, 70 %) as a yellow oil.

Rf 0.26 (light petroleum:EtOAc 7:3); δH (200 MHz, CDCl₃): 2.29-2.51 (2H, m, C7-H₂), 2.41 (3H, s, SCH₃), 3.25 (3H, s, OCH₃), 3.37-3.64 (5H, m, C1-H, C2-H, C7a-H, C3-H, C5-H), 3.71-3.80 (2H, m, C3-CH₂), 3.98-4.07 (2H, m, C5-CH₂), 4.59-4.65 (8H, m, 8 x OCH₂), 5.97 (1H, dt, J = 6.6, 5.1, C6-H), 7.25 (15H, m, 3 x Ph); δc (100 MHz): 18.9 (SCH₃), 35.5 (CH₂-7), 55.4 (OCH₃), 61.7 (CH-3), 63.5 (CH-5), 65.9 (C5-CH₂), 66.5 (CH-7a), 71.9 (OCH₂), 72.6 (OCH₂), 73.2 (OCH₂), 73.4 (OCH₂), 85.1 (CH-6), 85.4 (CH-1), 86.7 (CH-2), 95.8 (OCH₂O), 127.4 (CH₆), 127.5 (CH₆), 127.51 (CH₆), 127.57 (CH₆), 127.7 (CH₆), 128.2 (CH₂), 128.3 (CH₂), 137.9 (C₆), 138.4 (C₆), 138.6 (C₆); IR νmax 3087.7, 3062.5, 3029.7, 2923.3, 2855.5, 1495.9, 1453.5, 1363.8, 1212.0, 1148.3, 1104.1, 1069.9, 1039.0, 966.0, 917.5, 819.0, 735.9, 697.8 cm⁻¹; LRMS (ES): 623.2, 503.2, 394.2, 332.2, 274.1, 108.1; HRMS (ES): 623.2370 [M⁺]; C₃₄H₄₁NO₆S₂ requires 623.2370.

(1R,2R,3R,5S,7aR)-2-(Benzyloxy)-3,5-bis(benzyloxymethyl)-1-(methoxymethoxy)hexahydro-1H-pyrrolizine (307)

Method 1:
To a solution of mesylate 311 (800 mg, 1.31 mmol) in anhydrous THF (10 mL) was added LiAlH₄ (56 mg, 1.48 mmol) and the mixture heated at reflux for 12 h. The reaction was
quenched with the careful addition of NaOH and partitioned between EtOAc (10 mL) and water (10 mL). The organic phase was separated and aqueous phase extracted with EtOAc (2 x 5 mL). The combined organic phases were concentrated and purified by column chromatography (light petroleum:EtOAc 8:2) yielded the product 307 (18 mg, 2 %) as a yellow oil.

Method 2:

To a solution of mesylate 311 (600 mg, 0.98 mmol) in anhydrous THF (40 mL) was added super hydride (1M, 5 mL, 5 mmol) at ambient temperature and the reaction left to stir for 24 h. The reaction was quenched with addition of water and the organic phase extracted with EtOAc (30 mL) and concentrated. Purification by column chromatography (light petroleum:EtOAc 8:2) yielded the product 307 (48 mg, 9 %) as a yellow oil.

Method 3:

To a solution of xanthate 312 (600 mg, 0.96 mmol) in toluene (21 mL) at reflux was added a mixture of AIBN (60 mg, 0.36 mmol) and Bu3SnH (0.618 mL, 2.29 mmol) in toluene (3 mL) over 2 h. The mixture was left at reflux for a further 1 h after addition then allowed to cool to ambient temperature. The solvent was then removed under reduced pressure to produce a brown oil. Purification by column chromatography (light petroleum:EtOAc 8:2) yielded product 307 (291 mg, 59 %) as a yellow oil.

Rf 0.29 (light petroleum:EtOAc 8:2); δH (400 MHz, CDCl3): 1.78-1.87 (2H, m, C6-H2), 1.88-1.99 (2H, m, C7-H), 3.29 (3H, s, OCH3), 3.38 (2H, br. s, C3-H, C5-H), 3.46-3.49 (3H, m, C5-CH2, C7a -H), 3.58-3.43 (2H, m, C3-CH2), 3.89 (1H, t, J = 5.0, C1-H), 3.98 (1H, t, J =
5.0, C2-H), 4.43 (2H, s, OCH$_2$), 4.48 (2H, s, OCH$_2$), 4.58 (2H, s, OCH$_2$O), 4.59-4.64 (2H, m, OCH$_2$), 7.25-7.40 (15H, m, 3 x Ph); δ$_c$ (100 MHz): 28.7 (CH$_2$-7), 28.3 (CH$_2$-6), 55.4 (OCH$_3$), 61.1 (CH-3), 61.2 (CH-5), 68.5 (CH-7a), 70.7 (C3-CH$_2$), 71.9 (C5-CH$_2$), 72.7 (OCH$_2$), 73.1 (OCH$_2$), 73.2 (OCH$_2$), 85.4 (CH-1), 87.3 (CH-2), 95.6 (OCH$_2$O), 127.4 (CH$_{ar}$), 127.41 (CH$_{ar}$), 127.47 (CH$_{ar}$), 127.5 (CH$_{ar}$), 127.6 (CH$_{ar}$), 127.7 (CH$_{ar}$), 128.1 (CH$_{ar}$), 128.2 (CH$_{ar}$), 128.24 (CH$_{ar}$), 128.27 (CH$_{ar}$), 128.3 (CH$_{ar}$), 138.3 (C$_{ar}$), 138.55 (C$_{ar}$), 138.59 (C$_{ar}$); IR $\nu_{max}$ 3087.7, 3062.9, 3030.0, 2924.5, 2854.7, 1496.1, 1453.6, 1363.6, 1209.3, 1103.0, 1038.0, 918.5, 819.2, 736.2, 697.9 cm$^{-1}$;

