Total synthesis of eleven hyacinthacine C-type analogues and the correction of the structures of natural hyacinthacine C1 and C5

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Total synthesis of eleven hyacinthacine C-type analogues and the correction of the structures of natural hyacinthacine C₁ and C₅

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B. Med. Chem (Hons)

Principal Supervisor: Prof. Stephen G. Pyne

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The hyacinthacine C-type alkaloids are polyhydroxylated pyrrolizidine alkaloids that were first isolated in 1999 from the fruits and stalks of *Hyacinthoides non-scripta* and later in 2007 from the bulbs of *Scilla socialis*. Today they comprise hyacinthacines C₁, C₂, C₃, C₄ and C₅ and have attracted considerable attention within the large community of those interested in iminosugars, since they exhibit inherent glycosidase inhibitory activities. Their low to moderate IC₅₀ values (13 – 100 µM) against various α-glucosidases makes the hyacinthacine C-type alkaloids important scaffolds towards finding medicines that help treat type-2 diabetes as well as the increasing obesity epidemic.

The hyacinthacine C-type alkaloids can contain up to seven possible stereogenic centres. This means that there are 128 unique stereoisomers (64 diastereomers and their enantiomers) containing a 3-hydroxymethyl-5-methylpyrrolizidine-1,2,6,7-tetraol core that have potentially different glycosidase inhibitory activities. Their constitutional complexity has resulted in structural insecurities in four of the five naturally occurring hyacinthacines, namely hyacinthacines C₁, C₃, C₄ and C₅. Their configurational ambiguity coupled with their relatively low glycosidase inhibitory activity presents an enticing challenge for many chemists and so has led to an increasing number of publications that detail synthetic work towards this class of compounds.

To shed light on the current inconsistencies in the literature, the work presented in this Ph.D. describes the successful total synthesis and consequent correction of the structures for hyacinthacines C₁ (Chapter 5), and C₅ (Chapter 4B). More specifically, it was found that the true structure for hyacinthacine C₁ is the C8 epimer of the purported structure. Moreover, the true structure for hyacinthacine C₅ has the opposite configuration at the three contiguous stereogenic centres, C₅, C₆, and C₇, to that of the originally proposed structure.

The successful pathway that resulted in these compounds was designed around the Petasis borono-Mannich acid reaction between 3,5-di-O-benzyl-α,β-L-xylofuranose, 3-amino-1-butene-hydrochloride and (E)-β-styrenylboronic acid. This led to the synthesis of two key amino-diol motifs that when strategically subjected to key steps such as ring-closing metathesis, *cis*-dihydroxylation, cyclic sulfate synthesis, regioselective cyclic sulfate ring-
opening and Swern oxidation/stereoselective reduction, resulted in the synthesis of an additional nine hyacinthacine C-type analogues.

The eleven hyacinthacine C-type analogues synthesized as a part of this study were then evaluated as glycosidase inhibitors and compared against the structurally analogous casuarine (one of the most powerful glucosidase inhibitors) and a useful SAR was developed. With respect to casuarine, hyacinthacine C-type analogues (−)-6-epi-hyacinthacine C₈ 156 and (+)-7-epi-hyacinthacine C₈ 155 displayed comparable inhibition against the α-glucosidase of rat intestinal sucrase (IC₅₀ values of 13 μM and 9.9 μM, respectively) and are suitable scaffolds towards investigating potential drug candidates for anti-diabetic and anti-obesity medications.
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Col 1:16 – 18 “For by Him were all things created, that are in heaven, and that are in earth, visible and invisible, whether they be thrones, or dominions, or principalities, or powers: all things were created by Him, and for Him: And He is before all things, and by Him all things consist.”
Certification

I, Anthony Walter Carroll, declare that this thesis submitted in fulfilment of the requirements for the conferral of the degree Doctor of Philosophy, from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Anthony Walter Carroll

February 2019
List of Abbreviations

[M+] molecular ion
[α]D specific rotation
µM micromolar
Ac acetyl
aq. aqueous
AUD Australian dollar
Bn benzyl
Boc tert-butylxocarbonyl
cat. catalyst
Cbz benzyloxy carbonyl
d.r. diastereotopic ratio
Da dalton
DBU 1,8-diazabicyclo[5.4.0]undec-7-ene
DCE 1,2-dichloroethane
DDQ 2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DFT density functional theory
DIPEA N,N-diisopropylethylamine
DMAP 4-dimethylaminopyridine
DMF dimethylformamide
DNJ deoxynojirimycin
eq. equivalence
EtOAc ethyl acetate
Fmoc fluorenylmethylxocarbonyl
GC-MS gas chromatography–mass spectrometry
gCOSY gradient correlated spectroscopy
gHMBC gradient heteronuclear multiple bond correlation
gHSQC gradient heteronuclear single quantum correlation
HPLC high-performance liquid chromatography
HRESIMS high-resolution electrospray ionization mass spectra
HTS high throughput screening
Hz hertz
IC₅₀ half maximal inhibitory concentration
LRESIMS low-resolution electrospray ionization mass spectra
M molar
m/z mass-to-charge ratio
mCPBA meta-chloroperoxybenzoic acid
MHz  megahertz
mol %  mole percent
MOM  methoxymethyl
Ms  methanesulfonyl
MW  microwave
NBS  \(n\)-bromosuccinimide
NI  no inhibition
NIDDM  non-insulin dependent diabetes mellitus
NMO  \(n\)-methylmorpholine-\(n\)-oxide
NMR  nuclear magnetic resonance
NOE  nuclear Overhauser effect
NOESY  nuclear Overhauser effect spectroscopy
PA  polyhydroxylated alkaloid
pH  potential hydrogen
ppm  parts per million
R&D  research and development
RCM  ring-closing metathesis
R\(f\)  relative mobility
ROESY  rotating frame Overhauser effect spectroscopy
SAR  structure-activity-relationship
rt  room temperature
SET  single electron transfer
TBAF  tetra-\(n\)-butylammonium fluoride
TBAI  tetra-\(n\)-butylammonium iodide
TBDDS  \(\text{tert}\)-butyldiphenylsilyl
TBS  \(\text{tert}\)-butyldimethylsilyl
TFA  trifluoroacetic acid
THF  tetrahydrofuran
TLC  thin-layer chromatography
TMEDA  tetramethylethlenediamine
TMS  tetramethylsilane
\(v/v\)  volume per volume
\(\delta\)  nmr chemical shift in ppm
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Chapter 1: History and development of the hyacinthacine C-type Alkaloids

1.1. The importance of natural products

A natural product can be defined as a substance that is produced by life. Such compounds are initially isolated from a natural source and if useful, they can be later prepared by chemical synthesis in a scalable manner. Whether perceived as good or bad, natural products are undeniably one of the most influential compound classes for living and are undoubtedly fundamental to the lifestyles of all species.

