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 A_1 and hyacinthacine B_2 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 A_1 and B_2, where the stereochemistry at C-1 and C-2 is *cis*.

Hyacinthacine B_2
R=CH_2OH
Hyacinthacine A_1
R=H
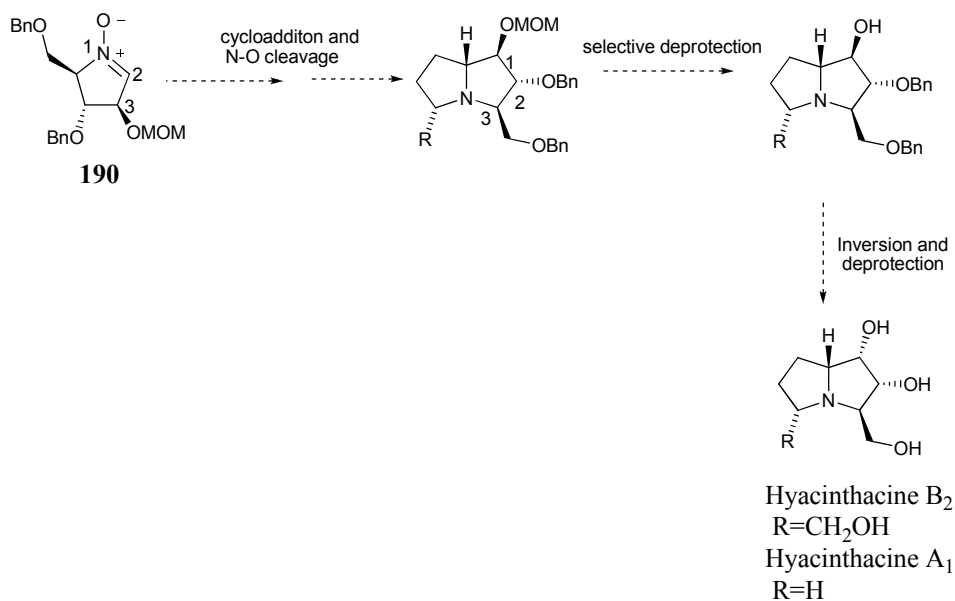
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Ac	acetyl
AIBN	Azobisisobutyronitrile
AIDS	Acquired Immune Deficiency Syndrome
Bn	benzyl
Bu	butyl
Boc	tert-Butyloxycarbonyl
Cbz	Carboxybenzyl
COSY	correlated spectroscopy
d	doublet
DCM	dichloromethane
dd	double doublet
ddd	double double doublet
DEAD	diethyl azodicarboxylate
DIBAL	diisobutylaluminium hydride
DMJ	deoxymannojirimycin
DMSO	dimethyl sulfoxide
DNJ	deoxynojirimycin
dt	doublet of triplets
ES	electrospray
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
h	hour
HIV	Human Immunodeficiency Virus
HOMO	highest occupied molecular orbital
*i*Pr	*iso*-propyl
IR	infrared
LUMO	lowest occupied molecular orbital
m	multiplet
Me	methyl
MeOH	methanol
min	minute(s)

mmol	milimole
MOM	methoxymethayl
m.p.	melting point
Ms	methanesulfonyl
MS	mass spectrometry
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
Ph	phenyl
Py	pyridine
q	quartet
RCM	ring closing metathesis
R_f	retention factor
rt	room temperature
s	singlet
t	triplet
TBAF	tetrabutylammonium fluoride
TBAP	Tetrabutylammonium perchlorate
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butlydiphenylsilyl
THF	tetrahydrofuran
TMS	trimethyl silyl
t.l.c	thin layer chromatography
TPAP	Tetrapropylammonium perruthenate

1   INTRODUCTION

1.1 Iminosugars

Iminosugars have the potential to block or modifiy 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.

1.2.1 Monocyclic structures

There are two classes of monocyclic iminosugars, piperidines and pyrrolidines, with the general structures 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.[3] As a hemiacetal, NJ is an inherently unstable structure and therefore its corresponding 1-deoxy analogue, 1-deoxynojirimycin (DNJ; **2**) [figure2], was synthesised by reduction with NaBH₄ by Paulsen and coworkers.[4] DNJ was later isolated from the roots of mulberry leaves and was called moranoline.[5] 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 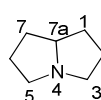C-2 has the opposite stereochemistry, mimicking the pyranose form of D-mannose. DMJ was found to be produced by *Streptomyces lavendulae* SF-425 and has recently been extracted from *Adenophora triphylla*.[5] A number of other piperidine iminosugars have since been isolated from a range of plants and bacterial cultures and these are shown in Figure 2.[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*)[6] as well as the Moreton Bay Chestnut and *Castanospermum australe*.[7] 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*.[8] Fagomine (**6**) and 6-deoxyfagomine (**9**) have been isolated from the roots of *Lycium Chinese* (Solanaceae)[9] and 2*S*-carboxy-3*R*,4*R*,5*S*-trihydroxypiperidine (**10**) was isolated from the seeds of the legume *Baphhia racemosa*.[10] The first naturally occurring DNJ derivative with a carbon substituent at C-1, α-homonojirimycin (α-HNJ, **11**), was isolated from *O. diandra*[11] and more recently has been isolated from the bulbs of *Hyacinthus orientalis* (Hyacinthaceae).[12] 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*[13] including two new compounds, α-7-deoxyhomonojiromycin (**17**) and α-7-deoxyhomomannojiromycin (**18**).
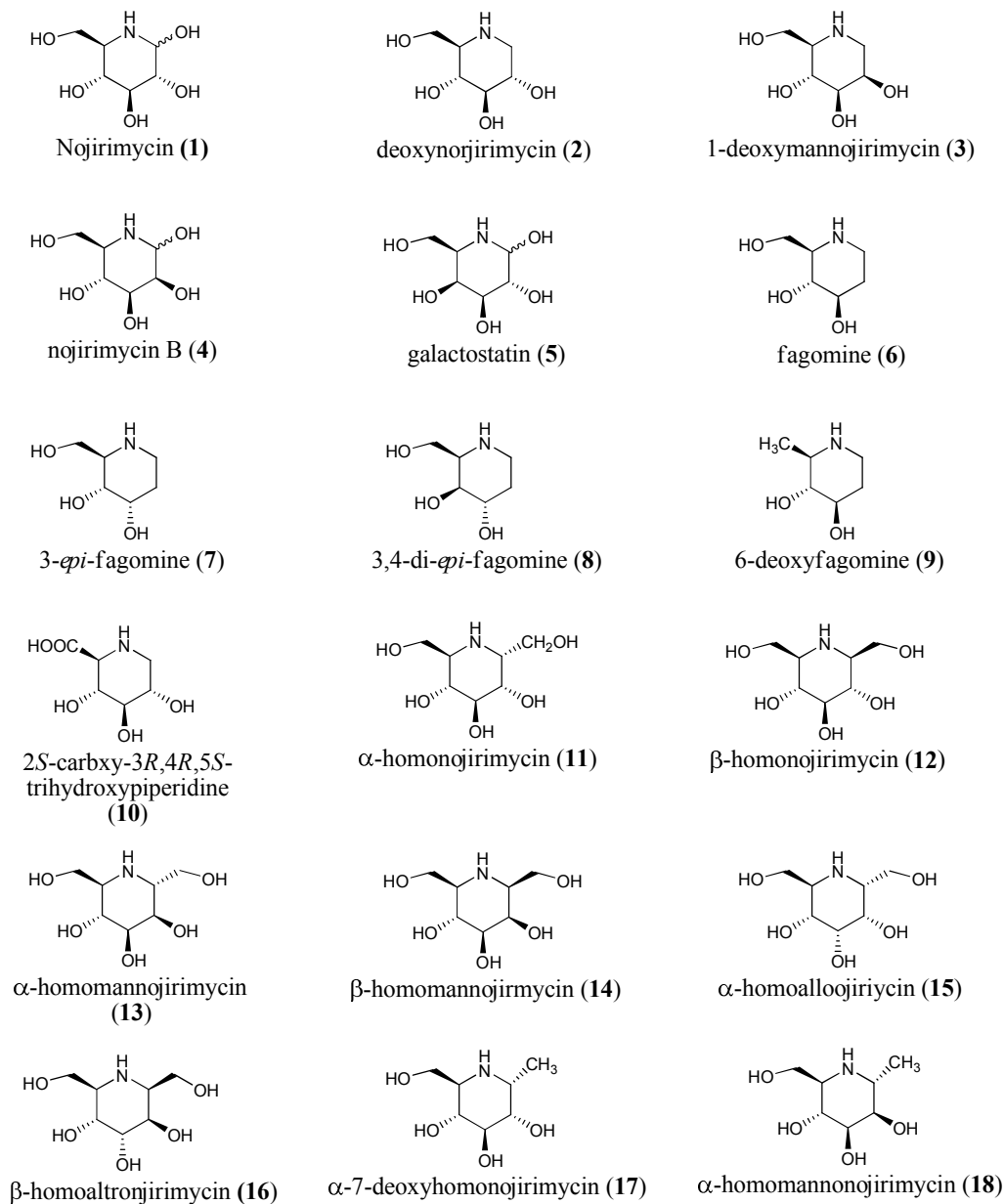
Figure 2. Naturally occurring piperidines

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*

elliptica.[14, 15] This compound is a very common metabolite as it has since been isolated from various other plants and microorganisms.[16] 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 *Angylocalyx boutiqueanus* but, like DMDP, has since been isolated from different species of plants.[17, 18]
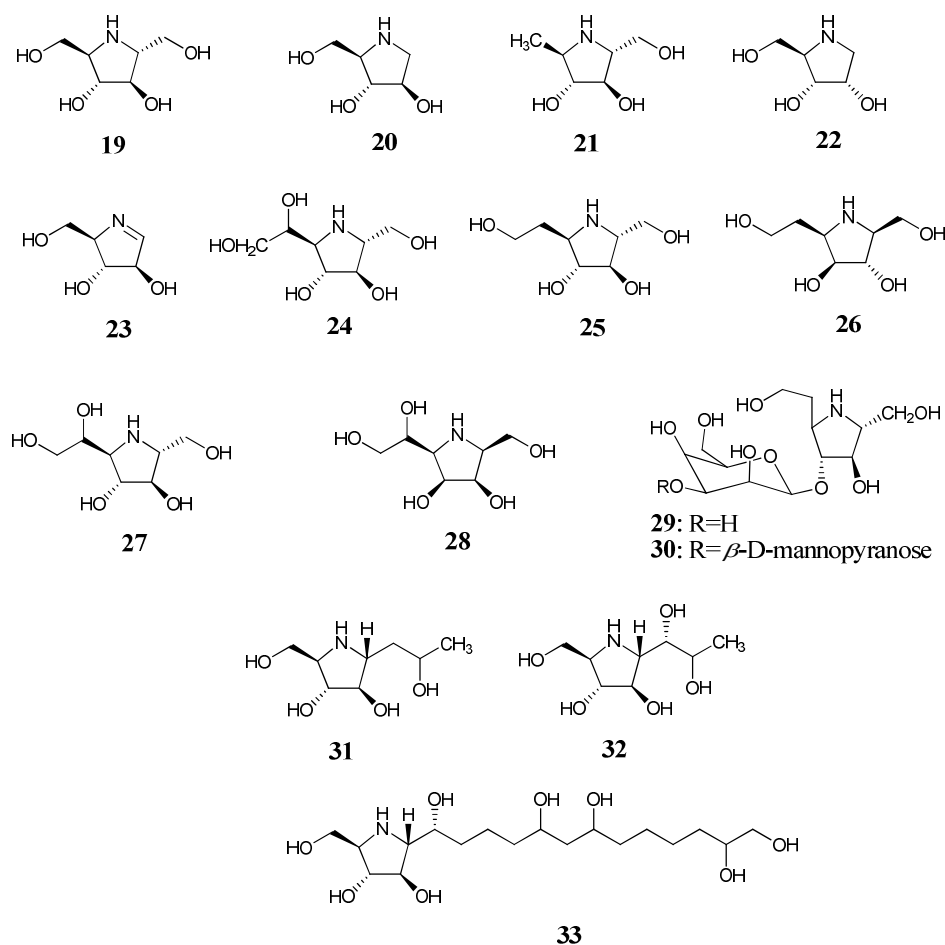
Figure 3. Naturally occurring pyrrolidine iminosugars

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 *Anglocalyx pynaertii*.[19] 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*.[2] 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*).[20] More recently, homoDMDP has been

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found in the bulbs of hyacinths (*Hyacinthus orientalis*) along with 2,5-imino-2,5,6-trideoxy-D-*manno*-heptitol (6-deoxy-homoDMDP, **25**) and 2,5-imino-2,5,6-trideoxy-D-*gulo*-heptitol (**26**).[12] In 2002, homo-DMDP (**24**), 6-deoxy-homo-DMDP (**25**) and 2,5-imino-2,5,6-trideoxy-D-*gulo*-heptitol (**26**) were isolated from *Scilla sibirica*[13] along with four new pyrrolidines (**27-30**). These new pyrrolidines were determined to be 7-deoxy-homoDMDP (2,5-imino-2,5,7-trideox-*glycero*-D-*manno*-heptitol **27**), 2,5-dideoxy-2,5-imino-*glycero*-D-*galacto*-heptitol (**28**), 4-*O*-β-D-mannopyranosyl-6-deoxy-homoDMDP (**29**), and 4-*O*-[β-D-mannopyranosyl-(1→4)-β-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 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-1α position and were therefore identified as α-1-*C*-(1,5,7,12,13-pentahydroxytridecyl)-D-AB1 (**31**), α-1-*C*-(1,2-dihydroxypropyl)-D-AB1 (**32**) and α-1-*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].[22] 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*.[23] 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*-canstanospermine (**36**), 7-deoxy-6-*epi*-castanospermine (**37**) and 6,7-di-*epi*-castanospermine (**38**) the structures of which are shown in Figure 4.*[23]* The leaves of *Astragalus Lentiginous* produce lentiginosine (**39**) and 2-*epi*-lentiginosine (**40**).[24]
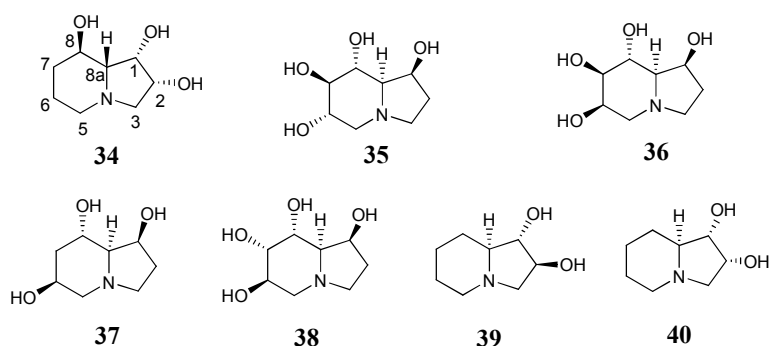
Figure 4. Naturally occurring indolizidine iminosugars

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*.[25] 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.[2] 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).[26]   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*.[27]
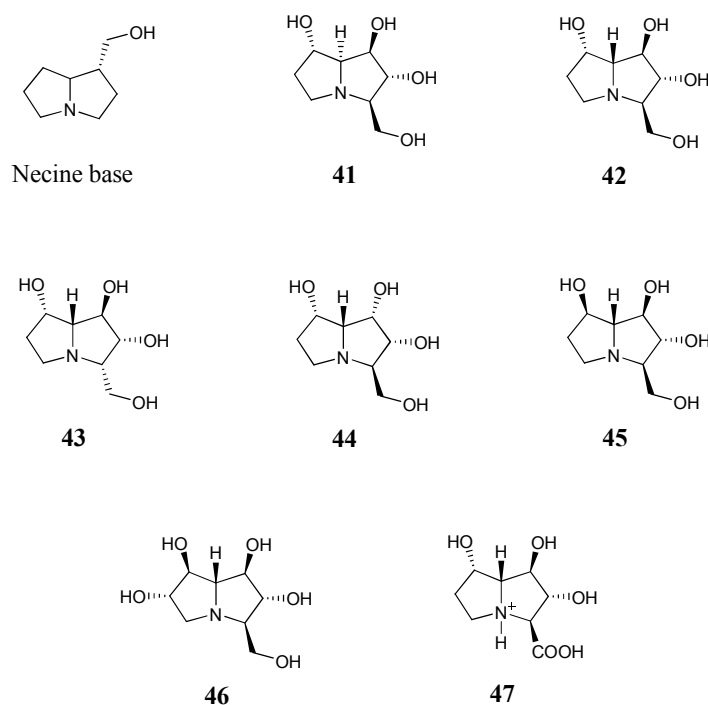
Figure 5. Examples of polyhydroxylated pyrrolizidine alkaloids isolated from *Castanospermum australe* and other sources and structural comparison with necine

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.*[28]   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**).[29]   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**).[13] Four new pyrrolizidines, with longer side chains at the C-5 position, were isolated from *Scillia peruviana* in 2004.[21] These were, α-5-*C*-(3-hydroxbutyl)-7-*epi*-australine (**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_7 (**66**), C_2 (**67**), C_3 (**68**), C_4 (**69**), C_5 (**70**), and α-5-*C*-(3-hydroxybutyl)hyacinthacine A_2 (**71**).[30] The known hyacinthacines A_3 (**53**), A_5 (**56**), B_3 (**54**), and B_4 (**59**) were also found in *Scillia solcialis*.
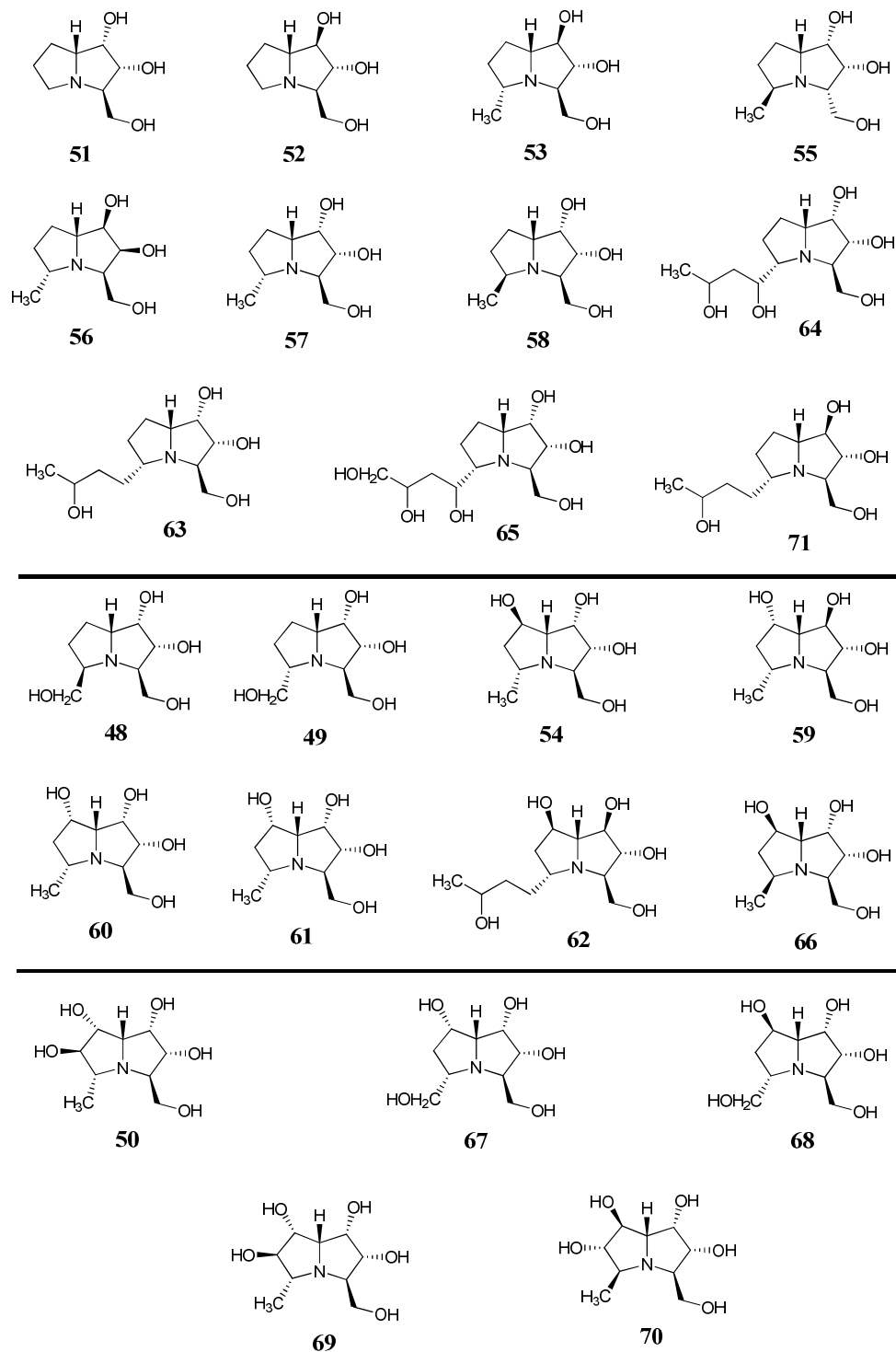
Figure 6. Hyacinthacine family of polyhydroxylated pyrrolizidines arranged according to hyacinthacine letter grouping; A, B or C.

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

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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.[31] A shown in Figure 7, Calystegines have a nortropane ring system, two to four hydroxyl functions of varying stereochemistrics and position and a tertiary alcohol group at the bridgehead carbon C-1 of the bicyclic ring. The first calystegines, A_3 (**72**), B_1 (**73**) and B_2 (**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.[31, 32] Examination of *M. bombycis* and *M. alba* both of the Moraceae family resulted in the discovery of calystegines B_2 and C_1 (**75**).[33,34] Later, calystegines A_3, A_5 (**76**), B_1, B_2, and B_3 (**77**) were isolated from the roots of *Physalis alkekengi* var. *francheti*.[35] Calystegines A_3, B_1, and B_2 have now been found in the Solanaceae plant family genera *Atropa*, *Datura*, *Duboisia*, *Hyoscyamus* and *Scopolia*.[36] Low amounts of calystegines were detected in *Datura wrightii leaves*[37] and *D. stramonium* roots and leaves.[38] Calystegines A_5, A_6 (**78**), B_1, B_2, B_3, and N_1 (**79**) were subsequently isolated from *Hyoscyamus niger*.[39] Calystegines A_3, A_5, B_1, B_2, B_3, B_4 (**80**), C_1 and calystegines B_1, B_2, B_4, C_1, and C_2 (**81**) were found in *Scopolia japonica*[40] and in *Duboisia leichhardtii* respectively.[41] More recently, new calystegines, A_8 (**82**) and B_6 (**83**), were isolated from *H. niger* and *S. Japonica* and it was discovered that *Lycium chinense* produced *N*-methyl-calystegines B_2 (**84**) and C_1 (**85**), and calystegines A_7 (**86**) and B_5 (**87**).[42] 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.[43]
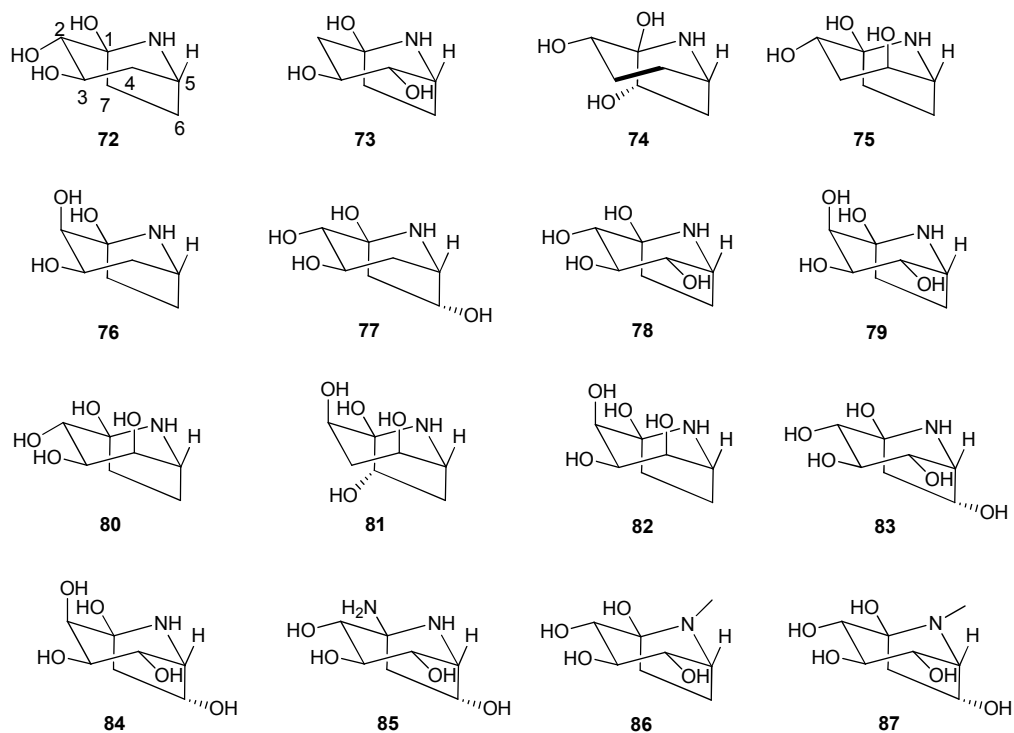
Figure 7: Structures of polyhydroxylated nortropanes.

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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 glycoconujugates (glycoproteins, glycolipids, proteoglycans) in nearly all life forms.[44] The first example involved the first isolated iminosugar NJ (**1**) which was reported to inhibit both α-glucosidases and β-glucosidases.[3] 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.[2, 15] Castanospermine (**35**) has been shown to be a powerful inhibitor of the α- and β-glucosidases in the mammalian gut.[23] Australine was found to be a good inhibitor of the α-glucosidases, amylglucosidase and subsequently the glycoprotein enzyme glucosidase I.[45]

Unsurprisingly, the inhibition of glycosidase and glycosyltransferase enzmes can affect the digestion and metabolism of polysaccharides as well as the maturation, transportation, and secretion of glycoconjugates. Glycocongugates 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.[15] DNJ (**2**) was subsequently found to have a good inhibitory effect on mammalian α-glucosidase *in vitro* and was thus considered to be a promising agent for treatment of diabetes.[46] Unfortunately, the compound lacked efficacy *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.[47] Migitol (GLYSET^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.[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).[49]

89
Isofagomine

6
fagomine

88
Migitol
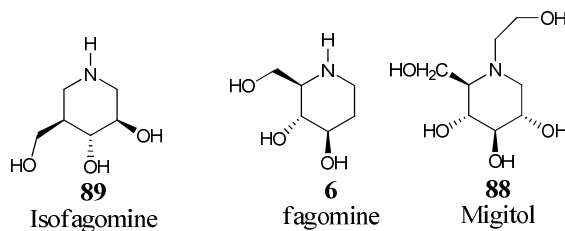
Figure 8: Structure showing specificity of inhibitory action on digestive glucosidase.

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.[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,[51] and *N*-butyl DNJ (**90**) [Figure 9], marketed as Zavesca[®], is now used in the treatment of type 1 Gaucher's disease.
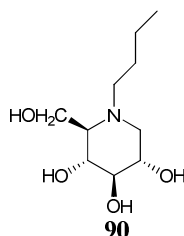
Figure 9: N-butyl DNJ

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 hydrolyses as models for studying the pathogenesis of lysosomal storage disease.[52,53] 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 orfan 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.[23,54] The *N*-hydroxyethyl derivative of DNJ also induces the accumulation of glycogen, as demonstrated in normal human fibroplasts and the polarized HepG2 cells.[2] 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 glycotype 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.
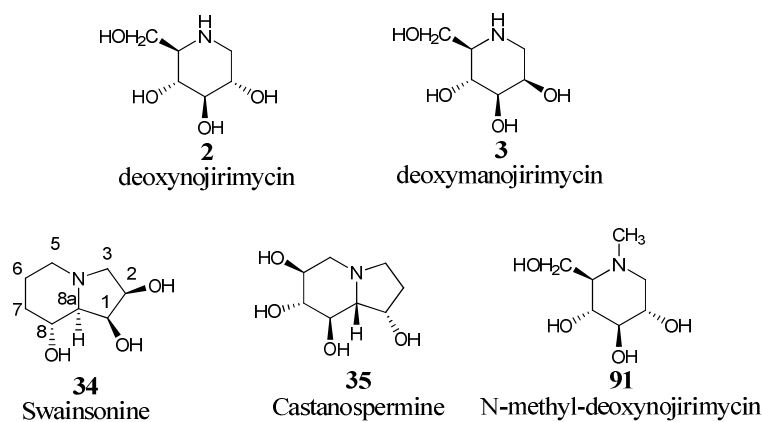
Figure 10

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.[15] 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 *in vitro* than their parent compounds. The effect of the common glycoprotein processing inhibitors on the biosynthesis, intracelluar 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.[57] Gp-120 is generated in the Golgi apparatus together with a transmembrane glycoprotein, gp-41, by endoproteolytic cleavage of a precursor protein,

gp-160.[58] 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.[59] 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**),[60] DMDP (**19**)[61] and swainsonine (**34**)[62] have also been reported to prevent the processing of the influenza viral glycoprotein (hemagglutinin) that is responsible for adsorption of the inflenza virion to host cells. One of the most 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.[63]

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 sp^2 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).[64] 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.[65] 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 glycose with inversion of anomeric configuration (Fig. 11A). Retaining glycosidases convert the glycoside to glycose 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.
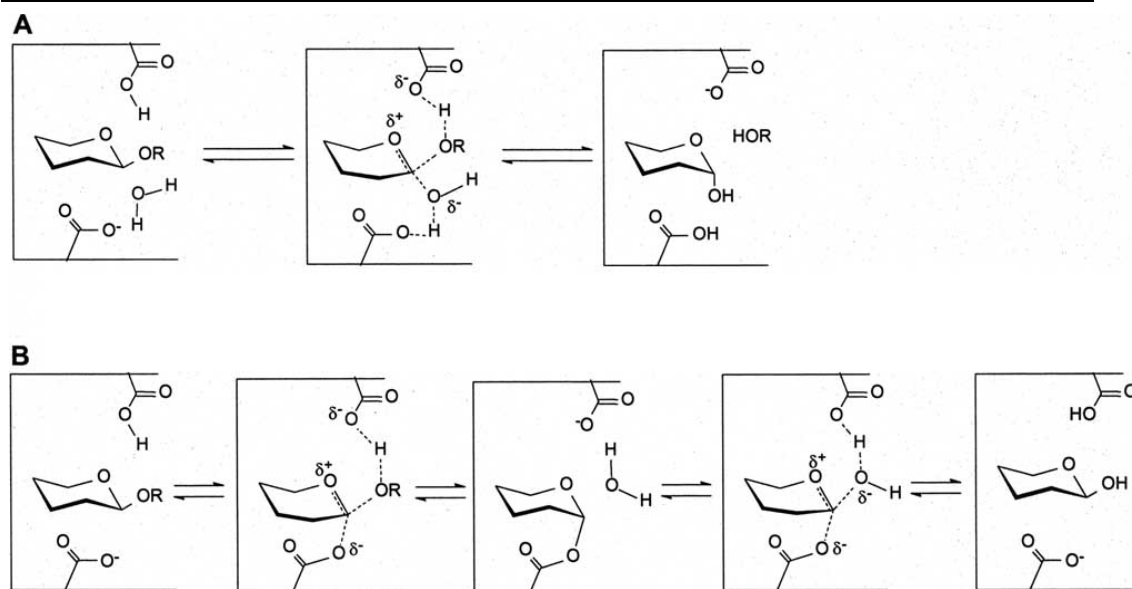
Figure 11: Inverting (A) and retaining (B) glycosidase mechanisms.

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 α-glucosidase and α-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 α-glucosidases and not the expected α-mannosidase.[64] With the exception of DMJ (**3**), α-D-mannosidase inhibitors in reality generally do not have structures that closely resemble mannose. For example, swainsonine (**34**) exhibits potent inhibition of α-mannosidases although its structural resemblance to α-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 α-D-mannose analogues and mannosidase inhibitors.[69] These calculations and studies showed that there are two optimized half chair geometries of the mannosyl cation, a "flap up" form (**93**) and the "flap down" form (**94**), 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-*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.[70]
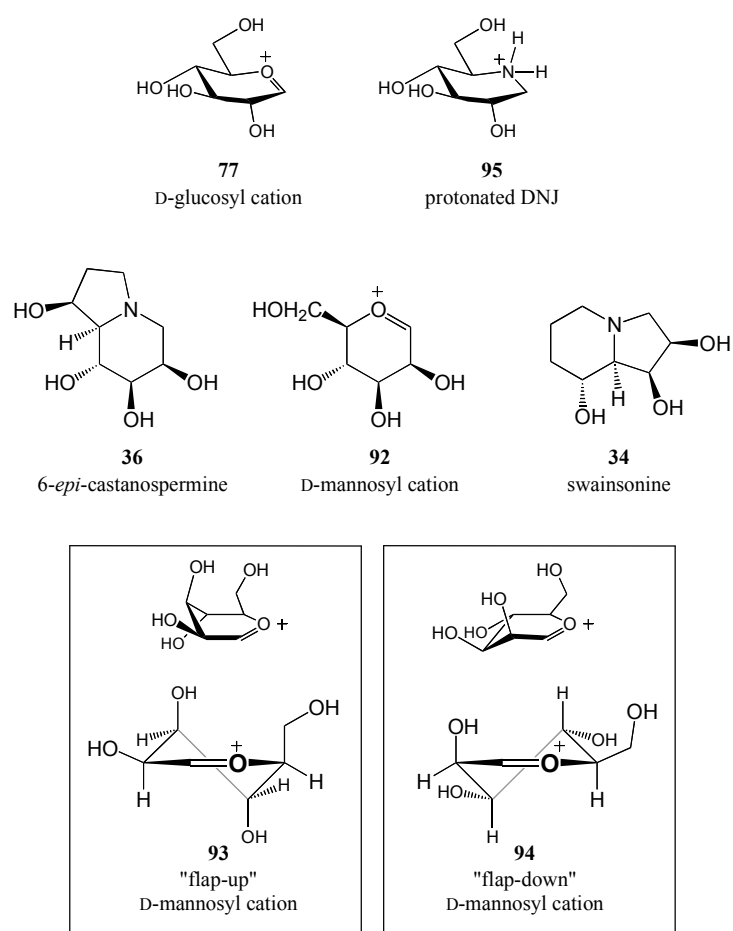

Figure 12: Structural resemblance between selected iminosugars and carbohydrate cations

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 wth improved activity and selectivity.

1.5   Synthetic routes to hyacinthancines

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.
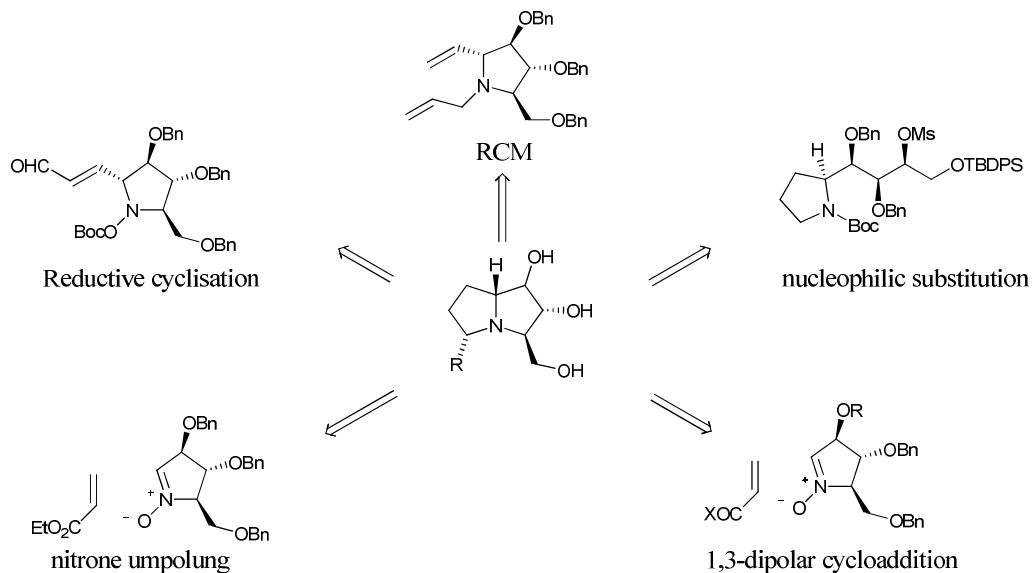
Figure 13 Synthetic strategies for synthesis of hyacinthacines

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 A$_2$ (**97**, Scheme 1) and 5,7a-di*epi*-hyacinthacine A$_3$ (**98**, Scheme 2).[75]
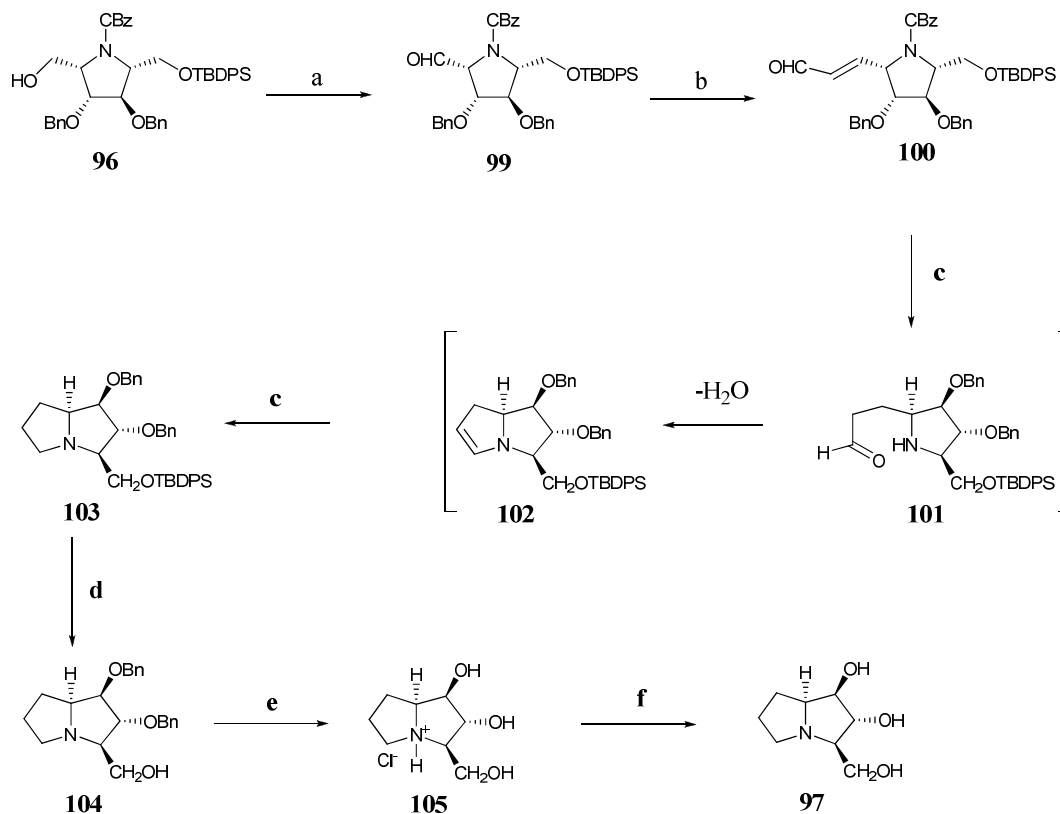
Scheme 1: (a) TPAP,NMO, CH_2Cl_2, 4 Å MS, (95%); (b) $Ph_3P=CHCHO$, CH_2Cl_2, (44%); (c) H_2, 10% Pd/C, (45%); (d) TBAF, (59%); (e) H_2, 10% Pd/C, MeOH, HCl, (93%); (f) MeOH, Amberlite IRA-400 (OH^- form), (76%).