(1$R$,2$R$,3$R$,5$S$,7$a$R)-2-(Benzyloxy)-3,5-bis(benzyloxymethyl)hexahydro-1H-pyrrolizin-1-ol (308)

![Chemical structure](image)

To a solution of 307 (270 mg, 0.52 mmol) in DCM (10 mL) was added TFA (1 mL, 12.91 mmol). The reaction mixture was stirred at ambient temperature for 48 h. The mixture was washed with sodium bicarbonate (2 mL) and extracted with DCM (3 x 3 mL). The Organic phase was dried over MgSO$_4$, filtered and the filtrate concentrated to give brown residue. Purification by column chromatography (EtOAc) yielded the product 308 as an orange oil (200 mg, 81%).

R$_f$ 0.21 (EtOAc); δ$_H$ (200 MHz, CDCl$_3$): 1.78-1.86 (2H, m, C6-H$_2$), 1.88-1.95 (2H, m, C7-H$_2$), 3.38-3.41 (2H, br. s, C3-H, C5-H), 3.46-3.58 (3H, m, C5-CH$_2$, C7a-H), 3.58-3.67 (2H, m, C3-CH$_2$), 3.89 (1H, t, $J$ 5.0, C1-H), 3.98 (1H, t, $J$ 5.0, C2-H), 4.43 (2H, s, OCH$_2$Ph), 4.48 (2H, s, OCH$_2$Ph), 4.59-4.63 (2H, m, OCH$_2$Ph), 7.25-7.38 (15H, m, 3 x Ph); δ$_c$ (100 MHz): 26.28 (CH$_2$-7), 26.98 (CH$_2$-6), 63.79 (CH-3), 64.70 (CH-5), 68.49 (C3-CH$_2$), 70.93 (C5-CH$_2$), 71.70 (OCH$_2$), 73.12 (OCH$_2$), 73.29 (CH-7a), 73.56 (OCH$_2$), 77.92 (CH-1), 88.45 (CH-2), 127.47 (CH$_{ar}$), 127.72 (CH$_{ar}$), 127.79 (CH$_{ar}$), 127.95 (CH$_{ar}$), 128.10 (CH$_{ar}$), 128.39
(CH$_2$), 137.61 (C$_{ar}$), 137.65 (C$_{ar}$), 137.00 (C$_{ar}$); IR $v_{max}$ (br. OH), 3301.58, 3087.6, 3062.4, 3030.4, 2869.0, 1672.9, 1454.8, 1365.6, 1199.1, 1098.0, 1070.3, 1026.7, 736.2, 697.9 cm$^{-1}$.

**1-epi-hyacinthacine B$_2$ (293)**

![Diagram of 1-epi-hyacinthacine B$_2$ (293)]

To a solution of benzyl ether 308 (40 mg, 0.085 mmol) in methanol was added HCl (4 drops). The mixture was hydrogenated in the presence of 10 % Pd/C (25 mg) for 48 hr. The catalyst was filtered off and the filter washed well with methanol. The solvent was removed under reduced pressure to produce the free pyrrolizidine 293 (15 mg, 88 %) as a clear oil.

R$_f$ 0.12 (EtOAc); $\delta$$_{H}$ (400 MHz, D$_2$O): 2.15-2.31 (4H, m, C6- H$_2$ & C7- H$_2$), 3.74-3.79 (1H, m, C3-H), 3.85-3.92 (1H, m, C5-H), 3.9-4.0 (4H, m, C3-H$_2$ & C5- H$_2$), 4.07-4.09 (2H, m, C1-H & C7a-H), 4.11-18 (1H, m, C2-H). $\delta$$_{C}$ (100 MHz): 24.77 (CH$_2$-6), 26.34 (CH$_2$-7), 57.14 & 57.82 (CH$_2$-8 & CH$_2$-9), 64.78 (CH-3), 68.44 (CH-5), 71.22 (CH-7a), 74.59 (CH-2), 77.27 (CH-1); IR $v_{max}$ (br. OH), 3301.58, 3087.6, 3062.4, 3030.4, 2869.0, 1672.9, 1454.8, 1365.6, 1199.1, 1098.0, 1070.3, 1026.7, 736.2, 697.9 cm$^{-1}$; LRMS (ES): 204.2; HRMS (ES) 204.1242 [M$^+$; C$_9$H$_{18}$NO$_4$ requires 204.1236]. Anal. Caled for C$_9$H$_{18}$NO$_4$: C, 52.93; H, 8.88; N, 6.86. Found: C, 52.89; H, 8.91; N, 6.83.
4 APPENDICES
Table 4. Crystal data and structure refinement for ASM111A x82103_0m.