Among humans, the impact of natural products can be seen through the example of a cup of tea or coffee. These brewed solutions enjoyed by most are rich sources of alkaloids. More specifically, theophylline and caffeine (Figure 1.1) are the natural products that most would recognize as the active ingredients in both drinks, respectively.

![Fig 1.1: Natural products theophylline and caffeine.](image1)

Another example of a natural product and its impact on lifestyle is nicotine: a component of tobacco blends (Figure 1.2). Similarly, this natural product is generally accepted to be the potent parasympathomimetic stimulant alkaloid responsible for inducing relaxation after it is inhaled.
Even with advancements in medical technology to allow the design of potent drugs for medical use, natural products are still actively and widely used on large commercial scales. An example of this are the recently introduced Coles™ brand of cold and flu, pain relief and decongestant tablets, whereby they solely contain natural product extracts from *Andrographis paniculate* (Green chirayta), *Echinacea purpurea* (Eastern purple coneflower), *Salix alba* (white willow) and *Sambucus nigra* (Black elderberry) (Figure 1.3).

Regardless of lifestyle or medicinal use, these examples only scratch the surface of the importance and widespread abundance of natural products in modern lifestyles. For the remainder of this Chapter however, the broader focus of this research will address modern natural product-derived medicines.
1.2. Drug discovery and development

New drug discovery has been largely influenced through the examination of natural product medicines that have been used for centuries. Interest and investigation into naturally occurring medicines excelled in the early 1980s, and by 2002, it was found that 75% of new drugs for infectious disease and 60% of new drugs for cancer, stemmed from natural products. However, this statistic was short lived. More specifically, between 2001 and 2005, only 23 new drugs succeeded from natural product to market to be used as: immunosuppressive agents, pain relief, anti-bacterial and anti-fungal agents, treating infections, cancer, diabetes, dyslipidemia, atopic dermatitis, Alzheimer’s disease and other genetic diseases (tyrosinemia and Gaucher’s disease). This 20-year low is perceived as the reason for a large number of pharmaceutical companies having reduced or cancelled funding towards natural product research programs. In addition to this, the late 1990s brought a significant paradigm shift amongst pharmaceutical companies and their approach to sourcing first-in-class drugs. Technologies such as combinatorial chemistry and high-throughput screening (HTS) allowed for high speed assays of large synthetic compound libraries, but could not effectively be applied to natural product screening on a similar magnitude.

Today, the research and drug discovery model includes modern molecular biological methods (understanding disease mechanisms, biomarkers, surrogate endpoints), HTS, structure-based drug design, combinatorial and parallel chemistry, and the sequencing of the human genome. This latest research and development (R&D) model is largely influenced by the result of six decades of inflation-adjusted costs. On average, introducing a new drug to the market doubles in cost approximately every 9 years. Despite such innovations, there are many doubts and raised concerns over the current R&D model simply because the number of drug approvals by the US Food and Drug Administration (FDA) are not increasing proportionally with the advancements of the aforementioned technologies. Furthermore, with less emphasis on natural product research and more interest in developing large compound libraries, it is unsurprising that a notable reduction in the discovery of new chemical entities has also been observed.

These alarming facts suggest that the current R&D model should be revised. This does not necessarily need to become more advanced, rather pharmaceutical natural product
research programs should be reconsidered. During the 1980s, natural product research rarely considered aqueous extracts. If aqueous extracts of natural products were investigated for the following reasons, perhaps similar success observed in the ‘golden era’ of new drug discovery would be achieved.

1.3. Polyhydroxylated Alkaloids- Distribution in Nature:

Unlike traditional preparations, drug discovery programs largely extracted natural products and promising medicinal agents with the aid of organic solvents such as methanol, dichloromethane, chloroform and hexane. However, folkloric medicines are almost exclusively brewed as water soluble extracts of natural products, and so any ascribed therapeutic benefit is likely to be attributed to water soluble components, including polyhydroxylated carbohydrate analogues. Of these, the polyhydroxylated alkaloids (PAs) prove particularly interesting for organic and medicinal chemists. More specifically, they display high stereoselectivity and activity for biological systems. In conjunction with a low molecular weight (~250 Da), heterocyclic nature and large stereoconfiguration scope, this class of compound make them an ideal synthetic target.

Polyhydroxylated alkaloids can also be referred to as iminosugars, azasugars or sugar-shaped alkaloids, largely owing to their structural resemblance to monosaccharides. As their name suggests, their chief difference is that they contain a ring nitrogen in place of oxygen. After their initial finding in 1966 (norjirimycin), this class of alkaloid was thought to be restricted in Nature, however, today they can be subdivided into six ring systems: pyrrolines, pyrrolidines, piperidines, pyrrolizidines, indolizidines and nortropanes. These can either be monocyclic or bicyclic structures (Selected examples of each are found in Table 1.1).
Table 2.1: Selected examples of the six polyhydroxylated alkaloid classes, along with their distribution and biological activity.

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Source</th>
<th>Figure</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>noririmycin (iminopyranose)</td>
<td>The first PA and iminopyranose to be discovered as an isolate from <em>Streptomyces roseochromogenes</em> R-468 and <em>Streptomyces nojiriensis</em> SF-426 between 1966 - 1967.</td>
<td>[Image]</td>
<td>Inhibitor of α- and β-glucosidases as well as antibiotic action.¹⁷</td>
</tr>
<tr>
<td>2,5-dihydroxymethyl-3,4-dihydroxy.pyrrolidine (DMDP) (iminofuranose)</td>
<td>The first naturally occurring iminofuranose to be discovered and isolated from the leaves of <em>Derris elliptica</em> (legume) in 1976.</td>
<td>[Image]</td>
<td>Inhibitor of glycoprotein processing. Inhibits α- and β-glucosidases, human lysosomal β-mannosidase, β-galactosidase and β-xylosidase.²⁰⁻²⁵</td>
</tr>
<tr>
<td>nectrisine (pyrrole)</td>
<td>The first naturally occurring pyrrole to be discovered and isolated from the fungus <em>Nectria lucida</em> F-4490 (Asscomycetes) in 1988.</td>
<td>[Image]</td>
<td>An immunomodulator and inhibitor of α-glucosidases, α- and β-mannosidases, β-glycosidases, β-N-acetylglucosaminidase and trimming glucosidase 1.²⁶</td>
</tr>
<tr>
<td>alexine (pyrrolizidine)</td>
<td>One of the first examples of a naturally occurring polyhydroxylated pyrrolizidine with a hydroxymethyl substituent at the C3 position. Alexine was isolated in 1988 from <em>Alexa leiopetala</em> (Leguminosae).</td>
<td>[Image]</td>
<td>Inhibitor of disaccharidase-type-α-glucosidase (trehalase, amyloglucosidase) and β-galactosidase.²⁸,²⁹</td>
</tr>
</tbody>
</table>
One of the first examples of an isolated polyhydroxylated nortropane from *Calystegia sepium* in 1988.\textsuperscript{31,32}