The pyrrolizidine precursor (**96**) was prepared from D-fructose in 11 steps.[76] Oxidation of the primary alcohol group catalysed by TPAP produced aldehyde **99**. Wittig olefination of the aldehyde with (triphenylphosphoranylidine)acetaldehyde 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 A_2 (**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-di*epi*-hyacinthacine A_3 (**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-di*epi*-hyacinthacine A_3 (**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**.
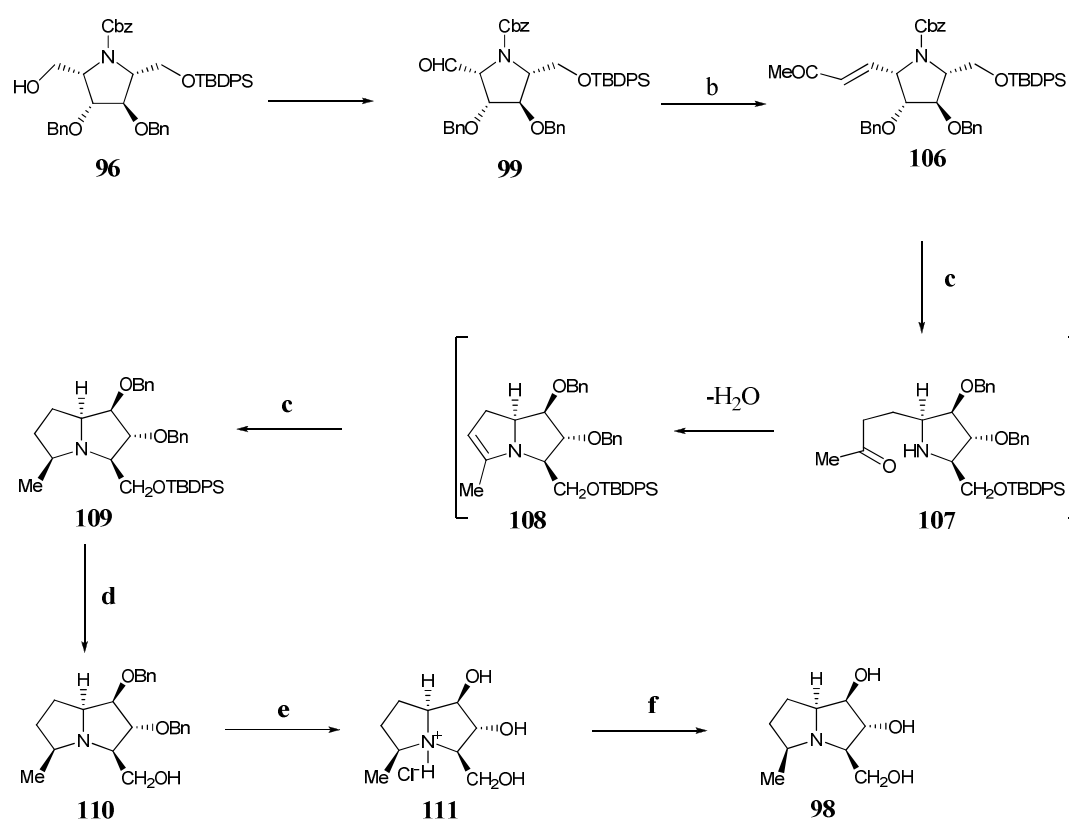
Scheme 2: (a) TPAP, NMO, CH_2Cl_2, 4 Å MS (85%); (b) Ph_3P=$CHCOCH_3$, CH_2Cl_2 (57%); (c) H_2, 10% Pd/C (60% of **109** from **106**); (d) TBAF; (85%) (e) H_2, 10% Pd/C, MeOH, HCl; (f) Amberlite IRA-400 (OH⁻ form) (83% of **98** from **110**).

The Izquierdo group then applied the methodology developed in Schemes 1 and 2 to the synthesis of hyacinthacine A_3 (**53**, Scheme 3)[77] and hyacinthacine A_2 (**52**, Scheme 4).[78] Access to these members of the hyacinthacine 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

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difference in the pyrrolidine precursor (**112**) used for synthesis of hyacinthacines A_3 and A_2 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 A_3 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**. Hydrogenaton from the less hindered β-face of **116** proceeded with very high steroselectivity to complete the first synthesis of hyacinthacine A_3 (**53**).
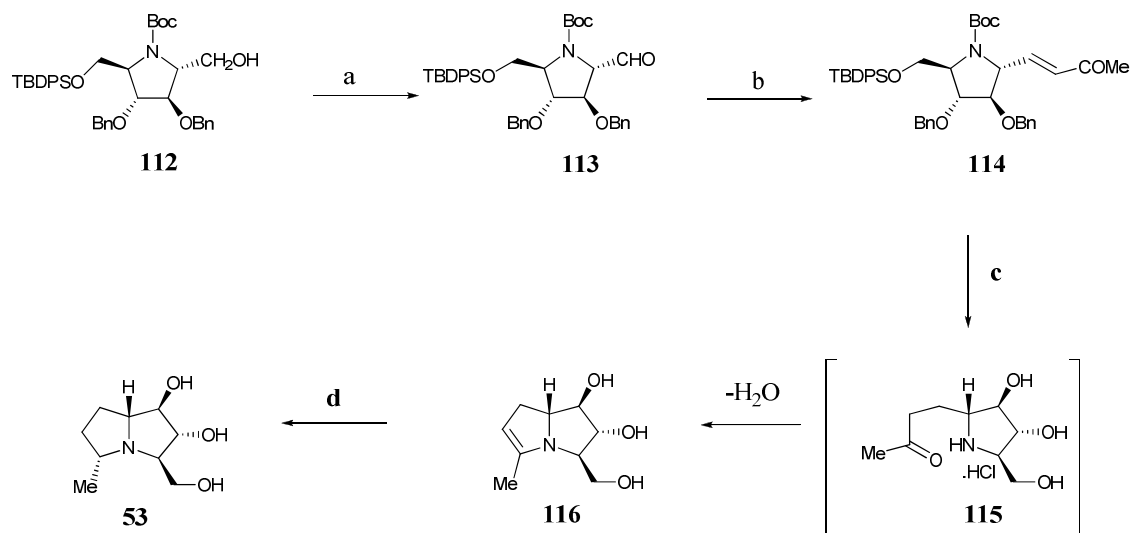
Scheme 3: (a) TPAP, NMO, CH_2Cl_2, 4 Å MS (90%); (b) $Ph_3P{=}CHCOCH_3$, toluene (100%); (c) H_2, 10% Pd/C, MeOH, HCl then Amberlite IRA-400 (OH⁻ form); (d) H_2, 10% Pd/C, MeOH, HCl then Amberlite IRA-400 (OH⁻ form), (68% of **53** from **114**).

Shortly after disclosing the synthesis of hyacinthacine A_3, Izquierdo *et al.* described the application of the DMDP derivative **112** in the synthesis of hyacinthacine A_2 (**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 A_2 (**52**) isolated in the free base form after treatment with basic resin.
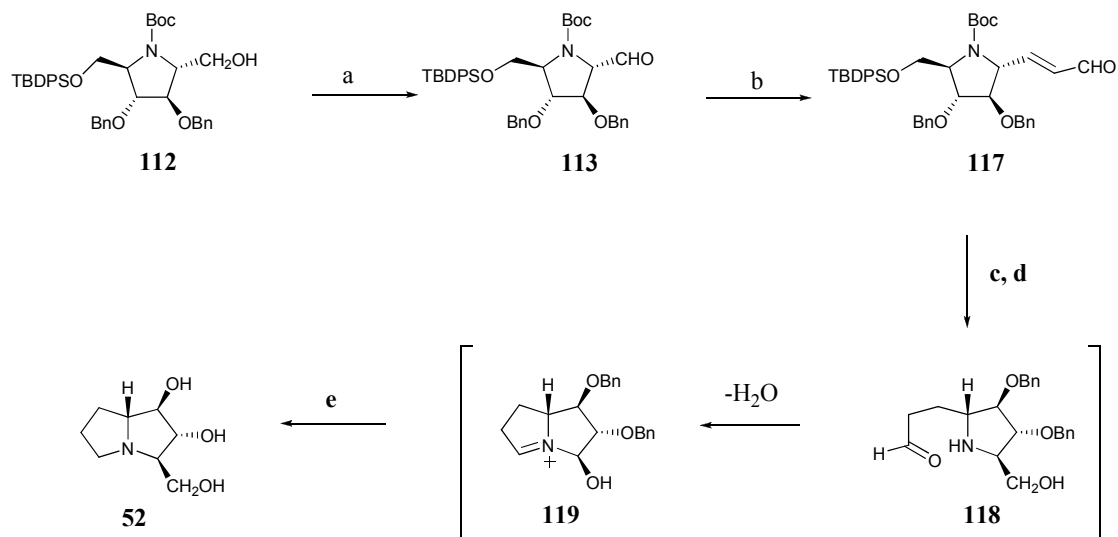
Scheme 4: (a) TPAP, NMO, CH_2Cl_2, 4 Å MS; (b) $Ph_3P=CHCHO$, toluene (57%); (c) H_2, 10% Pd/C; (d) HCl, then Amberlite IRA-400 (OH^- form); (e) H_2, 10% Pd/C, MeOH, HCl, then Amberlite IRA-400 (OH^- form) (26% of **52** from **113**).

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 A_3 (**120**) and 3-*epi*-hyacinthacine A_2 (**121**),[79] and three dihydroxypyrrolizidines (**122**, **123** and **124**) (Figure 14).[80] Synthesis of casuarine (**46**), 6,7-di*epi*-casuarine (**125**),[81] 3-*epi*-hyacinthacine A_5 (**126**) and (+)-3-*epi*-hyacinthacine A_5 (**127**),[82] (+)-5-*epi*-hyacinthacine A_5 (**128**) and ent-3-*epi*-hyacinthacine A_4 (**129**)[83] have also been descibed.
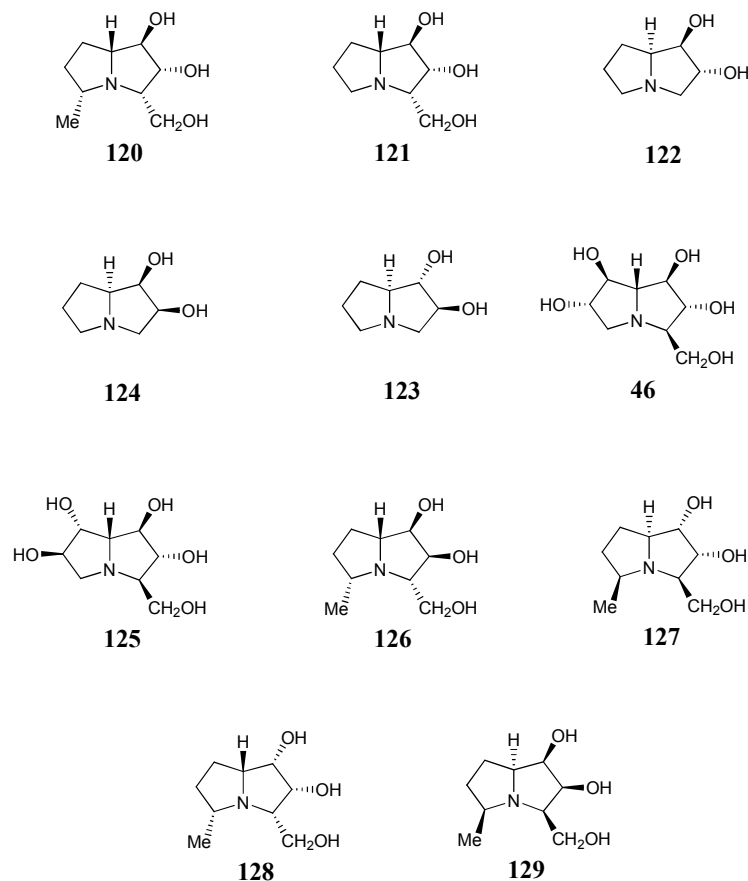
Figure 14. Series of pyrrolizidines synthesized by Izquierdo *et al.*[83]

The earlier routes investigated by the Izquierdo group all targeted polyhydroxylated prrolizidines with *trans* relative stereochemistry across the C-1 and C-2 hydroxlated 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 pyrollidine derivative (**130**) possessing three orthogonal protecting groups synthesised in multiple steps from D-fructose.[84,85] Protection of the free hydroxymethyl side chain by benzoylation followed by desilylation to remove the TBDPS protecting group exposed the required hydroxmethyl 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-

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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 A_1 (**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 givie 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.
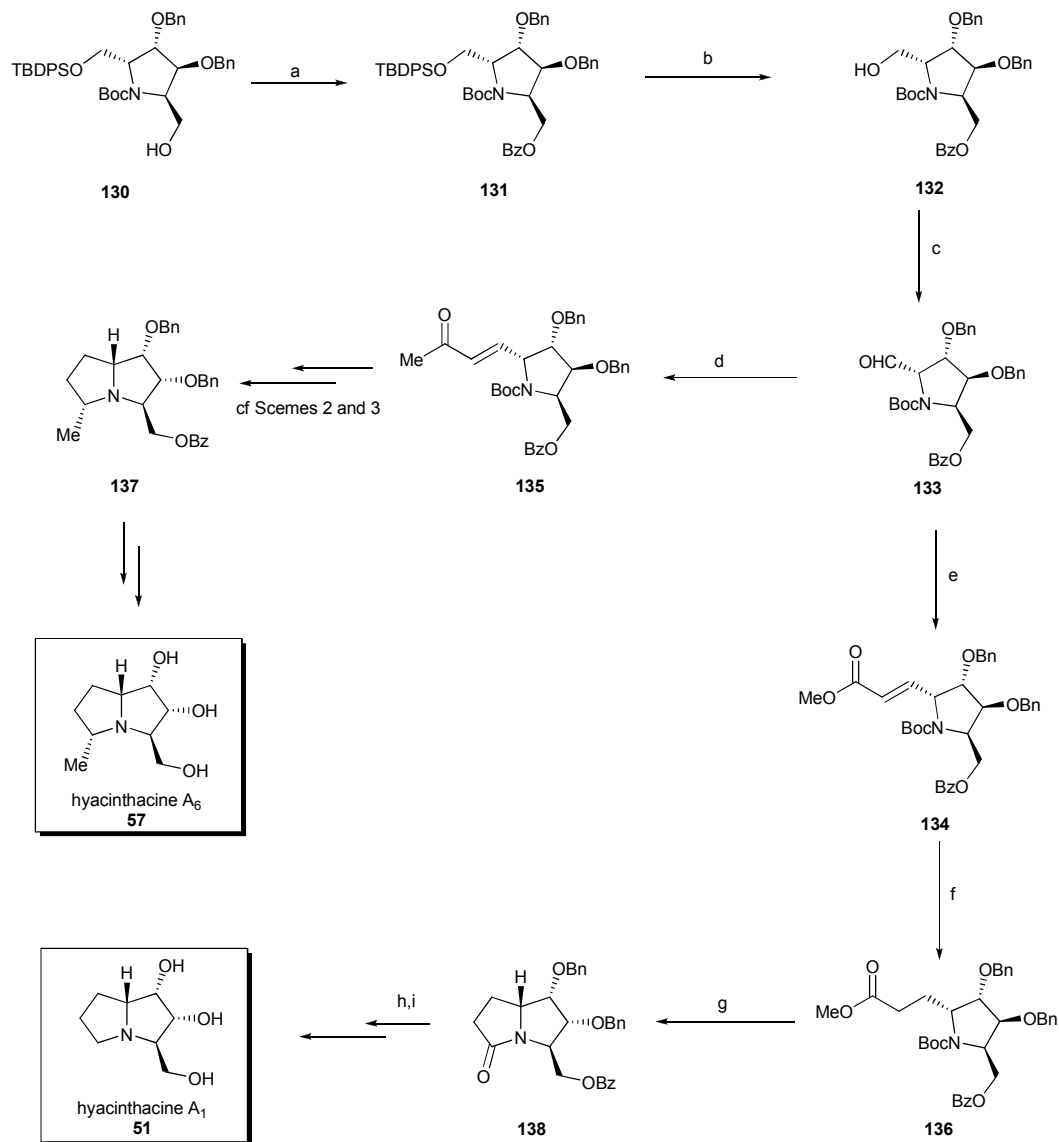
Scheme 5: (a) BzCl, CH$_2$Cl$_2$, TEA (78%); (b) TBAF, THF (88%); (c) NMO, TPAP, CH$_2$Cl$_2$, 4 Å MS (59%); (d) Ph$_3$P=CHCOMe, toluene, 80 °C (71%); (e) Ph$_3$P=CHCO$_2$Me, toluene, 80 °C (59%); (f) H$_2$, 10% Pd/C, MeOH; (g) i. TFA, DCM, ii. neutralization with MeONa in MeOH, iii. MeONa (cat.), MeOH, Δ to cyclise lactam (86% from **133**); (h) H$_3$B.SMe$_2$, THF then MeOH (59%); (i) H$_2$, 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]
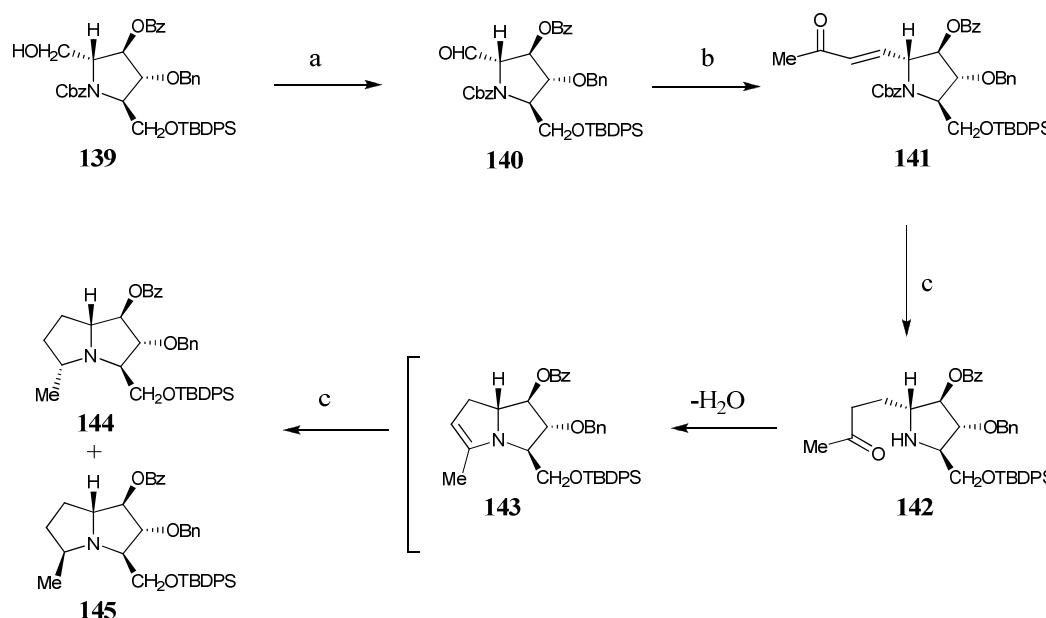
Scheme 6: (a) TPAP, NMO, CH_2Cl_2, 4 Å MS (89%); (b) $Ph_3P=CHCOCH_3$, toluene, 80 °C (78%); (c) H_2, 10% Pd/C, MeOH, 60 psi (44% of **145** and 40% of **144**).

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 stereocontrol 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 debenzoylated to give alcohol **146** (Scheme 7). Further sequential deprotection of the remaining two masked hydroxyl groups led to 1-*epi*-hyacinthacine A$_7$ (**150**). Implementation of an inversion strategy for the C-1 alcohol in **146** allowed access to hyacinthacine A$_7$ (**58**) itself however. Unsuccessful initial attempts to invert the alcohol were made under Mitsunobu conditions (BzOH, DEAD, Ph$_3$P, 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 A$_7$ (**58**).
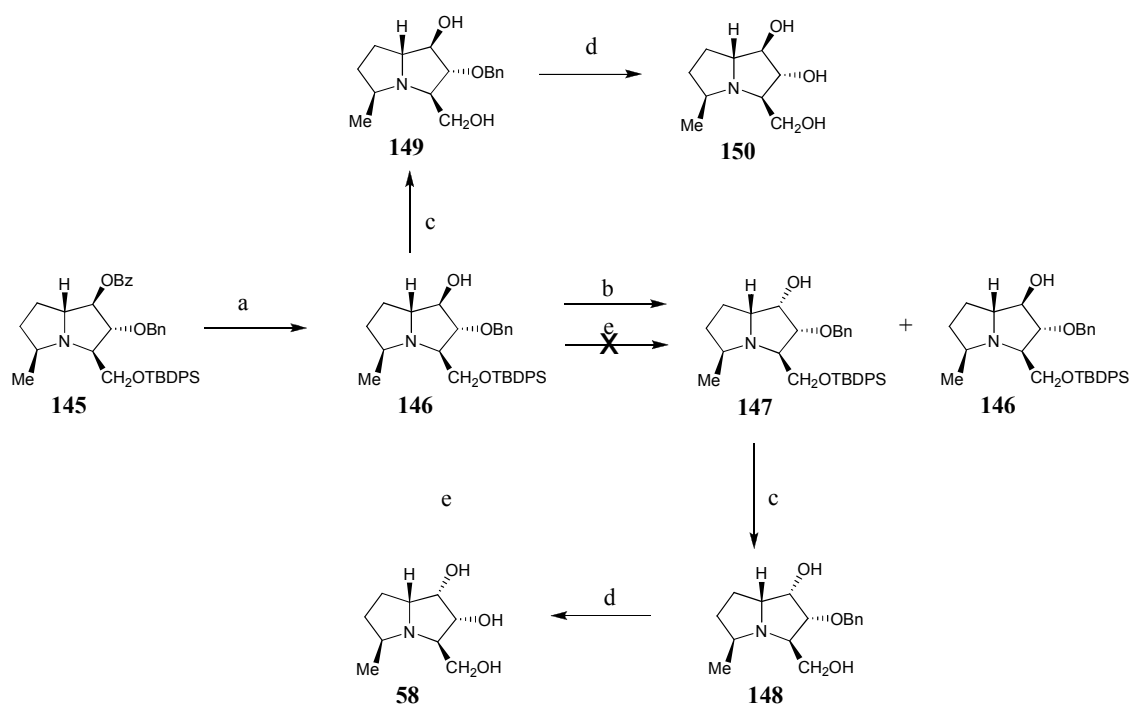
Scheme 7: (a) MeONa (cat.),MeOH (86%); (b) i. oxalyl chloride, DMSO, TFA, DCM, -78°C ii. NaBH$_4$, MeOH (46% of **147** and 29% of **146**); (c) TBAF, THF (65% for **148** and 85% for **149**); (d) H$_2$, 10%, Pd/C, MeOH, HCl then Amberlite IRA-400 (OH$^-$ form) (76% for **58** and 63% for **150**); (e) i. BzOH, DEAD, Ph$_3$P, 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 A$_6$ (**57**) and 1-*epi*-hyacinthacine A$_6$ (**151**) (Figure 15).
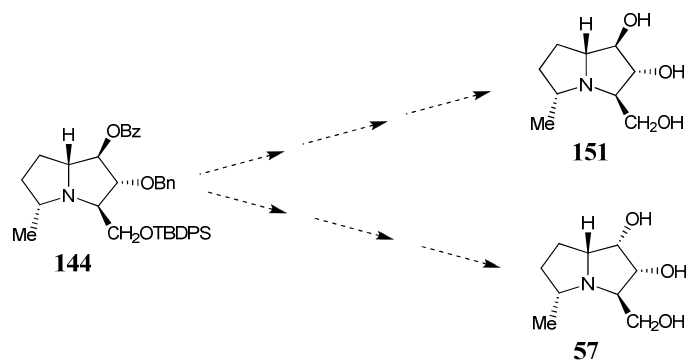
Figure 15. Potential hyacinthacine products from intermediate **134**.

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).[87]
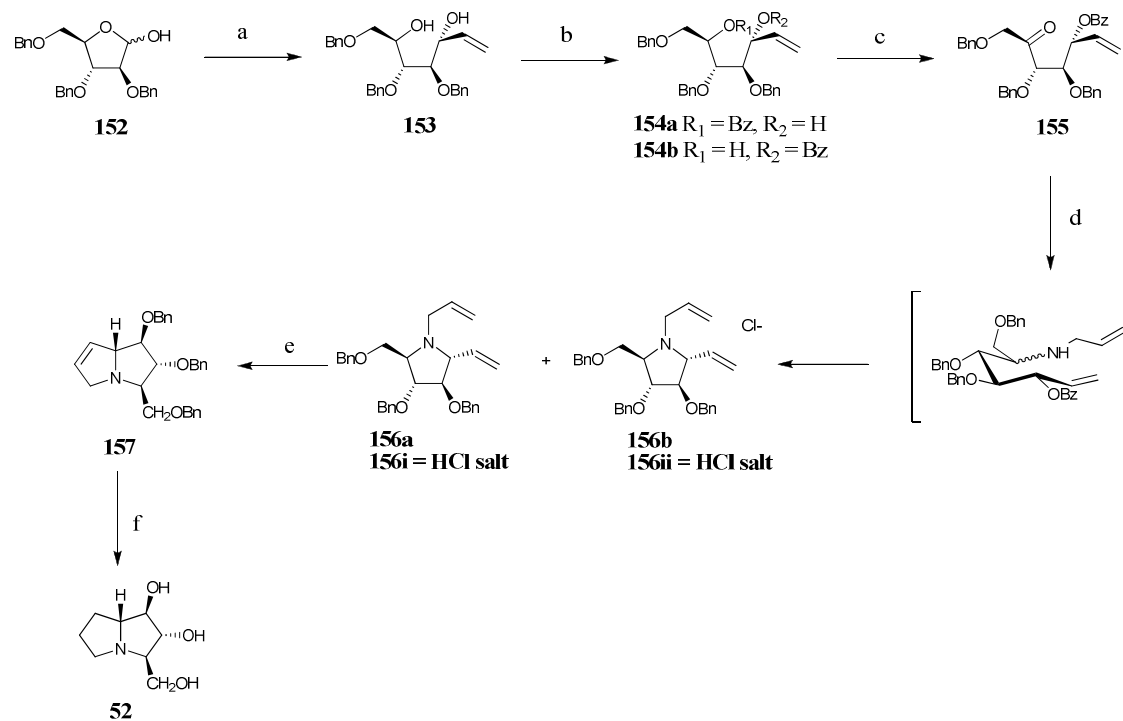
Scheme 8: (a) $(CH_2=CH)_2Zn$ (95%); (b) BzCl, *n*-Bu$_4$NI, CH_2Cl_2 / 1 N NaOH (1:1); (c) oxalyl chloride, DMSO, TFA, DCM, -78°C (63% from **153**) ; (d) allylamine, AcOH, NaBH$_3$CN, 3 Å MS, MeOH (78%); (e) Grubbs I catalyst, toluene (30%); (f) H_2, Pd/C, MeOH, THF, 6N HCl (82%).

The synthesis started by addition of divinylzinc to commercially available D-arabinose derivative **152** to give **153**. Benzoylation 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 A$_2$ (**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 A$_2$ (**52**) starting from a D-arabinose derived nitrone (**158**) has been reported in which the RCM step has a much improved yield (Scheme 9).[88]
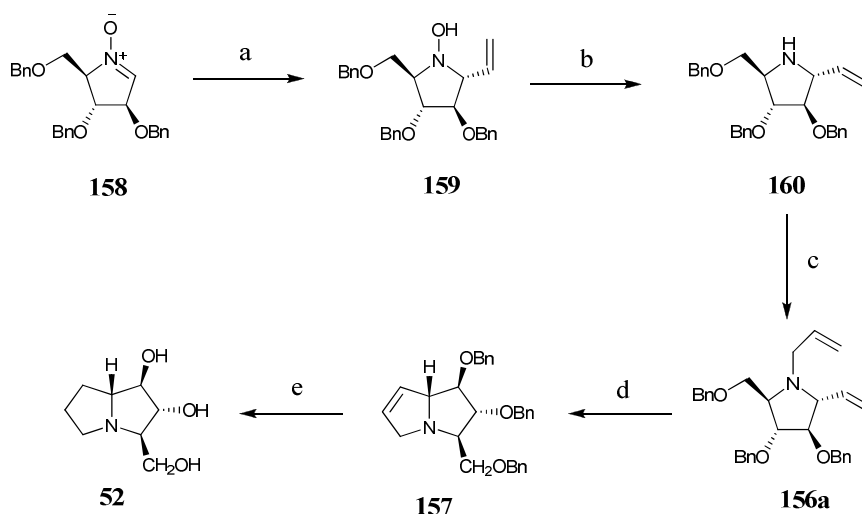
Scheme 9: (a) vinylmagnesium bromide, Et$_2$O (98%); (b) Zn, AcOH (100%); (c) allyl bromide, DMF, K$_2$CO$_3$, Bu$_4$NI (81%); (d) Grubbs second generation catalyst, toluene (87%); (e) H$_2$, 3 bar, Pd(OH)$_2$-C, MeOH-HCl, then Dowex (96%).

Nucleophilic addition of vinylmagnesium 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 A$_2$ (**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). [89]
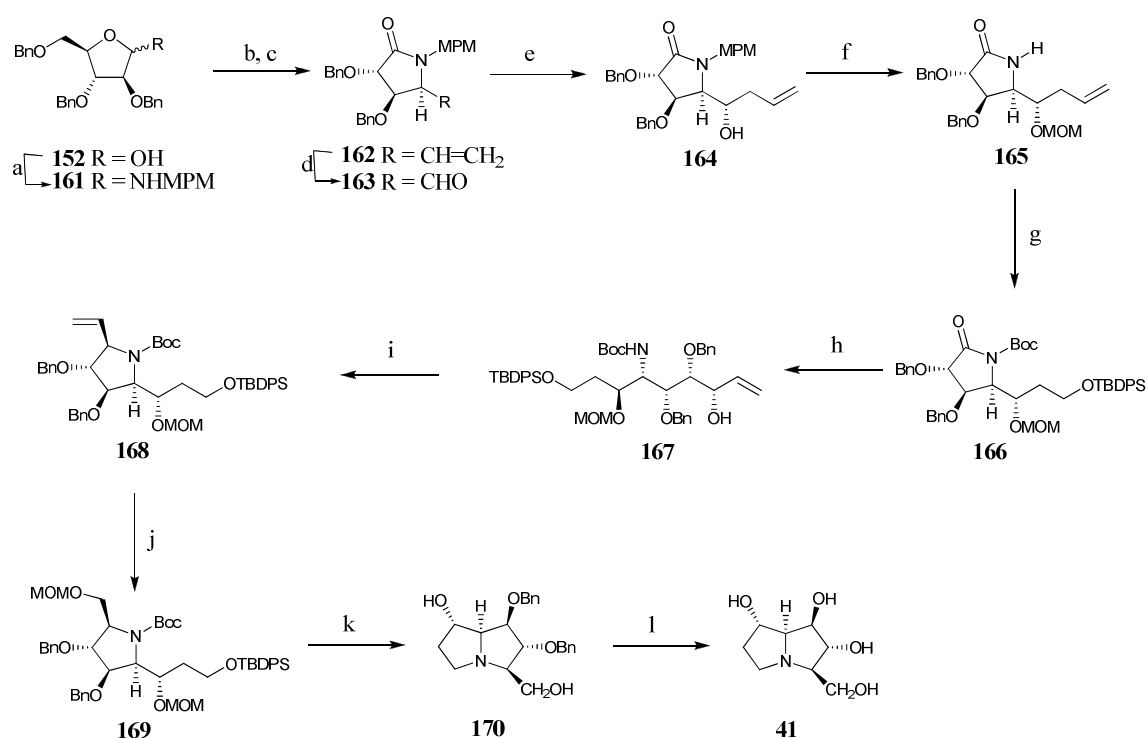
Scheme 10: (a) MPMNH$_2$, benzene-CHCl$_3$ (1:1), 4 Å MS, (quant.) (b) vinylmagnesium bromide, THF, (70%); (c) PCC, CH$_2$Cl$_2$, (98%); (d) i.OsO$_4$, NMO, acetone-water (1:1); ii. NaIO$_4$, Et$_2$O-water (1:1); (e) allyltrimethylsilane, BF$_3$.OEt$_2$, CH$_2$Cl$_2$, (82 % over 2 steps); (f) i. CAN, CH$_3$CN-water (9:1), (71%); ii. MOMCl, *N,N*-diisopropylethylamine, CH$_2$Cl$_2$, (75%); (g) i. OsO$_4$, NMO, acetone-water (1:1), (91%); ii. NaIO$_4$, Et$_2$O-water (1:1), iii. NaBH$_4$, EtOH (90% over 2 steps); iv. TBDPSCl, imidazole, DMF, (quant.); (h) i. vinylmagnesium bromide, THF; ii. NaBH$_4$-CeCl$_3$, MeOH (66% over 2 steps); (i) i. MsCl, TEA, DCM; ii, *t*-BuOK, THF, (84% over 2 steps); (j) i. OsO$_4$, NMO, acetone-water(1:1), (92%); ii. NaIO$_4$, Et$_2$O-water (1:1), iii. NaBH$_4$, EtOH, (74% over 2 steps); iv. MOMCl, *N,N*-diisopropylethylamine, CH$_2$Cl$_2$, (99%); (k) i. TBAF, THF, (quant.); ii TsCl, pyridine, (92%); iii. c. HCl, MeOH; iv. K$_2$CO$_3$, MeOH, (94% over 2 steps); (l) H$_2$, 10% Pd/C, EtOH, (70%).

The synthesis started with conversion of D-arabinose tribenzyl ether **152** into the aminal (**161**) by reaction with 4-methylbenzyl amine (MPMMH$_2$). 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

intermediate (**163**), which was subjected to Lewis acid induced alkylation with allytrimethylsilane in the presence of boron trifluoride; this selectively led to the corresponding allylic alcohol (**164**) by attack of the carbonyl from the least hindered face. Removal of the MPM group and protection of the secondary alcohol function as its MOM ether gave **165**. Oxidative cleavage of the alkene group of **165** followed by reduction produced the primary alcohol, which upon treatment with TBDPSCl and then (Boc)$_2$O gave the *N*-Boc lactam (**166**). Addition of vinylmagnesium bromide to **166** and subsequent reduction of the resulting enone, from the least hindred face, gave the allylic alcohol **167** with 5 contigous 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 A$_2$ (7-deoxyalexine, **97**), Scheme 11.[90]
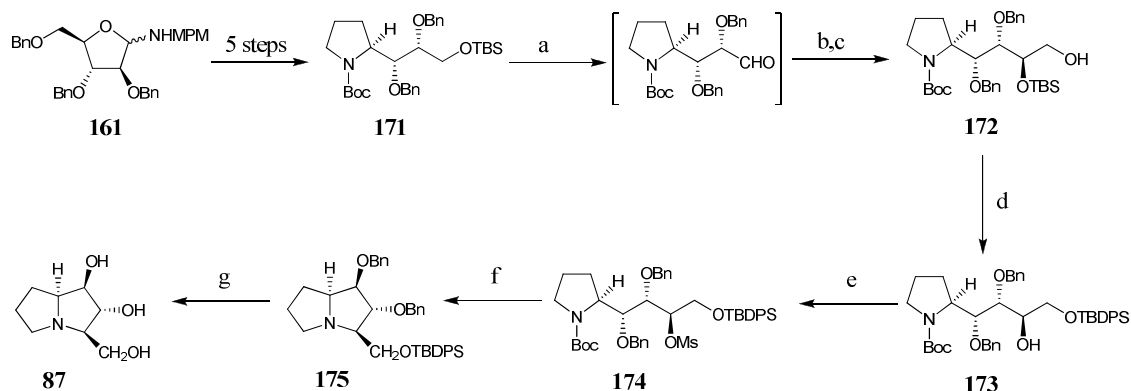
Scheme 11: (a) i. TBAF; ii. DMSO, DCM, -78 °C; (b) vinylmagnesium bromide, SmCl$_3$; (c) i. TBDMSCl; ii. OsO$_4$, NMO; iii. NaIO$_4$; iv. NaBH$_4$ (52 % from **171**); (d) i. TBAF; ii. TBDPSCl (87 %); (e) i. MsCl, TEA, CH$_2$Cl$_2$ (98 %); (f) i. BF$_3$.OEt$_2$; iii. KOH (73 %); (g) i. c. HCl; ii. H$_2$, 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 $SmCl_3$. 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 7a-*epi*-hyacinthacine A_2 (7-deoxyalexine, **87**).

The synthesis of both alexine (**41**) and 7-*epi*-alexine (**42**) has recently been reported from L-xylose using similar methodology to that presented above.[91] Syntheses of hyacinthacines B_1 (**48**) and B_2 (**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).[92]
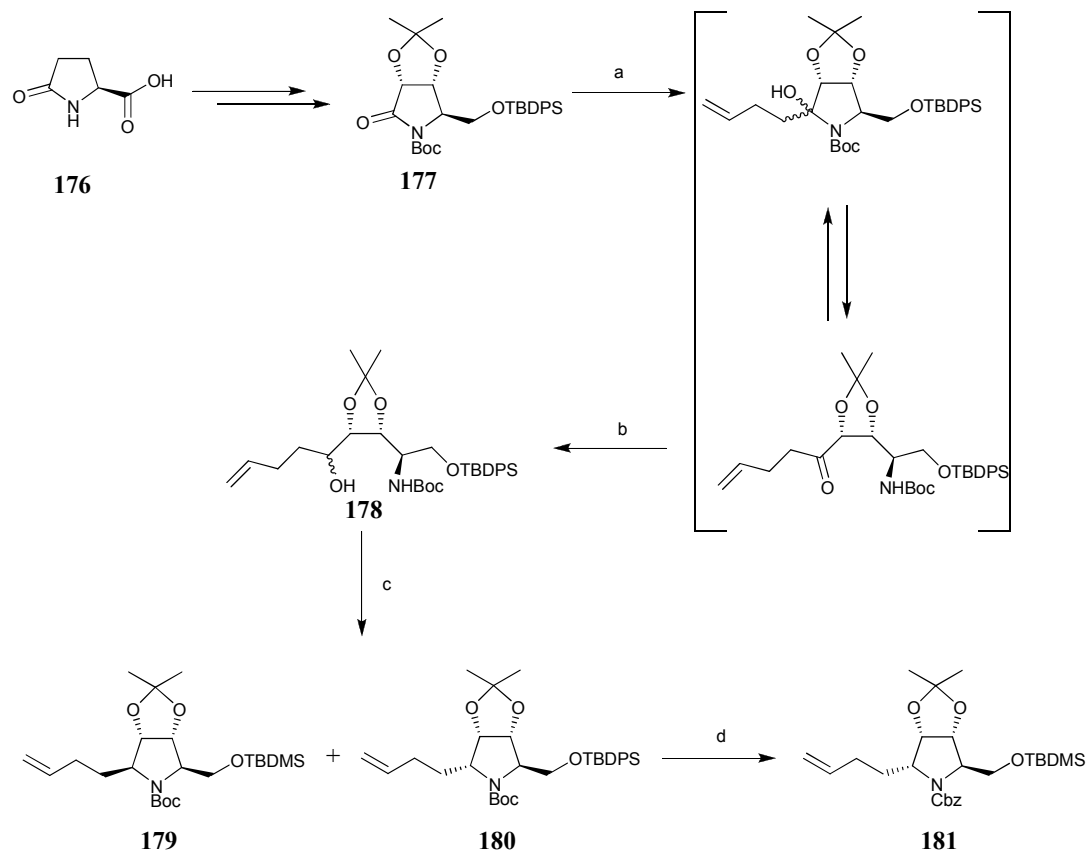
Scheme 12: (a) $CH_2=CH(CH_2)_2MgBr$, THF, (83%); (b) $NaBH_4$, $CeCl_3$, EtOH (quantitative over 2 steps); (c) i. MsCl, Et_3N, CH_2Cl_2 (90%); ii. *t*-BuOK, THF (91% of **180** and 5% **179** from **178**); (d) i. TBAF, THF (quantitative); ii. NaH, THF (quantitative); iii. CbzCl, $NaHCO_3$,(97%); TBDMSCl, imidazole, DMF (97%).