<table>
<thead>
<tr>
<th>Identification code</th>
<th>x82103_0m</th>
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<tbody>
<tr>
<td>Empirical formula</td>
<td>C19 H20 O5</td>
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</tr>
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<td>100(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P2(1)</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 9.0996(14) Å</td>
</tr>
<tr>
<td></td>
<td>□ = 90°.</td>
</tr>
<tr>
<td></td>
<td>b = 5.1326(9) Å</td>
</tr>
<tr>
<td></td>
<td>□ = 93.602(10)°.</td>
</tr>
<tr>
<td></td>
<td>c = 17.537(3) Å</td>
</tr>
<tr>
<td></td>
<td>□ = 90°.</td>
</tr>
<tr>
<td>Volume</td>
<td>817.4(2) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.334 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.096 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>348</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.81 x 0.68 x 0.08 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>2.46 to 29.21°.</td>
</tr>
</tbody>
</table>
Appendices

Index ranges  
-12<=h<=12, -7<=k<=7, -24<=l<=23

Reflections collected  
19320

Independent reflections  
2439 [R(int) = 0.0414]

Completeness to theta = 25.00°  
99.8 %

Absorption correction  
Semi-empirical from equivalents

Max. and min. transmission  
0.9923 and 0.8489

Refinement method  
Full-matrix least-squares on F^2

Data / restraints / parameters  
2439 / 1 / 218

Goodness-of-fit on F^2  
1.124

Final R indices [I>2sigma(I)]  
R1 = 0.0395, wR2 = 0.0856

R indices (all data)  
R1 = 0.0474, wR2 = 0.0888

Largest diff. peak and hole  
0.248 and -0.206 e.A^-3

Table 5. Bond lengths [Å] and angles [°] for x82103_0m.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length [Å]</th>
</tr>
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<td>O(1)-C(2)</td>
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<tr>
<td>O(1)-C(5)</td>
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</tr>
<tr>
<td>C(2)-O(2)</td>
<td>1.204(2)</td>
</tr>
<tr>
<td>C(2)-C(3)</td>
<td>1.528(2)</td>
</tr>
<tr>
<td>C(3)-O(3)</td>
<td>1.4093(19)</td>
</tr>
<tr>
<td>C(3)-C(4)</td>
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<tr>
<td>C(3)-H(3)</td>
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</tr>
<tr>
<td>O(3)-H(3A)</td>
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</tr>
<tr>
<td>C(4)-O(4)</td>
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<td>C(4)-C(5)</td>
<td>1.539(2)</td>
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<tr>
<td>C(4)-H(4)</td>
<td>1.0000</td>
</tr>
<tr>
<td>O(4)-C(6)</td>
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<tr>
<td>C(5)-C(13)</td>
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<tr>
<td>C(5)-H(5)</td>
<td>1.0000</td>
</tr>
<tr>
<td>C(6)-C(7)</td>
<td>1.499(3)</td>
</tr>
<tr>
<td>C(6)-H(6A)</td>
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</tr>
<tr>
<td>C(6)-H(6B)</td>
<td>0.9900</td>
</tr>
<tr>
<td>C(7)-C(8)</td>
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</tr>
<tr>
<td>C(7)-C(12)</td>
<td>1.382(3)</td>
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<tr>
<td>C(8)-C(9)</td>
<td>1.381(3)</td>
</tr>
<tr>
<td>C(8)-H(8)</td>
<td>0.9500</td>
</tr>
<tr>
<td>C(9)-C(10)</td>
<td>1.376(3)</td>
</tr>
</tbody>
</table>
C(9)-H(9)  0.9500
C(10)-C(11)  1.376(3)
C(10)-H(10)  0.9500
C(11)-C(12)  1.380(3)
C(11)-H(11)  0.9500
C(12)-H(12)  0.9500
C(13)-O(14)  1.417(2)
C(13)-H(13A)  0.9900
C(13)-H(13B)  0.9900
O(14)-C(15)  1.435(2)
C(15)-C(16)  1.507(3)
C(15)-H(15A)  0.9900
C(15)-H(15B)  0.9900
C(16)-C(21)  1.388(3)
C(16)-C(17)  1.393(3)
C(17)-C(18)  1.377(3)
C(17)-H(17)  0.9500
C(18)-C(19)  1.383(4)
C(18)-H(18)  0.9500
C(19)-C(20)  1.390(4)
C(19)-H(19)  0.9500
C(20)-C(21)  1.387(3)
C(20)-H(20)  0.9500
C(21)-H(21)  0.9500
C(2)-O(1)-C(5)  109.77(13)
O(2)-C(2)-O(1)  120.96(15)
O(2)-C(2)-C(3)  128.15(16)
O(1)-C(2)-C(3)  110.89(15)
O(3)-C(3)-C(4)  118.49(15)
O(3)-C(3)-C(2)  112.35(14)
C(4)-C(3)-C(2)  100.54(14)
O(3)-C(3)-H(3)  108.3
C(4)-C(3)-H(3)  108.3
C(2)-C(3)-H(3)  108.3
C(3)-O(3)-H(3A)  109.5
O(4)-C(4)-C(3)  117.22(14)
O(4)-C(4)-C(5)  109.58(13)
C(3)-C(4)-C(5)   102.00(14)
O(4)-C(4)-H(4)   109.2
C(3)-C(4)-H(4)   109.2
C(5)-C(4)-H(4)   109.2
C(4)-O(4)-C(6)   111.77(13)
O(1)-C(5)-C(13)  110.47(14)
O(1)-C(5)-C(4)   103.35(13)
C(13)-C(5)-C(4)  116.09(14)
O(1)-C(5)-H(5)   108.9
C(13)-C(5)-H(5)  108.9
C(4)-C(5)-H(5)   108.9
O(4)-C(6)-C(7)   109.41(14)
O(4)-C(6)-H(6A)  109.8
C(7)-C(6)-H(6A)  109.8
O(4)-C(6)-H(6B)  109.8
C(7)-C(6)-H(6B)  109.8
H(6A)-C(6)-H(6B) 108.2
C(8)-C(7)-C(12)  118.49(18)
C(8)-C(7)-C(6)   120.40(18)
C(12)-C(7)-C(6)  121.06(19)
C(7)-C(8)-C(9)   121.0(2)
C(7)-C(8)-H(8)   119.5
C(9)-C(8)-H(8)   119.5
C(10)-C(9)-C(8)  120.1(2)
C(10)-C(9)-H(9)  120.0
C(8)-C(9)-H(9)   120.0
C(9)-C(10)-C(11) 119.3(2)
C(9)-C(10)-H(10) 120.3
C(11)-C(10)-H(10) 120.3
C(10)-C(11)-C(12) 120.5(2)
C(10)-C(11)-H(11) 119.7
C(12)-C(11)-H(11) 119.7
C(11)-C(12)-C(7)  120.6(2)
C(11)-C(12)-H(12) 119.7
C(7)-C(12)-H(12)  119.7
O(14)-C(13)-C(5)  110.67(15)
O(14)-C(13)-H(13A) 109.5
C(5)-C(13)-H(13A)  109.5
O(14)-C(13)-H(13B)  109.5
C(5)-C(13)-H(13B)  109.5
H(13A)-C(13)-H(13B)  108.1
C(13)-O(14)-C(15)  110.60(14)
O(14)-C(15)-C(16)  111.91(14)
O(14)-C(15)-H(15A)  109.2
C(16)-C(15)-H(15A)  109.2
O(14)-C(15)-H(15B)  109.2
C(16)-C(15)-H(15B)  109.2
H(15A)-C(15)-H(15B)  107.9
C(21)-C(16)-C(17)  119.16(19)
C(21)-C(16)-C(15)  121.11(18)
C(17)-C(16)-C(15)  119.71(19)
C(18)-C(17)-C(16)  120.6(2)
C(18)-C(17)-H(17)  119.7
C(16)-C(17)-H(17)  119.7
C(17)-C(18)-C(19)  119.8(2)
C(17)-C(18)-H(18)  120.1
C(19)-C(18)-H(18)  120.1
C(18)-C(19)-C(20)  120.4(2)
C(18)-C(19)-H(19)  119.8
C(20)-C(19)-H(19)  119.8
C(21)-C(20)-C(19)  119.4(2)
C(21)-C(20)-H(20)  120.3
C(19)-C(20)-H(20)  120.3
C(20)-C(21)-C(16)  120.6(2)
C(20)-C(21)-H(21)  119.7
C(16)-C(21)-H(21)  119.7

Symmetry transformations used to generate equivalent atoms:

Table 6. Torsion angles [°] for x82103_0m.

<table>
<thead>
<tr>
<th>Torsion Angles</th>
<th>Value</th>
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<tbody>
<tr>
<td>C(5)-O(1)-C(2)-O(2)</td>
<td>179.07(15)</td>
</tr>
<tr>
<td>C(5)-O(1)-C(2)-C(3)</td>
<td>-1.18(19)</td>
</tr>
<tr>
<td>O(2)-C(2)-C(3)-O(3)</td>
<td>31.5(3)</td>
</tr>
</tbody>
</table>
Appendices