![Calystegia sepium](image1.jpg)

Inhibitor of β-glucosidases, α- and β-galactosidases, and trehalase.\textsuperscript{34}

The first polyhydroxylated indolizidine isolated from *Swainsona canescens*.\textsuperscript{35}

![Swainsona canescens](image2.jpg)

Inhibitor of α-mannosidase.\textsuperscript{37}

To date, hundreds of novel polyhydroxylated alkaloids have and continue to be isolated by a simple means of reinvestigating the aqueous fractions of previously characterised natural products.\textsuperscript{13} Their initially proposed distribution in Nature is misleading because of inadequate extraction methods previously employed. This suggests a huge potential to find new subclasses of polyhydroxylated alkaloids in plants, micro-organisms and taxa not previously considered to be alkaloid producing.\textsuperscript{13} More importantly, their analogous structure to glycosides -like glucose and mannose- mean that the polyhydroxylated alkaloids can interact with enzymes known as glycosidases and might therefore have important therapeutic potential towards treating diseases such as viral infections, cancer and diabetes.

### 1.4. Polyhydroxylated alkaloids as glycosidase inhibitors

Essential for existence and found in all domains of life, glycosidases are enzymes that catalyse the hydrolysis of carbohydrates and complex glycosides to release simpler monosaccharides and oligosaccharides. This large and diverse group of enzymes has been extensively studied since the early 1830s.\textsuperscript{38} Today, these enzymes are well characterised and facilitate many functions which range from the digestion of large polysaccharides through to developing cellular structural integrity.\textsuperscript{13,20}

To ensure a range of unique functions, individual glycosidases must display distinct affinity for certain carbohydrate molecules and also for specific anomeric configurations
of that sugar.\textsuperscript{13} It is therefore reasonable that through mimicry of pyranosyl or furanosyl moieties, polyhydroxylated alkaloids display the potential to be highly potent and specific inhibitors of glycosidases. Furthermore, the number, position and configuration of the hydroxy groups of each alkaloid can influence the type of glycosidase inhibited.\textsuperscript{13}

The above rationale certainly paved way for the development of the oral antidiabetic drug, miglitol. This evolution of a naturally occurring polyhydroxylated alkaloid to its marketable drug should certainly be explored. However, before this case study is examined, we must first consider the mechanisms of enzymatic glycoside hydrolysis.

1.5. Glycosidase hydrolysis

Glycosidases are an extremely efficient class of enzymes. In 1998, Wolfenden \textit{et al.} were able to demonstrate this, where they found that glycosidases were able to enhance the rate of catalysis \(10^{17}\) fold faster than the uncatalyzed reaction (noting that efforts to preserve paper (cellulose) books and other rare documents is somewhat futile).\textsuperscript{39} Additionally, this figure correlates to an estimated dissociation constant for the transition state to be no more than \(10^{-22}\) M, which suggests that the substrate must have a very strong binding affinity for the enzyme.\textsuperscript{39-40} Therefore, the ideal mechanism-based glycosidase inhibitor should mimic the transition state formed during glycosidase hydrolysis such that it binds strongly and also reversibly to the glycosidase active site.\textsuperscript{41}

Glycosidases can be segregated into two main groups based on whether the stereochemical outcome of the anomic configuration of the hydrolysed monosaccharide is either retained or inverted (Figure 1.4).\textsuperscript{41-44} For instance, if a carbohydrate enters the active site of a retaining glycosidase (Figure 1.4A), the anomic carbon of the terminal glucose unit undergoes a ‘double inversion’ and retains the original stereochemistry. More specifically, after an initial attack at the anomic carbon by a carboxylate nucleophile, a neighbouring carboxylic acid residue concurrently donates a proton to the leaving group oxygen. The proton deficient carboxylate can then expedite a second nucleophilic attack of a water molecule at the anomic carbon which subsequently displaces the monomeric sugar unit, but retains the original stereochemistry.\textsuperscript{45-46} Comparatively, inverting glycosidases react through a single step (S\textsubscript{N}2) mechanism (Figure 1.4B). To facilitate this process, one
carboxylate residue acts as an acid and protonates the glycosidic oxygen. The other carboxylic acid residue acts as a base and can deprotonate a water molecule, resulting in a nucleophilic attack towards the anomeric carbon. Common to both glycoside hydrolysis pathways is the formation of an oxocarbenium-ion-like transition state which proves to be an important characteristic when considering the design of specific glycosidase inhibitors. Additionally, through the advancements of an increasing number of structural and mechanistic studies, glycoside hydrolases have, and continue to be, subcategorized based on their specific mechanism of action. Since the PAs resemble the oxocarbenium-ion-like transition state at certain pH levels, scientists are able to exploit these scaffolds and design extremely potent and specific inhibitors of glycosidases. To gain insight into this process, we will investigate the development of the non-insulin dependent diabetes mellitus (NIDDM) drug, miglitol.
Figure 2.4: Mechanisms of A) retaining and B) inverting glycosidases.

1.6. Case study: Deoxynorjirimycin to antidiabetic drug miglitol

At a physiological pH, PAs become protonated at their nitrogen atom which leads to the formation of a stable complex within the glycosidase active site. As seen with the
iminosugar 1-deoxynojirimycin (DNJ) in Figure 1.5, its adoption of a comparable oxocarbenium-ion-like shape causes the enzyme to have a high affinity for the inhibitor. When inside the active site cleft of the enzyme, the iminosugar can form an ion pair with the carboxylate moieties resulting in a physical blockage of disaccharides that would otherwise be hydrolysed. This partial bond allows for reversibility, where the strength and specific inhibition will be largely dependent on the shape, size and stereoconfiguration of the iminosugar, as well as the target enzyme.