The route to hyacinthacines B_1 and B_2 involved construction of the key pyrrolidine (**177**) as a common intermediate. Thus, (*S*)-(–)-2-pyrrolidone-5-carboxylic acid (**176**) was converted into **177**[93], 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 B_1 (**48**) and B_2 (**49**) (Scheme 13).
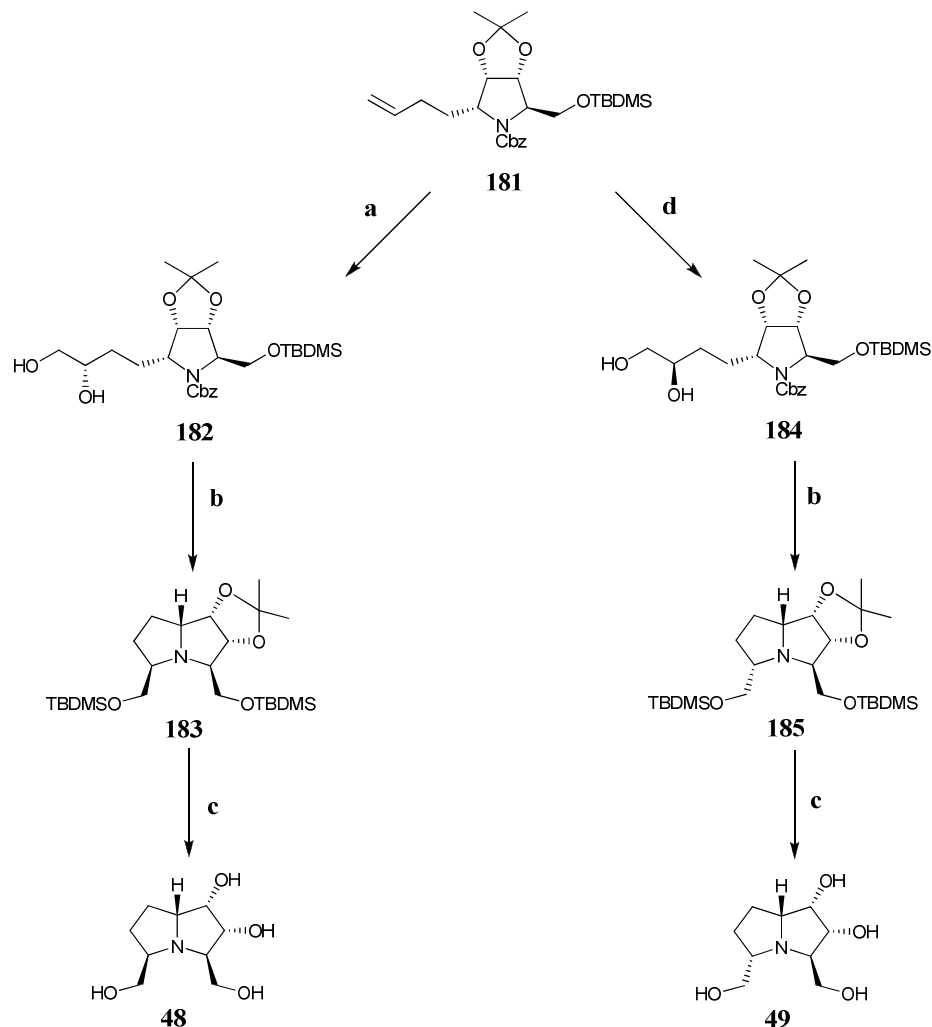
Scheme 13: (a) AD-mix-α[(DHQ)$_2$PHAL ligand], *t*-BuOH/H$_2$O (1:1) (98%); (b) i. TBDMSCl, Et$_3$N, DCM (97%); ii. MsCl, Et$_3$N, DCM (92%); iii. H$_2$, Pd/C, EtOH (69% for **183** and 28% for **185**); (c) i. TBAF, THF (97%); ii. TFA, H$_2$O (92%); (d) AD-mix-β[(DHQD)$_2$PHAL ligand], *t*-BuOH/H$_2$O (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)$_2$PHAL 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 isopropylidene group produced hyacinthacine B$_1$ (**48**). The asymmetric dihydroxylation of **181** with AD-mix-β [(DHQD)$_2$PHAL 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.[71] This nitrone has, as discussed previously (*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-xylose; 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).[95]
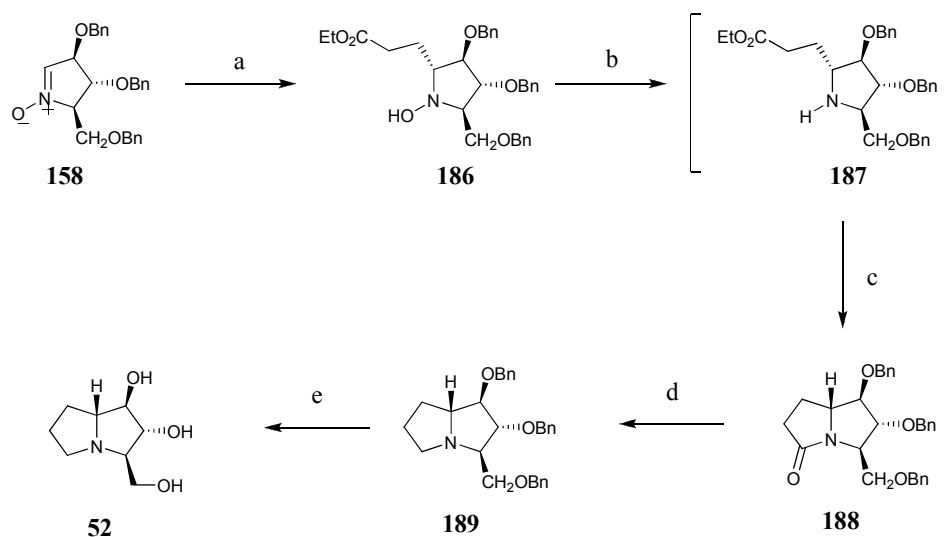
Plausible mechanism:
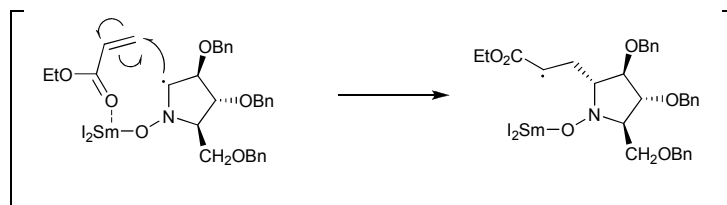
Scheme 14: (a) ethyl acrylate, SmI_2, H_2O (64%); (b) SmI_2, THF; (c) K_2CO_3 (59% from **186**); (d) $LiAlH_4$, THF (79%); (e) H_2, Pd/C, MeOH, HCl, DOWEX 1 x 8 (79%).

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 A_2 (**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).[96]
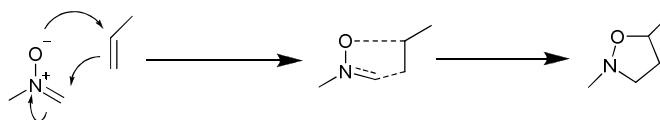
Scheme 15: Nitrone-alkene 1,3-dipolar cycloaddition

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.[97] As with the Diels-Alder reaction, the thermally allowed 1,3-dipolar cycloaddition is a suprafacial $[_\pi2_s+_\pi4_s]$ process. Regio- and stereocontrol in nitrone 1,3-dipolar cycloadditions have been reviewed in detail in the literature[96, 98] 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. [99]
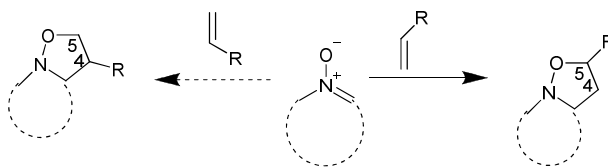
Figure 16. Regiochemical outcomes of 1,3-dipolar cycloaddition.

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).[100] Reactions of monosubstituted alkenes with 5-membered ring nitrones, such as 1-pyrroline-*N*-oxide, behave accordingly and 5-substituted isoxazolidines are predominantly formed. [101]
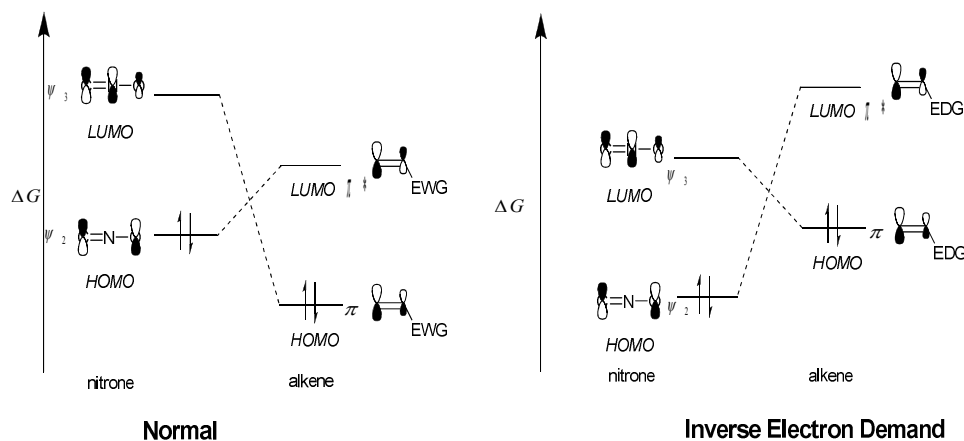
Figure 17. Frontier molecular orbital interactions in 1,3-dipolar cycloadditions.

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.[102] 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 (*endo* 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).[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 nitrones, the interaction of the N-nitrone p_z orbital with a vicinal p_z orbital is small.[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 nitrones generally occurs *via* an *exo* transition state therefore.
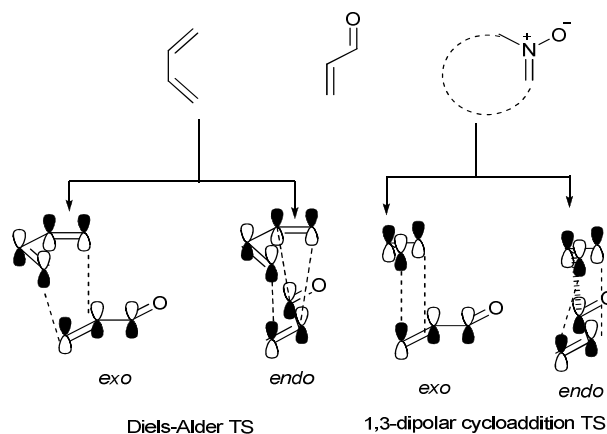
Figure 18. Orbital interactions of *exo* and *endo* approach.

The stereochemical outcome of the cycloaddition is further complicated for cyclic nitrones bearing an existing stereogenic feature in the form of a substituent at one of the ring sp^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. [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.
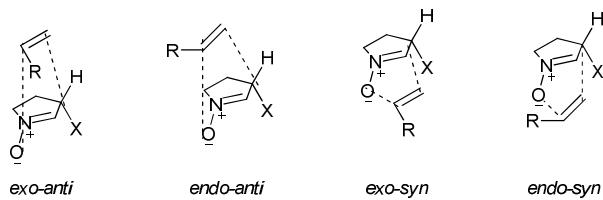
Figure 19. Possible approaches of dipolarophile in 1,3-dipolar cycloaddition.

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).
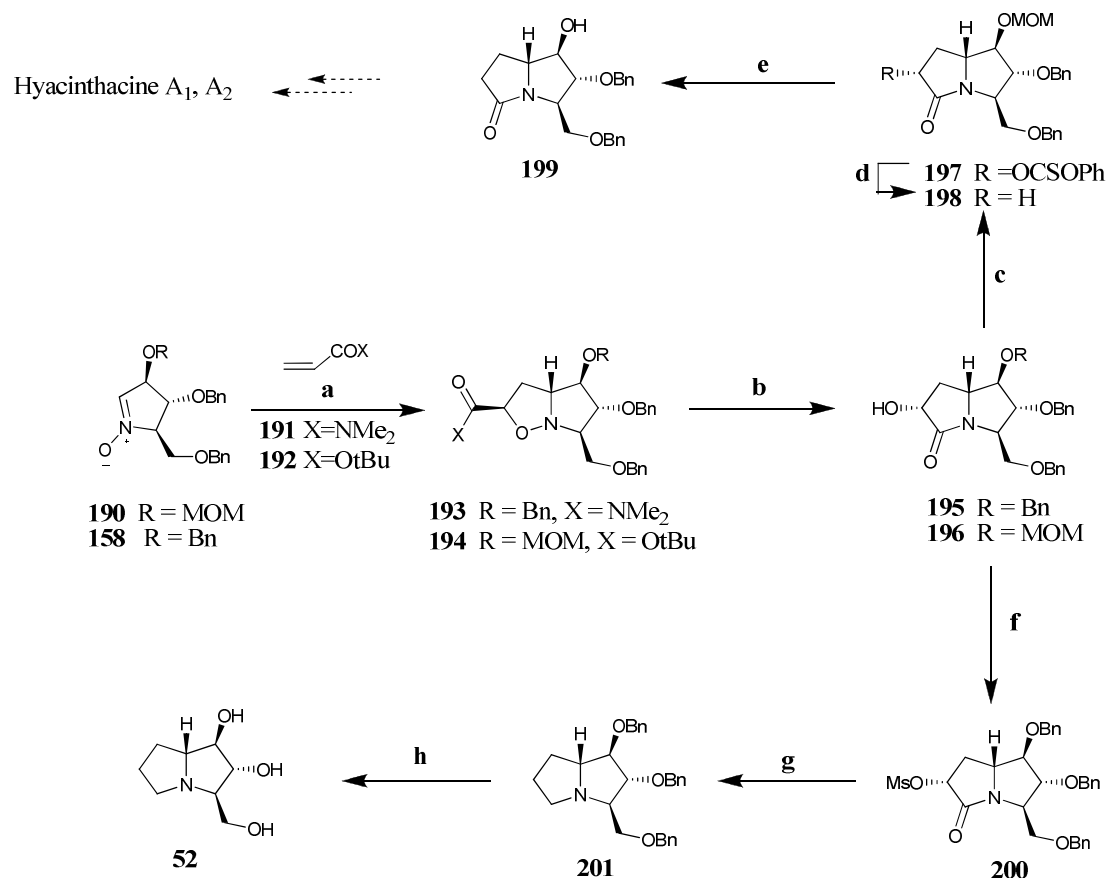
Scheme 16: (a) **191** in CH$_2$Cl$_2$, or **192** in C$_6$H$_6$ (78% **193**), (64% **194**); (b), Zn, AcOH-H$_2$O (80% **195**), (64% **196**); (c), PhOCSCl, py, DMAP, CH$_2$Cl$_2$ (80%); (d), Bu$_3$SnH, AIBN, C$_6$H$_6$, Δ (58%); (e), 3 N HCl, MeOH (84%); (f), MsCl, TEA, CH$_2$Cl$_2$, (100%); (g) 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.[110] 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 A_1 and A_2 but this was not reported. The route to hyacinthacine A_2 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 A_2 (**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]. [111] 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.
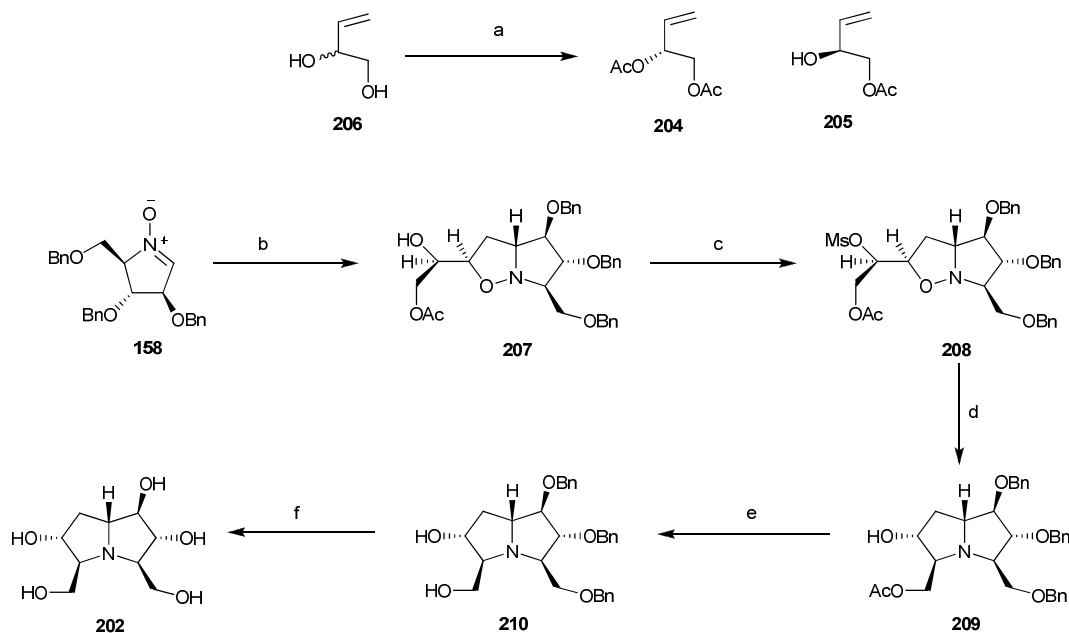
Scheme 17: (a) vinyl acetate, Chirazyme® L-2, TBME (44%, 64% ee, **204**), (46%, 61% ee, **205**); (b) **205**, CH$_2$Cl$_2$, 70 °C, 2.5 h, (70%); (c) MsCl, py, CH$_2$Cl$_2$ (74%); (d) Zn, AcOH/H$_2$O (9:1), 60 °C (60%); (e) Ambersep 900 (OH$^-$), MeOH (83%); (f) i.Pd/C, H$_2$, c. HCl, ii. Dowex 50W X8 NH$_4$OH (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$^-$ form) 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).
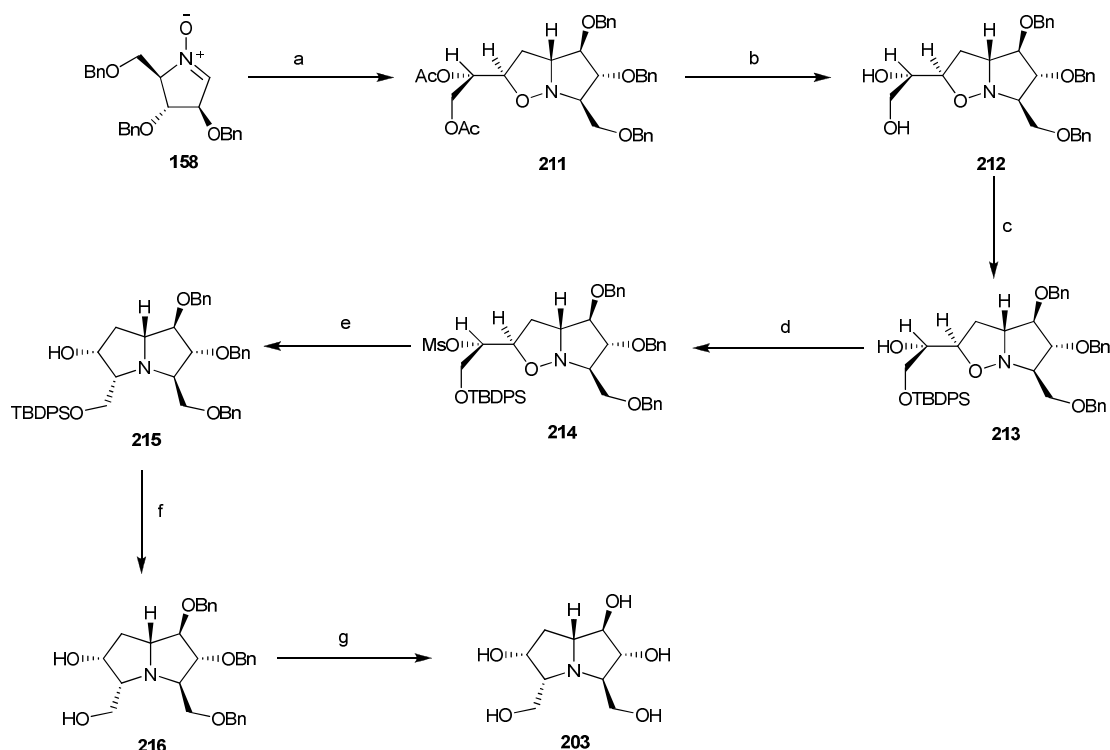
Scheme 18: (a) **204**, DCM, 70 °C, 4, 74%; (b) Ambersep 900 (OH⁻), MeOH (83%) (c) TBDPSCl, imidazole, DMF, (92%); (d) MsCl, py, DCM (81%); (e) Zn, AcOH/H₂O (9:1), 60°C (76%); (f) TBAF, THF (61%); (g) i. Pd/C, H₂, c.HCl, ii. Dowex 50W X8 NH₄OH (85%).

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-xylose 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.
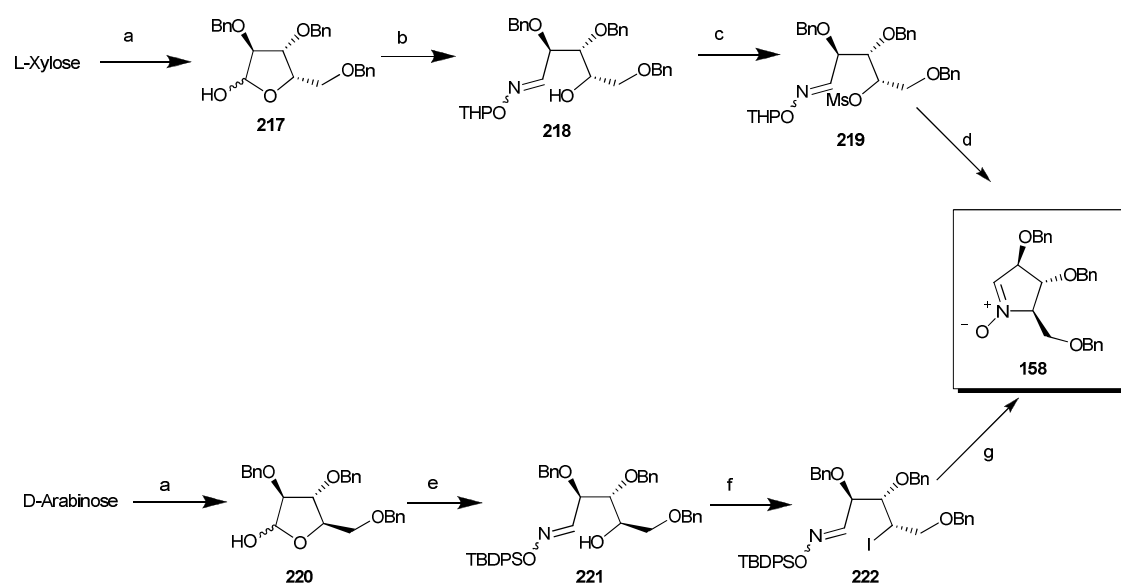
Scheme 19: (a) i. MeOH/H$_2$SO$_4$, anhydr. Na$_2$SO$_4$, rt, 21 h; ii BnCl/KOH, Na$_2$SO$_4$, reflux, 8 h; iii. 6 N HCl, CH$_3$COOH, 60-70 °C (50% over 3 steps); (b), NH$_2$OTHP, no solvent, r.t., 6 d, (100%); (c) MsCl, TEA, CH$_2$Cl$_2$, r.t., 24 h (50-70%); (d) i. Dowex 50W X8, MeOH, r.t., 24 h (96%); 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-xylose and D-arabinose respectively following the procedure reported by Fleet and Smith.[112] Compound **217** was converted into tetrahydropyranyloxime **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 nitrone **158**.

Py *et al.* have also synthesized nitrone **158** from L-xylose (Scheme 20).[95] 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 TBDPSONH$_2$ 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 nitrone **158**.
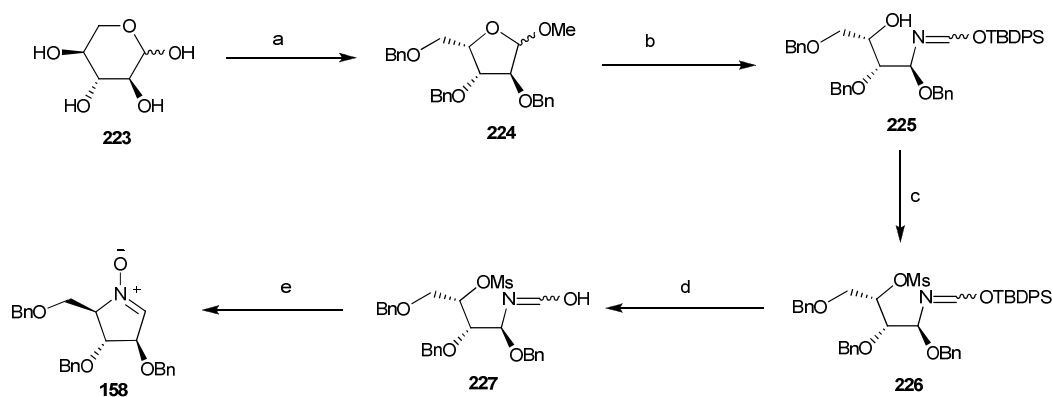
Scheme 20: (a) i. 0.5% MeOH, MeOH, r.t, 15 h; i. NaH, BnBr, *n*-Bu$_4$NI, DMF/THF, r.t, 48 h (90% over 2 steps); (b) i. AcOH/1 M H$_2$SO$_4$, 100 °C, 1h; ii. TBDPSONH$_2$, cat. PPTS, MgSO$_4$, toluene, 110 °C, 30 min (79% over 2 steps); (c) MsCl, TEA, DCM, 0 °C, (93%); (d) TBAF, THF, 0 °C, 30 min (93%); (e) NH$_2$OH.HCl, NaHCO$_3$, MeOH/H$_2$O 4:1, 65 °C, 15h (92%).

1.6.2   Route to nitrone 190

As mentioned earlier, Ishibashi *et al.*[113] have used nitrone **190** in the synthesis of a pyrrolizidine lactam precursor (**199**, Scheme 16) to hyacinthacine A$_2$. Nitrone **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 nitrone **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 nitrone and a terminal alkene can be utilised for the synthesis of bicyclic pyrrolizidine alkaloids. Thus, the 1,3-dipolar cycloaddition of a suitable nitrone 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,[113] Goti [114] and in our group where cycloaddition reactions of functionalised cyclic nitrones have been used to prepare hydroxylated pyrrolizidines.[115]
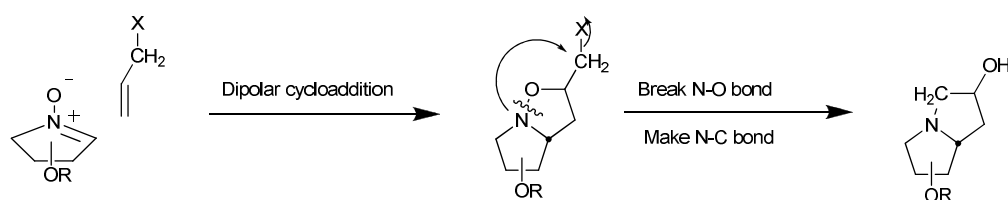
Scheme 21: Route to pyrrolizidines *via* 1,3-dipolar cycloadditions between a cyclic nitrone and a terminal alkene bearing a leaving group in the allylic position.

The aim of this PhD project was to investigate routes to hyacinthacine A_1 and hyacinthacine B_2 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 nitrone **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 A_1 and B_2, where the stereochemistry at C-1 and C-2 is *cis*. A nitrone 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. Nitrone **190** was therefore selected as the preferred cyclic nitrone for

our work as high stereo- and regioselectivity had already been established for cycloaddition reactions employing its use (Section 1.5.5.2).
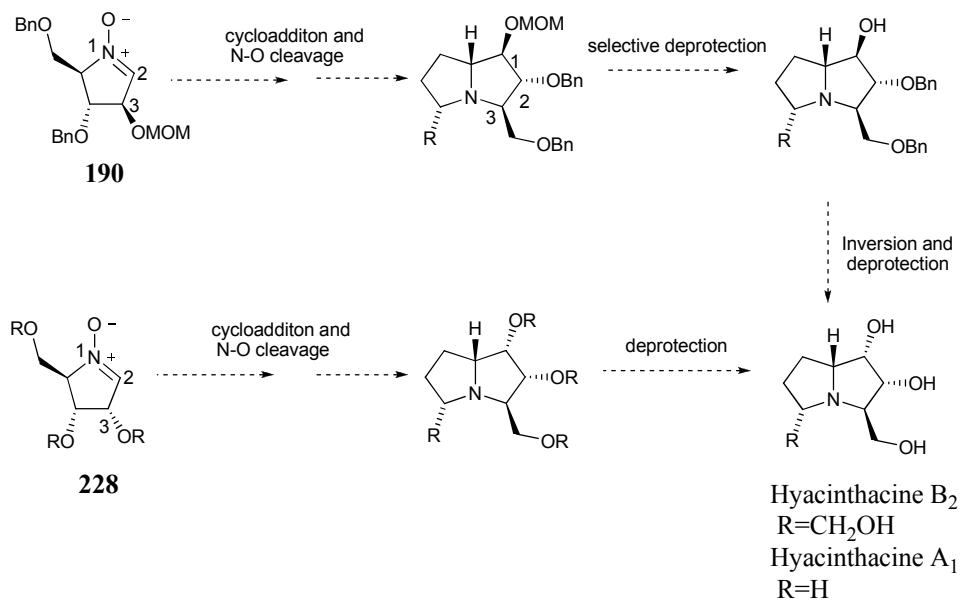
Scheme 22: Potential routes to hyacinthacine A_1 and hyacinthacine B_2 *via* 1,3-dipolar cycloadditions of nitrone **190** or nitrone **228**.

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 A_1 and B_2, efficient synthetic access was required. A synthesis starting from L-xylose has been discussed.[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
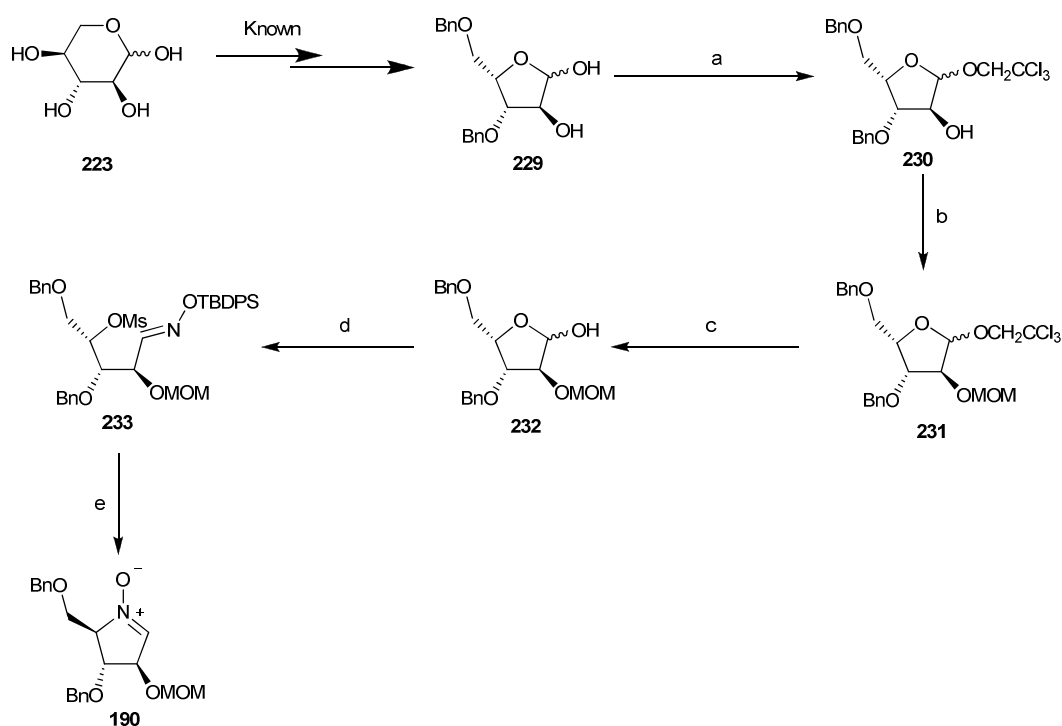
Scheme 23: (a) 2,2,2-trichloroethanol, cat. TsOH, DCM, 39 °C (72%); (b) NaH, MOMCl, THF-DMF, -20 °C; (c) Zn, NaOAc, AcOH, (80%); (d) i. NH₂OTBDPS, cat. PPTS, MgSO₄, benzene, reflux; ii. MsCl, Et₃N, toluene, 0 °C, (98%); (e) TBAF (1.05 equiv.), 4 Å MS, THF, reflux, 15 min, (72%).

Ishibashi *et al.*[113] have synthesised nitrone **190** from L-xylose (Scheme 23) *via* the known derivative (**229**).[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

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 $NH_2OTBDPS$ 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 itssubsequent differential protection by benzylation. Installation of the isopropylidine group was achieved by acid-catalysed reaction of L-xylose (**223**) with acetone.[117] The hydroxyl functions remaining at the C-3 and C-5 positions of **234** were then protected as dibenzyl ethers to give **235**.[116] 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.
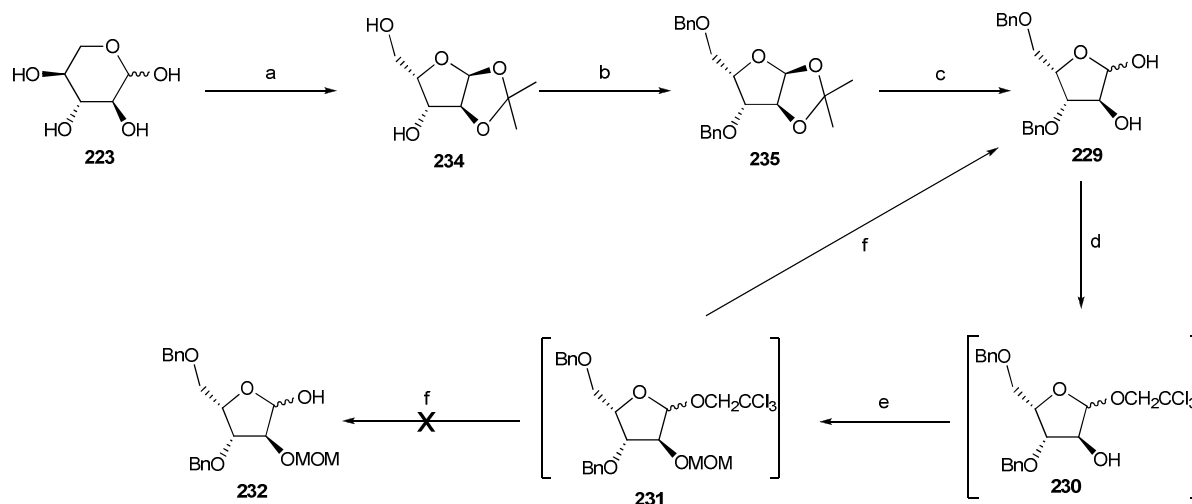
Scheme 24: (a) acetone, c. H_2SO_4, rt, 24 h (75%); (b) NaH, *n*-Bu$_4$NI, dry THF, BnBr, 0 °C to rt, 24 h, (84%); (c) acetic acid:water (1:1), 80 °C, 24 h, (86%); (d) 2,2,2-trichloroethanol, cat. TsOH, DCM, 39 °C, (72)%; (e) NaH, MOMCl, THF-DMF, -20 °C, (92%); (f) Zn, NaOAc, AcOH, (0 % for **232** and (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 anomeric position would be temporarily masked as a lactone [Scheme 25], allowing its regeneration under non-acidic conditions following installation of the MOM protecting group.
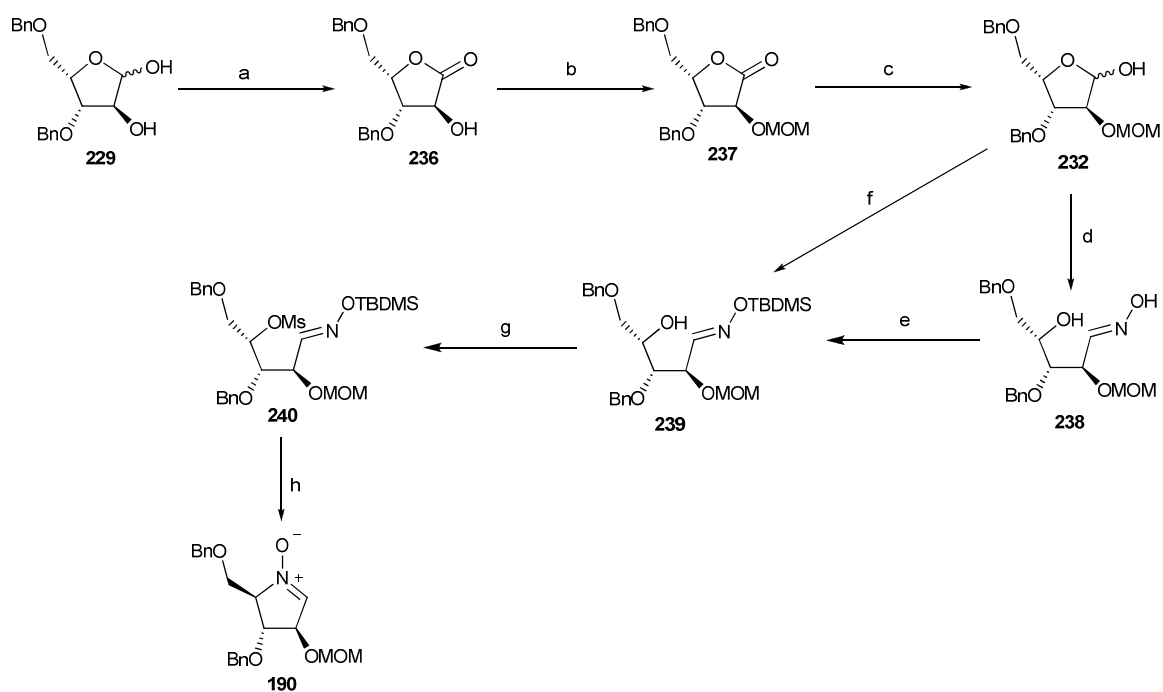
Scheme 25: (a) Ag$_2$CO$_3$/celite® (large excess), toluene, 80 °C, 1 h, (99%); (b) dimethoxymethane (large excess), chloroform dried over P$_2$O$_5$, 30 min, (92%); (c) DIBAL (8 equiv.), THF, -78 °C, 3 h, (78%); (d) NH$_2$OH.HCl, NaOMe, MeOH, r.t., 18 h, (82%); (e) TBDMS, dry py, 18 h, (73%); (f) H$_2$NOTBDMS, MgSO$_4$, dry toluene, reflux, 30 min, (89%); (g) MsCl, Et$_3$N, 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]
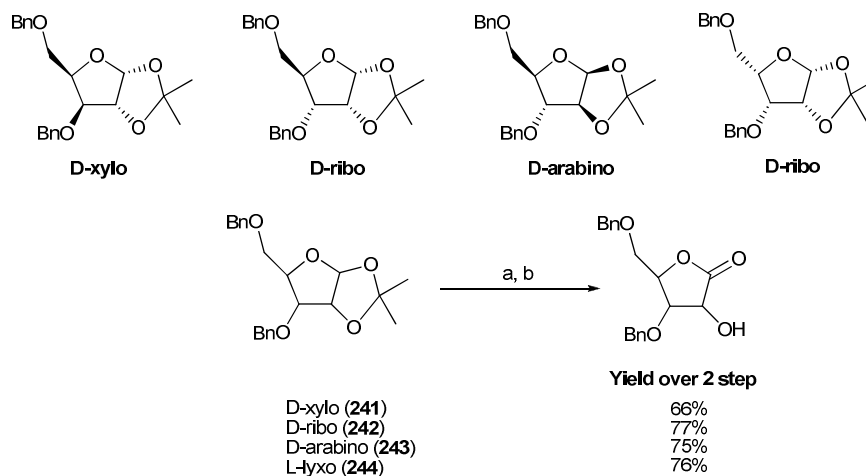
Scheme 26: (a) aqueous CF$_3$CO$_2$H; (b) Br$_2$, BaCO$_3$ in aqueous dioxane, (66-76%).