O(1)-C(2)-C(3)-O(3) = -148.26(15)
O(2)-C(2)-C(3)-C(4) = 158.42(17)
O(1)-C(2)-C(3)-C(4) = -21.30(17)
O(3)-C(3)-C(4)-O(4) = -84.24(18)
C(2)-C(3)-C(4)-O(4) = 153.00(13)
O(3)-C(3)-C(4)-C(5) = 156.12(14)
C(2)-C(3)-C(4)-C(5) = 33.36(15)
C(3)-C(4)-O(4)-C(6) = 76.8(2)
C(5)-C(4)-O(4)-C(6) = -167.64(17)
C(2)-O(1)-C(5)-C(13) = -101.62(16)
C(2)-O(1)-C(5)-C(4) = 23.20(18)
O(4)-C(4)-C(5)-O(1) = -160.20(14)
C(3)-C(4)-C(5)-O(1) = -35.31(16)
O(4)-C(4)-C(5)-O(1) = -39.1(2)
C(3)-C(4)-C(5)-C(13) = 85.78(18)
C(4)-O(4)-C(6)-C(7) = 172.32(16)
O(4)-C(6)-C(7)-C(8) = -130.9(2)
O(4)-C(6)-C(7)-C(12) = 51.8(3)
C(12)-C(7)-C(8)-C(9) = 0.2(3)
C(6)-C(7)-C(8)-C(9) = -177.2(2)
C(7)-C(8)-C(9)-C(10) = -0.5(4)
C(8)-C(9)-C(10)-C(11) = 0.5(4)
C(9)-C(10)-C(11)-C(12) = -0.3(4)
C(10)-C(11)-C(12)-C(7) = 0.1(4)
C(8)-C(7)-C(12)-C(11) = 0.0(3)
C(6)-C(7)-C(12)-C(11) = 177.4(2)
O(1)-C(5)-C(13)-O(14) = 51.02(19)
C(4)-C(5)-C(13)-O(14) = -66.2(2)
C(5)-C(13)-O(14)-C(15) = -178.38(13)
C(13)-O(14)-C(15)-C(16) = -73.4(2)
O(14)-C(15)-C(16)-C(21) = 109.8(2)
O(14)-C(15)-C(16)-C(17) = -68.7(2)
C(21)-C(16)-C(17)-C(18) = 0.8(3)
C(15)-C(16)-C(17)-C(18) = 179.3(2)
C(16)-C(17)-C(18)-C(19) = -0.8(4)
C(17)-C(18)-C(19)-C(20) = 0.5(4)
C(18)-C(19)-C(20)-C(21) = -0.3(4)
Table 7. Hydrogen bonds for x82103_0m [Å and °].

<table>
<thead>
<tr>
<th>D-H...A</th>
<th>d(D-H)</th>
<th>d(H...A)</th>
<th>d(D...A)</th>
<th>&lt;(DHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(3)-H(3A)...O(2)#1</td>
<td>0.84</td>
<td>1.93</td>
<td>2.7592(19)</td>
<td>167.6</td>
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</table>

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,y-1/2,-z
4.2 Appendix B: NOESY interactions for compound 267
Two molecules in the asymmetric unit, both molecules are of the same chiral hand, assigned on the basis of the enclosed chemical diagram.
Solution attempted in C2 several times (pseudo symmetry relationship between molecules)
but not successful. Very weakly diffracting crystal, two domains second rotated from first domain by 3.2 degrees.

Table 1. Crystal data and structure refinement for twin5. asm186b

<table>
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<th>Identification code</th>
<th>twin5</th>
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<tr>
<td>Temperature</td>
<td>100(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P1</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>$a = 4.5560(4)$ Å, $\beta = 77.689(5)^\circ$, $b = 10.5338(9)$ Å, $\gamma = 84.80(3)^\circ$, $c = 24.362(2)$ Å, $\delta = 89.82(3)^\circ$.</td>
</tr>
<tr>
<td>Volume</td>
<td>1137.45(17) Å$^3$</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.248 Mg/m$^3$</td>
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<td>Absorption coefficient</td>
<td>0.089 mm$^{-1}$</td>
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<tr>
<td>F(000)</td>
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<tr>
<td>Crystal size</td>
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<tr>
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<tr>
<td>Index ranges</td>
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<td>Reflections collected</td>
<td>2640</td>
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<tr>
<td>Independent reflections</td>
<td>2640 [R(int) = 0.0000]</td>
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<tr>
<td>Completeness to theta = 19.60°</td>
<td>85.9 %</td>
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<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.9964 and 0.9159</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on $F^2$</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>2640 / 406 / 546</td>
</tr>
<tr>
<td>Goodness-of-fit on $F^2$</td>
<td>1.023</td>
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<tr>
<td>Final R indices [I&gt;2sigma(I)]</td>
<td>$R1 = 0.0845$, $wR2 = 0.2068$</td>
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<tr>
<td>R indices (all data)</td>
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<tr>
<td>Largest diff. peak and hole</td>
<td>0.322 and -0.385 e Å$^{-3}$</td>
</tr>
</tbody>
</table>

Table 2. Bond lengths [Å] and angles [°] for twin5.