![Figure 2.5: Comparison of DNJ (left) and the oxocarbenium-ion-like transition state of glycoside hydrolysis (right).](image)

The importance of PA isolation and research can be clearly seen through the development of the pseudomonosaccharide α-glucosidase inhibitor miglitol. Although approved in 1996 as a NIDDM treatment, miglitol (marketed under the name Glyset™) can be traced back to the 1970s through its naturally occurring parent analogue DNJ (Figure 1.6).48-49 Prior to its isolation in 1976 from the roots of a Mulberry species (Moraceae), DNJ had already been the product of two syntheses reported in 1967 and 1968 by Paulsen et al. and Inouye et al. respectively.15, 49-50 Its synthetic attention at this time was largely due to the recent isolation of the closely related natural isolate, nojirimycin, an inherent antibiotic.51 It was found that relative to nojirimycin, the lack of an anomeric hydroxy group at C1 caused DNJ to act as a potent inhibitor of yeast and pancreas glycosidases.52
Although this was a noteworthy find, the impressive glycosidase inhibitory activity of DNJ could be considered an unsurprising result. In addition to its original isolation, DNJ has been well established as a dominant constituent of various Moraceae; a species with a rich history in East Asian, folklore-based use and medicine.\textsuperscript{22, 49, 53-54} In fact, brewed extracts of Mulberry leaves are considered a traditional remedy used to treat and prevent “Xiao-ke” (diabetes), particularly in China.\textsuperscript{54-55}

Its traditional use, in conjunction with inherent glycosidase inhibitory activity, convinced scientists that DNJ would be a promising NIDDM therapeutic. However, this was soon dismissed on the basis of \textit{in vivo} experiments, where DNJ displayed only moderate effects on small intestinal D-glucose uptake.\textsuperscript{56} In light of this result, $N$-alkyl derivatives of DNJ were synthesized on the basis of improved \textit{in vivo} activity, whereby the $N$-hydroxyethyl-DNJ analogue (miglitol) proved most effective for managing postprandial blood glucose and was consequently sold on the market as medicine for type-2 diabetes.\textsuperscript{57}

\textbf{Figure 2.6:} Development of the naturally derived\textsuperscript{58} DNJ and its analogous marketed NIDDM derivative, miglitol.\textsuperscript{59}

Although effective for managing type-2 diabetes, miglitol's mechanism and localization (small intestine) of glycosidase inhibition led to undesired side effects. As a result of preventing the digestion of polysaccharides, flatulence and gastrointestinal discomfort were notable in patients and are today the largest deterrents for using this medication.\textsuperscript{60} However, it has also been observed that abdominal discomfort is dose-related and can be better tolerated with continued use.\textsuperscript{61-62}

The development of miglitol from the naturally occurring DNJ is a convincing example of just how important it is for the present R&D model to include the investigation of natural
products as a source for drug scaffolds. The polyhydroxylated nature of DNJ and miglitol validate the investigation into unexplored water-soluble components of natural products. Although all classes of PAs should be of interest, the polyhydroxylated pyrrolizidine alkaloids have gained considerable attention in the literature for their noteworthy glycosidase inhibitory activities. For this reason, this class of PA will be the broader focus for the remainder of this Chapter.

1.7. Polyhydroxylated Pyrrolizidine Alkaloids

Prior to discussing the polyhydroxylated pyrrolizidine alkaloids, it is worth noting pyrrolizidine alkaloids, particularly those that contain a hydroxymethyl substituent at C1, as they have been of exponentially growing interest since the 1960s.63

![General structure for pyrrolizidine alkaloids.](image)

**Figure 2.7:** General structure for pyrrolizidine alkaloids.

Their appreciation is largely a result of the increasing number of deaths to livestock that have consumed plants rich in pyrrolizidine alkaloids.63 A memorable example occurred in Australia in the late 1970s, whereby a large number of chicken poisonings were reported.64 On closer inspection, researchers found that the ill-thrift, ascites and degenerative lesions on the chickens, were the result of a commercial poultry feed containing heliotrine and lasiocarpine: pyrrolizidine alkaloids found in the seeds of *Heliotropium stenophyllum* (Figure 1.8).64 It is more alarming however that many cases of liver disease and cancer in humans have been the direct result of pyrrolizidine alkaloid consumption.65-68 This wide spread occurrence is not surprising since there have been recorded findings of pyrrolizidine alkaloids detected in wheat flour, honey and also cow’s milk.69-71 At least from an economic point of view, it remains important that improved methods of detection, chromatographic separation and chiefly, natural product characterization are continually explored.
Figure 2.8: *Heliotropium stenophyllum* along with pyrrolizidine alkaloids heliotrine and lasiocarpine.

By the 1980s, pyrrolizidine alkaloids were deemed pernicious upon ingestion and were consequently flagged as a world-wide health concern. Their toxicity is a result of the 1,2-unsaturated bond, in combination with an ester at the C9 position (Figure 1.7). After consumption, hepatic mixed function oxidases convert these pyrrolizidine alkaloids to their counter pyrrole derivatives. These metabolic products are highly reactive and can scar various organs in the body, particularly rich in thiol containing amino acids.

During this time, only a few examples existed of pyrrolizidine alkaloids that displayed anti-tumour activity and neuromuscular blocking activity and so this class of alkaloid was generally thought to be toxic. However, by the late 1980s, this view drastically changed after the discovery of the polyhydroxylated pyrrolizidine alkaloids containing a hydroxymethyl substituent at the C3 position. The compound first discovered of this nature was alexine and was appropriately named after its source, the legume *Alexia leiopetala* (Figure 1.9). Its structure was unequivocally confirmed by X-ray crystallography and just a few months later, its C7a epimer (also crystalline), australine, was reported as a constituent of *Castanospermum australe* seeds (Figure 1.9). Following these discoveries, a number of closely related epimers were also isolated from *C. australe*. Interestingly, alexine and australine along with their related epimers displayed notable anti-viral properties as well as low IC50 values towards specific glycosidases. These findings attracted significant attention to the polyhydroxylated pyrrolizidine alkaloids, because contrary to their non-hydroxylated analogues, they proved to be medicinally useful.
Prior to the discovery of alexine and australine, it was perceived that the only glycosidase inhibitors were restricted to polyhydroxylated indolizidines (swainsonine), polyhydroxylated pyrrolidines (DMDP) and polyhydroxylated piperidines (nojirimycin). The polyhydroxylated pyrrolizidine alkaloids unique structure and hydroxy substitution patterns gives rise to their reported antiviral properties and other medicinal applications. For this reason, it has become apparent that related analogues of alexine and australine, synthetic or naturally derived, are of considerable interest for medicinal chemists globally. 