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.

Solvent	Br$_2$ mol equiv.	Br$_2$ addition	Isolated Yield (%)	% bromobutanol in isolated product
Dioxane	1.5	Br$_2$ water	51	0
Dioxane	2	Br$_2$ water	49	0
Dioxane	3	Br$_2$ water	36	0
Dioxane	1.5	Br$_2$ in ether	24	0
Dioxane	2	Br$_2$ in ether	22	0
Dioxane	3	Br$_2$ in ether	18	0
Diethyl ether	1.5	Br$_2$ water	16	0
Diethyl ether	2	Br$_2$ water	15	0
Diethyl ether	3	Br$_2$ water	21	0
Diethyl ether	1.5	Br$_2$ in ether	18	0
Diethyl ether	2	Br$_2$ in ether	24	0
Diethyl ether	3	Br$_2$ in ether	19	0
THF/water (1:3)	1.5	Br$_2$ in THF	5	5
THF/water (1:3)	2	Br$_2$ in THF	31	5
THF/water (1:3)	3	Br$_2$ in THF	34	6
THF/water (1:3)	3	Br$_2$	2	7
THF/water (1:3)	3	Br$_2$ in ether	6	3

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 Br_2 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.[121] 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.[122] 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®.[123] The resultant Ag_2CO_3/celite® reagent is a versatile and useful oxidising reagent and has been thoroughly investigated by Mikillop and Young.[124] This reagent is commercially available but it was prepared in our laboratory using the procedure originally reported by Fetizon.[123] Pleasingly, treatment of lactol **229** with a large excess of the silver carbonate reagent in toluene produced lactone **236** in quantitative yield as a white crystalline solid. X-ray crystalography confirmed the structure of lactone **236** (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 P2(1) space group, with a zig-zag chain along the b axis. Pi stacking of the aromatic rings was not observed but intermolelcular hydrogen bonding is present between the hydroxyl hydrogen and the lactone oxygen atom.
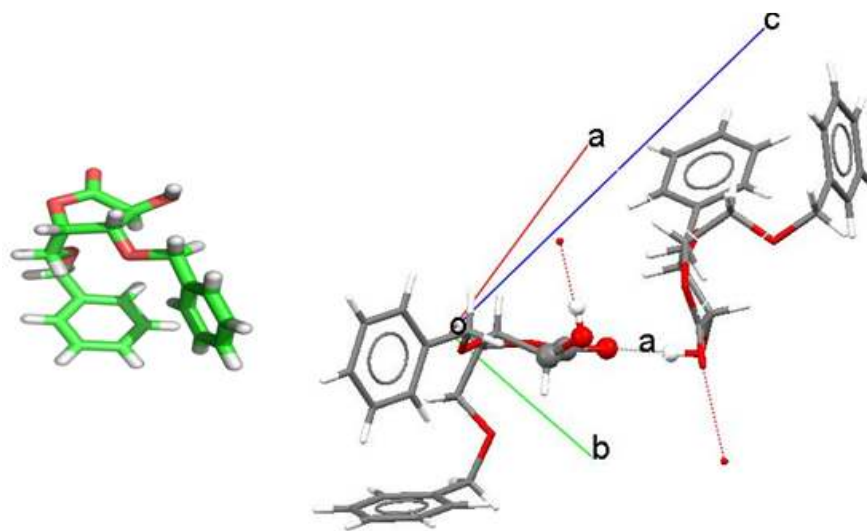
Figure 20: Crystal structure of lactone **236**.

The free hydroxyl group in lactone **236** was then protected as the methoxymethyl ether using 10 equivalents of dimethoxymethane and P_2O_5 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 ^{1}H 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 ^{1}H NMR spectrum, but was more readily evident in the ^{13}C 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 ^{13}C 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 H$_2$NOTBDMS, which can be prepared by a known procedure.[125] In order to explore this we therefore prepared H$_2$NOTBDMS 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 H$_2$NOTBDPS, MgSO$_4$ and a catalytic amount of pyridinum *p*-toluenesulfonate in benzene (Scheme 23). The formation of *O*-TBDMS protected oxime **239** (Scheme 25) by similar treatment of **232** with H$_2$NOTBDMS was unsuccessfuland 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**) sucessfully 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.*[127] described optimum cyclisation conditions for their analogue (**233**) of **240** involving the use of tetrabutylammonium triphenyldifluorosilicate (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.[128] 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).
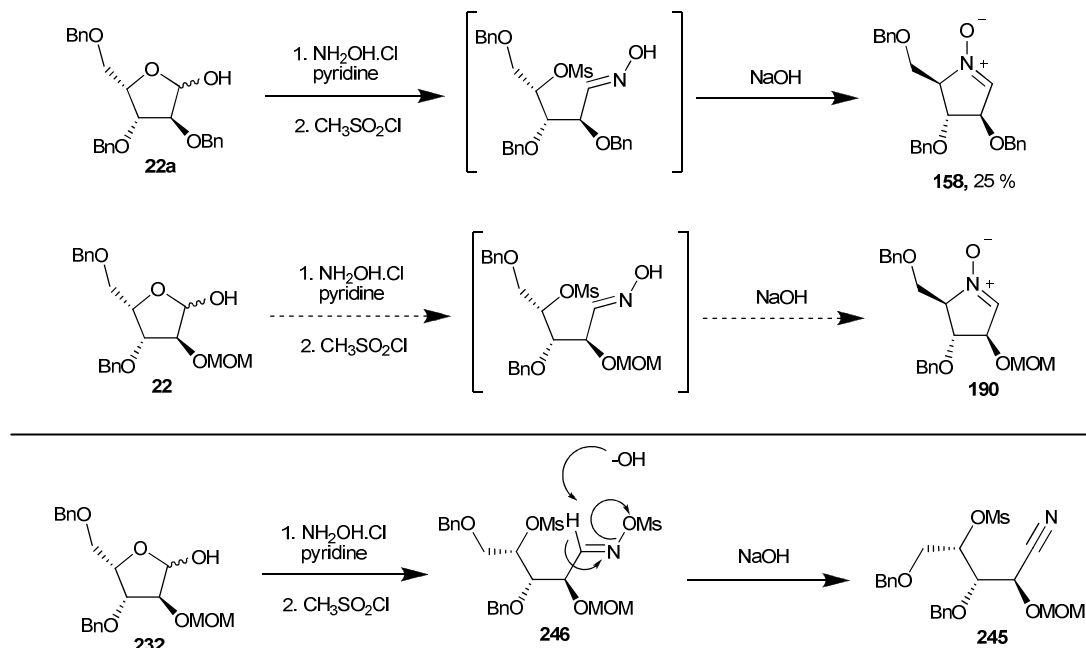
Scheme 27: Attempted preparation of nitrone **190** and mechanistic formulation for the generation of nitrile **245**.

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.
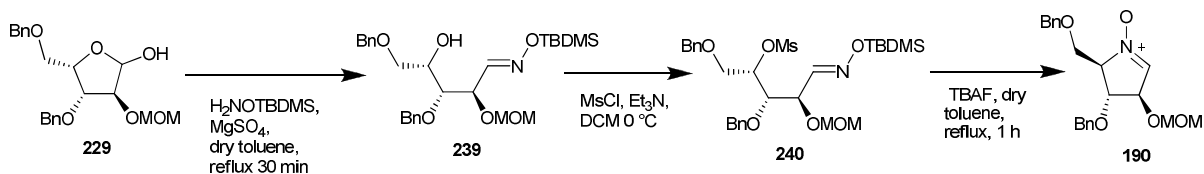
Scheme 28: Telescoping the lactol to nitrone transformation

69

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-xylose-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-xylose 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).
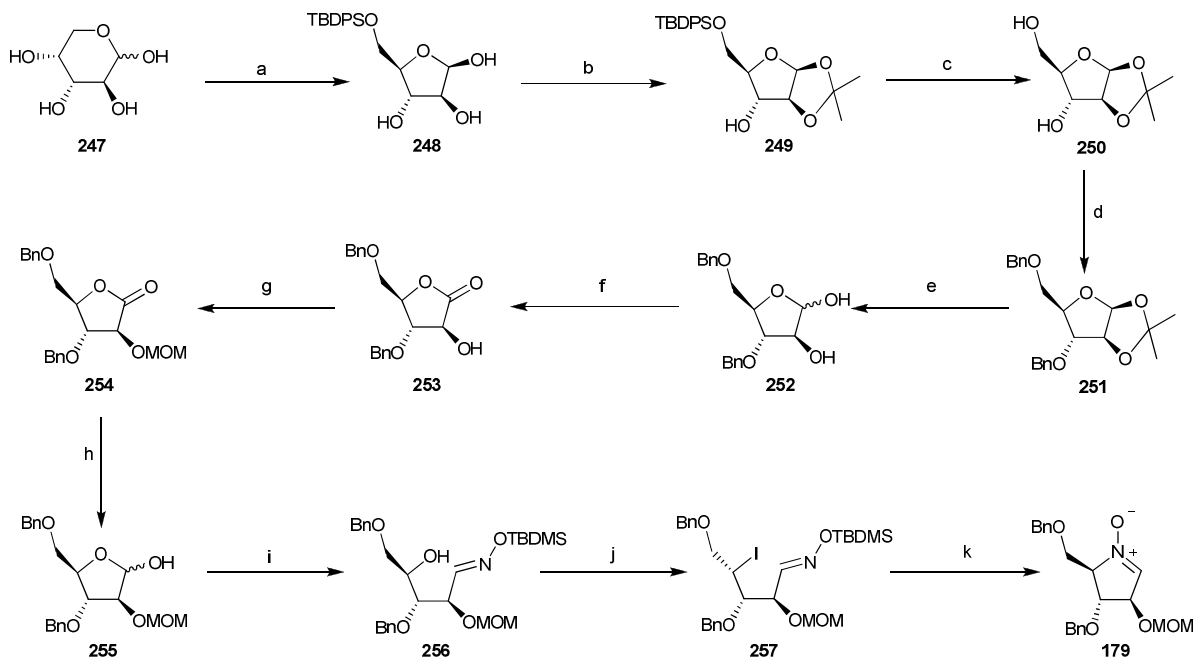
Scheme 29: (a) TBDPSCl, imidazole, DMF (55%); (b) acetone, CuSO$_4$, H$_2$O (38%); (c) TBAF, THF (75%); (d) BnCl, Bu$_4$NI, NaH, DMF (62%); (e) 20% aqueous AcOH, 60 °C, 5 h (92%); (f) Br$_2$, BaCO$_3$, THF-H$_2$O (85%); (g) CH$_2$(OMe)$_2$, P$_2$O$_5$, CHCl$_3$ (92%); (h) DIBAL, THF, -78 °C (98%); (i) NH$_2$OTBDMS, toluene, MgSO$_4$, reflux, 5 min (72%); (j) PPh$_3$, I$_2$, 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 TBDPSCl. 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 nitrone 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 nitrone **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 anomeric 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 $H_2NOTBDMS$ 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_N2 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.*[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 materialL-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_1, hyacinthacine B_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_1 and B_2 will be discussed in this thesis. The application of nitrone **190** in the first total synthesis of 1-*epi*-hyacinthacine B_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 A_1

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 A_1 (**51**) was chosen as the first synthetic target (Figure 21).
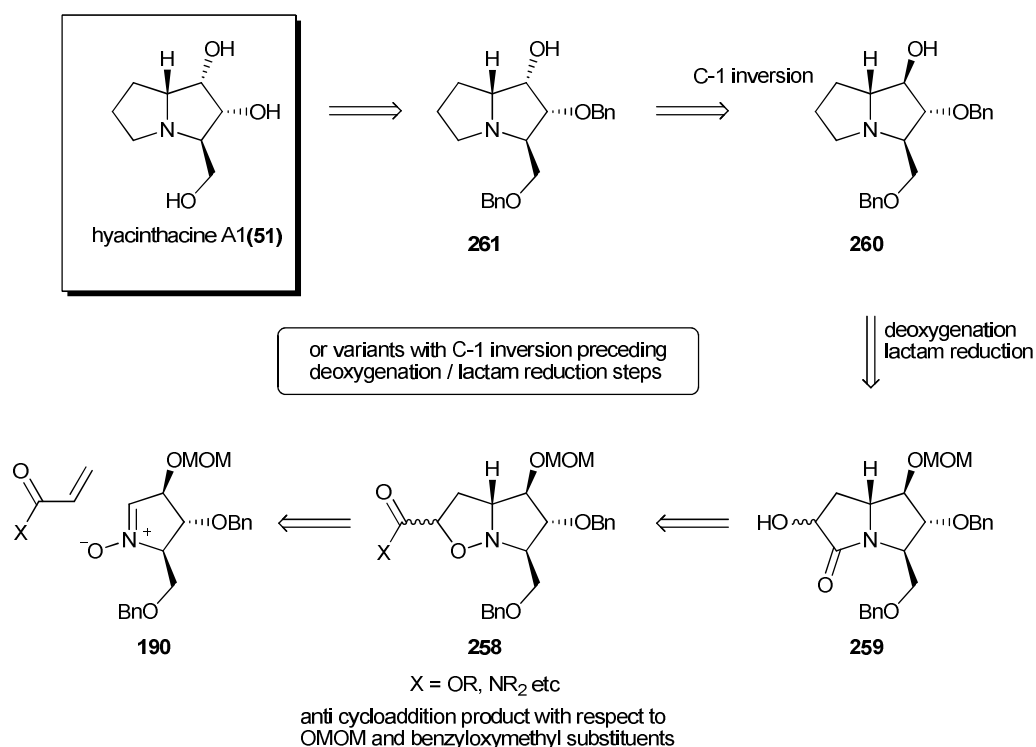
Figure 21: Reterosynthetic route to Hyacinthacine A_1.

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 A_1 through subsequent inversion of the stereochemistry at C-1 and reduction of the lactam.[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].
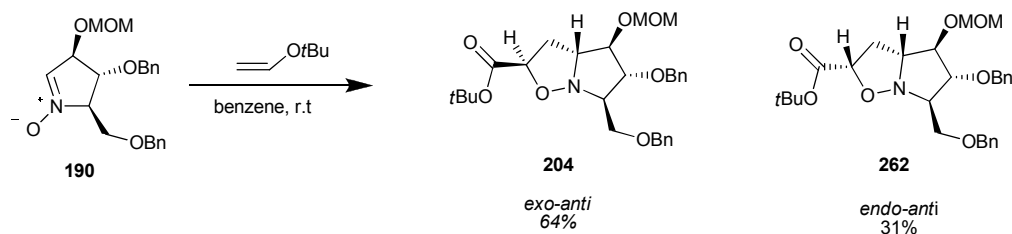
Scheme 30: Outcome of Ishibashi's 1,3-dipolar cycloaddition between nitrone **190** and alkene **192**.

The Goti group have investigated the 1,3-dipolar cyclcaddition products formed upon reaction of a related nitrone (**158**) with maleic and acrylic acid derivatives **191**, **263**, and **264** (Scheme 31).[109] 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** occured 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).
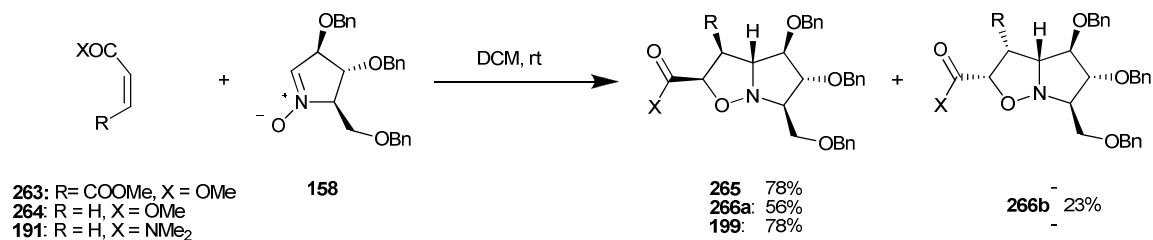
Scheme 31: 1,3-dipolar cycloadditions of nitrone **158** with **263**, **244** and **191** carried out by Goti *et al.*[109]

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
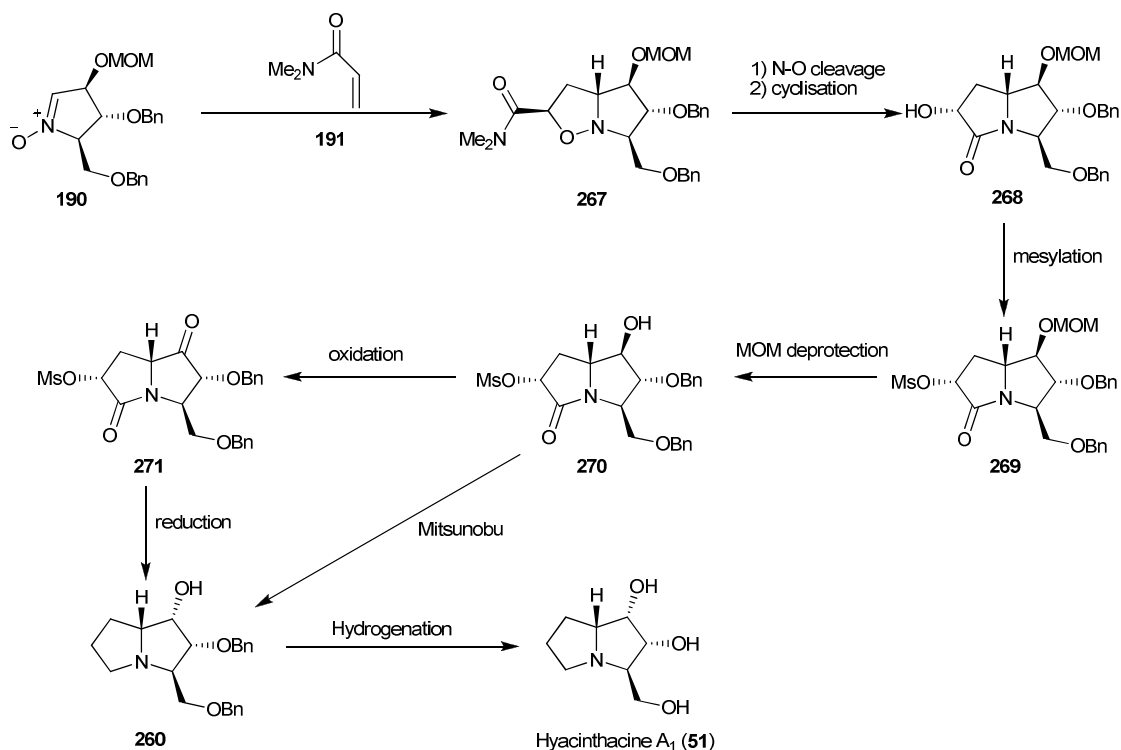
Scheme 32: Planned route to hyacinthacine A$_1$ (**51**) – Route A.

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 A$_1$ 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 A$_1$ (**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 A$_1$ by the route shown in Scheme 11, although it would be preferable to take a single isomer forward.

exo-anti   *endo-anti*

exo-syn   *endo-syn*
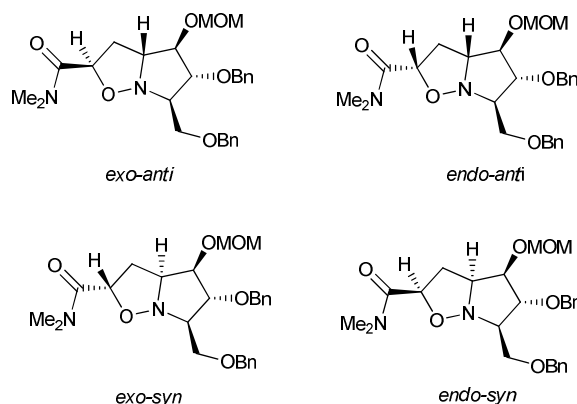
Figure 22. Possible cycloadducts from reaction between nitrone **190** and acrylamide **191**

Pleasingly, when nitrone **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 A$_1$ 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 *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.

anti-exo approach   *anti-endo* approach

syn-exo approach   *syn-endo* approach

Figure 24: Chem3D image showing the possible approaches of dipolarophile **191** to nitrone **190** in the cycloaddition reaction.

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,[129] and reduction using zinc in acetic acid,[130] or using lithium aluminium hydride.[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 asymmeteric unit and are linked via OH...H hydrogen bonds into a discrete dimer
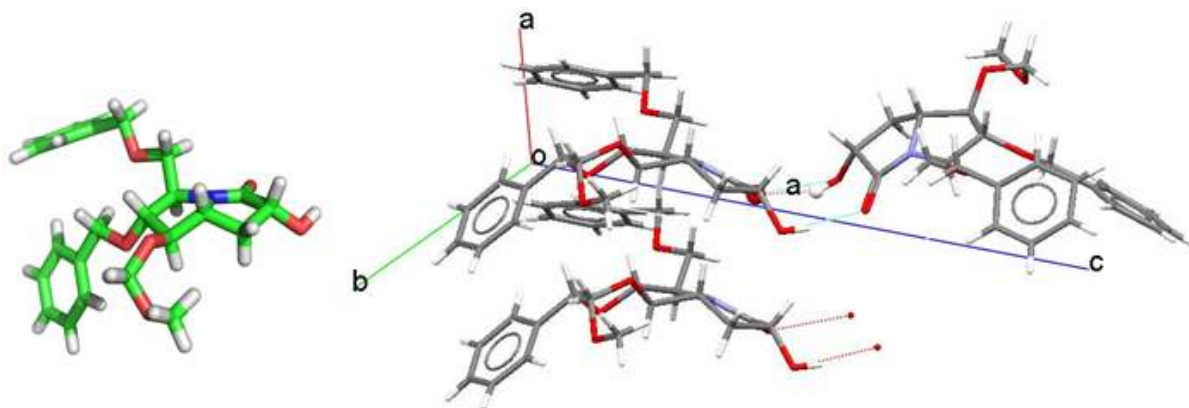
Figure 25: Crystal structure of **268**.

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 hydroxypyrrolizidinone (**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.[132] 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_1 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_2 (**52**) [Scheme 16].[109] Moreover, Izquierdo and co-workers had also successfully applied the redox inversion procedure to an analogous pyrrolizidinone intermediate (**146**) [Scheme 7].[86] 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.
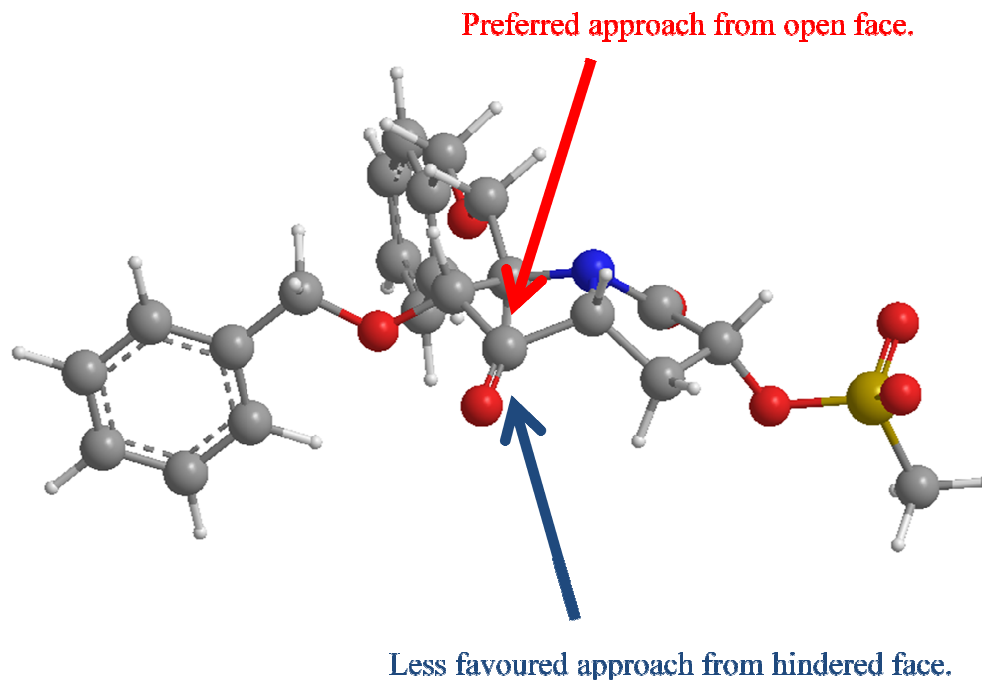
Figure 26: Expected preferred approach of reducing agent to ketone **271**.

Common reagents for the oxidation of a secondary alcohol to a ketone are potassium dichromate, Dess-Martin periodinane[133] and Swern conditions.[134] Initially Dess-Martin oxidation of alcohol **270** to ketone **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 **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 ^{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 **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 nsucessful and the alcohol was returned.
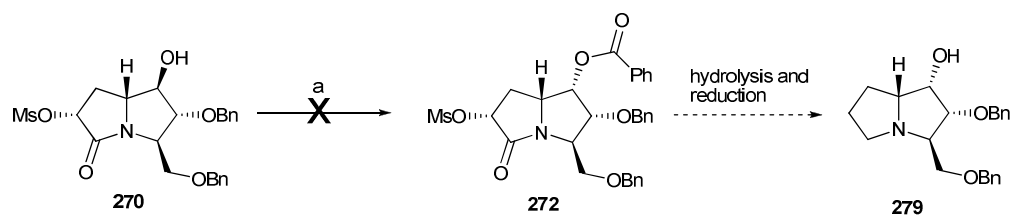
Scheme 33: (a) BzOH, PPh$_3$, DEAD, THF (0%).

As a last resort it was decided to further modify the planned route to hyacinthacine A$_1$ (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.
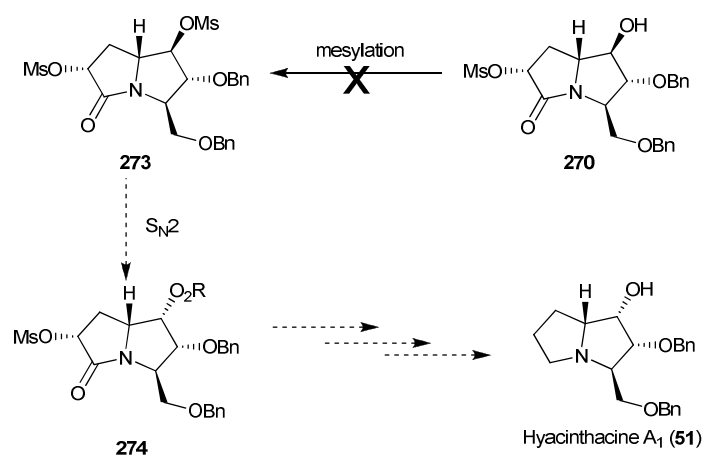
Scheme 34: MsCl, TEA, DCM (0%).

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.[86] This yielded the inverted alcohol (**147**) and starting alcohol (**146**) as 1.5:1 mixture which was separable by chromatography.
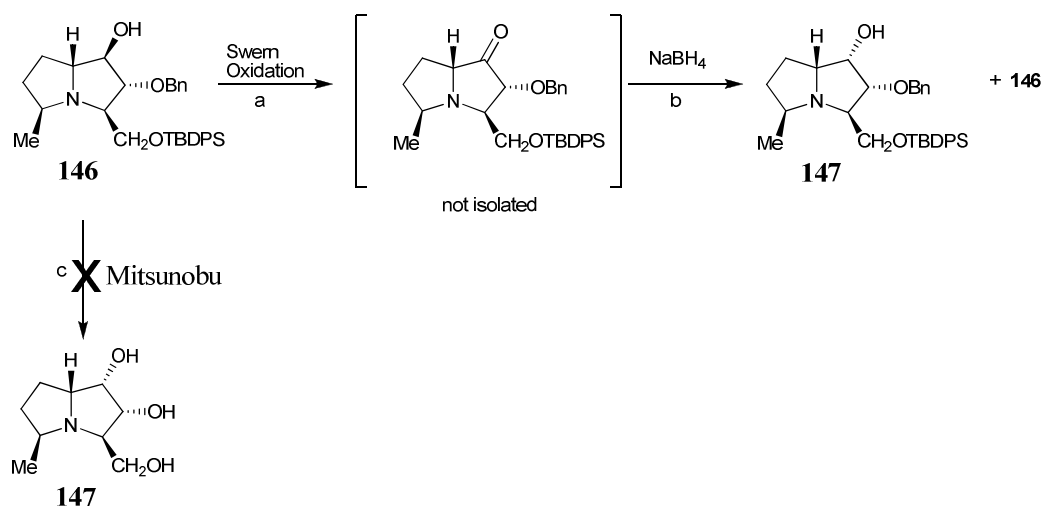
Scheme 35: (a) Swern oxidation, (not isolated); (b) NaBH$_4$, MeOH; **147** (46%) and **146** (29%) (1.5:1); (c) i. BzOH, DEAD, Ph$_3$P, THF, ii. MeONa (cat.), MeOH, (no reaction).

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 A_1 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 A_2 (**52**). Rather than commit our material to this synthesis however, we focused attention on the inversion chemistry required to access hyacinthacine A_1 (**51**).
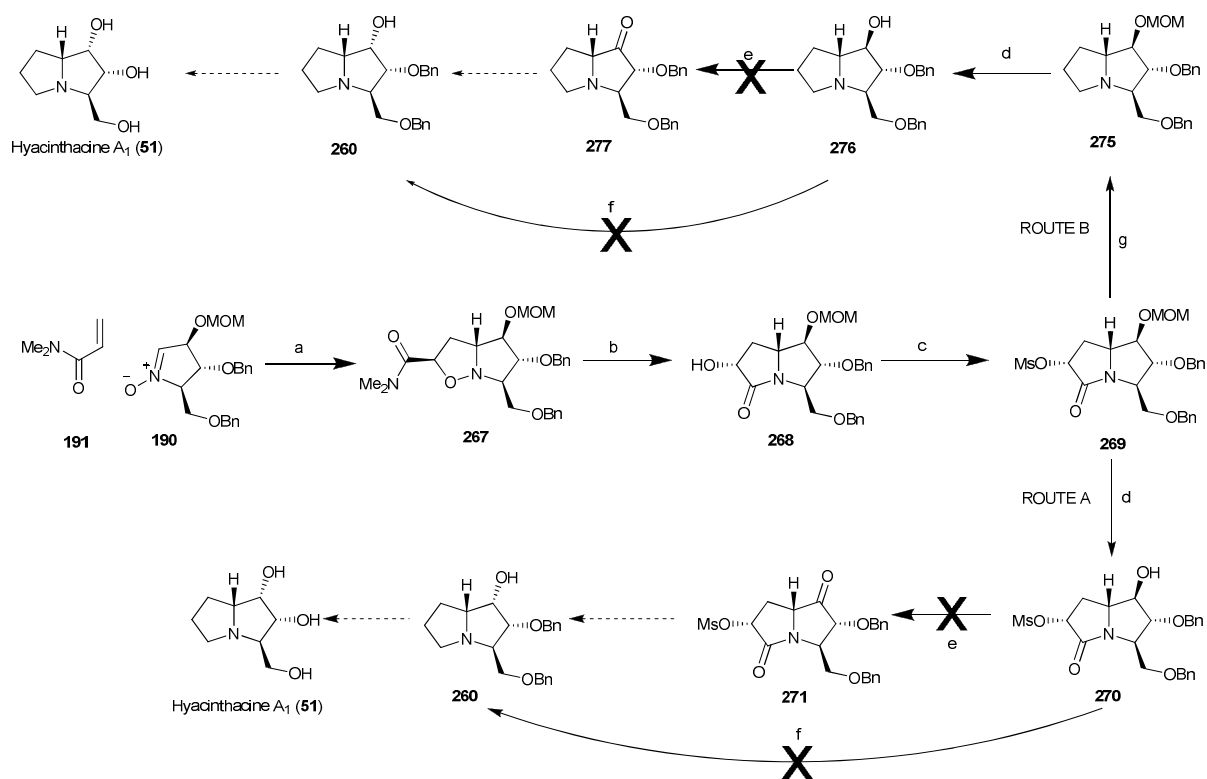
Scheme 36: (a) DCM, r.t., 2 h (100%); (b) Zn/AcOH, Δ, 3 h (57%); (c) MsCl, TEA, DCM, 0°C, 0.5 h (90%); (d) TFA, DCM, r.t., (87% for **270**, 86% for **276**); (e) Swern oxidation, (0%); (f) i. BzOH, DEAD, Ph$_3$P, THF, ii. MeONa (cat.), MeOH, (0%); (g) LiAlH$_4$, THF, Δ, 1.5 h (89%).

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 (PPh$_3$/DEAD/benzoic acid) but again no reaction occurred and alcohol **276** was returned unreacted.
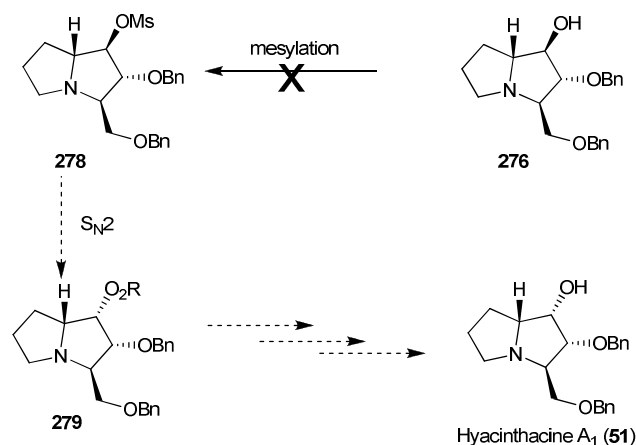
Scheme 37: Attempted mesylation of alcohol **253**; MsCl, TEA, DCM, 0 °C, (0%).

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 cycloadduct (**267**) prior to cleavage of the isoxazolidine N-O bond and pyrrolizidine formation.
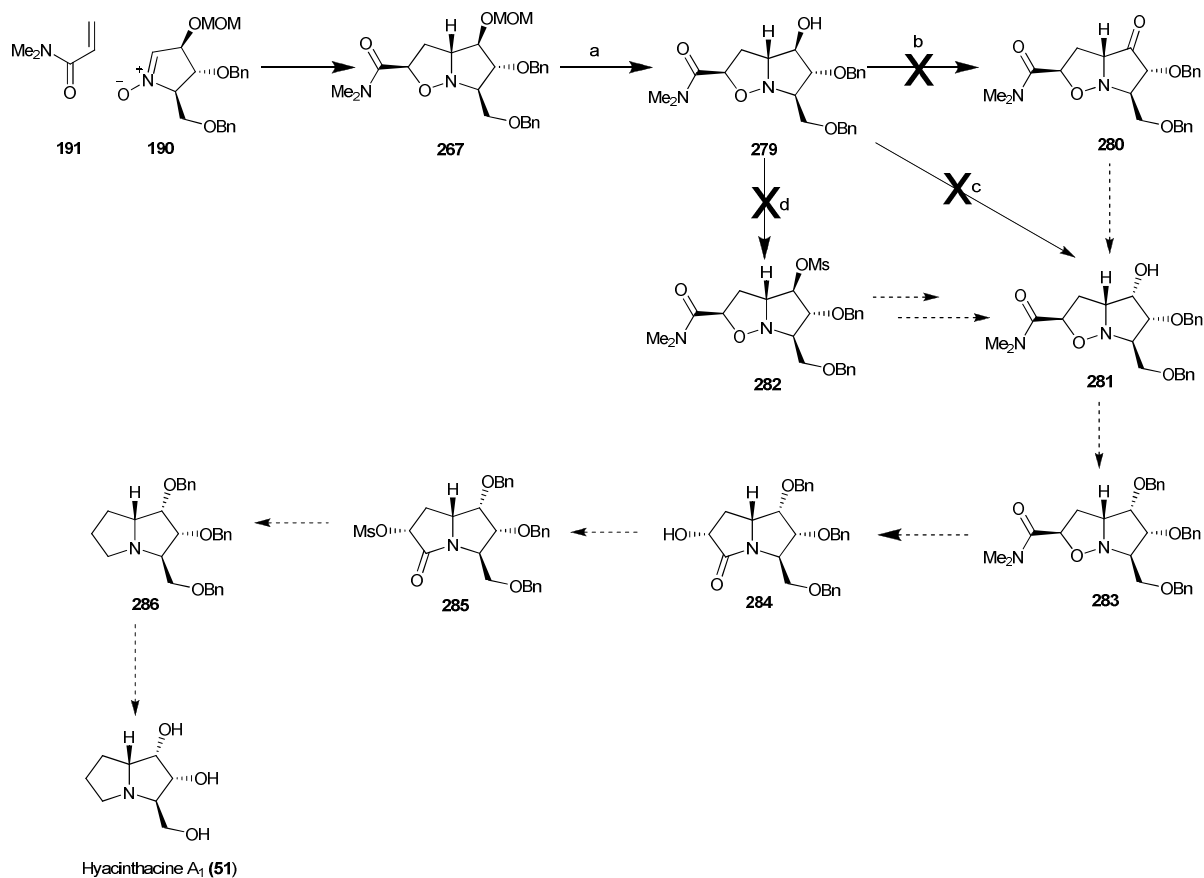
Scheme 38: Route C to Hyacinthacine A$_1$ (**51**); (a) TFA, DCM, r.t., (78%); (b) Swern oxidation, (no reaction); (c) i. BzOH, DEAD, Ph$_3$P, THF, (no reaction); ii. MeONa (cat.); (d) MsCl, DCM, TFA, (no reaction).

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 (PPh$_3$/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 A$_1$ (**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 proceeed. 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 S$_N$2 inversion and subsequent manipulation in mind, was similarly unsuccessful with each alcohol being returned unreacted. The failure of the inversion attempts on alcohols **270**, **276** and **279** means that differentially protected nitrone **190** is not a suitable precursor in the synthesis of hyacinthacine A$_1$ (**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).
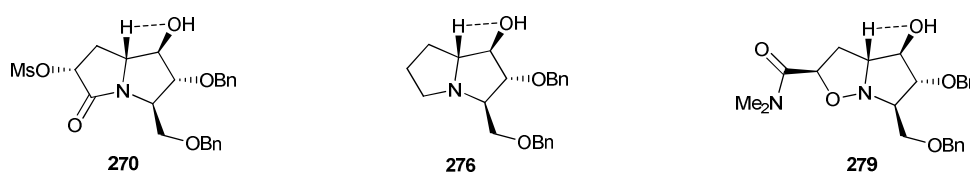
Figure 27: Substrates investigated for inversion showing potential hydrogen bonding possibly explaining the lack of reactivity of these compounds..