| C(1A)-O(9A)     | 1.45(2) |
| C(1A)-C(2A)     | 1.47(3) |
C(1A)-C(8A) 1.60(2)
C(1A)-H(1A) 1.0000
C(2A)-N(3A) 1.48(2)
C(2A)-C(17A) 1.59(3)
C(2A)-H(2A) 1.0000
N(3A)-C(4A) 1.32(2)
N(3A)-C(7A) 1.46(2)
C(4A)-O(4A) 1.24(2)
C(4A)-C(5A) 1.60(2)
C(5A)-O(5A) 1.42(2)
C(5A)-C(6A) 1.52(3)
C(5A)-H(5A1) 1.0000
O(5A)-H(5A) 0.8400
C(6A)-C(7A) 1.55(3)
C(6A)-H(6A1) 0.9900
C(6A)-H(6A2) 0.9900
C(7A)-C(8A) 1.51(3)
C(7A)-H(7A) 1.0000
C(8A)-O(26A) 1.41(2)
C(8A)-H(8A) 1.0000
O(9A)-C(10A) 1.469(19)
C(10A)-C(11A) 1.55(3)
C(10A)-H(10A) 0.9900
C(10A)-H(10B) 0.9900
C(11A)-C(12A) 1.39(3)
C(11A)-C(16A) 1.44(3)
C(12A)-C(13A) 1.42(3)
C(12A)-H(12A) 0.9500
C(13A)-C(14A) 1.42(3)
C(13A)-H(13A) 0.9500
C(14A)-C(15A) 1.36(3)
C(14A)-H(14A) 0.9500
C(15A)-C(16A) 1.45(3)
C(15A)-H(15A) 0.9500
C(16A)-H(16A) 0.9500
C(17A)-O(18A) 1.44(2)
C(17A)-H(17C) 0.9900
C(17A)-H(17D)  0.9900
O(18A)-C(19A)  1.48(2)
C(19A)-C(20A)  1.55(3)
C(19A)-H(19C)  0.9900
C(19A)-H(19D)  0.9900
C(20A)-C(21A)  1.36(3)
C(20A)-C(25A)  1.43(3)
C(21A)-C(22A)  1.41(3)
C(21A)-H(21A)  0.9500
C(22A)-C(23A)  1.39(3)
C(22A)-H(22A)  0.9500
C(23A)-C(24A)  1.38(3)
C(23A)-H(23A)  0.9500
C(24A)-C(25A)  1.37(2)
C(24A)-H(24A)  0.9500
C(25A)-H(25A)  0.9500
O(26A)-C(27A)  1.38(2)
C(27A)-O(28A)  1.42(3)
C(27A)-H(27C)  0.9900
C(27A)-H(27D)  0.9900
O(28A)-C(29A)  1.44(2)
C(29A)-H(29D)  0.9800
C(29A)-H(29E)  0.9800
C(29A)-H(29F)  0.9800
C(1B)-O(9B)  1.47(2)
C(1B)-C(2B)  1.54(3)
C(1B)-C(8B)  1.60(3)
C(1B)-H(1B)  1.0000
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C(2B)-C(17B)  1.52(3)
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N(3B)-C(7B)  1.48(2)
C(4B)-O(4B)  1.29(2)
C(4B)-C(5B)  1.488(16)
C(5B)-O(5B)  1.44(2)
C(5B)-C(6B)  1.55(3)
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C(12B)-C(11B)-C(16B)  119(2)
C(12B)-C(11B)-C(10B)  124.0(17)
C(16B)-C(11B)-C(10B)  116.7(18)
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C(20B)-C(19B)-H(19A)  109.9
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C(22B)-C(23B)-C(24B)  120.0
C(22B)-C(23B)-H(23B)  120.0
C(24B)-C(23B)-H(23B)  120.0
C(25B)-C(24B)-C(23B)  120.0
C(25B)-C(24B)-H(24B)  120.0
C(23B)-C(24B)-H(24B)  120.0
C(24B)-C(25B)-C(20B)  120.0
C(24B)-C(25B)-H(25B)  120.0
C(20B)-C(25B)-H(25B)  120.0
C(8B)-O(26B)-C(27B)  114.1(15)
O(28B)-C(27B)-O(26B)  114.6(15)
O(28B)-C(27B)-H(27A)  108.6
O(26B)-C(27B)-H(27A)  108.6
O(28B)-C(27B)-H(27B)  108.6
O(26B)-C(27B)-H(27B)  108.6
H(27A)-C(27B)-H(27B)  107.6
C(27B)-O(28B)-C(29B)  108.8(15)
O(28B)-C(29B)-H(29A)  109.5
O(28B)-C(29B)-H(29B)  109.5
H(29A)-C(29B)-H(29B)  109.5
O(28B)-C(29B)-H(29C)  109.5
H(29A)-C(29B)-H(29C)  109.5
H(29B)-C(29B)-H(29C)  109.5

Symmetry transformations used to generate equivalent atoms:

Table 3. Torsion angles [°] for twin5.