It was therefore an exciting discovery when the first pentahydroxylated pyrrolizidine alkaloid, casuarine, was isolated from the bark of *Casuarina equisetifolia* L (Figure 1.10) reported by Nash et al. in 1994. Nash and colleagues were initially interested in the bark of *C. equisetifolia* L because of its traditional use as a medicine to treat diarrhoea, dysentery and colic. Perhaps a more convincing reason for Nash et al. to examine this natural source, was due to previous reports of prescribed *C. equisetifolia* L bark extract for the treatment of breast cancer in Western Samoa. Their investigation started with an GC-MS analysis of the extract, where a pentahydroxy- nitrogen-containing species, later established to be casuarine, was identified to be one of the major compounds present. Moreover, it contained the highest oxidation level to be found in any related naturally occurring iminosugar. Glycosidase inhibition studies of casuarine found it to be a highly potent glycosidase inhibitor.
Casuarine has also been identified as a common constituent within the leaves and seeds of *Eugenia jambolana* (Figure 1.11), a plant native to India known particularly for its various medicinal uses including the management of diabetes and bacterial infections. In the related species, *Eugenia uniflora*, isolates initially labelled as uniflorine A and B were assigned structures containing an indolizidine core. However, by members from the Pyne research group, it was later found that both uniflorine A and B were incorrectly assigned and were in fact casuarine along with its C6 epimer, respectively (Figure 1.11). The only other naturally occurring casuarine epimer reported in the literature is the C3 epimer which was isolated from *Myrtus communis* L. in 2006 by Fleet et al. Compared to casuarine, its notably weaker inhibition of $\alpha$-D glucoside is a result of its single stereochemical difference.
Since their discovery, casuarine, alexine, australine and related structures have been largely sought after due to their inherently selective glycosidase inhibition and are consequently perceived as ideal leads for drug candidature. Although no marketable drug has yet resulted from this class of compound, scientists have deduced that the polyhydroxylated pyrrolizidine alkaloids containing a C3 hydroxymethyl are medicinally useful (unlike their unsaturated analogues). Moreover, the absolute configuration as well as the degree of oxidation is crucial for selectivity and potency against specific glycosidase inhibition.

1.8. The Hyacinthacine Alkaloids: Isolation and Biological activity

One recently discovered subclass of polyhydroxylated pyrrolizidine alkaloid that has attracted considerable attention for their inherent glycosidase inhibitory activities are the hyacinthacine alkaloids. Despite their analogous structure to casuarine, alexine and australine, these alkaloids have been named after their original source, namely the Hyacinthaceae subfamily within the Asparagaceae. Shown in Figure 1.12, the hyacinthacine alkaloids can be divided into three classes: Hyacinthace A, B and C;
whereby the letter assignment denotes whether 0, 1 or 2 hydroxy/hydroxymethyl substituents are found on the B-ring of the alkaloid, respectively.

Figure 2.12: Hyacinthacines A₁, B₁, C₁, containing 0, 1 and 2 hydroxy or hydroxymethyl substituents on the B-ring of the pyrrolizidine (red), respectively.

The discovery of the hyacinthacine alkaloids can be traced back to the late 1960s and is considered an unprecedented find, largely because Asparagaceae is unrelated to the Leguminosae, Casuarinaceae and Mytaceae plant families.¹⁰¹ In 1967 a report from The Veterinary Record written by Thursby-Pelham, detailed the toxicological effects on livestock after consuming Hyacinthoides non-scripta (Figure 1.13).¹⁰² More specifically, Thursby-Pelham found that ingestion led to abdominal pain and dysentery in horses and lethargy and dullness in cows.¹⁰² The plants metabolites however remained a mystery until 1997, where, prompted by this report and perhaps also the availability of this material, Fleet et al. identified the presence of five pyrrolidine alkaloids present in the ethanolic fraction of the leaves.¹⁰³ This was the first report of any such alkaloids from the Hyacinthaceae species and although the plant was considered detrimental to livestock health, Fleet et al. were able to demonstrate that the isolated pyrrolidine alkaloids were potential therapeutic leads. Glycosidase inhibition studies of these isolates found that they might serve as useful chemotherapeutic agents against cancers, viruses and also diabetes.¹⁰¹
Two years after this novel finding, several of the same authors led by Asano, reported their investigation into the fruits and stalks of the *H. non-scripta*.\textsuperscript{105} To further explore the natural abundance of similar compounds, their research also detailed the analysis of the bulbs of a related species, *Scilla campanulata* (Figure 1.14).\textsuperscript{105}

In addition to the isolation of six pyrrolidine alkaloids, three novel polyhydroxylated pyrrolizidine alkaloids labelled as hyacinthacine alkaloids were reported (See Table 1.2).\textsuperscript{105} More specifically, from the 50\% ethanoic extracts of the *H. scripta* stalks, hyacinthacine C\textsubscript{1} was isolated, and from the 50\% ethanoic extract of the *S. campanulata* bulbs, hyacinthacine B\textsubscript{2} was isolated.\textsuperscript{105} Hyacinthacine B\textsubscript{1} was common in both plants and thus found as a constituent in both extracts.\textsuperscript{105} Although not necessarily potent, these three alkaloids gained considerable interest for their selective glycosidase inhibition.
Hyacinthacine B₁ and B₂ both displayed weak inhibition of almond β-glucosidase (IC₅₀ values of 320 µM and 100 µM, respectively) and bovine liver β-galactosidase (IC₅₀ values of 110 µM and 160 µM, respectively). Both alkaloids also showed selective inhibition for rat intestinal lactase β-galactosidase, however hyacinthacine B₂ proved far more active (IC₅₀ = 3.6 µM) than hyacinthacine B₁ (IC₅₀ = 270 µM). Interestingly, hyacinthacine C₁ proved moderately active against the amylglucosidase of *Aspergillus niger* (IC₅₀ = 84 µM), whereas hyacinthacine B₁ and B₂ displayed no inhibition for these two glycosidases.

Given these noteworthy glycosidase inhibitory activities, the therapeutic potential warranted further investigation of the hyacinthacine alkaloids. Since there had not yet been a proven synthetic route to access these novel structures, natural product investigation was the only viable option. To increase the chance of finding similar novel hyacinthacine alkaloids, the same authors reported in 2000, their investigation into the bulbs of the related *Muscari armeniacum* whereby they identified four novel hyacinthacine type alkaloids. More specifically, Asano and co-workers successfully isolated (from 60% ethanoic extracts) hyacinthacine C₁ as well as new Hyacinthacines A₁, A₂, A₃ and B₃ (See Table 1). When tested against a panel of glycosidases, hyacinthacines A₁, A₂, A₃ and B₃ displayed weak to moderate activity against rat intestinal lactase β-galactosidase (IC₅₀ values of 4.4 µM, 73 µM, 160 µM and 18 µM, respectively) and also *A. niger* amylglucosidase (IC₅₀ values of 25 µM, 8.6 µM, 17 µM and 51 µM, respectively). Hyacinthacine A₁ proved to be the only alkaloid active against rat epididymis α-L-fucosidase (IC₅₀ = 46 µM) and rice α-glucosidase (IC₅₀ = 240 µM). Hyacinthacines A₁ and A₂ also displayed activity against almond β-glucosidase with IC₅₀ values of 250 µM and 150 µM, respectively.