2.3.2   Routes to hyacinthacine B$_2$

Hyacinthacines B$_1$ (**48**) and B$_2$ (**49**) are identical to hyacinthacine A$_1$ (**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 B$_1$ and B$_2$ through cycloaddition with appropriate 4-carbon dipolarophiles. The strategy is illustrated in Figure 28 for hyacinthacine B$_2$, 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 B$_2$ (**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 B$_2$ 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 A$_1$, 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 B$_2$ (**293**), a polyhydroxylated pyrrolizidine that, to the best of our knowledge, has not previously been synthesised.
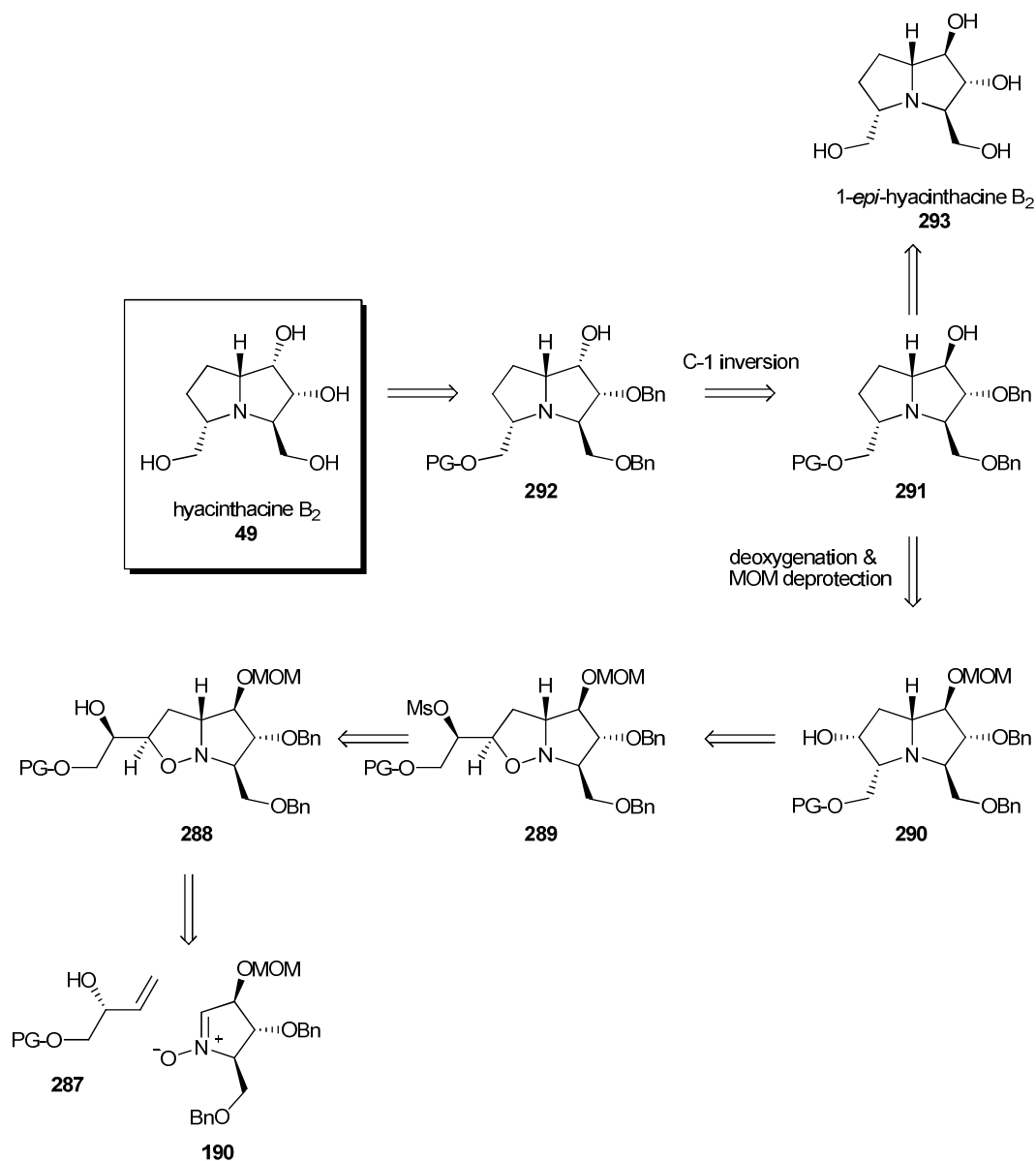
Figure 28: Potential route from nitrone **190** to hyacinthacine B$_2$.

Before commencing the route to hyacinthacine B$_2$, 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).
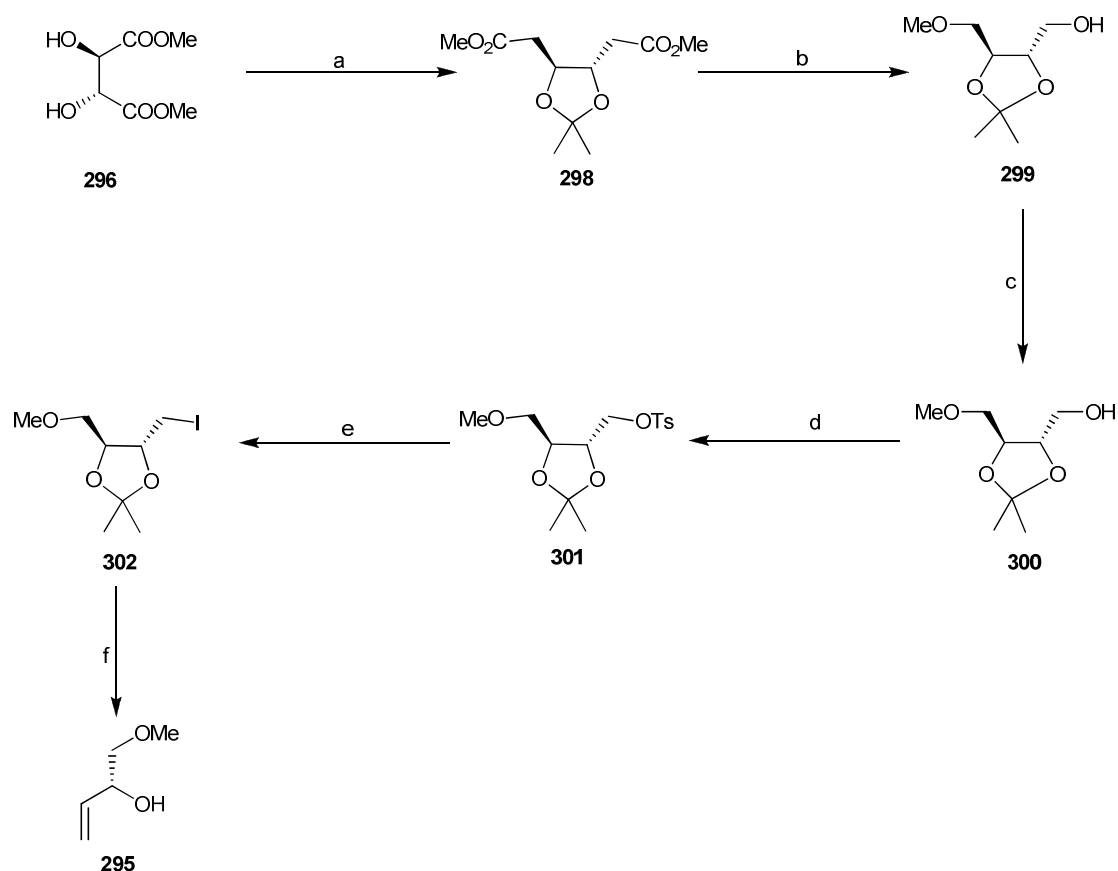
Scheme 39: (a) 2,2-dimethoxypropane, DCM, *p*-toluenesulfonic acid, Δ, 4 h, (85%); (b) LiAlH$_4$, Et$_2$O, -10 °C, 4 h, (70%); (c) NaH, MeI, DMF, r.t., 1 h, (65%); (d) TsCl, py, 0 °C, 1 h, (88%); (e) acetonitrile, NaI, Δ, 5 h, (93%); (f) Zn, EtOH, Δ, 5 h, (70%).

(*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 trimethylsulfonium ylide to give alcohol **295** directly (Scheme 40).
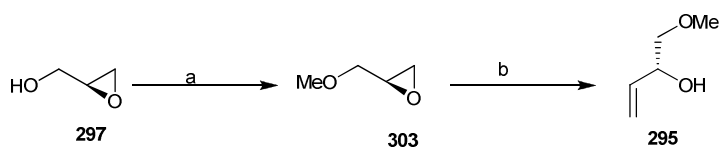
Scheme 40: (a) MeI, Ag$_2$O, DCM, Δ, 24 h, (60%); (b) Me$_3$S$^+$I$^-$, BuLi, THF, -15 °C, (76%).

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 B$_2$ (Scheme 41).
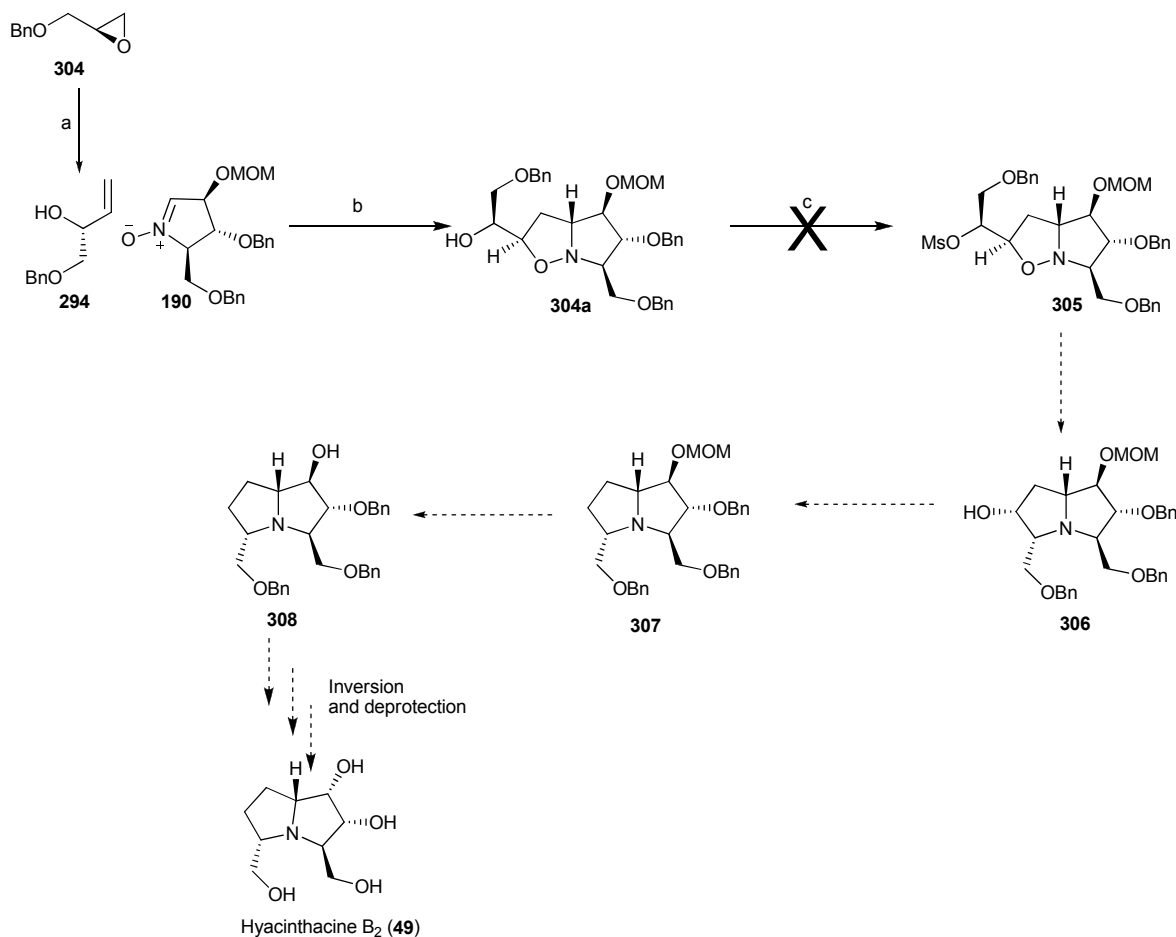
Scheme 41: (a) Me$_3$S$^+$I$^-$, BuLi, THF, -15 °C, (91%); (b) **190**, toluene, Δ, 24 h, (100% as inseparable mixture); (c) MsCl, TEA, DCM, 0 °C, 24 h (0%).

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

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 B_2 (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*).
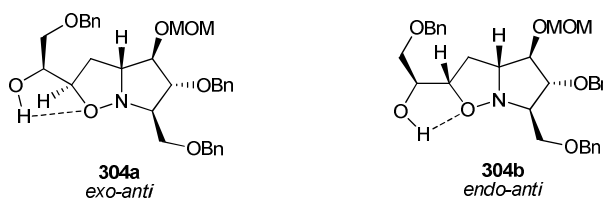
Figure 29: Structure of assumed cycloadducts **304a** and **304b** provided from the reaction of nitrone **190** with alkene **294**; an intramolecular hydrogen bond in the major adduct is tentatively proposed to account for the failure of the compound's conversion into its mesylate derivative.

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 B_2.
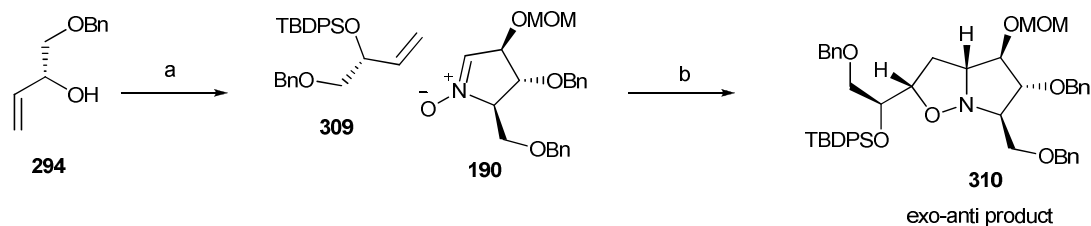
Scheme 42: (a) TBDPSCl, imidazole, THF, r.t., 24 h, (96%); (b) toluene, reflux, 24 h, (92%).

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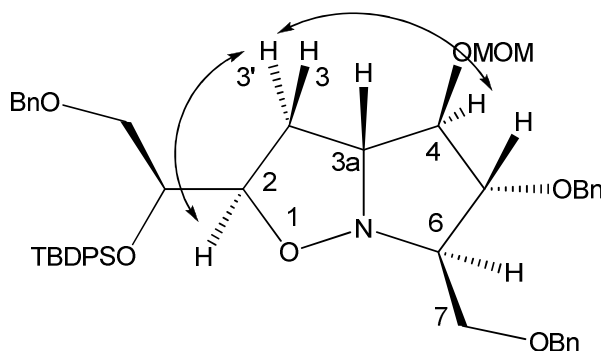
Figure 30: Key NOESY interactions in cycloadduct **310**.

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 B_2 (Scheme 41). The silyl ether was successfully cleaved under standard conditions with

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– firstly, 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 A_1, 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/PPh$_3$/DEAD), but again no reaction took place, and the configurationally inverted carboxylate intermediate was not formed. As with the attempted synthesis of hyacinthacine A_1, all inversion attempts of C-1 on alcohol **308** were unsuccessful.
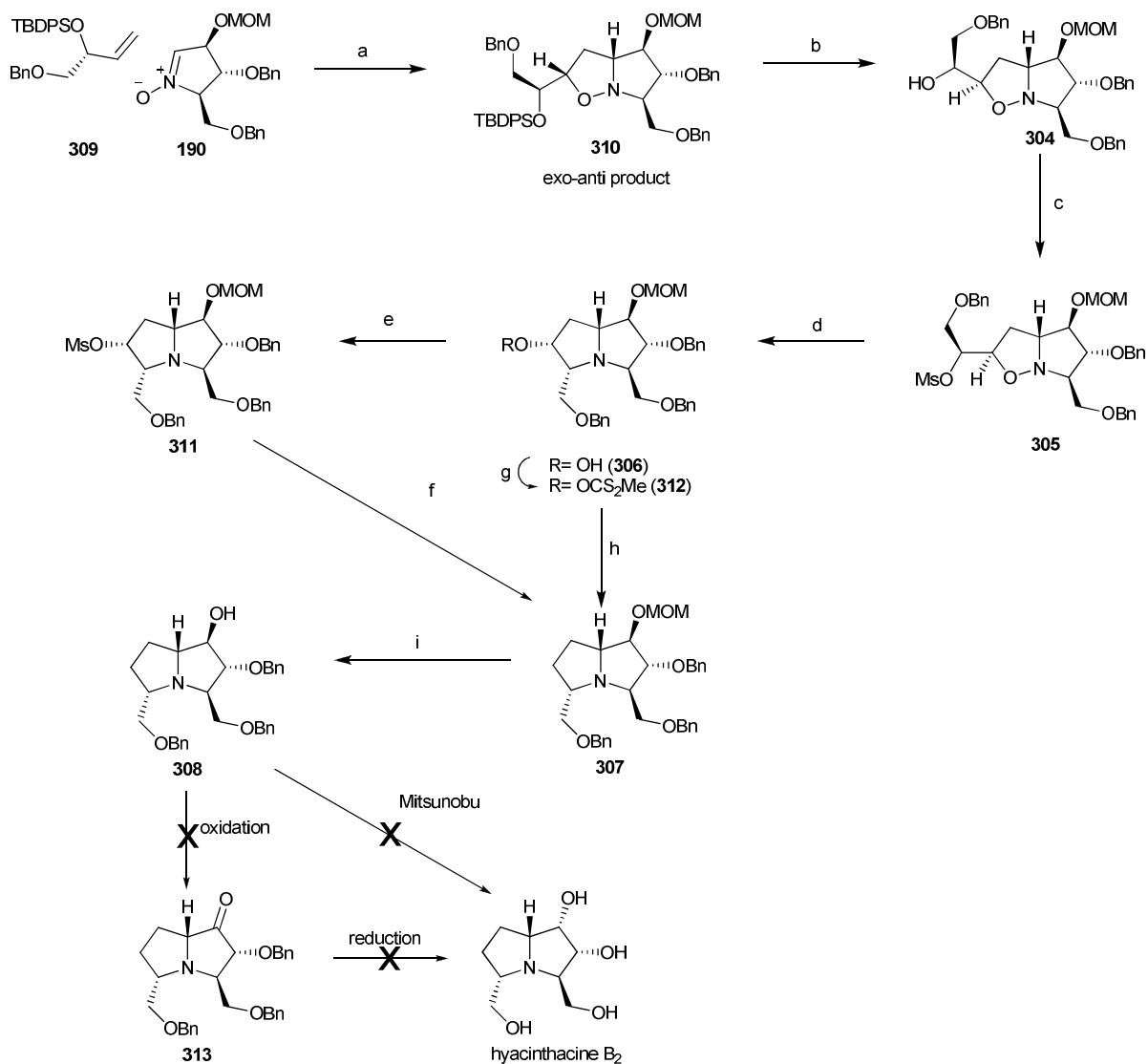
Scheme 43: Synthetic route to hyacinthacine B$_2$; (a) **309** and **190**, toluene, reflux, 18 h, (92%); (b) TBAF, THF, r.t., 1 h, (85%); (c) MsCl, TEA, DCM, r.t., (99%); (d) Zn dust, 1/1 acetic acid/water, 85 °C, (87%); (e) MsCl, TEA, DCM, r.t., (99%); (f) Super-Hydride®, THF,

r.t., 24 h, (10%); (g) KH, CS$_2$, MeI, THF, r.t., 20 min, (70%); h) AIBN, Bu$_3$SnH, toluene, reflux, 1 h, (59%); (i) TFA, DCM, r.t., 48 h, (81%).

2.3.3   Synthesis of 1-*epi*-hyacinthacine B$_2$.

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 B$_2$ (**293**) (Scheme 44). Initially the hydrogenation was attempted using a ThalesNano H-cube^{TM}, 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 B$_2$ (**293**), was obtained in 88% yield following ion exchange chromatography over a basic resin.
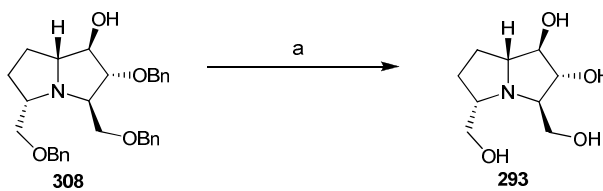
Scheme 44: (a) i. H$_2$/Pd/C, MeOH, HCl, r.t., 48 h; ii. Amberlite resin IR-400 (OH$^-$ form) (88%).

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.
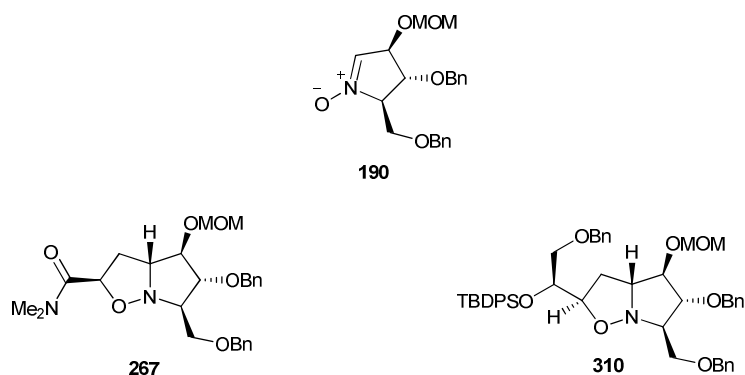
Figure 31

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 A_1 and B_2 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

(1*S*,2*R*,3*R*,7a*R*)-absolute stereochemistry in the right-hand ring of these hyacinthacine alkaloids.
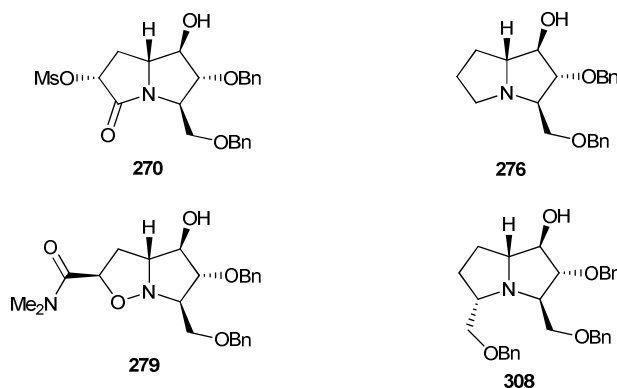
Figure 32. Substrates on which inversion was attempted.

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 A$_1$, 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 A$_1$ and B$_2$. 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 B$_2$ (**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 B$_2$, which has yet to be isolated from a natural source and would therefore be an interesting candidate for biological testing.
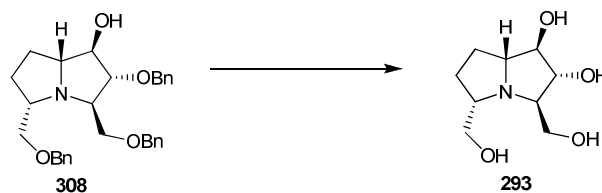
Figure 33: Formation of 1-epi-hyacinthacine B$_2$.

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).
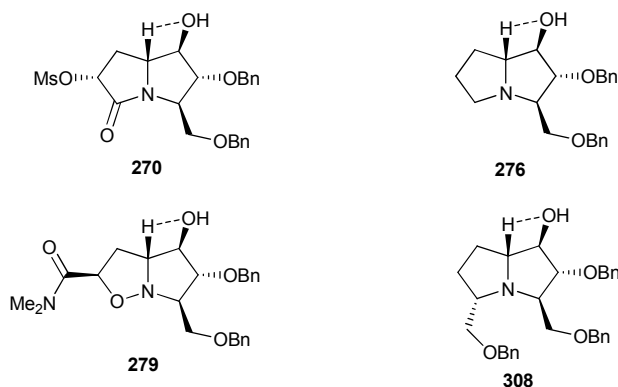
Figure 34: Potential Hydrogen bonding of invesion substrates.

Since the oxidation (Swern, and Dess-Martin) and inverstion stratagies (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 sustrates would provide novel compounds (Figure 35).
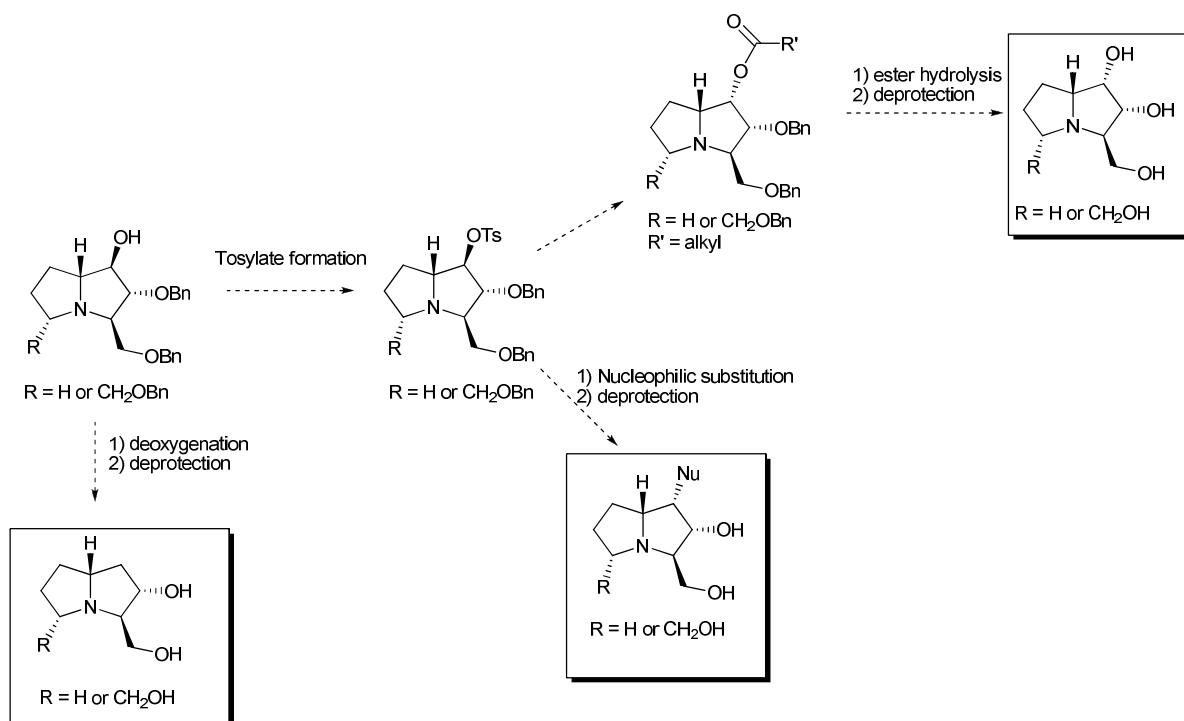
Figure 35: Further reactivity of inversion substrates to be explored leading to desired and alternative structures.

3   EXPERIMENTAL

Instrumentation

NMR spectra were recorded on a Bruker AC200 at 200 MHz (^{1}H) and 50 MHz (^{13}C), or a Bruker DPX400 at 400 MHz (^{1}H) and 100 MHz (^{13}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 ℃. 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-*O*-Isopropylidene-α-L-xylofuranose (234)
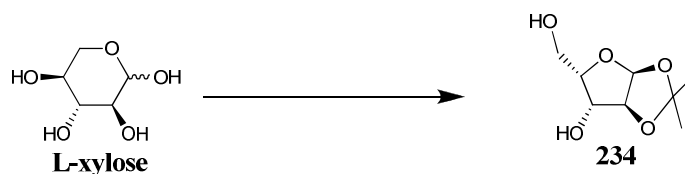
L-xylose (5.00g, 33.32 mmol) was added to acetone (130 mL) containing conc. H_2SO_4 (5 mL) and the mixture was stirred at room temperature for 30 minutes. A solution of Na_2CO_3 (6.50 g) in water (50 ml) was added with external cooling and the mixture was left to stir for a further 5 h. The mixture was then neutralised with Na_2CO_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)[136]; δ_H (200 MHz, CDCl$_3$): 1.23 (3H, s, CMe$_2$), 1.42 (3H, s,CMe$_2$), 2.83 (1H, b. s, OH-3), 3.93-4.19 (4H, m, C4-H, C5-H$_2$, 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); δ_c (50 MHz): 26.4 (CH$_3$), 27.0 (CH$_3$), 61.2 (CH$_2$), 77.0 (C-3), 79.1 (C-4), 85.8 (C-2), 105.1 (C-1), 112.1 (C); IR ν_{max} 3401.4 (br OH), 2988.7, 1377.4, 1217.6, 1165.4, 1074.3, 1016.8 cm^{-1}; LRMS (ES): 208.2, 191.1, 173.1, 150.1, 133.0, 115.1, 76.3; HRMS (ES): 208.1177 [(M+NH$_4$)$^+$; requires 208.1179]; Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.54. Found: C, 50.72; H, 7.53.

3,5-Di-*O*-benzyl-1,2-iso-*O*-propylidene-α-L-xylofuranose (235)
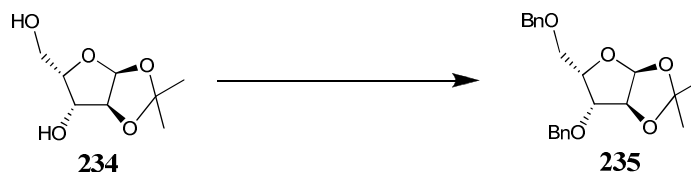
Compound **234** (4.10 g, 23.67 mmol) in THF (10 mL) was added to a suspension of NaH (60% dispersion in oil, 3.00 g, 25.60 mmol) and *n*-Bu$_4$NI (6.80 g, 18.41 mmol) in anhydrous THF (100 mL) at 0 °C. The mixture was stirred at this temperature for 20 min then allowed

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 MgSO$_4$, filtered and evaporated to produce an orange oil. The cude 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 0.36 (light petrol/ethyl acetate, 5:1); δ_H (200 MHz, CDCl$_3$): 1.29 (3H, s, CCH$_3$), 1.46 (3H, s, CCH$_3$), 3.75 (2H, m, C5-H$_2$), 3.96 (1H, d, J = 3.2 , C3-H), 4.27 (1H, dt, J = 6.1, 3.2, C4-H), 4.56 (5H, m, 2 x O*CH$_2$*Ph, C2-H), 6.18 (1H, d, J = 3.05 , C1-H), 7.47-7.51 (10H, m, Ph); δ_c (50 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 ν_{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 cm^{-1}. Anal. Calcd for C$_{22}$H$_{26}$O$_5$: C, 71.33; H, 7.07. Found: C, 71.02; H, 7.01.

3, 5-Di-*O*-benzyl-L-xylofuranose (229) [113]
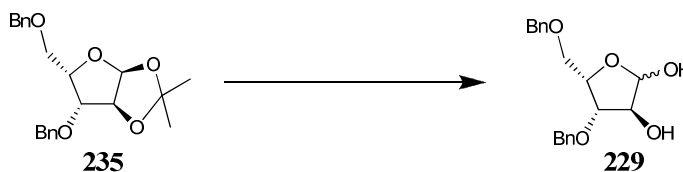
A stirred solution of 3,5-di-*O*-benzyl-1,2-iso-*O*-propylidene-α-L-xylose **235** (4.01 g, 10.81 mmol) in acetic acid-water (1:1, 30 mL) was heated to 80 °C and stirred for 24 h. The mixture was cooled, diluted with water (30 ml), and extracted with DCM (60 ml). The organic layer was washed with saturated sodium bicarbonate (20 ml), brine (20 ml), dried over Na$_2$SO$_4$, filtered and concentrated to give the diol **229** (3.09 g, 86 %) as a white wax.

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

4.33-4.61 (4H, m, 2 x OCH_2Ph), 5.12 (α-anomer, s, C1-H), 5.39 (β-anomer, d, J = 3.3, C1-H), 7.13-7.33 (10H, m, Ph); δ$_c$ (50 MHz): 68.5 & 68.7 (α & β CH$_2$), 71.6 & 72.5 (α & β CH$_2$), 73.2 & 73.4 (α & β CH$_2$), 77.24 & 79.1 (α & β CH), 82.5 & 83.2 (α & δ CH), 95.7 (α C1-H), 103.2 (δ CH), 127.3, 127.4, 127.5, 127.6, 127.8, 128.1 & 128.3 (CH$_{ar}$), 137.5 & 137.6 (c); IR ν$_{max}$ 3365.8 (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^{-1}. LRMS (ES): 348.3, 330.3, 240.3, 198.2, 168.2, 108.2, 91.2; HRMS (ES): 348.1805 [(M+NH$_4$)$^+$; requires 348.108]. Anal. Calcd for C$_{19}$H$_{22}$O$_5$: C, 69.07; H, 6.71. Found: C, 68.88; H, 6.66.

3,5-Di-*O*-benzyl-L-xylono-1,4-lactone (236)
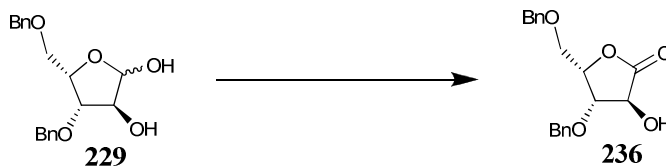
Method 1:

Barium carbonate (490 mg, 2.48 mmol) and bromine (100 µL, 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$^®$ 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$_2$CO$_3$/Celite$^®$ (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

chromatograpy, 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** (1.32g, 3.99 mmol) and Ag_2CO_3/Celite[®] (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 (100ml). The filtrate was then evaporated and the residue was purified by flash chromatograpy, 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 recrystalised 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; δ_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,), 3.53 (2H, 2 dd, $J = 10.9, 2.7$, C5b-H), 4.15-4.72 (6H, m, 2 O*CH$_2$*Ph, C3-H, C4-H), 7.02-7.25 (10H, m, Ph); δ_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 ν_{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_2CO_3/Celite[®] reagent[124]

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 (500ml). 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_2CO_3.

3,5-Di-*O*-benzyl-2-*O*-methoxymethyl-L-xylono-1,4-lactone (237)

236   **237**
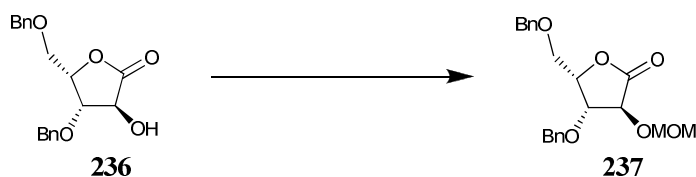
To a stirred solution of **236** (0.23g, 0.97 mmol) in chloroform (22 mL) at ambient temperature was added dimethoxymethane (4.40 mL, 48.41 mmol) and phosphorous pentoxide (20.24 g, 5.72 mol). The mixture was stirred for 30 min, the solids decanted and the filtrate poured in to an ice cold solution of 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 Na_2SO_4, and evaporated in *vacuo* to give the ether **237** (354 mg, 98 %) as a yellow oil.

R_f 0.76 (light petroleum/ethyl acetate 1:1); δ_H (200 MHz, $CDCl_3$): 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_2Ph, OCH_2O, C3-H, C4-H), 4.93 (1H, d, J=6.65, C2-H), 7.01-7.29 (10H, m, Ph); δ_c (50 MHz): 55.81 (OCH_3), 66.66 (C-5), 71.92 (OCH_2), 72.59 (OCH_2), 73.22 (C-3), 76.98 (C-2), 79.14 (C-4), 95.74 (OCH_2O), 127.22, 127.38, 127.44, 127.67, 127.82, 128.06, 128.16 & 128.24 (CH_{ar}) 136.78 (C), 137.20 (C), 172.595 (C=O); IR ν_{max} 2898.3, 1789.9, 1496.5, 1453.8, 1244.8, 1165.8, 1123.7, 1023.3, 736.8 cm^{-1}. 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+NH$_4$)$^+$; $C_{21}H_{28}NO_6$ requires 390.1911]. Anal. Calcd for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 67.91; H, 6.78.

3,5-Di-*O*-benzyl-2-*O*-methoxymethyl-L-xylofuranose (232)[113]

237   **232**

110
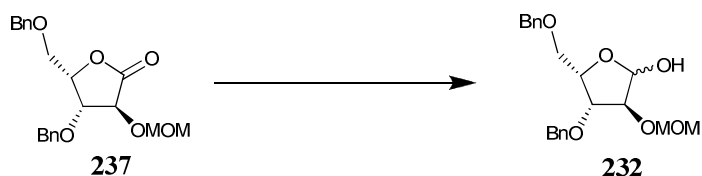
Lactone **237** (1.00 g, 2.69 mmol) was dissolved in THF (80 mL) and cooled to -78 °C. A solution of DIBAL in toluene (1.5M, 3.60 mL, 5.40 mmol) was added dropwise over 30 min and the mixture allowed to stir for 1.5 h. A further portion of the DIBAL solution (5.00 mL, 7.50 mmol) was then added dropwise 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 (light petroleum/ethylacetate 1:1); δ_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$_2$Ph & OCH$_2$O), 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); δ_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$_2$O), 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 ν_{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^{-1}.

3,5,-Di-*O*-benzyl-2-*O*-methoxymethyl-L-xylose oxime (238)
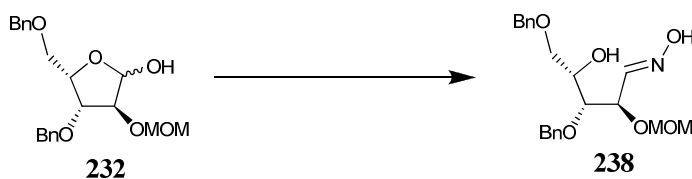
A solution of Lactol **232** (158 mg, 0.422 mmol), hydroxylamine hydrochloride (553 mg, 7.91 mmol), and sodium methoxide (442 mg, 7.92 mmol) were stirred in methanol (15 mL) for 16 h at ambient temperature. The reaction mixture was then concentrated *in vacuo* and the residue was dissolved in DCM (20ml). The resulting solution was washed with water (2 x 20ml) and the combined organic layers were dried over Na$_2$SO$_4$ and filtered. The filtrate was concentrated *in vacuo* to give **238** (134mg, 82 %) as a dark yellow oil.

R_f 0.27 (light petroleum/diethyl ether 1:1); δ_H (200 MHz, CDCl$_3$): 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 (1H, m, C4-H), 3.75 -3.91 (1H, m, C3-H), 4.09-4.23 (1H, m, C2-H), 4.28-4.77 (6H, m, 2 x O*CH$_2$*Ph, O*CH$_2$*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); δ_c (50 MHz): 55.6 (*E*-isomer, OCH$_3$), 55.9 (*Z*-isomer, OCH$_3$), 68.9 (CH), 69.6 (CH), 70.6 (*Z*-isomer, CH$_2$), 71.0 (*E*-isomer, OCH$_2$), 73.2 (OCH$_2$), 74.4 (*E*-isomer, CH$_2$), 74.8 (*Z*-isomer, CH$_2$), 78.5 (CH), 94.9 (*E*-isomer, OCH$_2$O), 95.8 (*Z*-isomer, OCH$_2$O), 127.6, 127.8, 128.2 & 128.2 (CHar), 137.6 (*E*-isomer, C), 137.6 (*Z*-isomer, C); IR ν_{max} 3327.7 (br. OH), 3080.4, 3024.8, 2865.8, 1458.8, 1093.1, 1067.4, 732.6, 695.7 cm^{-1}.