<table>
<thead>
<tr>
<th>Bond/Rotation</th>
<th>Torsion Angle [°]</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(9A)-C(1A)-C(2A)-N(3A)</td>
<td>-128.5(15)</td>
</tr>
<tr>
<td>C(8A)-C(1A)-C(2A)-N(3A)</td>
<td>-9.7(19)</td>
</tr>
<tr>
<td>O(9A)-C(1A)-C(2A)-C(17A)</td>
<td>108(2)</td>
</tr>
<tr>
<td>C(8A)-C(1A)-C(2A)-C(17A)</td>
<td>-133.3(16)</td>
</tr>
<tr>
<td>C(1A)-C(2A)-N(3A)-C(4A)</td>
<td>154(2)</td>
</tr>
<tr>
<td>C(17A)-C(2A)-N(3A)-C(4A)</td>
<td>-75(2)</td>
</tr>
<tr>
<td>C(1A)-C(2A)-N(3A)-C(7A)</td>
<td>-10(2)</td>
</tr>
<tr>
<td>C(17A)-C(2A)-N(3A)-C(7A)</td>
<td>121.4(17)</td>
</tr>
<tr>
<td>C(7A)-N(3A)-C(4A)-O(4A)</td>
<td>176(2)</td>
</tr>
<tr>
<td>C(2A)-N(3A)-C(4A)-O(4A)</td>
<td>13(4)</td>
</tr>
<tr>
<td>C(7A)-N(3A)-C(4A)-C(5A)</td>
<td>-6(2)</td>
</tr>
<tr>
<td>C(2A)-N(3A)-C(4A)-C(5A)</td>
<td>-169.6(17)</td>
</tr>
<tr>
<td>O(4A)-C(4A)-C(5A)-O(5A)</td>
<td>-38(3)</td>
</tr>
<tr>
<td>N(3A)-C(4A)-C(5A)-O(5A)</td>
<td>144.9(16)</td>
</tr>
</tbody>
</table>
O(4A)-C(4A)-C(5A)-C(6A)  -160(2)
N(3A)-C(4A)-C(5A)-C(6A)  23(2)
O(5A)-C(5A)-C(6A)-C(7A)  -148.1(15)
C(4A)-C(5A)-C(6A)-C(7A)  -28.9(19)
C(4A)-N(3A)-C(7A)-C(8A)  -140.1(17)
C(2A)-N(3A)-C(7A)-C(8A)  27(2)
C(4A)-N(3A)-C(7A)-C(6A)  -12(2)
C(2A)-N(3A)-C(7A)-C(6A)  154.6(14)
C(5A)-C(6A)-C(7A)-N(3A)  25.8(19)
C(5A)-C(6A)-C(7A)-C(8A)  143.2(17)
N(3A)-C(7A)-C(8A)-O(26A) -153.6(15)
C(6A)-C(7A)-C(8A)-O(26A)  90(2)
N(3A)-C(7A)-C(8A)-C(1A)  -30.3(19)
C(6A)-C(7A)-C(8A)-C(1A)  -146.4(17)
O(9A)-C(1A)-C(8A)-O(26A) -88.1(18)
C(2A)-C(1A)-C(8A)-O(26A)  145.7(16)
O(9A)-C(1A)-C(8A)-C(7A)  151.0(15)
C(2A)-C(1A)-C(8A)-C(7A)  24.8(19)
C(2A)-C(1A)-O(9A)-C(10A) -78(2)
C(8A)-C(1A)-O(9A)-C(10A)  162.6(14)
C(1A)-O(9A)-C(10A)-C(11A)  179.3(15)
O(9A)-C(10A)-C(11A)-C(12A)  10(3)
O(9A)-C(10A)-C(11A)-C(16A) -166.4(16)
C(16A)-C(11A)-C(12A)-C(13A)  0(3)
C(10A)-C(11A)-C(12A)-C(13A)  -176.3(19)
C(11A)-C(12A)-C(13A)-C(14A)  -2(3)
C(12A)-C(13A)-C(14A)-C(15A)  0(3)
C(13A)-C(14A)-C(15A)-C(16A)  3(4)
C(12A)-C(11A)-C(16A)-C(15A)  4(3)
C(10A)-C(11A)-C(16A)-C(15A)  180.0(17)
C(14A)-C(15A)-C(16A)-C(11A)  -5(3)
C(1A)-C(2A)-C(17A)-O(18A)  -60(2)
N(3A)-C(2A)-C(17A)-O(18A)  177.0(14)
C(2A)-C(17A)-O(18A)-C(19A)  170.9(15)
C(17A)-O(18A)-C(19A)-C(20A)  173.8(15)
O(18A)-C(19A)-C(20A)-C(21A)  77(3)
O(18A)-C(19A)-C(20A)-C(25A)  -108(2)
C(25A)-C(20A)-C(21A)-C(22A) 7(3)
C(19A)-C(20A)-C(21A)-C(22A) -178(2)
C(20A)-C(21A)-C(22A)-C(23A) -7(3)
C(21A)-C(22A)-C(23A)-C(24A) 5(4)