![Muscari armeniacum](image)

*Figure 2.15: Muscari armeniacum.*
Despite what could be considered a small compound library, Asano and co-workers remarked on the difficulty to predict the specificity and potency of glycosidase inhibition based on absolute configuration and also the degree of substitution on the pyrrolizidine ring. Concluding statements from this body of work highlighted the need for a variety of highly oxygenated or substituted pyrrolizidine alkaloids to better understand the structural requirements for glycosidase inhibition. Concurrently, glycosidase inhibitors were also receiving considerable attention as therapeutic agents. In addition to potential treatment for NIDDM (type II diabetes), glycosidase inhibitors were also established as possible treatments for tumour metastasis, viral infections, and various lysosomal storage disorders. For these reasons, investigation into the hyacinthacine type alkaloids also received increased attention and resulted in perhaps the most profitable recordings of novel hyacinthacine alkaloids isolated from a single species to date by Asano et al. in 2002. Their report detailed the analysis of the bulbs of the related Scilla sibirica (Figure 1.16) that were originally purchased from a flower shop in 1999. Specifics of this report include 9 kg of S. sibirica bulbs being homogenized in 60% aqueous ethanol, and further extracted to give seven new hyacinthacine alkaloids appropriately named hyacinthacines A₄, A₅, A₆, A₇, B₄, B₅ and B₆ (See Table 1.2). Although the majority of these hyacinthacine alkaloids proved relatively inactive towards the selected panel of glycosidase enzymes, they still added to the understanding of the structure-activity relationships of these and related compounds. In summary, the only active hyacinthacine alkaloids were A₅, B₄, and B₅, where they showed inhibitory activity towards the amylglucosidase of Aspergillus niger with IC₅₀ values of 110 µM, 89 µM and 110 µM, respectively. In addition to this, hyacinthacine B₄ displayed a moderate inhibition of bovine epididymis α-L-fucodisase with an IC₅₀ value of 23 µM.
Two years later, Asano and co-workers reported findings of long side chain pyrrolidines and pyrrolizidines in a closely related Asparagaceae, *Scilla peruviana* (Figure 1.17). Asano *et al.* isolated three novel hyacinthacine A₁-type related alkaloids along with one novel australine type related alkaloid. These long-chain hyacinthacine derivatives were isolated from 5 kg of bulb plant material using 50% aqueous ethanoic extracts and were accordingly named α-5-C-(3-hydroxybutyl)hyacinthacine A₁, α-5-C-(1,3-dihydroxybutyl)hyacinthacine A₁, α-5-C-(1,3,4-trihydroxybutyl)hyacinthacine A₁ and α-5-C-(3-hydroxybutyl)-7-epi-australine (See Table 1.2). When tested against a panel of glycosidases, α-5-C-(1,3-dihydroxybutyl)hyacinthacine A₁ and α-5-C-(3-hydroxybutyl)-7-epi-australine displayed relatively good activity against yeast α-glucosidase (IC₅₀ values of 3.6 µM and 6.6 µM, respectively), however only the former alkaloid, along with α-5-C-(1,3,4-trihydroxybutyl)hyacinthacine A₁, proved relatively low inhibition against almond β-glucosidase and *C. saccharolyticum* β-glucosidase with IC₅₀ values of 9.5 µM, 25.4 µM and 5.1 µM, 11.4 µM, respectively.
The most recent addition to the naturally occurring hyacinthacine alkaloids is the 2007 reporting of six new isolates from *Scilla socialis* (Figure 1.18).\textsuperscript{117} Kato and co-workers initially purchased *S. socialis* plants in 2003 and cultivated this species in a heated greenhouse. Once grown, 2.3 kg of the plant bulbs were homogenized in a 50% ethanol solution and further investigated whereby α-5-C-(3-hydroxybutyl)hyacinthacine A\textsubscript{2}, hyacinthacine B\textsubscript{7}, C\textsubscript{2}, C\textsubscript{3}, C\textsubscript{4} and C\textsubscript{5} were isolated and tested against a panel of selected glycosidases (See Table 1.2). Of these results, hyacinthacine C\textsubscript{2}, C\textsubscript{3} and C\textsubscript{5} displayed noteworthy activity against *C. saccharolyticum* β-glucosidase with IC\textsubscript{50} values of 13 µM, 25 µM and 48 µM, respectively. Hyacinthacine C\textsubscript{4} proved to be the only active compound against rice α-glucosidase (IC\textsubscript{50} = 110 µM), but shared similar activities with hyacinthacine C\textsubscript{5} against rat α-glucosidase (IC\textsubscript{50} = 45 µM and 77 µM, respectively) and the amyloglucosidase of *A. niger* (IC\textsubscript{50} = 57 µM for both C\textsubscript{4} and C\textsubscript{5}). Hyacinthacine C\textsubscript{3} displayed a unique inhibition of bovine liver β-galactosidase with an IC\textsubscript{50} value of 52 µM, whilst hyacinthacine C\textsubscript{2} proved the only alkaloid capable of inhibiting human placenta α-L-fucosidase with an IC\textsubscript{50} value of 17 µM.\textsuperscript{117}
Table 2.2: Concentration (µM) of the naturally isolated hyacinthacine alkaloids giving 50% inhibition of various glycosidases (IC\textsubscript{50} values).

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\textsuperscript{a}: Not tested, \textsuperscript{b}: NI : No inhibition (less than 50% inhibition at 1000 µM)
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1.9. Structural assignment issues with the Hyacinthacine alkaloids

Excluding the three long-chain hyacinthacine A-type analogues, 19 hyacinthacine type alkaloids have been isolated and reported between 1999 and 2007. In most cases, 2D NMR experiments (gCOSY, HSQC, HMBC) were the sole basis of structure elucidation. Moreover, NOE correlations as well as $^3J_{H,H}$ coupling patterns and constants were used to determine the relative configuration but were limited such that they were unable to identify the absolute configuration of the isolated hyacinthacines. With no X-ray crystallographic structures of these complex natural isolates, this has understandably led to inconsistencies among the hyacinthacine structures and suggests that their assigned configurations are not secure. An immediately obvious example of this can be seen in the proposed structures of hyacinthacines C$_1$ and C$_4$. Although reported nearly eight years apart, both alkaloids were assigned with identical structures despite their prominent differences in both $^1$H and $^{13}$C NMR spectroscopic data and their specific rotations. Despite plausible NOE correlations presented for both structures (Figure 1.19), the aforementioned differences between the spectroscopic data suggests at least one of these structures to be incorrect.$^{105,117}$