3,5-Di-*O*-benzyl-2-*O*-methoxymethyl-L-xylose-*O*-(*tert*-butyldimethylsilyl)oxime (239)

Method 1:

238   **239**
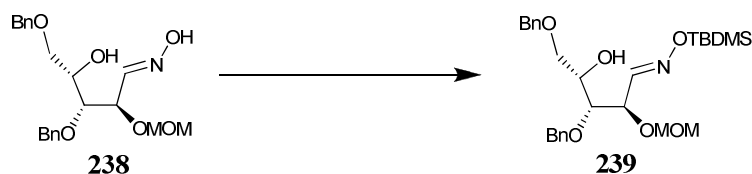
Oxime **238** (118 mg, 304 mmol) was dissolved in dry pyridine (35 mL) and *tert*-butyldimethylsilyl choride (46 mg, 304 mmol) was added. The mixture was allowed to stir for 12 h at ambient temperature. Concentration left a residue which was dissolved in DCM (50 ml) and washed with water (2 x 20 ml). The organic layers were washed with 1M HCl (5 ml), Sodium Bicarbonate (10ml) and brine (20 ml) before being dried over MgSO$_4$, filtered and evaporated *in vacuo* to give **239** (0.262 g, 34 %) (E:Z, 5:1) as a white/green oil.

Method 2:

232   **239**
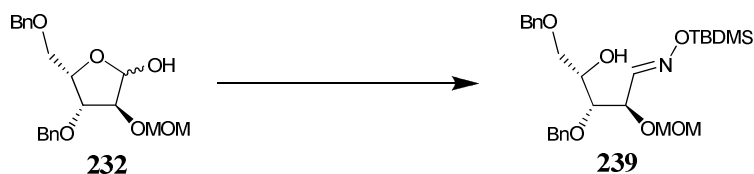
To a stirred solution of lactol **232** (577 mg, 1.54 mmol) in dry toluene (6 mL) was added MgSO$_4$ (670 mg). The suspension was brought to reflux for 5 min then *tert*-butyldimethylsilylhydroxylamine (340 mg, 1.54 mmol) was added. The reaction mixture was left at reflux for a further 30 min before being allowed to cool. The filtrate was washed with saturated sodium bicarbonate (5ml), brine (10ml) then dried over MgSO$_4$ and fitered. The filtrate was concentrated to give **239** (0.114 g, 89%) (E:Z, 5:1) as a yellow/green oil.

R$_f$ 0.68 (light petroleum/diethyl ether 1:1); δ$_H$ (200 MHz, CDCl$_3$): 0.00 (6H, s, SiMe$_2$), 0.78 (9H, s, SiCMe$_3$), 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 OCH$_2$Ph & OCH$_2$O), 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 CH$_3$), 17.9 (*C*Me$_3$), 25.8 (3 x CH$_3$), 55.6 (OCH$_3$), 69.1 (CH-4), 69.9 (CH$_2$), 73.1 (CH$_2$), 73.8 (CH-3), 74.1 (CH$_2$), 77.5 (CH-2), 94.7 (OCH$_2$O), 127.5, 127.6, 127.7, 128.0 & 128.2 (CH$_{ar}$), 137.5 & 137.7 (C), 152.0 (CH-1); 148.8 (*E*-isomer, H-1), 150.0 (*Z*-isomer, H-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*-butyldimethylsilylhydroxylamine[125]

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*-butyldimethylsilylhychloride (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, CDCl$_3$): 0.00 (6H, s, SiMe$_2$), 0.79 (9H, s, CMe$_3$), 4.98 (2H, s, br. NH$_2$).

3,5-Di-*O*-benzyl-4-*O*-methanesulfonyl-2-*O*-methoxymethyl-L-xylose-*O*-(*tert*-butyldimethylsilyl) oxime (240)[113]

239 → **240**
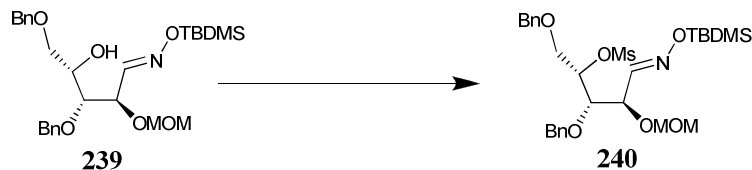
Compound **239** (0.26 g, 0.52 mmol) was dissolved in DCM (1.5 mL), cooled to 0 °C and stirred under an argon atmosphere. Triethylamine (113 µL, 0.81 mmol), and methanesulfonyl chloride (49 µL, 0.62 mmol) were added successively. The mixture was stirred for 30 min at 0 °C and then water (0.5 mL) was 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$_4$, filtered and evaporated to give **240** (0.270 g, 93%) (E:Z, 5:1) as a yellow oil.

R_f 0.72 (light petroleum/diethyl ether 1:1); δ_H (200 MHz, CDCl$_3$): 0.00 (6H, s, SiMe$_2$), 0.79 (9H, s, CMe$_3$), 2.78 (3H, s, SO$_2$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$_2$Ph, OCH$_2$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_c (50 MHz): (*E*-isomer) -7.9 (2 x CH$_3$), 18.5 (*C*Me$_3$), 26.4 (3 x CH$_3$), 38.7 (SCH$_3$), 56.6 (OCH$_3$), 68.9 (C-5), 73.6 (CH), 73.80 (OCH$_2$), 75.5 (OCH$_2$), 79.1 (CH), 81.2 (CH) 94.7 (OCH$_2$O), 128.3, 128.3, 128.5 128.7 & 128.9 (CH$_{ar}$), 137.6 & 137.7 (C), 152.62 (CH-1); IR ν_{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^{-1}.

5-*O-tert*-Butyldiphenylsilyl-D-arabinofuranose (248)[137]
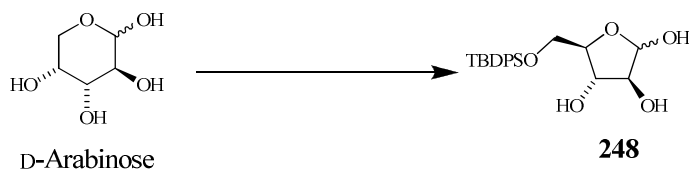
D-Arabinose → **248**

D-Arabinose (7.67 g, 51.06 mmol) was added to a solution of *tert*-butyldiphenylsilyl 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 $MgSO_4$, 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.

R_f 0.10 (3:2 Light petroleum:EtOAc); δ_H (200 MHz): 1.03 (9H, s, α-anomer C*Me*$_3$), 1.05 (9H, s, β-anomer, C*Me*$_3$), 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, CH$_{ar}$), 7.62-7.74 (2x 4H, m, CH$_{ar}$); δ_C (50 MHz): 18.9 (α-anomer CSi), 19.0 (β-anomer CSi), 26.6 (α-anomer C*Me*$_3$), 26.7 (β-anomer C*Me*$_3$), 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 (CH$_{ar}$), 131.6 & 131.8 (α-anomer C), 132.0 & 132.2(β-anomer C), 135.5 & 135.5 (CH$_{ar}$); IR υ_{max} 3400.3 (br. OH), 3072.3, 2931.7, 2858.5, 1659.9, 1428, 1113.2, 1069.4, 702.7 cm^{-1}.

5-*O*-(*tert*-Butyldiphenylsilyl)-1,2-*O*-isopropylidene-β-D-arabino-furanose (249)[136]
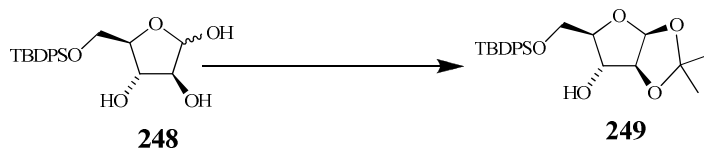
Anhydrous copper (II) sulfate (7.97 g, 49.93 mmol) and sulfuric acid (sp. gr. 1.84, 0.36 mL, 6.40 mmol) were added to a solution of **248** (7.02 g, 18.07 mmol) in anhydrous acetone (80 mL). After stirring for 17 h at ambient temperature, the blue reaction mixture was filtered and neutralised with ammonia 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_f 0.32 (4:1 Light petroleum:EtOAc); δ_H (200 MHz): 1.06 (9H, s, CMe$_3$), 1.28 & 1.32 (2x 3H, 2s, CMe$_2$), 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$_{ar}$), 7.62-7.70 (4H, m, CH$_{ar}$); δ_C (50 MHz): 19.1 (C-Si), 26.0 and 26.7 (CMe$_2$), 63.5 (CH$_2$), 76.2 (C-4), 86.9 (C-3), 87.3 (C-2), 105.5 (C-1), 112.4 (CMe$_2$), 127.7 (CH), 129.7 (CH), 133.0 (C), 135.5 (CH); IR υ_{max} 3452.2 (br. OH), 3071.9, 2932.8, 2858.3, 1428.1, 1113.6, 1066, 1018.5, 702.7 cm^{-1}.

1,2-*O*-Isopropylidene-β-D-arabinofuranose (250)[136]

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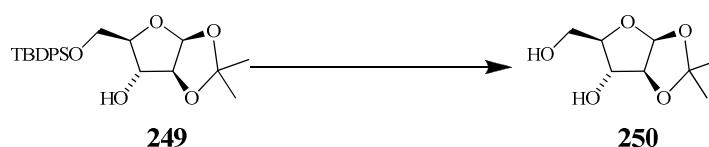
To a solution of **249** (2.88 g, 6.72 mmol) in dry THF (120 mL) was added tetra-*n*-butylammonium fluoride in THF (1M, 13.44 mL, 13.44 mmol). The reaction mixture was stirred for 1 h at ambient temperature, then evaporated to give a brown oil. The crude residue was purified by flash chromatography (100%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

R_f 0.34 (100% EtOAc); m.p.: 113-116 ℃ (no lit. m.p given); δ_H (200 MHz, CD$_3$OD): 1.30 & 1.48 (2x 3H, 2s, CMe$_2$), 3.68 (2H, d, J =6.8, C5-H), 3.97 (1H, dt, J_4 = 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_C (50 MHz, CD$_3$OD): 26.3 (CH$_3$), 27.2 (CH$_3$), 63.5 (C-5), 76.6 (CH), 88.4 (CH), 89.9 (CH), 107.2 (C-1), 113.5 (C); IR υ_{max} 3391.7 (br. OH), 2940.2, 1378.4, 1213.6, 1163.3, 1066.1, 1015.1 cm^{-1}.

3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-β-D-arabinofuranose (251)[136]

250 → **251**
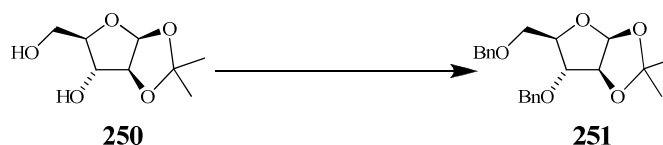
To an ice cold solution of **250** (5.52g, 31.52 mmol) in DMF (100 mL) was added NaH in mineral oil (60% w/w, 3.64 g, 91.0 mmol). The mixture was stirred for 15 min, allowed to warm to ambient temperature and stirred for a further 15 min. Tetrabutylammonium iodide (11.2 g, 30.32 mmol) was added, followed 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 Na_2SO_4, 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, CMe_2), 3.64 (2H, d, *J* 6.2, C5-H), 4.03 (1H, d, *J* = 3.3, C3-H), 4.27 (1H, dt, *J* = 6.2, 2.9, C4-H), 4.54 (1H, d, *J* = 12.0, O*CH*$_2$Ph), 4.56 (1H, d, *J* = 11.6, O*CH*$_2$Ph), 4.60 (1H, d, *J* = 12.0, O*CH*$_2$Ph), 4.63 (1H, d, *J* = 11.6, O*CH*$_2$Ph), 4.63 (1H, d, *J* = 3.7, C2-H), 5.90 (1H, d, *J* = 3.7, C1-H), 7.22-7.37 (10H, m, Ph); δ_C (50 MHz): 26.2 (CH_3), 26.9 (CH_3), 69.9 (C-5), 71.5 (CH_2), 73.2 (CH_2), 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 (CH_{ar}), 137.2 & 137.9 (C); IR υ_{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]
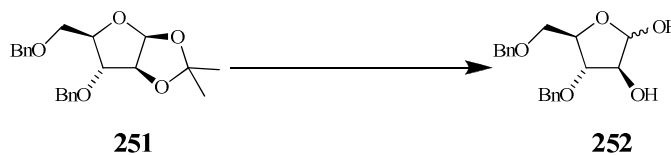
A solution of the isopropylidene derivative **251** (6.60 g, 17.83 mmol) in acetic acid-water (1:1, 50 mL) was heated at 60 ºC for 5 h. The reaction mixture was cooled to ambient temperature, diluted with water (125 mL), and extracted with DCM (2 x 125 mL). The combined organic layers were washed with saturated sodium bicarbonate (125 mL) and brine (125 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give the diol **252** (5.38 g, 92%) (1:1, α:β) as a colourless syrup.

R_f 0.56 (1:1 Light petroleum:EtOAc); δ_H (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, OCH_2Ph), 5.24 (α-anomer, s, C1-H), 5.30 (β-anomer, d, J = 3.3, C1-H), 7.15-7.33 (10H, m, Ph); δ_C (50 MHz): 69.1 & 69.9 (α and β CH_2), 71.4 & 71.8 (α and β CH_2), 73.5 (CH_2), 75.4 & 76.2 (α and β CH), 80.7, 82.9, 83.2 & 84.2 (α and β 2x CH), 97.3 (β-anomer C-1), 103.6 (α-anomer C-1), 127.3, 127.5, 127.8, 128.0 & 128.2 (CH_{ar}), 136.1, 136.1, 136.3 & 137.1 (C); IR υ_{max} 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]

252   **253**
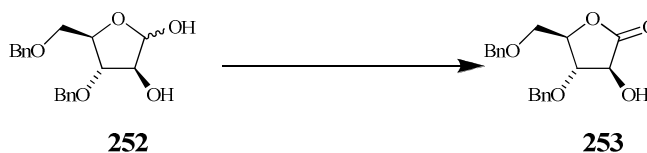
To an ice cold solution of **252** (3.90 mg, 12 mmol) in THF-water (1:3, 200 mL), was added barium carbonate (39 g, 19 mmol) and bromine (8 mL, 16 mmol). The reaction mixture was allowed to warm to ambient temperature and was left stirring overnight. The mixture was filtered through Celite$^®$ and the 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.

R_f 0.36 (7:3 Light petroleum:EtOAc); m.p.: 83-85 ºC; δ_H (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, $J_{3,2}$ = 6.6, C3-H), 4.34 (1H, ddd, J= 6.6, 3.7, 2.5, C4-H), 4.43 (1H, d, J =12.0, OCH_2Ph), 4.44 (1H, dd, J = 6.6, C2-H) 4.50 (1H, d, J = 12.0, OCH_2Ph), 4.52 (1H, J = 11.6, OCH_2Ph), 4.72 (1H, J = 11.6, OCH_2Ph), 7.17-7.32 (10H, m, Ph); δ_C (50 MHz): 67.9 (C-5), 72.5 (CH_2), 73.7 (CH_2), 74.3 (C-4), 79.8 (C-3), 80.2 (C-2), 127.9, 128.1, 128.2 & 128.6 (CH_{ar}), 137.1 (C), 137.2 (C), 174.6 (C=O); IR υ_{max} 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)[136]

253   **254**
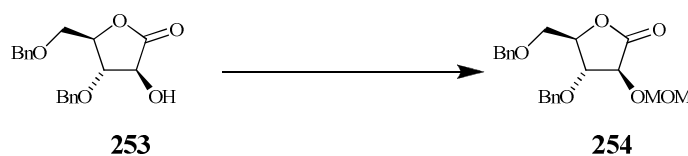
To a solution of **253** (3.00 g, 8.8 mmol) in dry chloroform (200 mL) at ambint temperature was added dimethoxymethane (40 mL, 440 mmol) and phosphorus pentoxide (184 g, 52.8 mmol). After stirring for 30 min, the mixture was decanted and the filtrate poured into an ice-cold saturated sodium bicarbonate 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_2SO_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); $[\alpha]_D$ +23.88 (*c* 0.49 $CHCl_3$); δ_H (200 MHz): 3.47 (3H, s, OMe), 3.56 (1H, dd, *J* = 11.2, 3.7, C5-H$_a$), 3.71 (1H, dd, *J* =11.2, 2.9, C5-H$_b$), 4.29-4.37 (2H, m, C2-H& C3-H), 4.49-4.77 (5H, m, C4-H & O*CH$_2$*Ph), 4.75 (1H, d, *J* =6.6, O*CH$_2$*O), 5.09 (1H, d, *J* =6.6, O*CH$_2$*O), 7.18-7.37 (10H, m, Ph); δ_C (50 MHz): 55.9 (OCH_3), 67.6 (C-5), 72.6 (CH_2), 73.2 (CH_2), 76.1 (CH), 78.7 (CH), 79.1 (CH), 95.7 (OCH_2O), 127.4, 127.6, 127.9, 128.2 & 128.2 (CH$_{ar}$), 126.9 (C), 137.1 (C), 171.8 (C=O); IR υ_{max} 2899.3, 1791.8, 1454.9, 1211.6, 1151.4, 1123.7, 1026.4, 739.4, 689.6 cm^{-1}.

3,5-Di-*O*-benzyl-2-*O*-methoxymethyl-D-arabinofuranose (255)[136]
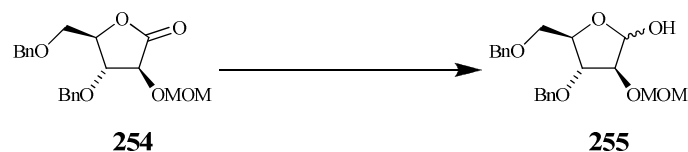
254   **255**

Lactone **254** (2.52 g, 9 mmol) was dissolved in anhydrous THF (120 mL) and cooled to -78 ºC. 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®, 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_f 0.43 (1:1 Light petroleum:EtOAc); δ_H (200 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 O*CH₂*Ph & O*CH₂*O), 5.17-5.32 (1H, m, C1-H); δ_C (50 MHz): 55.7 & 55.9 (α and β OCH_3), 70.0 & 70.2 (α and β C-5), 71.9, 72.2, 73.3 & 73.5 (α and β 2x CH_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_2O), 101.6 (α-anomer C-1), 127.7, 127.9, 128.3, 128.5 & 128.5 (CH_{ar}), 137.3 & 137.8 (C); IR υ_{max} 3413.9 (br. OH), 2926.7, 1453.8, 1366, 1151.9, 1104.7, 1035.2, 738.3, 698.2 cm^{-1}.

3,5-Di-*O*-benzyl-2-*O*-methoxymethyl-D-arabinose-*O*-(*tert*-butyldimethylsilyl)oxime (256)[136]
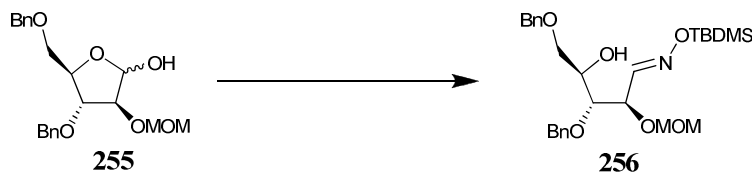
255　　　　**256**

To a solution of Lactol **255** (2.5 g, 6.68 mmol) in dry toluene (35 mL) was added $MgSO_4$ (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 $MgSO_4$ 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_f 0.42 (light petroleum:EtOAc 1:1); δ_H (200 MHz, CDCl₃): δ_H (200 MHz): 0.11 (6H, SiMe₃, *E*-isomer), 0.13 (6H, SiMe₃, *E*-isomer), 0.81 (9H, s, *Z*-isomer, 3x CH₃), 0.85 (9H, s, *E*-isomer, 3x CH₃), 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, O*CH$_2$*Ph), 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); δ_C (50 MHz): 25.4 (*C*Me$_3$, *Z*-isomer), 25.5 (*C*Me$_3$, *Z*-isomer), 55.5 (O*C*H$_3$) 68.9 (C-4), 69.9, 70.8, 73.3 & 74.0 (CH$_2$), 75.9 (CH), 79.9 (CH), 94.6 & 96.1 (2 x O*C*H$_2$O *E*- and *Z*-isomers), 125-140 (CH$_{ar}$), 154.1 (C-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}.

(2*R*,3*S*,4*S*,*E*)-3,5-Bis(benzyloxy)-4-iodo-2-(methoxymethoxy)pentanal *O*-tert-butyldimethylsilyl oxime (257)[136]
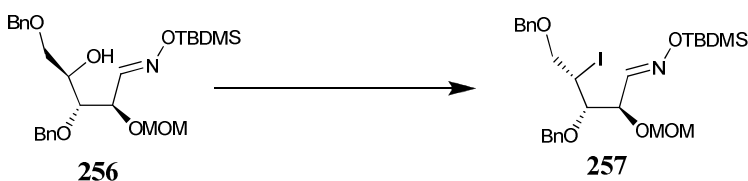
A suspension of alcohol **256** (2.20 g, 4.35 mmol), triphenylphosphine (4.57 g, 19.85 mmol), imidazole (1.18 g, 17.41 mmol) and iodine (3.32 g, 13.06 mmol) in toluene (175 mL) was heated at reflux for 1.5 h. The dark brown reaction mixture was then cooled to 0 °C. Sodium bicarbonate (175 mL) was added 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.

E-isomer

R$_f$ 0.49 *E*-isomer (8:1 Light petroleum:EtOAc); δ_H (200 MHz): 0.3 (6H, s, SiMe$_2$), 1.18 (9H, s, SiMe$_3$), 3.41 (OCH$_3$), 3.61 (1H, dd, *J* = 7.5, 2.9, C3-H), 3.79-3.91 (2H, m, C5-2H), 4.22-

4.28 (2H, m, C4-H and C2-H), 4.45 (2H, s, OCH$_2$Ph), 4.51-4.71 (2H, m, OCH$_2$Ph), 5.45 (2H, s, OCH$_2$O), 7.19-7.46 (10H, m, Ph), 7.63 (1H, d, J = 8.2, C1-H); δ_C (50 MHz): 18.5 (SiC), 26.5 (SiMe$_3$), 31.7 (CH), 56.1 (0CH$_3$), 72.9 (CH$_2$), 73.1 (CH$_2$), 75.1 (CH$_2$), 78.1 (CH), 78.8 (CH), 95.5 (OCH$_2$O), 125.2, 127.6, 127.7, 127.9, 128.2, 128.4, 128.9 & 129.7 (CH$_{ar}$), 133.0 (C), 133.2 (C), 135.5 (CH$_{ar}$), 137.4, 137.6 & 138.3 (C), 151.9 (C-1); IR υ_{max} 3069.8, 3030.6, 2930.8, 2857.8, 1428, 1115, 1071.7, 922, 739.3, 699.1 cm^{-1}.

Z-isomer

R$_f$ 0.44 *Z*-isomer (8:1 Light petroleum:EtOAc); δ_H (200 MHz): 0.13 (6H, s, SiMe$_2$), 0.88 (9H, s, SiMe$_3$), 3.21 (OCH$_3$), 3.45 (1H, dd, J = 7.5, 2.9, C3-H), 3.62-371 (2H, m, C5-H$_2$), 3.87 (1H, t, J = 5.0, C3-H), 4.20-4.30 (1H, m, C4-H), 4.39-4.61 (2H, m, OCH$_2$Ph), 4.71- 4.75 (2H, m, OCH$_2$Ph), 5.26 (1H, m, C2-H), 7.07 (1H, d, J = 6.2, C1-H), 7.36-7.44 (10H, m, Ph); δ_C (50 MHz): 18.1 (SiC), 26.1 (SiMe$_3$), 31.4 (C4-H), 56.1 (0CH$_3$), 72.6 (CH$_2$), 74.7 (CH$_2$), 74.8 (CH$_2$), 78.4 (CH), 78.5 (CH), 95.5 (OCH$_2$O), 125.2, 127.6, 127.7, 127.9, 128.2, 128.4, 128.9 & 129.7 (CH$_{ar}$), 133.04 (C), 133.24 (C), 135.49 (CH$_{ar}$), 137.40, 137.55 & 138.25 (C), 154.1 (C-1); IR υ_{max} 3069.8, 3030.6, 2930.8, 2857.8, 1428, 1115, 1071.7, 922, 739.3, 699.1 cm^{-1}.

(3*R*,4*R*,5*R*)-3-Methoxymethyl-4-benzyloxy-5-benzyloxymethyl-Δ-pyrroline-N-Oxide (179)[113]

Method 1:

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:

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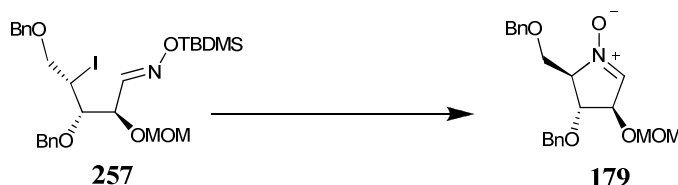
To a solution of **257** (528 mg, 0.86 mmol) in dry THF (25 mL) was added tetrabutylammonium fluoride 1M solution in THF (1.3 mL, 1.3 mmol). The mixture was refluxed for 10 minutes, cooled to ambient temperature then concentrated in *vacuo* to give a brown residue. The residue was purified by flash chromatography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

R_f 0.15 (light petroleum/ethyl acetate 1:1); δ_H (200 MHz, CDCl$_3$): 3.28 (3H, s, OMe), 3.65 (1H, dd, J = 2.22, 9.71, C5'-H$_a$), 3.94 (1H, m, C5'-H$_b$), 4.23-4.54 (7H, m, 2 x PhCH$_2$O, C3-H, C4-H& C5-H), 6.91 (d, J = 1.92, C2-H), 7.19-7.31 (10H, m, Ph); δ_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 (O*CH$_2$*O), 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-(benzyloxymethyl)-4-(methoxymethoxy)-*N*,*N*-dimethylhexahydropyrrolo[1,2-b]isoxazole-2-carboxamide (267)

179 → **267**
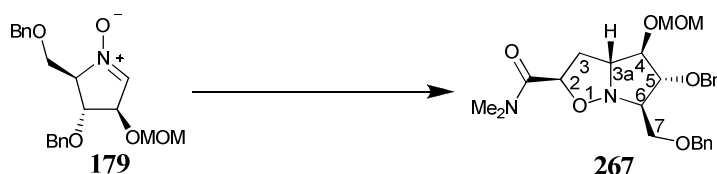
To a solution of nitrone **179** (1.50 g, 4.04 mmol) in DCM (29 mL) was added *N, N*-Dimethyacrylamide (0.43 mL, 4.04 mmol). The mixture was stirred at ambient temperature overnight then concentrated *in vacuo* producing a brown residue. Purification by flash chromatography (EtOAc) yielded a isoxazole **267** as a brown oil (2.21 g, 100 %).

R_f 0.33 (ethyl acetate); δ_H (400 MHz, CDCl$_3$): 2.33 (1H, m, C3-H), 2.92(3H, s, N*CH$_3$*), 2.93-3.02 (1H, m, C3'-H), 3.03 (3H, s, N*CH$_3$*), 3.30-3.33 (1H, m, C6-H), 3.34 (3H, s, O*CH$_3$*), 3.58-3.63 and 3.68-3.73 (2 x 1H, m, C7-*CH$_2$*), 3.75-3.80 (1H, m, C3a-H), 3.95-3.97 (1H, dd, *J* = 3.9, 2.2, C5-H), 4.09-4.11 (1H, t, *J* = 3.9, C4-H), 4.54-4.61 (4H, m, 2 x O*CH$_2$*Ph), 4.62 (2H, s, O*CH$_2$*O), 4.82 (1H, t, *J* = 7.1, C2-H), 7.19-7.31 (10H, m, Ph); δ_c (100 MHz): 35.8 (N*CH$_3$*), 35.9 (CH$_2$-3), 36.9 (N*CH$_3$*), 55.6 (O*CH$_3$*), 68.8 (CH-3a), 69.98 (CH-6*CH$_2$*), 70.0 (CH-6), 72.3 (O*CH$_2$*Ph), 73.3 (O*CH$_2$*Ph), 74.5 (CH-2), 83.9 (CH-5), 84.7 (CH-4), 95.8 (O*CH$_2$*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 v_{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^{-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$_{26}$H$_{35}$N$_2$O$_6$ requires 471.2490].

(1*R*,2*R*,3*R*,6*R*,7a*R*)-2-(Benzyloxy)-3-(benzyloxymethyl)-6-hydroxy-1-(methoxymethoxy)-hexahydropyrrolizin-5-one (268)
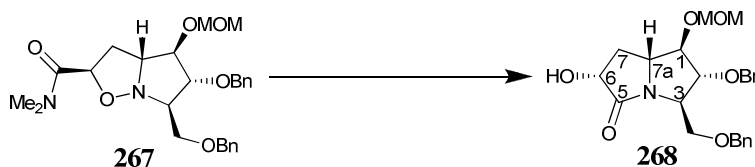
To compound **267** (1.8 g, 3.83 mmol) was added 10M AcOH (12 mL, 120 mmol) and Zinc dust (6.00 g, 91.22 mmol). The mixture was stirred at 80 °C for 3 hrs and then the pH was adjusted to 14 using aqueous NaOH. The product was extracted into DCM (2x 10ml) and the combined organic layers were dried over MgSO$_4$, filtered and concentrated. Purification by column chromatography (EtOAc) yielded **268** a white solid (0.793 g, 57 %). The solid was

recrystalised form heptanes and ethyl acetate to provide crystals for x-ray crystallography analysis.

R_f 0.46 (ethyl acetate); m.p 135-138 °C; δ_H (400 MHz, CDCl$_3$): 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, OCH$_3$), 3.53, (1H, dd, *J* = 9.9, 3.9, C3-CH$_{2\text{-Ha}}$'), 3.66 (1H, dd, *J* = 9.9, 3.9, C3-CH$_{2\text{-Hb}}$'), 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, OCH$_2$Ph, C6-H), 4.62-4.65 (2H, m, C5-CH$_2$OCH$_2$Ph), 4.67 (2H, m, OCH$_2$O), 7.28-7.42 (10H, m, Ph); δ_c (100 MHz): 36.9 (CH$_2$-7), 55.6 (OCH$_3$), 58.2 (CH-3), 59.6 (CH-7a), 68.7 (C3-CH$_2$), 71.8 (CH-6), 72.5 (OCH$_2$Ph), 72.6 (OCH$_2$Ph), 85.6 (CH-2), 87.7 (CH-1), 96.6 (OCH$_2$O), 127.6, 127.6, 127.7, 127.8, 128.4 & 128.4 (CH$_{Ar}$), 137.8 & 137.9 (C$_{ar}$), 174.2 (C=O); IR ν_{max} 3350.5, 3030.6, 2892.3, 1675.9, 1458.6, 1360.5, 1319.1, 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)$^+$; C$_{24}$H$_{30}$NO$_6$ requires 428.2068]; Anal. Calcd for C$_{24}$H$_{29}$NO$_6$: C, 67.43; H, 6.84; N, 3.28. Found: C, 67.38; H, 6.82; N, 3.21.

(1*R*,2*R*,3*R*,6R,7a*R*)-2-(Benzyloxy)-3-(benzyloxymethyl)-1-(methoxymethoxy)-5-oxo-hexahydro-7H-pyrrolizin-6-yl methanesulfonate (269)
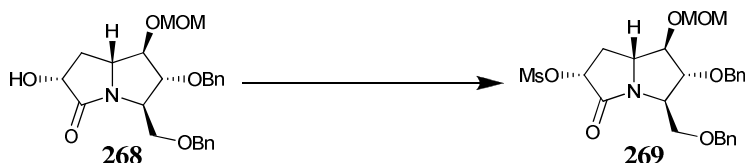
268 (0.76 g, 1.77 mmol) was dissolved in DCM (10 mL) and stirred at 0 °C under an argon atmosphere. Triethylamine (0.44 mL, 3.88 mmol), and methanesulfonyl chloride (2 mL, 3.00 mmol) were added successively. The mixture was stirred for 30 min and water (5 mL) was added and the layers separated. The aqueous layer was extracted with DCM (2 x 10ml) and the combined organic layers were washed with brine (10ml), dried over MgSO$_4$, filtered and evaporated to give mesylate **269** (0.270 g, 100 %).

R$_f$ 0.33 (ethyl acetate); δ$_H$ (400 MHz, CDCl$_3$): 2.12 (1H, m, C7-H), 2.97 (1H, ddd, J = 13.0, 8.3, 6.2, C7'-H), 3.31 (3H, s, CH_3SO$_3$), 3.37 (3H, s, OCH$_3$), 3.52, (1H, dd, J = 10.0, 3.7, C3-CH$_{2-Ha}$'), 3.67, (1H, dd, J = 9.9, 4.6, CH-3CH_{2-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, OCH_2Ph), 4.64-4.66 (2H, m, CH$_2$OCH_2Ph), 4.67-71 (2H, m, OCH$_2$O), 5.40 (1H, dd, J 9.8, 8.6), 7.28-7.42 (10H, m, Ph); δ$_c$ (100 MHz): 34.6 (CH$_2$-7), 39.8 (SO$_2$CH$_3$), 55.6 (OCH$_3$), 58.4 (CH-3), 59.4 (CH-7a), 68.4 (C3-CH$_2$), 72.6 (OCH$_2$Ph), 73.2 (OCH$_2$Ph), 78.5 (CH-6), 85.4 (CH-2), 87.9 (CH-1), 96.7 (OCH$_2$O), 127.5, 127.6, 127.7, 127.71, 127.8, 128.84 & 128.9 (CH$_{Ar}$), 137.6 & 137.7 (C$_{ar}$), 167.9 (C=O); IR ν$_{max}$ 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)$^+$; C$_{25}$H$_{32}$NO$_8$S requires 506.1843]. Anal. Calcd for C$_{25}$H$_{31}$NO$_8$S: C, 59.39; H, 6.18; N, 2.77. Found: C, 59.31; H, 6.15; N, 2.68.

(1*R*,2*R*,3*R*,6*R*,7a*R*)-2-(Benzyloxy)-3-(benzyloxymethyl)-1-hydroxy-5-oxo-hexahydro-7*H*-pyrrolizin-6-yl methanesulfonate (270)
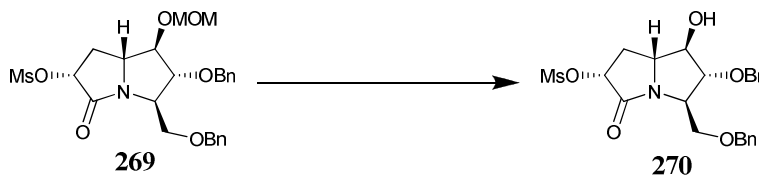
To a solution of **269** (100 mg, 0.198 mmol) in DCM (2.5 mL) was added TFA (0.2 mL). The reaction mixture was stirred at ambient temperature for 48 hours. The mixture was washed with sodium bicarbonate (2 mL) and extracted with DCM (3 x 3 mL). The Organic phase was dried over MgSO4, filtered and the filtrate concentrated. Purification by column chromatography (petrol/EtOAc 1:1) yielded **270** (78 mg, 86 %) as a yellow oil.

R$_f$ 0.20 (heptane:ethyl acetate, 1:1); δ$_H$ (400 MHz, CDCl$_3$): 2.07 (1H, m, C7-H), 2.95 (1H, m, C7'-H), 3.26 (3H, s, SO$_2$$CH_3$), 3.64 (1H, dd, t, J = 10.0, 2.8, C3CH_{2-Ha}), 3.68 (1H, dd, J 5.6, 3.3, C7a-H), 3.72 (1H, dd, t, J = 10.0, 3.5, C3CH_{2-Hb}), 3.97 (1H, t, J = 3.7, C1-H), 4.10 (1H, t, J = 3.1, C2-H), 4.23 (1H, q, J = 3.0, C3-H), 4.54-4.58 (2H, m, OCH_2Ph), 4.59-4.63 (2H, d, J = 4.9, OCH$_2$Ph), 5.42 (1H, dd, J = 10.4, 8.0, C6-H), 7.32 (10H, m, Ph); δ$_c$ (100 MHz): 34.9

(CH$_2$-7), 39.8 (SO$_2$CH$_3$), 59.7 (CH-3), 62.8 (CH-7a), 70.0 (C3-CH$_2$), 71.9 (OCH$_2$Ph), 73.8 (OCH$_2$Ph), 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 (CH$_{Ar}$), 136.7 & 137.3 (C$_{ar}$), 169.46 (C=O); HRMS (ES): 462.1584 [(M+H)$^+$; C$_{23}$H$_{28}$NO$_7$S requires 462.1581]. Anal. Calcd for C$_{23}$H$_{28}$NO$_7$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-(Benzyloxy)-3-(benzyloxymethyl)-1,5-dioxo-hexahydro-7H-pyrrolizin-6-yl methanesulfonate (271)
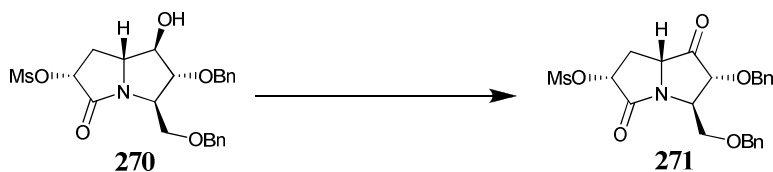
Method 1: Dess-Martin Periodinane Oxidation

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 mnitored by TLC (petrol/EtOAC 1:1) and on completion was diluted with ether (3 mL). 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 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 %).

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

MsO''' ... OH ... OBn ... OBn **271** → MsO''' ... OCOPh ... OBn ... OBn **272**
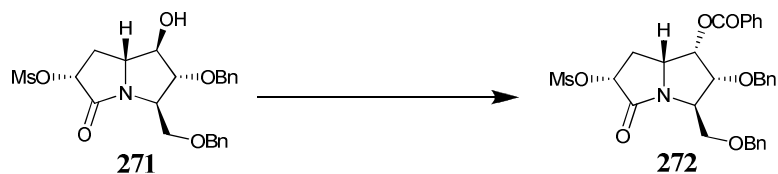
A solution of alcohol **271** (50 mg, 0.11 mmol) in PPh₃ (83 mg, 0.32 mmol) and benzoic acid (15 mg, 0.13 mmol), in dry THF (4 mL) was stirred at 0 °C under an atmosphere of nitrogen. DEAD (50 µL, 0.32 mmol) was added dropwise and the mixture stirred at 0 °C for 1 h, allowed to warm to ambient temperature 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.

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

MsO''' ... OMOM ... OBn ... OBn **269** → ... OMOM ... OBn ... OBn **275**
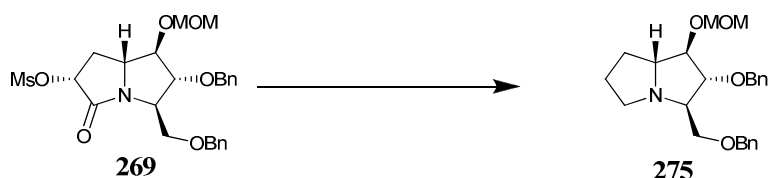
To a solution of amide **269** (450 mg, 0.89 mmol) in anhydrous THF (5 mL) was added LiAlH₄ (118 mg, 3.12 mmol) and mixture heated at reflux for 1.5 hr. The reaction was quenched by addition of NaOH, the solids were solids filtered off and the filtrate partitioned between EtOAc (5 mL) and water (5 mL). 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.