C(22A)-C(23A)-C(24A)-C(25A) -3(4)
C(23A)-C(24A)-C(25A)-C(20A) 3(3)
C(21A)-C(20A)-C(25A)-C(24A) -4(3)
C(19A)-C(20A)-C(25A)-C(24A) 180.0(18)
C(7A)-C(8A)-O(26A)-C(27A) -154.7(16)
C(1A)-C(8A)-O(26A)-C(27A) 90(2)
C(8A)-O(26A)-C(27A)-O(28A) 65(2)
O(26A)-C(27A)-O(28A)-C(29A) 69(2)
O(9B)-C(1B)-C(2B)-N(3B) -129.7(15)
C(8B)-C(1B)-C(2B)-N(3B) -13.5(18)
O(9B)-C(1B)-C(2B)-C(17B) 110.3(19)
C(8B)-C(1B)-C(2B)-C(17B) -133.5(18)
C(17B)-C(2B)-N(3B)-C(4B) -79(3)
C(1B)-C(2B)-N(3B)-C(4B) 155.1(19)
C(17B)-C(2B)-N(3B)-C(7B) 118.9(18)
C(1B)-C(2B)-N(3B)-C(7B) -7.3(19)
C(7B)-N(3B)-C(4B)-O(4B) 175.7(18)
C(2B)-N(3B)-C(4B)-O(4B) 13(3)
C(7B)-N(3B)-C(4B)-C(5B) -8(2)
C(2B)-N(3B)-C(4B)-C(5B) -170.2(17)
O(4B)-C(4B)-C(5B)-O(5B) -41(3)
N(3B)-C(4B)-C(5B)-O(5B) 142.9(16)
O(4B)-C(4B)-C(5B)-C(6B) -160(2)
N(3B)-C(4B)-C(5B)-C(6B) 24(2)
O(5B)-C(5B)-C(6B)-C(7B) -150.8(14)
C(4B)-C(5B)-C(6B)-C(7B) -29.3(18)
C(4B)-N(3B)-C(7B)-C(8B) -139.2(16)
C(2B)-N(3B)-C(7B)-C(8B) 26(2)
C(4B)-N(3B)-C(7B)-C(6B) -11(2)
C(2B)-N(3B)-C(7B)-C(6B) 154.3(15)
C(5B)-C(6B)-C(7B)-N(3B) 24.6(18)
C(5B)-C(6B)-C(7B)-C(8B) 141.1(18)
N(3B)-C(7B)-C(8B)-O(26B) -151.3(14)
C(6B)-C(7B)-C(8B)-O(26B) 93(2)
N(3B)-C(7B)-C(8B)-C(1B) -32.3(18)
C(6B)-C(7B)-C(8B)-C(1B) -147.8(17)
O(9B)-C(1B)-C(8B)-O(26B) -91.7(18)
C(2B)-C(1B)-C(8B)-O(26B) 146.1(16)
O(9B)-C(1B)-C(8B)-C(7B) 151.1(15)
C(2B)-C(1B)-C(8B)-C(7B) 29.0(19)
C(2B)-C(1B)-O(9B)-C(10B) -77.3(19)
C(8B)-C(1B)-O(9B)-C(10B) 165.1(14)
C(1B)-O(9B)-C(10B)-C(11B) -179.9(16)
O(9B)-C(10B)-C(11B)-C(12B) 9(3)
O(9B)-C(10B)-C(11B)-C(16B) -169.7(15)
C(16B)-C(11B)-C(12B)-C(13B) 0(3)
C(10B)-C(11B)-C(12B)-C(13B) -179.4(19)
C(11B)-C(12B)-C(13B)-C(14B) 4(3)
C(12B)-C(13B)-C(14B)-C(15B) -6(3)
C(13B)-C(14B)-C(15B)-C(16B) 4(3)
C(14B)-C(15B)-C(16B)-C(11B) -1(3)
C(12B)-C(11B)-C(16B)-C(15B) -1(3)
C(10B)-C(11B)-C(16B)-C(15B) 177.7(19)
N(3B)-C(2B)-C(17B)-O(18B) 177.7(14)
C(1B)-C(2B)-C(17B)-O(18B) -66(2)
C(2B)-C(17B)-O(18B)-C(19B) 175.1(18)
C(17B)-O(18B)-C(19B)-C(20B) 177.2(15)
O(18B)-C(19B)-C(20B)-C(21B) 69.9(18)
O(18B)-C(19B)-C(20B)-C(25B) -107.7(15)
C(25B)-C(20B)-C(21B)-C(22B) 0.0
C(19B)-C(20B)-C(21B)-C(22B) -177.5(13)
C(20B)-C(21B)-C(22B)-C(23B) 0.0
C(21B)-C(22B)-C(23B)-C(24B) 0.0
C(22B)-C(23B)-C(24B)-C(25B) 0.0
C(23B)-C(24B)-C(25B)-C(20B) 0.0
C(21B)-C(20B)-C(25B)-C(24B) 0.0
C(19B)-C(20B)-C(25B)-C(24B) 177.6(13)
C(7B)-C(8B)-O(26B)-C(27B) -155.4(15)
C(1B)-C(8B)-O(26B)-C(27B) 92.0(19)
C(8B)-O(26B)-C(27B)-O(28B) 65.6(19)
O(26B)-C(27B)-O(28B)-C(29B)  67(2)