![NOE correlations for hyacinthacine C$_1$ and C$_4$](image)

**Figure 2.19:** The NOE correlations reported for hyacinthacine C$_1$ and C$_4$. $^{105,117}$

Therefore, to confirm their structures (as well as the proposed relative and absolute configurations) the hyacinthacine alkaloids have been of recent interest for total synthesis. Furthermore, total synthesis also allows access to the core structure of these complex alkaloids and so presents a great significance to the large community interested in the preparation of glycosidase inhibitors and consequent therapeutic iminosugars.
To date, 14 of the natural hyacinthacines, $A_1$, $A_2$, $A_3$, $A_6$, $A_7$, $B_1$, $B_2$, $B_3$, $B_4$, $B_5$, $B_7$, $C_2$, $C_3$ and $C_5$ have been synthesized, along with their related epimers and enantiomers.\textsuperscript{119-132} At this point, the author notes that an increasing number of reports suggest the confirmation of hyacinthacine $A_5$ through methods of total synthesis.\textsuperscript{129, 131, 133} To the best of our knowledge, hyacinthacine $A_5$ has not yet been synthesized and although epimers of the structure have been reported,\textsuperscript{134} the absolute configuration of the natural isolate is still yet to be confirmed. In addition to the aforementioned issues, recent synthetic studies have also revealed that the original structures proposed for hyacinthacines $B_7$, $C_3$ and $C_5$ are incorrect.\textsuperscript{126-130} It therefore becomes apparent that structural revision of the original isolates is necessary because as demonstrated in Table 1.2, hyacinthacine alkaloid epimers determine the degree of specificity as well as the potency for the inhibition of various glycosidases.

### 1.10. Total syntheses of the Hyacinthacine C-type analogues

Each subclass of the hyacinthacine alkaloids displays relatively low glycosidase inhibitory activities and so merits their total synthesis. Relative to A-type hyacinthacines, both B and C-type analogues have a greater constitutional complexity and as a result, have led to a greater number of insecure structural assignments. Synthetic work towards hyacinthacine A and B-type analogues has been included in a number of reviews,\textsuperscript{135-137} with a comprehensive analysis completed in 2012 by Pyne \textit{et al}.\textsuperscript{138} The hyacinthacine C-type analogues on the other hand have attracted considerable interest and there has been a number of publications towards their synthetic work that has not yet been summarised. When considered from a structural point of view, the hyacinthacine C-type analogues are the most diverse subclass of the hyacinthacines. They can contain up to seven possible stereogenic centres which means that there are 128 unique possible diastereomers (together with their enantiomers) containing a 3-hydroxymethyl-5-methylpyrrolizidine-1,2,6,7-tetraol core that can be potentially synthesized. Their high degree of oxidation presents a remarkably analogous structure to glucose and is therefore an enticing, but challenging synthesis for chemists interested in accessing medicinally useful iminosugars. For these reasons, the remainder of this Chapter will largely focus on the hyacinthacine C-type
alkaloids and will aim to deliver a comprehensive and collective review of all the different synthetic approaches towards this subclass of hyacinthacine.

1.10.1. Yoda et al. 2009

The first synthesis of hyacinthacine C-type analogues was the synthesis of hyacinthacine C\(_2\) and C\(_3\) along with their C5-epimers reported by Yoda et al. in 2009.\(^{128}\) This synthesis was an initial attempt to reveal information regarding structure-activity relationships between the hyacinthacine C-type alkaloids and their inhibition against various glycosidases. Their synthetic strategy (Scheme 1.1) starts with the preparation of the N-Boc lactam 1 from commercially available (S)-(−)-2-pyrrolidone-5-carboxylic acid. Lactam 1 was subject to a Grignard addition followed by a 1,2-reduction of the resulting unsaturated ketone formed by pyrrolidione ring opening to give the corresponding allyl alcohol. Subsequent mesylation-cyclization then afforded the N-Boc-2 product. Next, the Boc protecting group was replaced with Cbz in a high yielding, three-step procedure to give N-Cbz-3. The olefin moiety of N-Cbz-3 was then subject to oxidative cleavage with OsO\(_4\) and NaIO\(_4\) to give aldehyde 4 as a single isomer. A Reformatsky-type allylation of 4 returned a 79:21 separable mixture of the alcohols 5\(_a\) and 5\(_b\), respectively. To establish the methodology, alcohol 5\(_a\) then underwent TBS protection of the hydroxy group followed by a non-selective dihydroxylation of the terminal olefin moiety. The inseparable diol mixture was then subject to a selective TBS protection of the primary alcohol, affording a high yield (92\%) of a 1:1 mixture of separable diastereomers 6\(_a\) and 6\(_b\). In separate reactions, mesylation of the secondary alcohols of 6\(_a\) and 6\(_b\) followed by Cbz deprotection resulted in concerted cyclization to give the pyrrolizidines 7\(_a\) and 7\(_b\). Individual global deprotection of both pyrrolizidines afforded hyacinthacine C\(_2\) along with its epimer, 5-epi-hyacinthacine C\(_2\) 10 in 72\% and 71\% yields, respectively. Having established the synthetic pathway to hyacinthacine C\(_2\), Yoda and co-workers employed a similar synthetic methodology to alcohol 5\(_b\) where they also successfully synthesized hyacinthacine C\(_3\) along with its epimer, 5-epi-hyacinthacine C\(_3\) 11. When compared with the spectroscopic data of the natural isolates,\(^{117}\) Yoda et al. confirmed only the structure for natural hyacinthacine C\(_2\) was correct. They found that the characterization data of their synthetic sample of hyacinthacine C\(_3\) was inconsistent with the corresponding natural product.
Scheme 2.1: The first total synthesis of hyacinthacine C\textsubscript{2} and proposed structure of C\textsubscript{3} and their C5 epimers 10 and 11, respectively by Yoda et al.\textsuperscript{128}