R_f 0.15 (ethyl acetate); δ_H (400 MHz, CDCl₃): 1.83-1.91 (3H, m, C6-H_2, 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-H_2),

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$_2$O*CH$_2$*Ph, O*CH$_2$*Ph-H$_a$), 4.61-4.72 (3H, m, O*CH$_2$*Ph-H$_b$, OCH$_2$O7.28-7.42 (10H, m, Ph); δ$_c$ (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 (O*CH$_2$*Ph), 73.2 (C5-CH$_2$O*CH$_2$*Ph), 85.5 (CH-2), 86.6 (CH-1), 91.9 (O*CH$_2$*O), 127.4, 127.5, 127.6, 127.6, 127.6, 127.65, 127.68, 127.7, 128.2, 128.2, 128.3, 128.3, & 128.4 (CH$_{Ar}$), 138.42 & 138.5 (C$_{ar}$); IR ν$_{max}$ 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$_{12}$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.

(1*R*,2*R*,3*R*,7a*R*)-2-(Benzyloxy)-3-(benzyloxymethyl)hexahydro-1*H*-pyrrolizin-1-ol (276)
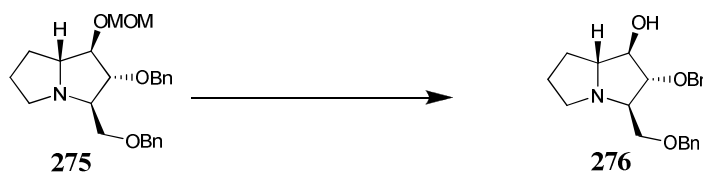
To a solution of **275** (297 mg, 0.75 mmol) in DCM (18 mL) was added TFA (1.75 mL, 22.74 mmol). The reaction mixture was stirred at ambient temperature for 48 h. The mixture was washed with sodium bicarbonate (2 mL) and extracted with DCM (3 x 3 mL). The combined organics were dried over MgSO$_4$, filtered 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); δ$_H$ (200 MHz, CDCl$_3$): 2.16-2.23 (4H, m, C6-H$_2$, C7-H$_2$), 2.83 (1H, dt, *J* = 10.7, 6.6, C5 -H$_a$), 2.98 (1H, ddd, *J* = 7.5, 7.0, 3.7, C3-H), 3.10 (1H, dt, *J* = 12.1, 6.1, C5-H$_b$), 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$_2$O*CH$_2$*Ph), 4.59-4.62 (1H, m, O*CH$_2$*Ph-H$_a$), 4.70-4.73 (1H, m, O*CH$_2$*Ph-H$_b$), 7.28-7.42 (10H, m, Ph); δ$_c$ (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 (O*CH$_2$*Ph), 73.2 (C5-CH$_2$O*CH$_2$*Ph), 85.5 (CH-2), 86.6 (CH-1), 91.9 (O*CH$_2$*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$_{Ar}$), 138.34 & 138.40 (C$_{ar}$); IR ν$_{max}$ 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): 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 (1*R*,2*R*,3*R*,7a*R*)-2-(Benzyloxy)-3-(benzyloxymethyl) hexahydro-1H-pyrrolizin-1-one (277)
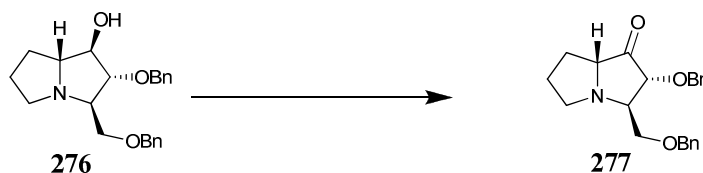

Method 1: Dess-Martin Periodinane Oxidation

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 mnitored 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 %).

Attempted preparation of (1*S*,2*R*,3*R*,7a*R*)-2-(Benzyloxy) -3- (benzyloxymethyl) hexahydro-1H-pyrrolizin-1-ol (260)
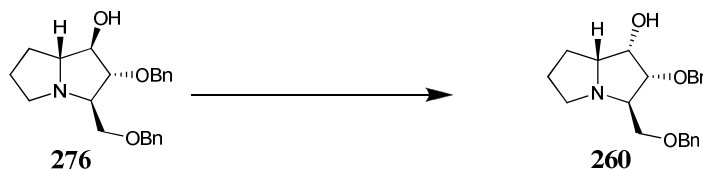
A solution of alcohol **276** (50 mg, 0.11 mmol), PPh₃ (83 mg, 0.32 mmol) and benzoic acid (15 mg, 0.13 mmol), in dry THF (4 mL) was stirred at 0 °C under an atmosphere of nitrogen. DEAD (50 µL, 0.32 mmol) was added dropwise and mixture stirred at 0 °C for 1 h then at ambient temperature overnight. The solvents were evaporated under reduced pressure to give an orange oil. No reacton was observed from NMR and TLC analysis of the crude product.

(2*R*,3a*R*,4*R*,5*R*,6*R*)-5-(Benzyloxy)-6-(benzyloxymethyl)-4-hydroxy-N,N-dimethylhexahydropyrrolo[1,2-b]isoxazole-2-carboxamide (279)
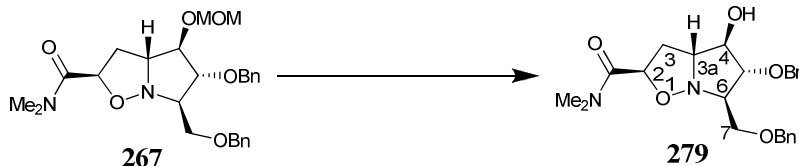
To a solution of **267** (150 mg, 0.32 mmol) in DCM (5 mL) was added TFA (0.5 mL). The reaction mixture was stirred at ambient temperature for 48 h. The mixture was washed with sodium bicarbonate (2 mL) and extracted with DCM (3 x 3 mL). The combined organic layers were dried over MgSO₄, filtered and the filtrate concentrated. Purification by column chromatography (petrol/EtOAc 1:1) yielded alcohol **279** (78 mg, 86 %) as a yellow oil.

R_f 0.33 (ethyl acetate); δ_H (400 MHz, CDCl₃): 2.38 (1H, ddd, *J* = 12.7, 7.5, 5.5, C3-H$_a$), 2.86 (1H, ddd, *J* = 12.4, 9.2, 7.3, C3-H$_b$), 2.94 (3H, s, N*CH₃*), 2.99 (3H, s, N*CH₃*), 3.38-3.43 (1H, m, C6-H), 3.69 (1H, dd, *J* = 9.6, 3.7, C7-*H$_a$*), 3.73 (1H, dd, *J* = 9.6, 3.7, C7-*H$_a$*), 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, O*CH₂*Ph), 4.66-4.73 (2H, m, O*CH₂*Ph), 4.83 (1H, t, *J* = 7.4, C2-H), 7.19-7.31 (10H, m,

Ph); δ_c (100 MHz): 35.8 (N*CH$_3$*), 36.3 (*CH$_2$*-3), 36.9 (N*CH$_3$*), 70.8 (CH-6*CH$_2$*), 72.1 (0*CH$_2$*Ph), 72.55 (CH-7a), 72.6 (CH-6), 73.7 (O*CH$_2$*Ph), 74.8 (CH-2), 79.3 (CH-5), 86.7 (CH-4), 127.75 (CH-Ar), 127.88 (CH-Ar), 128.5 (CH-Ar), 137.8 (CH-Ar), 137.4 (C-Ar), 137.6 (C-Ar), 168.3 (C=O); IR ν_{max} 3380.2 (br. OH), 3362.7, 3030.7, 2931.1, 2866.7, 1646.0 (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}; HRMS (ES) 427.2223 [(M+NH$_4$)$^+$; C$_{26}$H$_{35}$N$_2$O$_6$ requires 427.2227]; Anal. Calcd for C$_{26}$H$_{35}$N$_2$O$_6$: C, 66.22; H, 7.48; N, 5.94. 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)

279   **280**
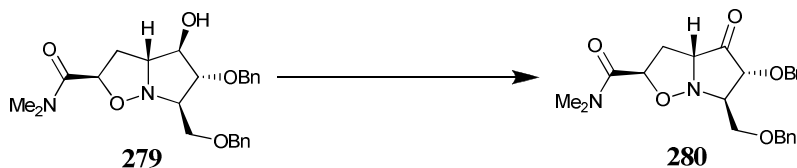
Method 1: Dess-Martin Periodinane Oxidation

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 mnitored 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 %).

Attempted preparation of (2R,3aR,4S,5R,6R)-5-(benzyloxy)-6-(benzyloxymethyl)-4-hydroxy-N,N-dimethylhexahydropyrrolo[1,2-b]isoxazole-2-carboxamide (281)
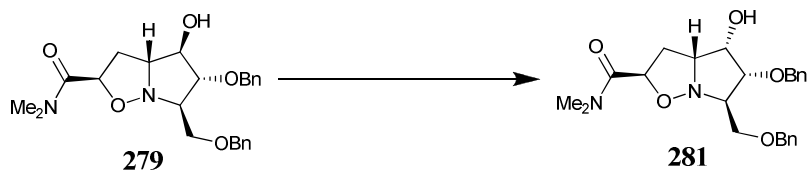
A solution of alcohol **279** (50 mg, 0.11 mmol) in PPh$_3$ (83 mg, 0.32 mmol) and benzoic acid (15 mg, 0.13 mmol), in dry THF (4 mL) was stirred at 0 °C under an atmosphere of nitrogen. DEAD (50 µL, 0.32 mmol) was added dropwise and mixture stirred at 0 °C for 1 h then at ambient temperature overnight. 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)[135]
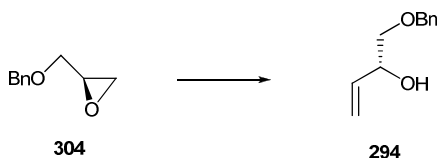
Trimethylsulfonium iodide (4.95 g, 24.23 mmol) was suspended in toluene (30 mL) and heated at reflux for 30 min on a Dean-Stark trap. Toluene was removed and residue dried under high vacuum. After flushing with nitrogen, THF (50 mL) was added and solution cooled to -15 °C in an ice/salt bath and butyllithium (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 (1g, 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.

R_f 0.17 (petroleum ether:EtOAc 8:1); δ_H (400 MHz, CDCl$_3$): 2.59 (1H, br. s, OH), 3.42 (1H, dd, J = 9.6, 7.9, C1-H$_a$), 3.58 (1H, dd, J = 9.6, 3,4, C1- H$_b$), 4.39 (1H, m, C2-H), 4.61 (2H, s, OCH_2Ph), 5.24 (1H, dt, J = 10.6, 1.5 Hz, C4-H$_a$), 5.40 (1H, dt, J = 17.3, 1.5 Hz, C4-H$_b$), 5.88 (1H, ddd, J = 17.3, 10.6, 5.6 Hz, C3-H), 7.39 (10H, m, Ph); δ_c (100 MHz): 71.5 (CH-2), 73.3 (OCH_2Ph), 73.9 (CH$_2$-1), 116.4 (CH$_2$-4), 127.72, 127.73, 127.79, 128.38, 128.43, 128.46, & 128.48 (CH$_{Ar}$), 136.5 (CH-3), 137.8 (C$_{ar}$); IR ν_{max} 3346 (br. OH), 3049.4, 2931.6, 2857.1, 1589.7, 1487.1, 1427.2, 1390.3, 1360.9, 1259.4, 1112.5, 1028.2, 998.2, 822.2, 739.2, 699.2, cm^{-1}; *m/z* (ES) 178.0992 [(M+H)$^+$; C$_{11}$H$_{14}$NO$_2$ requires 178.0994]. Anal. Calcd for C$_{11}$H$_{14}$O$_2$: 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)
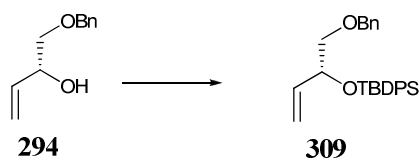
To a solution of alcohol **294** (0.9 g, 5.05 mmol) in anhydrous DMF (15 mL) and imidazole (1.02 g, 15 mmol) was added TBDPSCl (2.08 mL, 7.58 mmol) and mixture attired at room temperature overnight. The reaction was quenched with 1M HCl (20 mL), extracted with ethyl acetate (2 x 15 ml), washed with water (20 ml), sodium bicarbonate (20 ml) and brine (10 ml) then dried over MgSO$_4$, filtered and concentrated. Purification by flash chromatography (light petroleum ether: EtOAc 9:1) yielded **309** (2.03 g, 96 %) as a green/yellow oil.

R_f 0.58 (light petroleum ether: EtOAc 9:1); δ_H (200 MHz, CDCl$_3$): 0.99 (9H, s, 3 x CH$_3$), 3.27 (1H, dd, J = 9.7, 7.9, C1-H$_a$), 3.36 (1H, dd, J = 9.6, 3.4, C1-H$_b$), 4.26-4.31 (1H, m, C2-H), 4.29 (2H, s, OCH_2Ph), 4.99 (1H, dt, J = 10.8, 1.6 Hz, C4-H$_a$), 5.10 (1H, dt, J = 17.4, 1.6 Hz, C4-H$_b$), 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); δ_c (50 MHz): 19.1 (CSi), 26.7 (CH$_3$), 72.8 (CH-2), 73.0 (OCH_2Ph), 74.3 (CH$_2$-1), 115.4 (CH$_2$-4), 127.7, 127.73, 127.8, 128.4, 128.43, 128.46, & 128.48 (CH$_{Ar}$), 135.6 (CH-3), 133.9, 134.5 & 138.0 (C$_{ar}$); IR ν_{max} 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}.

(2*R*,3aR,4*R*,5*R*,6*R*)-5- (Benzyloxy)-2-((S)-2-(benzyloxy)-1-(tertbutyldiphenylsilyloxy) ethyl)-6-(benzyloxymethyl)-4-(methoxymethoxy)hexahydropyrrolo[1,2-b] isoxazole.
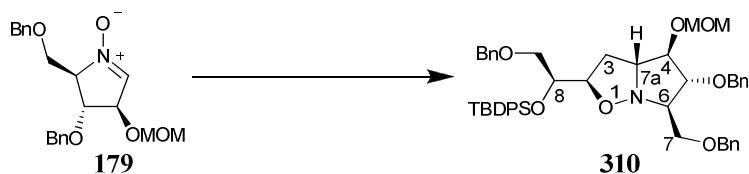
To a solution of nitrone **179** (800 mg, 2.15 mmol) in toluene (50 mL) was added alkene **309** (740 mg, 1.78 mmol) and the mixture was heated at reflux overnight. The mixture was cooled and concentrated *in vacuo* and purification by column chromatography (hexane: Et$_2$O, 7:3) yielded **310** (1.287 g, 92%) as a brown oil.

R$_f$ 0.28 (heptane: EtOAc, 7:3); δ$_H$ (400 MHz, CDCl$_3$): 1.09 (9H, s, SiMe$_3$), 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, OCH$_3$), 3.39, (2H, m, C8-*CH$_2$*O), 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, O*CH$_2$*O), 4.38 (1H, td, J= 7.5, 4.6, C2-H), 4.59-4.69 (6H, m, 3 x OCH$_2$Ph), 7.12-7.77 (35H, m, Ph); δ$_c$ (100 MHz): 19.4 (Si*C*), 26.9 (C*Me$_3$*), 35.4 (CH$_2$-3), 55.5 (O*CH$_3$*), 68.2 (CH-7a), 68.9 (CH-6), 69.76(CH-7*CH$_2$*), 69.77 (CH-6*CH$_2$*), 71.9 (CH$_2$), 72.3 (CH-5), 72.34 (OCH$_2$Ph), 72.9 (OCH$_2$Ph), 73.3 (O*CH$_2$*Ph), 83.5 (CH-2*CH*), 84.9 (CH-4), 95.9 (OCH$_2$O), 127.3 (CH$_{ar}$), 127.4 (CH$_{ar}$), 127.42 (CH$_{ar}$), 127.51 (CH$_{ar}$), 127.58 (CH$_{ar}$), 127.6 (CH$_{ar}$), 127.68 (CH$_{ar}$), 127.74 (CH$_{ar}$), 127.83 (CH$_{ar}$), 128.1 (CH$_{ar}$), 128.2 (CH$_{ar}$), 128.3 (CH$_{ar}$), 128.5 (CH$_{ar}$), 129.6 (CH$_{ar}$), 133.2 (C$_{ar}$), 134.4 (C$_{ar}$), 135.8 (CH$_{ar}$), 136.2 (CH$_{ar}$), 138.1 (C$_{ar}$), 138.2 (C$_{ar}$), 138.5 (C$_{ar}$); IR ν$_{max}$ 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)$^+$; C$_{48}$H$_{58}$NO$_7$Si requires 788.3977].

(S)-2-(Benzyloxy)-1-((2*R*,3a*R*,4*R*,5*R*,6*R*)-5-(benzyloxy)-6-(benzyloxymethyl)-4-(methoxymethoxy)hexahydropyrrolo[1,2-b]isoxazol-2-yl)ethanol (304)

310 → **304**

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 (0.283g, 85 %).

R_f 0.42 (heptane: EtOAc, 3:7); δ_H (200 MHz, CDCl$_3$): 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, CH$_2$O*CH$_3$*), 3.40-3.73 (5H, m, 2 x CH$_2$, C3a-H) 3.86-4.26 (4H, m, C5-H, C2-H, C8-H, C4-H), 4.44-4.64 (8H, m, O*CH$_2$*O, 3x O*CH$_2$Ph*), 7.12-7.77 (35H, m, Ph); δ_c (100 MHz): 34.4 (CH$_2$-b), 55.4 (O*CH$_3$*), 68.2 (CH-7a), 69.1 (CH-6), 69.3 (CH-6*CH$_2$*), 70.2 (CH-6), 70.9 (O*CH$_2$*Ph), 72.0 (O*CH$_2$*Ph), 73.1 (O*CH$_2$*Ph), 73.2 (O*CH$_2$*Ph), 76.8 (CH-5), 83.2 (CH-2*CH*), 85.2 (CH-4), 95.7 (O*CH$_2$*O), 127.3 (CH$_{ar}$), 127.5 (CH$_{ar}$), 127.51 (CH$_{ar}$), 128.0 (CH$_{ar}$), 128.1 (CH$_{ar}$), 128.2 (CH$_{ar}$), 137.6 (C$_{ar}$), 137.8 (C$_{ar}$), 138.0 (C$_{ar}$); IR ν_{max} 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)$^+$; C$_{32}$H$_{40}$NO$_7$ requires 550.280]. Anal. Calcd for C$_{32}$H$_{39}$NO$_7$: C, 69.92; H, 7.32; N, 2.55. Found: C, 69.88; H, 7.35; N, 2.59.

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

304 → **305**

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.

R_f 0.47 (heptane:EtOAc, 4:6); δ_H (200 MHz, CDCl₃): 2.23-2.56 (2H, m,C3-H₂), 3.01 (3H, s, S*Me*), 3.24-3.38 (1H, m, C6-H), 3.29 (3H, s, CH₂O*CH₃*), 3.45-3.70 (5H, m, CH₂, CH₂, 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, O*CH₂*O, 3x O*CH₂*Ph), 4.79 (1H, q, *J* = 5.2, C-2*CH*), 7.12-7.77 (35H, m, Ph); δ_c (100 MHz): 34.6 (CH₂-3), 38.2 (SO₂Me), 55.3 (O*CH₃*), 68.3 (CH-7a), 69.2 (CH₂), 69.3 (CH₂), 69.6 (CH-6), 71.9 (CH₂), 72.9 (CH₂), 73.1 (CH₂), 75.31 (CH-6), 80.02 (CH-5), 83.40 (C2-CH), 84.31 (CH-4), 95.57 (O*CH₂*O), 127.25 (CH_ar), 127.35 (CH_ar), 127.41 (CH_ar), 127.59 (CH_ar), 127.97 (CH_ar), 128.03 (CH_ar), 128.15 (CH_ar), 136.93 (C_ar), 137.49 (C_ar), 137.80 (C_ar); IR ν_max 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].

(1*R*,2*R*,3*R*,5*R*,6*R*,7a*R*)-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.

R_f 0.20 (EtOAc); δ_H (400 MHz, CDCl$_3$): 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, OCH$_3$), 3.49 (2H, m, C3-*CH$_2$*), 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-*CH$_2$*), 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 OCH$_2$), 7.28-7.35 (15H, m, 3 x Ph); δ_c (100 MHz): , 39.4 (CH$_2$-7), 55.4 (OMe), 62.9 (CH-3), 65.9 (CH-5), 67.2 (C5-CH$_2$), 67.8 (CH-7a), 71.9 (OCH$_2$), 72.6 (OCH$_2$), 72.9 (OCH$_2$), 73.4 (OCH$_2$), 74.3 (CH-6), 85.5 (CH-1), 86.4 (CH-2), 95.5 (O*CH$_2$*O), 127.4 (CH$_{ar}$), 127.6 (CH$_{ar}$), 127.61 (CH$_{ar}$), 127.8 (CH$_{ar}$), 127.88 (CH$_{ar}$), 127.89 (CH$_{ar}$), 128.2 (CH$_{ar}$), 128.22 (CH$_{ar}$), 128.3 (CH$_{ar}$), 128.36 (CH$_{ar}$) 128.4 (CH$_{ar}$), 137.9 (C$_{ar}$), 138.4 (C$_{ar}$); IR ν_{max} 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$^+$; C$_{32}$H$_{39}$NO$_6$ requires 533.2772]. Anal. Calcd for C$_{32}$H$_{39}$NO$_6$: C, 72.02; H, 7.37; N, 2.63. Found: C, 72.12; H, 7.42; N, 2.68.

(1*R*,2*R*,3*R*,5*R*,6*R*,7a*R*)-2-(Benzyloxy)-3,5-bis(benzyloxymethyl)-1-(methoxymethoxy) hexahydro-1H-pyrrolizin-6-yl methanesulfonate (311)
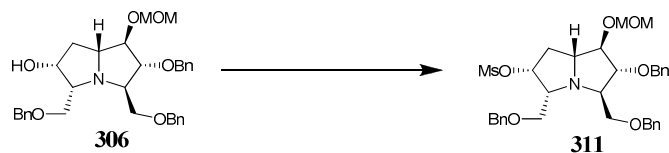
Alcohol **306** (400 mg, 1.12 mmol) in DCM (20 mL) was stirred at 0 °C under an atmosphere of argon. Et$_3$N (0.35 mL, 3.14 mmol) and MsCl (0.14 mL, 2.46 mmol) were successively added. The mixture was stirred at 0 °C for 30 min then the reaction was quenched by the addition of water (10 ml). The layers were separated and the aqueous layer extracted with DCM (2 x 10 ml), washed with brine (10ml), dried over MgSO$_4$, filtered and concentrated. Purification by flash chromatography (light petroleum:EtOAc, 3:7) yielded mesylate **311** (458 mg, 100 %) as a pale green oil.

R_f 0.34 (heptanes:EtOAc, 3:7); δ_H (200 MHz, CDCl$_3$): 2.35-2.41 (2H, m, C7-H$_2$), 2.83 (3H, s, SO$_2$*CH$_3$*), 3.31 (3H, s, O*CH$_3$*), 3.38-3.57 (5H,m, C3-*CH$_2$*, C7a-H, C3-H, C5-H), 3.78 (2H, d, J = 5.5, C5-*CH$_2$*), 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 x OCH_2), 4.59-4.63 (4H, m, 2 x OCH_2), 5.21 (1H, q, *J* 5.1 , H-6), 7.31-7.41 (15H, m, 3 x Ph); δ_c (100 MHz): 35.8 (CH_2-7), 38.2 (SCH_3), 55.4 (OCH_3), 61.6 (CH-3), 64.1 (CH-5), 65.7 (C5-CH_2), 66.6 (CH-7a), 72.2 (OCH_2), 72.4 (OCH_2), 73.3 (OCH_2), 73.5 (OCH_2), 82.8 (CH-6), 85.1 (CH-1), 86.6 (CH-2), 95.9 (OCH_2O), 127.6 (CH$_{ar}$), 127.7 (CH$_{ar}$), 127.9 (CH$_{ar}$), 128.3 (CH$_{ar}$), 137.8 (C$_{ar}$), 138.3 (C$_{ar}$), 138.4 (C$_{ar}$); IR ν_{max} 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$^+$; $C_{33}H_{41}NO_8S$ requires 611.2547]; Anal. Calcd for $C_{33}H_{41}NO_8S$: 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) hexahydro-1H-pyrrolizin-6-yl S-methyl carbonodithioate (312)
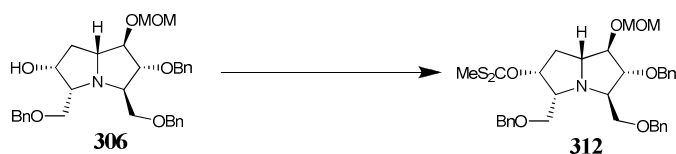
Method 1:

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 CS$_2$ (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 MgSO$_4$, 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 CS$_2$ (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 MgSO$_4$, 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.

R$_f$ 0.26 (light petroleum:EtOAc 7:3); δ$_H$ (200 MHz, CDCl$_3$): 2.29 -2.51 (2H, m, C7-H$_2$), 2.41 (3H, s, S*CH$_3$*), 3.25 (3H, s, O*CH$_3$*), 3.37-3.64 (5H, m, C1-H, C2-H, , C7a-H, C3-H, C5-H), 3.71-3.80 (2H, m, C3-*CH$_2$*), 3.98-4.07 (2H, m, C5-*CH$_2$*), 4.59-4.65 (8H, m, 8 x O*CH$_2$*), 5.97 (1H, dt, *J* = 6.6, 5.1, C6-H), 7.25 (15H, m, 3 x Ph); δ$_c$ (100 MHz): 18.9 (S*CH$_3$*), 35.5 (CH$_2$-7), 55.4 (OCH$_3$), 61.7 (CH-3), 63.5 (CH-5), 65.9 (C5-CH$_2$), 66.5 (CH-7a), 71.9 (OCH$_2$), 72.6 (OCH$_2$), 73.2 (OCH$_2$), 73.4 (OCH$_2$), 85.1 (CH-6), 85.4 (CH-1), 86.7 (CH-2), 95.8 (O*CH$_2$*O), 127.4 (CH$_{ar}$), 127.5 (CH$_{ar}$), 127.51 (CH$_{ar}$), 127.57 (CH$_{ar}$), 127.7 (CH$_{ar}$), 128.2 (CH$_{ar}$), 128.3 (CH$_{ar}$), 137.9 (C$_{ar}$), 138.4 (C$_{ar}$), 138.6 (C$_{ar}$); IR ν$_{max}$ 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$^+$; C$_{34}$H$_{41}$NO$_6$S$_2$ requires 623.2370].

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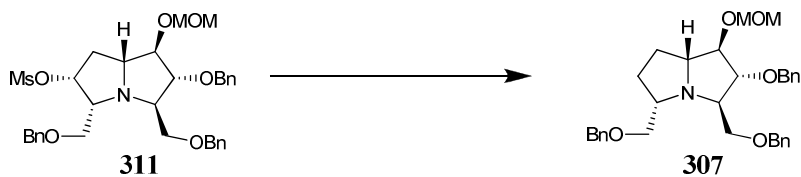
Method 1:

To a solution of mesylate **311** (800 mg, 1.31 mmol) in anhydrous THF (10 mL) was added LiAlH$_4$ (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:
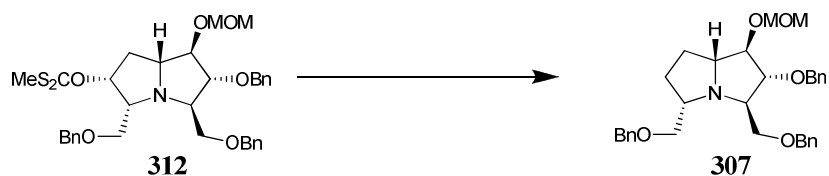
To a solution of xanthate **312** (600 mg, 0.96 mmol) in toluene (21 mL) at reflux was added a mixture of AIBN (60 mg, 0.36 mmol) and Bu₃SnH (0.618 mL, 2.29 mmol) in toluene (3 mL) over 2 h. The mixture was left at reflux for a further 1 h after addition then allowed to cool to ambient temperature. The 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.

R_f 0.29 (light petroleum:EtOAc 8:2); δ_H (400 MHz, CDCl₃): 1.78-1.87 (2H, m, C6-H₂), 1.88-1.99 (2H, m, C7-H), 3.29 (3H, s, O*CH₃*), 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_2O), 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 (0CH_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_2O), 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 v_{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*,7a*R*)-2-(Benzyloxy)-3,5-bis(benzyloxymethyl)hexahydro-1H-pyrrolizin-1-ol (308)
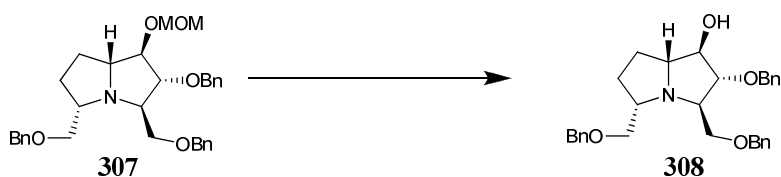
To a solution of **307** (270 mg, 0.52 mmol) in DCM (10 mL) was added TFA (1 mL, 12.91 mmol). The reaction mixture was stirred at ambient temperature for 48 h. The mixture was washed with sodium bicarbonate (2 mL) and extracted with DCM (3 x 3 mL). The Organic phase was dried over MgSO$_4$, filtered 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$_{ar}$), 137.61 (C$_{ar}$), 137.65 (C$_{ar}$), 137.00 (C$_{ar}$); IR ν$_{max}$ (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)

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); δ$_H$ (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). δ$_c$ (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 ν$_{max}$ (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. Calcd 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

4.1 Appendix A: X-ray data for compound 236
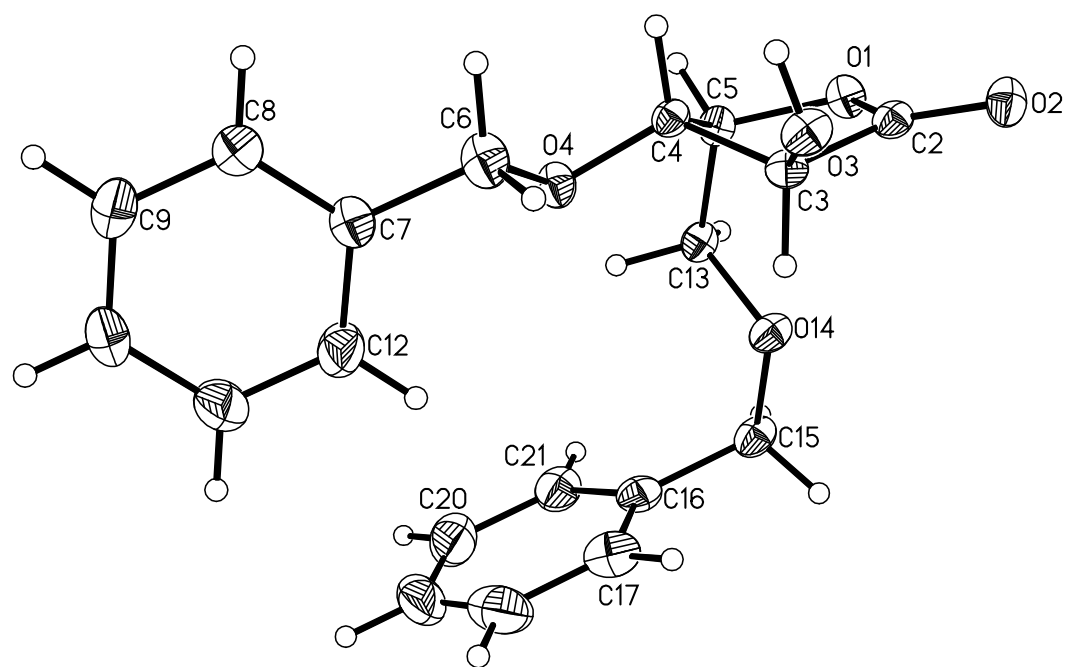
Table 4. Crystal data and structure refinement for ASM111A x82103_0m.

Identification code	x82103_0m
Empirical formula	C19 H20 O5
Formula weight	328.35
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)

Unit cell dimensions
$a = 9.0996(14)$ Å $\quad \square = 90°.$
$b = 5.1326(9)$ Å $\quad \square = 93.602(10)°.$
$c = 17.537(3)$ Å $\quad \square = 90°.$

Volume	817.4(2) Å^3
Z	2
Density (calculated)	1.334 Mg/m^3
Absorption coefficient	0.096 mm^{-1}
F(000)	348
Crystal size	0.81 x 0.68 x 0.08 mm^3
Theta range for data collection	2.46 to 29.21°.

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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.$Å^{-3}$

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

O(1)-C(2)	1.342(2)
O(1)-C(5)	1.461(2)
C(2)-O(2)	1.204(2)
C(2)-C(3)	1.528(2)
C(3)-O(3)	1.4093(19)
C(3)-C(4)	1.526(3)
C(3)-H(3)	1.0000
O(3)-H(3A)	0.8400
C(4)-O(4)	1.405(2)
C(4)-C(5)	1.539(2)
C(4)-H(4)	1.0000
O(4)-C(6)	1.4354(19)
C(5)-C(13)	1.503(2)
C(5)-H(5)	1.0000
C(6)-C(7)	1.499(3)
C(6)-H(6A)	0.9900
C(6)-H(6B)	0.9900
C(7)-C(8)	1.380(3)
C(7)-C(12)	1.382(3)
C(8)-C(9)	1.381(3)
C(8)-H(8)	0.9500
C(9)-C(10)	1.376(3)

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)

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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.

C(5)-O(1)-C(2)-O(2)	179.07(15)
C(5)-O(1)-C(2)-C(3)	-1.18(19)
O(2)-C(2)-C(3)-O(3)	31.5(3)

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)-C(13)	-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)

C(19)-C(20)-C(21)-C(16)	0.4(3)
C(17)-C(16)-C(21)-C(20)	-0.7(3)
C(15)-C(16)-C(21)-C(20)	-179.10(19)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for x82103_0m [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(3)-H(3A)...O(2)#1	0.84	1.93	2.7592(19)	167.6

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,y-1/2,-z

4.2   Appendix B: NOESY interactions for compound 267

4.3   Appendix C: X-ray data for compound 268

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)
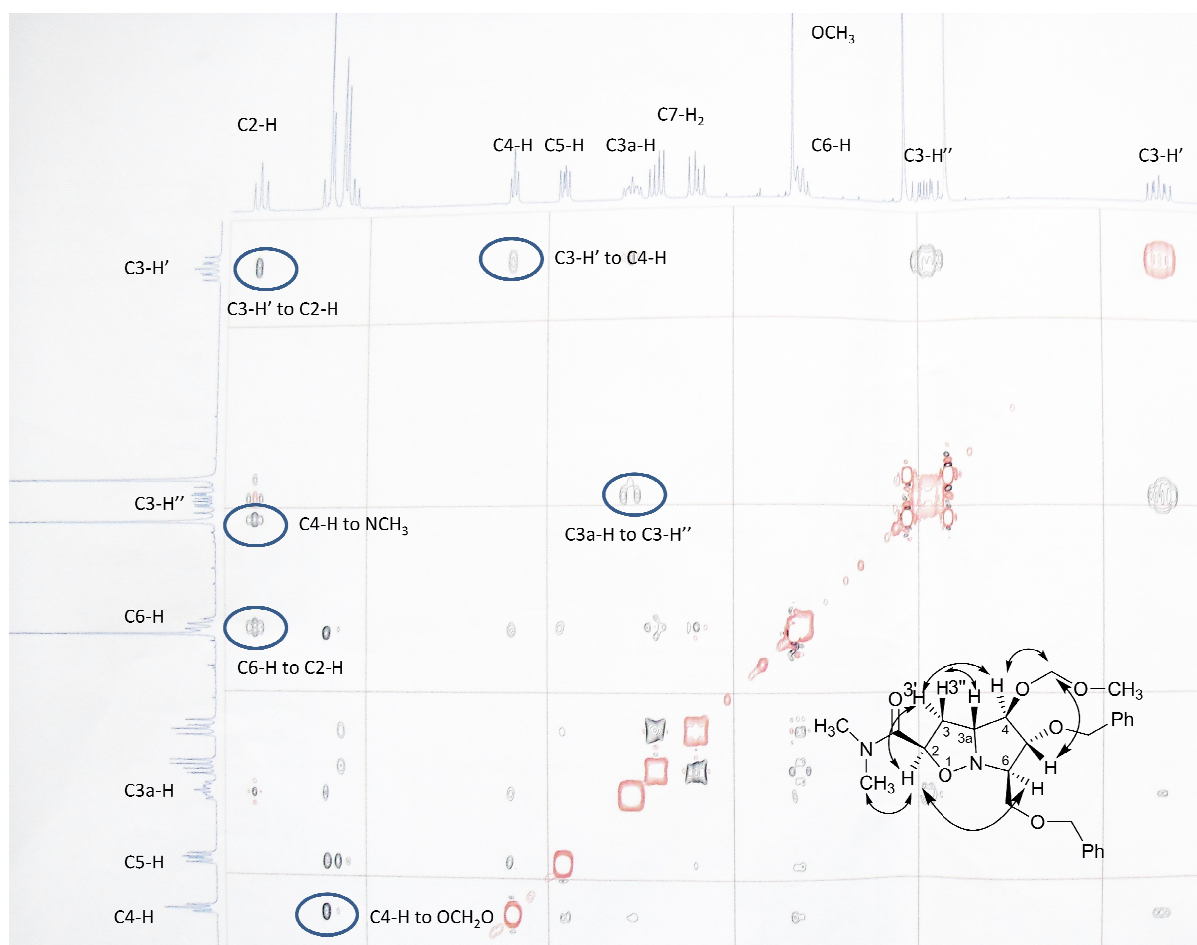
but not successful. Very weakly diffracting crystal, two domains second rotated from first domain by 3.2 degrees.

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

Identification code	twin5
Empirical formula	C24 H29 N O6
Formula weight	427.48
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P1

Unit cell dimensions:
a = 4.5560(4) Å $\square$= 77.689(5)°.
b = 10.5338(9) Å $\square$= 84.80(3)°.
c = 24.362(2) Å $\square$ = 89.82(3)°.