Symmetry transformations used to generate equivalent atoms:

Table 4. Hydrogen bonds for twin5 [Å and °].

<table>
<thead>
<tr>
<th>D-H...A</th>
<th>d(D-H)</th>
<th>d(H...A)</th>
<th>d(D...A)</th>
<th>&lt;(DHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(5A)-H(5A)...O(4B)#1</td>
<td>0.84</td>
<td>1.94</td>
<td>2.73(2)</td>
<td>157.1</td>
</tr>
<tr>
<td>O(5B)-H(5X)...O(4A)#2</td>
<td>0.84</td>
<td>1.95</td>
<td>2.786(19)</td>
<td>170.3</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms:
#1 x+1,y,z   #2 x-1,y,z
4.4 Appendix D: NOESY interactions for compound 310
4.5 Appendix E: Training record

Conferences attended


SCI postgraduate symposium – Speaker March 2009.

ACS National Meeting and Exposition – Speaker and poster presentation August 2009.

Courses Attended

Vitae UK Grad School – Bournemouth 2009

Educational Development Courses

Research writing
Communication skills for researchers
Introduction to presenting research
Project management for research students
How to be an effective researcher
Presenting your thesis
Viva preparation 1 and 2
LEADS 1

Undergraduate lecture courses

Advanced synthesis with pharmaceutical applications

Postgraduate courses

Advanced organic synthesis
Optics for chemists
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