Volume	1137.45(17) Å^3
Z	2
Density (calculated)	1.248 Mg/m^3
Absorption coefficient	0.089 mm^{-1}
F(000)	456
Crystal size	1.00 x 0.10 x 0.04 mm^3
Theta range for data collection	0.86 to 19.60°.
Index ranges	-4<=h<=4, -8<=k<=9, 0<=l<=22
Reflections collected	2640
Independent reflections	2640 [R(int) = 0.0000]
Completeness to theta = 19.60°	85.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9964 and 0.9159
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2640 / 406 / 546
Goodness-of-fit on F^2	1.023
Final R indices [I>2sigma(I)]	R1 = 0.0845, wR2 = 0.2068
R indices (all data)	R1 = 0.1464, wR2 = 0.2672
Largest diff. peak and hole	0.322 and -0.385 e.Å^{-3}

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

C(1A)-O(9A)	1.45(2)
C(1A)-C(2A)	1.47(3)

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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
C(2B)-N(3B)	1.50(2)
C(2B)-C(17B)	1.52(3)
C(2B)-H(2B)	1.0000
N(3B)-C(4B)	1.34(2)
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(5B)-H(5B)	1.0000
O(5B)-H(5X)	0.8400
C(6B)-C(7B)	1.56(2)
C(6B)-H(6B1)	0.9900
C(6B)-H(6B2)	0.9900
C(7B)-C(8B)	1.49(3)
C(7B)-H(7B)	1.0000
C(8B)-O(26B)	1.43(2)
C(8B)-H(8B)	1.0000
O(9B)-C(10B)	1.50(2)
C(10B)-C(11B)	1.50(3)
C(10B)-H(10C)	0.9900
C(10B)-H(10D)	0.9900
C(11B)-C(12B)	1.35(3)
C(11B)-C(16B)	1.43(3)
C(12B)-C(13B)	1.37(3)
C(12B)-H(12B)	0.9500
C(13B)-C(14B)	1.43(3)
C(13B)-H(13B)	0.9500
C(14B)-C(15B)	1.30(3)
C(14B)-H(14B)	0.9500
C(15B)-C(16B)	1.39(3)
C(15B)-H(15B)	0.9500
C(16B)-H(16B)	0.9500
C(17B)-O(18B)	1.40(2)
C(17B)-H(17A)	0.9900
C(17B)-H(17B)	0.9900
O(18B)-C(19B)	1.44(2)
C(19B)-C(20B)	1.512(19)
C(19B)-H(19A)	0.9900
C(19B)-H(19B)	0.9900
C(20B)-C(21B)	1.3900
C(20B)-C(25B)	1.3900
C(21B)-C(22B)	1.3900
C(21B)-H(21B)	0.9500
C(22B)-C(23B)	1.3900
C(22B)-H(22B)	0.9500

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C(23B)-C(24B)	1.3900
C(23B)-H(23B)	0.9500
C(24B)-C(25B)	1.3900
C(24B)-H(24B)	0.9500
C(25B)-H(25B)	0.9500
O(26B)-C(27B)	1.43(2)
C(27B)-O(28B)	1.41(2)
C(27B)-H(27A)	0.9900
C(27B)-H(27B)	0.9900
O(28B)-C(29B)	1.46(2)
C(29B)-H(29A)	0.9800
C(29B)-H(29B)	0.9800
C(29B)-H(29C)	0.9800
O(9A)-C(1A)-C(2A)	117.6(15)
O(9A)-C(1A)-C(8A)	105.7(15)
C(2A)-C(1A)-C(8A)	107.2(14)
O(9A)-C(1A)-H(1A)	108.7
C(2A)-C(1A)-H(1A)	108.7
C(8A)-C(1A)-H(1A)	108.7
C(1A)-C(2A)-N(3A)	106.2(13)
C(1A)-C(2A)-C(17A)	121.2(16)
N(3A)-C(2A)-C(17A)	108.0(14)
C(1A)-C(2A)-H(2A)	106.9
N(3A)-C(2A)-H(2A)	106.9
C(17A)-C(2A)-H(2A)	106.9
C(4A)-N(3A)-C(7A)	115.8(15)
C(4A)-N(3A)-C(2A)	132.2(15)
C(7A)-N(3A)-C(2A)	110.2(14)
O(4A)-C(4A)-N(3A)	125.5(16)
O(4A)-C(4A)-C(5A)	128.3(17)
N(3A)-C(4A)-C(5A)	106.1(17)
O(5A)-C(5A)-C(6A)	114.5(16)
O(5A)-C(5A)-C(4A)	110.2(15)
C(6A)-C(5A)-C(4A)	102.4(15)
O(5A)-C(5A)-H(5A1)	109.8
C(6A)-C(5A)-H(5A1)	109.8

C(4A)-C(5A)-H(5A1)	109.8
C(5A)-O(5A)-H(5A)	109.5
C(5A)-C(6A)-C(7A)	103.9(16)
C(5A)-C(6A)-H(6A1)	111.0
C(7A)-C(6A)-H(6A1)	111.0
C(5A)-C(6A)-H(6A2)	111.0
C(7A)-C(6A)-H(6A2)	111.0
H(6A1)-C(6A)-H(6A2)	109.0
N(3A)-C(7A)-C(8A)	105.5(14)
N(3A)-C(7A)-C(6A)	103.0(15)
C(8A)-C(7A)-C(6A)	121.2(18)
N(3A)-C(7A)-H(7A)	108.8
C(8A)-C(7A)-H(7A)	108.8
C(6A)-C(7A)-H(7A)	108.8
O(26A)-C(8A)-C(7A)	111.7(14)
O(26A)-C(8A)-C(1A)	115.3(14)
C(7A)-C(8A)-C(1A)	101.4(16)
O(26A)-C(8A)-H(8A)	109.4
C(7A)-C(8A)-H(8A)	109.4
C(1A)-C(8A)-H(8A)	109.4
C(1A)-O(9A)-C(10A)	113.5(13)
O(9A)-C(10A)-C(11A)	108.8(14)
O(9A)-C(10A)-H(10A)	109.9
C(11A)-C(10A)-H(10A)	109.9
O(9A)-C(10A)-H(10B)	109.9
C(11A)-C(10A)-H(10B)	109.9
H(10A)-C(10A)-H(10B)	108.3
C(12A)-C(11A)-C(16A)	124.5(19)
C(12A)-C(11A)-C(10A)	122.1(17)
C(16A)-C(11A)-C(10A)	113.3(18)
C(11A)-C(12A)-C(13A)	119(2)
C(11A)-C(12A)-H(12A)	120.6
C(13A)-C(12A)-H(12A)	120.6
C(12A)-C(13A)-C(14A)	119(2)
C(12A)-C(13A)-H(13A)	120.6
C(14A)-C(13A)-H(13A)	120.6
C(15A)-C(14A)-C(13A)	122(2)

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C(15A)-C(14A)-H(14A)   119.0
C(13A)-C(14A)-H(14A)   119.0
C(14A)-C(15A)-C(16A)   122(2)
C(14A)-C(15A)-H(15A)   118.9
C(16A)-C(15A)-H(15A)   118.9
C(11A)-C(16A)-C(15A)   114(2)
C(11A)-C(16A)-H(16A)   123.2
C(15A)-C(16A)-H(16A)   123.2
O(18A)-C(17A)-C(2A)   104.5(15)
O(18A)-C(17A)-H(17C)   110.8
C(2A)-C(17A)-H(17C)   110.8
O(18A)-C(17A)-H(17D)   110.8
C(2A)-C(17A)-H(17D)   110.8
H(17C)-C(17A)-H(17D)   108.9
C(17A)-O(18A)-C(19A)   111.7(14)
O(18A)-C(19A)-C(20A)   103.1(15)
O(18A)-C(19A)-H(19C)   111.1
C(20A)-C(19A)-H(19C)   111.1
O(18A)-C(19A)-H(19D)   111.1
C(20A)-C(19A)-H(19D)   111.1
H(19C)-C(19A)-H(19D)   109.1
C(21A)-C(20A)-C(25A)   117.0(18)
C(21A)-C(20A)-C(19A)   122.6(19)
C(25A)-C(20A)-C(19A)   120.1(19)
C(20A)-C(21A)-C(22A)   124(2)
C(20A)-C(21A)-H(21A)   117.8
C(22A)-C(21A)-H(21A)   117.8
C(23A)-C(22A)-C(21A)   115(2)
C(23A)-C(22A)-H(22A)   122.5
C(21A)-C(22A)-H(22A)   122.5
C(24A)-C(23A)-C(22A)   124(2)
C(24A)-C(23A)-H(23A)   118.2
C(22A)-C(23A)-H(23A)   118.2
C(25A)-C(24A)-C(23A)   119(2)
C(25A)-C(24A)-H(24A)   120.6
C(23A)-C(24A)-H(24A)   120.6
C(24A)-C(25A)-C(20A)   121(2)

C(24A)-C(25A)-H(25A)	119.6
C(20A)-C(25A)-H(25A)	119.6
C(27A)-O(26A)-C(8A)	116.1(15)
O(26A)-C(27A)-O(28A)	113.7(17)
O(26A)-C(27A)-H(27C)	108.8
O(28A)-C(27A)-H(27C)	108.8
O(26A)-C(27A)-H(27D)	108.8
O(28A)-C(27A)-H(27D)	108.8
H(27C)-C(27A)-H(27D)	107.7
C(27A)-O(28A)-C(29A)	108.1(15)
O(28A)-C(29A)-H(29D)	109.5
O(28A)-C(29A)-H(29E)	109.5
H(29D)-C(29A)-H(29E)	109.5
O(28A)-C(29A)-H(29F)	109.5
H(29D)-C(29A)-H(29F)	109.5
H(29E)-C(29A)-H(29F)	109.5
O(9B)-C(1B)-C(2B)	114.3(16)
O(9B)-C(1B)-C(8B)	105.1(16)
C(2B)-C(1B)-C(8B)	107.4(15)
O(9B)-C(1B)-H(1B)	109.9
C(2B)-C(1B)-H(1B)	109.9
C(8B)-C(1B)-H(1B)	109.9
N(3B)-C(2B)-C(17B)	109.4(15)
N(3B)-C(2B)-C(1B)	101.9(14)
C(17B)-C(2B)-C(1B)	118.5(16)
N(3B)-C(2B)-H(2B)	108.9
C(17B)-C(2B)-H(2B)	108.9
C(1B)-C(2B)-H(2B)	108.9
C(4B)-N(3B)-C(7B)	113.5(15)
C(4B)-N(3B)-C(2B)	131.6(16)
C(7B)-N(3B)-C(2B)	112.8(15)
O(4B)-C(4B)-N(3B)	123.7(15)
O(4B)-C(4B)-C(5B)	127.4(17)
N(3B)-C(4B)-C(5B)	108.7(18)
O(5B)-C(5B)-C(4B)	113.3(15)
O(5B)-C(5B)-C(6B)	109.6(14)
C(4B)-C(5B)-C(6B)	104.1(15)

O(5B)-C(5B)-H(5B)	109.9
C(4B)-C(5B)-H(5B)	109.9
C(6B)-C(5B)-H(5B)	109.9
C(5B)-O(5B)-H(5X)	109.5
C(5B)-C(6B)-C(7B)	102.0(14)
C(5B)-C(6B)-H(6B1)	111.4
C(7B)-C(6B)-H(6B1)	111.4
C(5B)-C(6B)-H(6B2)	111.4
C(7B)-C(6B)-H(6B2)	111.4
H(6B1)-C(6B)-H(6B2)	109.2
N(3B)-C(7B)-C(8B)	104.8(15)
N(3B)-C(7B)-C(6B)	102.9(15)
C(8B)-C(7B)-C(6B)	121.4(17)
N(3B)-C(7B)-H(7B)	109.0
C(8B)-C(7B)-H(7B)	109.0
C(6B)-C(7B)-H(7B)	109.0
O(26B)-C(8B)-C(7B)	109.5(15)
O(26B)-C(8B)-C(1B)	112.2(14)
C(7B)-C(8B)-C(1B)	102.1(17)
O(26B)-C(8B)-H(8B)	110.9
C(7B)-C(8B)-H(8B)	110.9
C(1B)-C(8B)-H(8B)	110.9
C(1B)-O(9B)-C(10B)	113.7(14)
C(11B)-C(10B)-O(9B)	107.1(15)
C(11B)-C(10B)-H(10C)	110.3
O(9B)-C(10B)-H(10C)	110.3
C(11B)-C(10B)-H(10D)	110.3
O(9B)-C(10B)-H(10D)	110.3
H(10C)-C(10B)-H(10D)	108.6
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)
C(11B)-C(12B)-C(13B)	120(2)
C(11B)-C(12B)-H(12B)	120.0
C(13B)-C(12B)-H(12B)	120.0
C(12B)-C(13B)-C(14B)	120(2)
C(12B)-C(13B)-H(13B)	119.8

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C(14B)-C(13B)-H(13B)	119.8
C(15B)-C(14B)-C(13B)	119(2)
C(15B)-C(14B)-H(14B)	120.3
C(13B)-C(14B)-H(14B)	120.3
C(14B)-C(15B)-C(16B)	122(2)
C(14B)-C(15B)-H(15B)	119.1
C(16B)-C(15B)-H(15B)	119.1
C(15B)-C(16B)-C(11B)	119(2)
C(15B)-C(16B)-H(16B)	120.5
C(11B)-C(16B)-H(16B)	120.5
O(18B)-C(17B)-C(2B)	107.2(16)
O(18B)-C(17B)-H(17A)	110.3
C(2B)-C(17B)-H(17A)	110.3
O(18B)-C(17B)-H(17B)	110.3
C(2B)-C(17B)-H(17B)	110.3
H(17A)-C(17B)-H(17B)	108.5
C(17B)-O(18B)-C(19B)	117.0(15)
O(18B)-C(19B)-C(20B)	108.8(15)
O(18B)-C(19B)-H(19A)	109.9
C(20B)-C(19B)-H(19A)	109.9
O(18B)-C(19B)-H(19B)	109.9
C(20B)-C(19B)-H(19B)	109.9
H(19A)-C(19B)-H(19B)	108.3
C(21B)-C(20B)-C(25B)	120.0
C(21B)-C(20B)-C(19B)	121.2(12)
C(25B)-C(20B)-C(19B)	118.8(12)
C(20B)-C(21B)-C(22B)	120.0
C(20B)-C(21B)-H(21B)	120.0
C(22B)-C(21B)-H(21B)	120.0
C(23B)-C(22B)-C(21B)	120.0
C(23B)-C(22B)-H(22B)	120.0
C(21B)-C(22B)-H(22B)	120.0
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

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Symmetry transformations used to generate equivalent atoms:

Table 3. Torsion angles [°] for twin5.

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O(9A)-C(1A)-C(2A)-N(3A)	-128.5(15)
C(8A)-C(1A)-C(2A)-N(3A)	-9.7(19)
O(9A)-C(1A)-C(2A)-C(17A)	108(2)
C(8A)-C(1A)-C(2A)-C(17A)	-133.3(16)
C(1A)-C(2A)-N(3A)-C(4A)	154(2)
C(17A)-C(2A)-N(3A)-C(4A)	-75(2)
C(1A)-C(2A)-N(3A)-C(7A)	-10(2)
C(17A)-C(2A)-N(3A)-C(7A)	121.4(17)
C(7A)-N(3A)-C(4A)-O(4A)	176(2)
C(2A)-N(3A)-C(4A)-O(4A)	13(4)
C(7A)-N(3A)-C(4A)-C(5A)	-6(2)
C(2A)-N(3A)-C(4A)-C(5A)	-169.6(17)
O(4A)-C(4A)-C(5A)-O(5A)	-38(3)
N(3A)-C(4A)-C(5A)-O(5A)	144.9(16)

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)

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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 °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(5A)-H(5A)...O(4B)#1	0.84	1.94	2.73(2)	157.1
O(5B)-H(5X)...O(4A)#2	0.84	1.95	2.786(19)	170.3

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
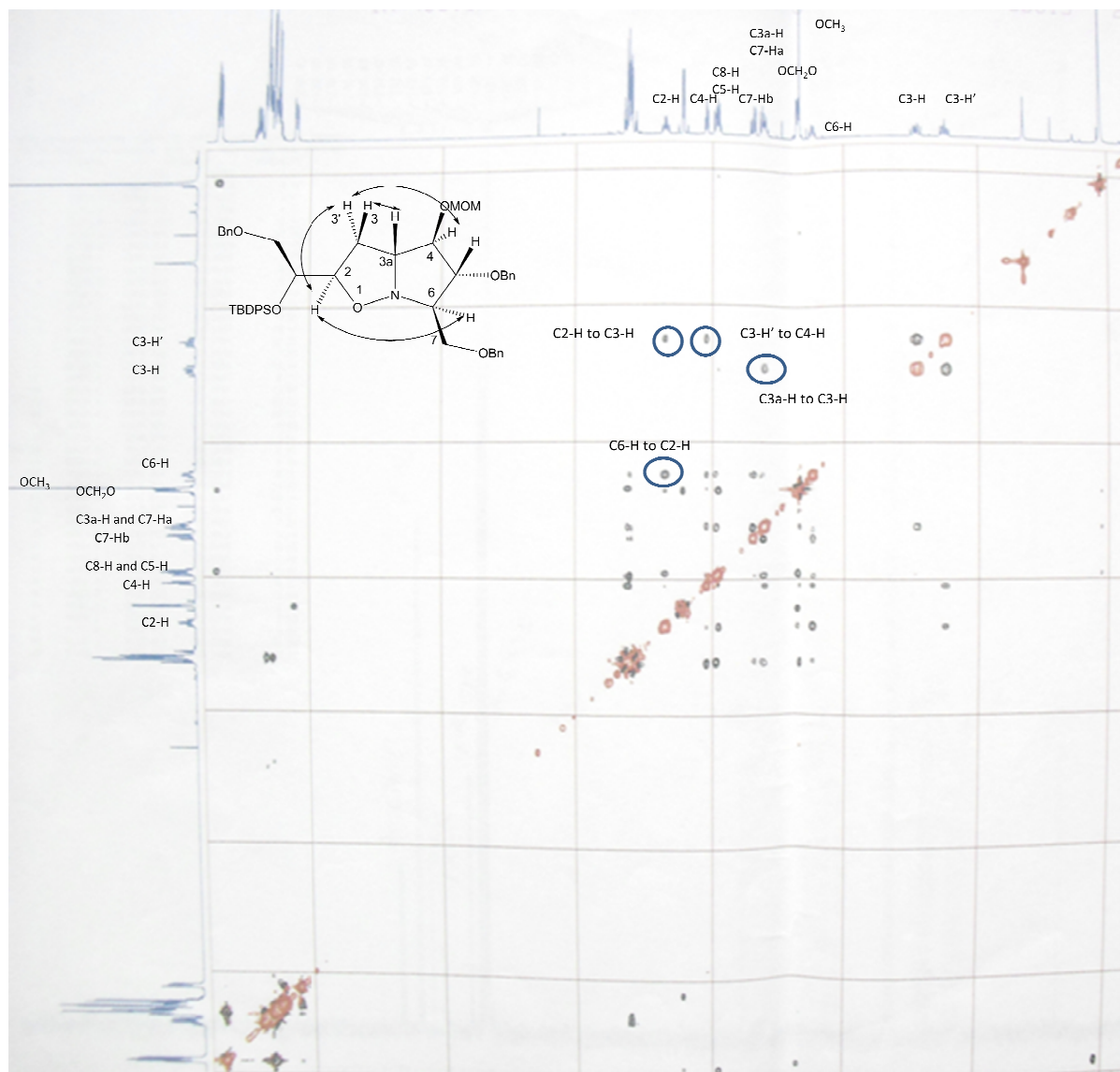
4.5   Appendix E: Training record

Conferences attended

Scottish regional meeting of the RSC organic division –poster presentation 2009, 2008, 2007, 2006.

SCI postgraduate snposium –Speaker March 2009.

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

Courses Attended

Vitae UK Grad School –Bournmouth 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

1   S.Inouye, T. Tsuruoka and T. Niida, *J. Antibiot.* 1966, **19**, 288.
2   N. Asano, R. J. Nash, R. J. Molyneux and G. W. J. Fleet, *Tetrahedron: Asymmetry* 2000, **11**, 1645 and ref. therein.
3   S. Inouyte, T. Tsuruoka and T. Niida, *Tetrahedron* 1968, **24**, 2125.
4   (a) H. Paulsen, I. Sangster and K. Heyns, *Chem. Ber.* 1967, **100**, 802; (b) H. Paulsen, K. Todt, *Adv. Carbohydr. Chem.* 1968, **23**, 115.
5   A. A. Watson, G. W. J. Fleet, N. Asano, R. J. Molyneux and R. J. Nash, *Phytochem* 2001, **56**, 265 and ref. therein.
6   M. Koyama and S. Sakamura, *Agric. Biol. Chem.* 1974, **38**, 1111
7   R. J. Molyneux, M. Benson, R. Y. Wong, J. H. Tropea and A. D. Elbein, *J. Nat. Prod.* 1988, **51**, 1198.
8   S. V. Evans, A. R. Hayman, L. E. Fellows, T. K. M. Shing, A. E. Derome and G. W. J. Fleet, *Tetrahedron Lett.* 1985, **26**, 1465.
9   N. Asano, A. Kato, M. Miyauchi, H. Kizu, T. Tomimori, K. matsui, R. J. Nash and R. J. Molyneux, *Eur. J. Biochem* 1997, **248**, 296.
10 I. Cenci di Bello, P. Dorling, L. E. Fellows and B. Winchester, *FEBS Lett.* 1984, **176**, 61.
11 G. C. Kite, L. E. Fellows, G. W. J. Fleet, S. P. Liu, A. M. Schofield and N. G. Smith. *Tetrahedron Lett.* 1988, **29**, 6483.
12 N. Asano, A. Kato, M. Miyauchi, H. Kizu, Y. Kameda, A. A. Watson, , R. J. Nash, and G. W.J. Fleet, *J. Nat. Prod.* 1998, **61**, 625.
13 T. Yamashita, K. Yasuda, H. Kizu, Y. Kameda, A. A. Watson, R. J. Nash, G. W. J. Fleet and N. Asano, *J. Nat. Prod.* 2002, **65**, 1875.
14 A. Welter, J. Jadot, G. Dardenne, M. Marlier and J. Casimir, *Phytochem.* 1976, **15**, 747.
15 B. Winchester and G. W. J. Fleet, *Glycobiology* 1992, **2**, 199R.
16 Nash, R. J.; Asano, N.and Watson, A. A. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Elsevier Science: Oxford, 1996; Vol. 11, pp. 345–376.
17 R.J. Nash, E.A. Bell and J.M. Williams, *Phytochem* 1985, **24**, 1620.
18 S.V.Evans, L.E. Fellows, T.K.M. Shing and G.W.J. Fleet, *Phytochem.* 1985, **24**, 1953.
19 R. J. Molyneux, Y. T. Pan, J. H. Tropea, A. D. Elbein, C. H. Lawyer, D. J. Hughes and G.W.J. Fleet, *J. Nat. Prod.* 1993, **56**, 1356.
20 A. A. Watson, R. J. Nash, M. R. Wormald, D. J. Harvey, S. Dealler, E. Lees, N. Asano, H. Kizu, A. Kato, R. C. Griffiths, A. J. Cairns and G. W. J. Fleet, *Phytochem.* 1997, **46**, 255.
21 N. Asano, K. Ikeda, Y. Arai and H. Kizu, *J. Nat. Prod.* 2004, **67**, 846.
22 For a review of Swainsonine and it's analogues, see: A. El Nemr, *Tetrahedron* 2000, **56**, 8579.
23 R. Saul, J. P. Chambers, R. J. Molyneux, A. D. Elbein, *Arch. Biochem, Biophys.* 1983, **221**, 593.
24 I. Pastuzak, R. J. Molyneux, L. F. James and A. D. Elbein, *Biochem.* 1990, **29**, 1886.
25 R. J. Nash, L. E. Fellows, J. V. Dring, G. W. J. Fleet, A. E. Derome, T. A. Hamer, A. M. Scofield and D. J. Watkin, *Tetrahedron Lett,* 1988, **29**, 2487.
26 R. M. Wormald, R. J. Nash, A. A. Watson, B. K. Bhadoria, R. Langford, M. Sims, G. W. J. Fleet, *Carbohydr. Lett.* 1996, **2,** 169.
27 A. C. de S. Pereira, M. A. C.Kaplan, J. G. S. Maia, O. R. Gottlieb, R. J. Nash, G. W. J

Fleet, L. Pearce, D. J Watkin and A. M. Scofield, *Tetrahedron* 1991,**47**, 5637.

28 A. Kato, I. Adachi, M. Miyauchi, K. Ikeda, T. Komae, H. Kizu, Y. Kameda, A. A. Watson, R. J. Nash, R. M. Wormald, G. W. J. Fleet and N. Asano, *Carbohydr. Res*. 1999, **316**, 95-103.

29 N. Asano, H. Kurori, H. Ikeda, H. Kizu, Y. Kameda, A. Koto, I. Adachi, A. A. Watson, R. J. Nash and G. W. J. Fleet, *Tetrahedron: Asymmetry* 2000, **11**, 1.

30 A. Kato, N. Kato, I. Adiachi, J. Hollinshead, G. W. J. Fleet, C. Kuriyama, H. Ikeda, N. Asano and R. J. Nash, *Nat. Prod*. 2007, **70**, 993.

31 D. Tepfer, A. Goldmann, N. Pamboukdjian, M. Maille, A. Lepingle, D. Chevalier, J. Denarie and C. J. Rosenberg, *Bacteriol.* 1988, **170**, 1153.

32 A. Goldmann, M.L. Milat, P.H. Ducrot, J.Y. Lallemand, M. Maille, A. Lepingle, I. Charpin, and D. Tepfer, *Phytochem.* 1990, **29**, 2125.

33 N. Asano, E. Tomioka, H. Kizu and K. Matsui, *Carbohydr. Res.* 1994, **253**, 235.

34 N. Asano, K. Oseki, E. Tomioka, H. Kizu and K. Matsui, *Carbohydr. Res.* 1994, **259**, 243.

35 N. Asano, A. Kato, K. Oseki, H. Kizu and K. Matsui, *Eur. J. Biochem*. 1995, **229**, 369.

36 B. Draeger, *Phytochem. Anal*. 1995, **6**, 31.

37 R. J. Nash, M. Rothschild, E. Porter, A. A. Watson, R. D. Waigh and P. G. Waterman, *Phytochemistry* 1993, **34**,1281.

38 B. Draeger, A. van Almsick and G. Mrachatz, *Planta Med*. 1995, **61**, 577.

39 N. Asano, A. Kato, Y. Yokoyama, M. Miyauchi, M. Yamamoto, H. Kizu and K. Matsui, *Carbohydr. Res.* 1996, **284**, 169.

40 N. Asano, A. Kato, H. Kizu, K. Matsui, A. A. Watson andR. J. Nash, *Carbohydr. Res*. 1996, **293**, 195.

41 A. Kato, N. Asano, H. Kizu, K. Matsui, S. Suzuki and M. Arisawa, *Phytochem.* 1997, **45**, 425.

42 N. Asano, A. Kato, M. Miyauchi, H. Kizu, T. Tomimori, K. Matsui, R. J. Nash and R. J. Molyneux, *Eur. J.Biochem*. 1997, **248**, 296.

43 N. Asano, A. Kato, K. Matsui, A. A. Watson, R. J. Nash, R. J. Molyneux, L. Hackett, J. Topping and B. Winchester, *Glycobiology* 1997, **7**, 1085.

44 N. Asano, *Glycobiology* 2003, **13**, 93R.

45 J. E. Tropea, R. J. Molyneux, G. P. Kaushal, Y. T. Pan, M.Mitchell and A.D. Elbein *Biochem*.1989, **28**, 2027.

46 B. Junge, M. Matzke and J. Stoltefuss, In *Handbook of Experimental Pharmacology*; J. Kuhlmann, W.Pils, Eds.; Springer: Berlin, Heidelberg, New York, 1996; Vol. 119, pp. 411–482.

47 M. Bollen and W. Stalmans, *Eur, J. Biochem*. 1989, **181**, 775.

48 K. Lundgren, A. Rassov, and M.Bols, *Diabetes* 1996, **45**, 521.

49 P. Jacobsen, J. M. Lundbeck, M. Kristiansen, J. Breinholt, H. Demuth, J. Pawlas, M. P. Torres Candela, B. Andersen, N. Westergaard, K. Lungren, N. Asano, *Bioorg. Med. Chem*. 2001, **9**, 733.

50 T. D. Butters, R. A. Dwek and F. M. Platt, *Curr. Med.Chem.* 2003, **3**, 561.

51 M.S. Pearson, M. Mathe-Allaonmat, V. Fargeas and J. Lebreton, *Eur. J. Org. Chem*. 2005, **11**, 2159.

52 P. R. Dorling, C. R. Huxtable and S. M. Colegate, *J. Biochem.* 1980, **191**, 649

53 P. R. Dorling, C. R. Huxtable and P. Vogel, *Neuropathol. Appl. Neurobiol.* 1978, **4**, 285.

54   R. Saul, J. Ghidoni, R. J. Molyneux and A. D. Elbein, *Proc. Natl. Acad. Sci .USA* 1985, **82**, 93.

55   E. M. Bomhard, In *Handbook of Experimental Pharmacology*; J. Kuhlmann, W. Puls, Eds; Springer: Berlin, Heidelberg, New York, 1996; Vol. 119, pp. 557–610.

56   For review see: U. Fuhrmann, E. Bause and H. Ploegh, *Biochem. Biophys. Acta*, 1985, **825**, 95.

57   R. A. Gruters, J. J. Neefjes, M. Tersmette, R. E. Y. de Goede, A. Tulp, H. G. Huisman, F.Miedema and H. L Ploegh, *Nature* 1987, **330**, 74.

58   J. M. McCune, L. B. Rabin, M. B Feinberg, M. Lieberman, J. C Kosek, G. R. Reyes and I. L. Weissman, *Cell* 1988, **53**, 55.

59   (a) L. A. Lasky, J. E. Groopman, C. W. Fennie, P. M. Benz, D. J. Capon, D. J. Dowbenko, G. R. Nakamura, W. M. Nunes, M. E. Renz and P. M. Berman, *Science* 1986, **233**, 209. (b) T. J. Matthews, K. J. Weinhold, H. K. Lyerly, A. J. Langlois, H.Wigzel and D. Bolognesi, *Proc. Natl. Acad. Sci. USA* 1987, **84**, 5424.

60   Y. T. Pan, H. Hori, R. Saul, B. A. Sanford, R. J. Molyneux and A. D. Elbein, *Biochem.*, 1983, **22**, 3975.

61   A. D. Elbein, M. Mitchell, B. A. Stanford, L. A. Fellows and S. V. Evans, *J. Bio. Chem*. 1984, **259**, 1240.

62   A. D. Elbein, P. R. Dorling, K. Vosbeck and M. Horisberger, *J. Bio. Chem.* 1983, **257**, 1573.

63   P. E. Goss, M. A. Baker, J. P. Carver and J. W. Dennis, *Clin. Cancer Res.*, 1995, **1**, 935

64   L. L. Lairson and S. G. Withers, *Chem. Commun*. **204**, 2243.

65   D. L. Zechel and S. G. Withers, *Acc. Chem. Res.* 2000, **33**, 11; (b) T. D. Heightman and A. T. Vasella, *Angew. Chem. Int. Ed*. 1999, **38**, 750.

66   M. L. Sinnott, *Chem. Rev.* 1990, **90**, 1171.

67   M. Bols. *Acc. Chem. Res.* 1998, **31**, 1.

68   S.V. Evans, L. E. Fellows, T. K. M. Shing and G. W. J. Fleet, *Phytochem.*, 1985, **24**, 1953.

69   D. A. Winkler and G. Holan, *J. Med. Chem*. 1989, **32**, 2084.

70   R. J. Molyneux, J. N. Roitman, G. Dunnheim, T. Szumilo and A. D. Elbein, *Arch. Biochem. Biophys*. 1986, **251**, 450.

71   A. E. Stutz (Ed.), *Iminosugars as glycosidase inhibitors: Nojirimycin and beyond*, Wiley-VCH, Weinheim, 1999, chapter 8.

72   M. D. Lopez, J. Cobo and M. Nogueras, *Curr. Org. Chem*. 2008, **12**, 718.

73   J. P. Michael, Natural Product Reports 1998, **11**, 17.

74   A. El Nemr, *Tetrahedron* 2000, **56**, 8579-9628.

75   I. Izquierdo, M. T. Plaza, R. Robles and F. Franco, *Tetrahedron: Asymmetry* 2001,**12**, 2481.

76   I. Izquierdo, M. T. laza, R. Robles and F. Franco, *Carbohydr. Res.* 2001, **330**, 401.

77   I. Izquierdo, M. T. Plaza and F. Franco, *Tetrahedron: Asymmetry* 2002, **13**, 1581.

78   I. Izquierdo, M. T. Plaza and F. Franco, *Tetrahedron: Asymmetry* 2003, **14**, 3933.

79   I. Izquierdo, M. T. Plaza and F. Franco, *Tetrahedron: Asymmetry* 2004, **15**, 1465.

80   I. Izquierdo, M. T. Plaza and F. Franco, *Tetrahedron: Asymmetry* 2004, **15**, 3635.

81   I. Izquierdo, M. T. Plaza and J. A. Tamayo, *Tetrahedron* 2005, **61**, 6527.

82   I. Izquierdo, M. T. Plaza, J. A. Tamayo, M. Rodriguez and A. Martos, *Tetrahedron* 2006, **62**, 6006.

83   Izquierdo, M. T. Plaza, J. A. Tamayo and F. Sanchez-Cantalejo, *Tetrahedron:Asymmetry*

2007, **15**, 2211.

84 Izquierdo, M. T. Plaza, J. A. Tamayo and F. Sanchez-Cantalejo, *Eur. J. Org. Chem.* 2007, 6078.

85 Izquierdo, M. T. Plaza, F. Franco and A. Matos, *Tetrahedron* 2005, **61**, 11697.

86 Izquierdo, M. T. Plaza, J. A. Tamayo, V. Yanez, D. Lo Re and F. Sanchez-Cantalejo, *Tetrahedron* 2008, **64**, 4613.

87 O. R. Martin, P. Compain and L. Rambaud, *Tetrahedron: Asymmetry* 2001,**12**, 1807.

88 I. Delso, T. Tejero, A. Goti and P. Merino, *Tetrahedron* 2010, **66**, 1220.

89 H. Yoda, H. Katoh and K. Takabe, *Tetrahedron. Lett.* 2000, **41**, 7661.

90 H. Yoda, F. Asai and K. Takabe, *Synlett.* 2000, 1001.

91 M. Takahashi, T. Maehara, T. Sengoku, N. Fujita, K. Takabe and H. Yoda, *Tetrahedron.* 2008, **64**, 5254.

92 T. Sengoku, Y. Satoh, M. Oshima, M. Takahashi and H. Yoda *Tetrahedron.* 2008, **64**, 8052.

93

94 T. Sengoku, Y. Satoh, M. Takahashi and H. Yoda *Tetrahedron Lett.* 2009, **50**, 4937.

95 S. Desvergnes, A. Py and Y. Vallee, *J. Org. Chem*. 2005, **70**, 1459.

96 A. Padwa (Ed.), 1, 3-Dipolar Cycloaddition Chemistry, Wiley-Interscience; New York, 1984.

97 K. N. Houk, J. González, Y. Li, *Acc. Chem. Res*. 1995, **28**, 81

98 K. B. Torssell, *Nitrile Oxides, Nitrones and Ntronates in Organic Systems*, VCH, 1988.

99 J. J. Tufariello, and S. k. Ali, *Tetrahedron Lett*. 1990, **31**, 3351.

100 J. J. Tufariello, "*Nitrones in 1,3-Dipolar Cycloaddition Chemistry*," A. Padwa, Ed., Vol. 2, John Wiley and Sons 1984, p. 83-167.

101 S. A. Ali and M. I. M. Wazeer*, J. Chem. Soc., Perkin Trans. 2* 1990, 1035.

102 A. Goti, S. Chicchi, F. M. Cordero, V. Fedi and A. Brandi, *Molecules* 1999, **4**, 1.

103 K. V. Gothelf, and K. A. Jørgensen, *Chem. Rev.* 1998, **98**, 863.

104 K. V. Gothelf, R. G. Hazell, K and A. Jørgensen, *J. Org. Chem*. 1996, **61**, 346.

105 a) F. Cardona, S. Valenza, A. Goti and A. Brandi, *Tetrahedron Lett*. 1997, **38**, 8097; b) F. Cardona, S. Valenza, S. Picasso, A. Goti and A. Brandi, *J. Org. Chem*. 1998, **63**, 7311.

106 F. Cardona, P. Salanski, M. Chimielewski, S. Valenza, A. Goti and A. Brandi, *Synlett* 1998, 1444.

107 F. J. Duff, V. Vivien and R. H. Wightman, *Chem. Commun.* 2000, 2127.

108 Toyao, O. Tamura, H. Takagi and H. Ishabashi, *Synlett* 2003, **1**, 35

109 F. Cardona, E. Faggi, F. Liguori, M. Cacciarini and A. Goti, *Tetrahedron Lett*. 2003, **44**, 2315.

110 D. H. R. Barton, J. Dorchak andJ. C. Jaszberenyi, *Tetrahedron* 1992, **48**, 7435.

111 J. A. Tamayo, F. Franco, D. Lo Re, F. Sanchez-Cantalejo, *J. Org. Chem*. 2009, **74**, 5679.

112 J. Fleet, P. Smith, *Tetrahedron* 1986, **42**, 5685.

113 Toyao, O. Tamura, H. Takagi and H. Ishabashi, *Synlett* 2003, **1**, 35

114 F. Cardona, E. Faggi, F. Liguori, M. Cacciarini and A. Goti, *Tetrahedron Lett*. 2003, **44**, 2315.

115 a) A.Hall, K. P. Meldrum, P. R. Therond and R. H. Wightman, *Synlett* 1997, 123; b) A. E. McCaig, K. P. Meldrum and R. H. Wightman, *Tetrahedron* 1998, **54**, 9429.

116 L. S. Jeong, H. R. Moon, Y. J. Choi, M. W. Chun and H. O. Kim, *J. Org. Chem.* 1998, **63**, 4821

117 J.Moracona, J. Copkora, and J. Skoinek, *Carbohydr. Res* 1994, **263**, 61.

118 K. Kefurt, Z. Kefurtova, V. Markova and K. Slivova, *Collect. Czech. Chem. Commun*

1996, **61**, 1027.

[119] K. Kefurt, M, Hamernikova, J. Havlicek and H. Votavova, *Collect. Czech. Chem. Commun.* 2002, **67**, 622.

[120] D.R. Witty, G. W. J. Fleet, K. Vogt, F. X. Wilson, Y. Wang, R. Storer, P. L. Myers and C. J. Wallis, *Tetrahedron Lett*. 1990, **31**, 4787.

[121] H. Rapaport and H. N. Reist, *J. Am. Chem. Soc*. 1955, **77**, 480.

[122] M. Fetizon and M. Golfier, *C. R. Acad. Sci. Ser. C*, 1968, **267**, 900.

[123] V. Balogh, M. Fetizon and M. Golfier, *J. Org. Chem.* 1971, **36**, 1339.

[124] A. Mikillop and D. W. Young, *Synthesis*, 1979, 401.

[125] J. Barttaro, C.D. Bedford and A. Dodge, *Synth. Commun.* 1985, **15**, 1333.

[126] Kenji Mori, H. Takikawa and M. Kido*, J. Chem. Soc. Perkin 1*, 1993,169.

[127] H. Ishibashi, A. Toyao and O. Tamura, *Synlett* 2002, **8**, 1344.

[128] S. Cicchi, M. Marradi, P. Vogel, and Goti. A. *J. Org. Chem*. **2006**, 71, 1614.

[129] E. C. Davidson and A. B. Holmes, *Tetrahedron Lett*. 1995, **36**, 9047.

[130] S. Chackalamannil and Y. Wang, *Tetrahedron*, 1997, **53**, 11203.

[131] R. Huisgen, R. Grasey, H. Hauck and H. Seidl, *Chem. Ber*. 1969, 102, 736.

[132] O.Mitsunobu and Y. Yamada. *Bull. Chem. Soc. Japan* 1967, **40**, 2380-2382.

[133] D. B. Dess and J. C. Martin. *J. Am. Chem. Soc.* 1991, **113**, 7277-7287.

[134] K. Omura and D. Swern. *Tetrahedron* 1978, **34**, 1651.

[135] J.Mulzer, W. felzmann, D. Castagnolo andD. Rosenbieger. *J. Org. Chem.* 2007, **72**, 2812.

[136] J. Moravcová, J Čapková and J. Staněk, *Carb. Res*. 1994, **263**, 61.

[137] T. Aslam, PhD thesis, Heriot-Watt University, 2006.