Reagents and conditions: (a) (i) vinylmagnesium bromide, THF, -78 °C, 83%; (ii) NaBH\textsubscript{4}, CeCl\textsubscript{3}, MeOH, -20 °C, 67%; (iii) MsCl, Et\textsubscript{2}N, 0 °C, 90%; (b) (i) TBAF, THF, 0 °C, then NaH, 98%; (ii) CbzCl, NaHCO\textsubscript{3}, MeOH, 97%; (iii) TBDDS, Imidazole, DMF, 97%; (c) (i) OsO\textsubscript{4}, aq NMO, acetone/I-BuOH, 97%; (ii) NaIO\textsubscript{4}, THF/H\textsubscript{2}O (2:1), 99%; (d) Zn, 3-bromopropene, THF/satd NH\textsubscript{4}Cl aq (1:5), 73% (5a), 19% (5b); (e) (i) TBSCI, imidazole, DMF, 99% (5a), 97% (5b); (ii) OsO\textsubscript{4}, aq NMO, acetone/I-BuOH, 98% (5a), 98% (5b); (iii) TBSCI, Et\textsubscript{3}N, CH\textsubscript{2}Cl\textsubscript{2}, 46% (6a), 49% (6b), 48% (8a), 46% (8b); (f) (i) MsCl, Et\textsubscript{3}N, CH\textsubscript{2}Cl\textsubscript{2}, 74% (from 6a), 71% (from 6b), 82% (from 8a), 75% (from 8b); (ii) H\textsubscript{2}, 5% Pd/C, EtOH, 89% (7a), 70% (7b), 91% (9a), 90% (9b); (g) (i) TBAF, THF; (ii) TFA/H\textsubscript{2}O (1:2), 73% (hyacinthacine C\textsubscript{2}), 84% (5-epi-hyacinthacine C\textsubscript{2} (10)), 72% (hyacinthacine C\textsubscript{2}), 71% (5-epi-hyacinthacine C\textsubscript{2} (11)) (two steps).
At around the same time as Yoda et al., Tamayo and co-workers published their work detailing the synthetic approach to making analogues of hyacinthacines C₂/C₃ from the arabinose derived nitrone 12 (Scheme 1.2). Their synthesis began with the 1,3-dipolar cycloaddition of the chemoenzymatically prepared 3-buten-1,2-diol derivatives 13a or 13b to nitrone 12 to afford cycloadducts 14 or 15, respectively. At this stage, methodological probing was performed with the pyrroloisoxazolidine 14, which was subsequently converted into the mesylate 16. Compound 16 underwent an N-O reduction with Zn/AcOH, which after a basic work up, spontaneously cyclized to give the pyrrolizidine 18. Deacetylation to give 19, followed by a catalytic hydrogenolysis under acidic conditions afforded the final product 20. After access to the pyrrolizidine core was established, Tamayo and co-workers focused their attention on cycloadduct 15. More specifically, deacetylation of 15, followed by a TBDPS protection of the primary alcohol afforded 17 in near quantitative yields. Using similar methodology (mesylation, N-O reduction, global deprotection), the 5-epimer 24 was obtained. Although they were not assessed as potential glycosidase inhibitors, access to these highly oxygenated iminosugars is still important for the broader community interested in synthesizing complex azasugars.
1.10.3. Tamayo et al. 2010

At this stage, hyacinthacines C₁, C₄ and C₃ proved to be insecure in their structural assignment. Their glycosidase inhibitory activities proved noteworthy, but difficult to predict, especially due to these inconsistencies. For these reasons, Tamayo and co-workers were compelled to investigate and report the synthesis of unnatural 7α-epi-hyacinthacine...
C₁ 36 and 5,7a-di-epi-hyacinthacine C₁ 37 in 2010. From their previous work towards the synthesis of hyacinthacine A-type analogues, Tamayo et al. recognized that using the common precursor, the α,β-unsaturated ketone 25, would afford access to both hyacinthacine C-type analogues (Scheme 1.3). More specifically, 25 was subject to a catalytic dihydroxylation using OsO₄ and NMO to afford a single diol diastereomer 26 in high yield. The high diastereoselectivity achieved is a result of the steric hindrance created by both the Boc protecting group at the amino moiety as well as the benzyl groups at C3 and C4. Diol 26 was then acetylated in good yield to afford the di-O-acetylated 27, which was subsequently reduced with NaBH₄ to afford a separable mixture of alcohols 28 and 29 in a 1:1 ratio (isolated yields of 25% and 22%, respectively). To prepare the pyrrolizidine core, alcohols 28 and 29 underwent separate mesylation reactions, followed by an acid-catalysed N-Boc deprotection to give the secondary amines 32 and 33, respectively. After heating at reflux in THF under basic conditions, both amines cyclised to give their respective pyrrolidine cores. To both pyrrolizidine mixtures was then added MeONa in MeOH to facilitate a one-pot debenzoyl- and deacetylation to afford triols 34 and 35, respectively. A final O-benzyl deprotection of 34 and 35 revealed the desired hyacinthacine C-type analogues, 7a-epi-hyacinthacine C₁ 36 and 5,7a-di-epi-hyacinthaicne C₁ 37, respectively. Although neither of the synthesized hyacinthacine C-type analogues were able to resolve the structural discrepancies in the literature, access to stereogenic variations of the heavily hydroxylated pyrrolizidine core proves important. Their therapeutic potential remains unclear as these compounds were not assessed for their glycosidase inhibitory activities.
**Scheme 2.3:** The synthesis of unnatural hyacinthacine C₁ analogues, 7a-epi-hyacinthacine C₁ 36 and 5,7a-di-epi-hyacinthacine C₁ 37 by Tamayo et al.¹⁴⁰

Reagents and conditions: (a) OsO₄, NMO, acetone/H₂O (8:1), 71%; (b) Ac₂O, pyridine, 86%; (c) NaBH₄, MeOH, 0°C, 25% (28), 22% (29); (d) MsCl, Et₃N, CH₂Cl₂, 75% (30), 83% (31); (e) TFA, CH₂Cl₂, 83% (32), 90% (33); (f) Et₃N, THF reflux, then NaOMe, 60% (34), 67% (35); (g) H₂, 10% Pd/C, HCl, MeOH, 68% (7a-epi-hyacinthacine C₁ (36), 70% (5,7a-di-epi-hyacinthacine C₁ (37)).

1.10.4. Yu et al. 2011

In the following year, Yu et al. published their findings regarding the synthesis of (−)-hyacinthacine Cs, 6-epi-(−)-hyacinthacine Cs and 7-epi-(−)-hyacinthacine Cs from the L-arabinose derived cyclic nitrone 38 (Scheme 1.4).¹²⁹ This not only expanded the number of hyacinthacine C-type analogues synthesized, but it was also the first synthesis of the enantiomer of natural isolate hyacinthacine Cs. Their synthesis began with the addition of the lithiated dithiane 39 to the cyclic nitrone 38 which returned the stereoselective hydroxylamine intermediate 40. Compound 40 was then subject to Cope-House
cyclization conditions which afforded the separable pyrrolizidine N-oxides 41 and 42 in a 1:1 ratio (55% combined yield). Pyrrolizidine N-oxides 41 and 42 were then separately reduced using Zn-HOAc to afford the pyrrolizidines 43 and 44, respectively. Initially focusing on the enantiomer of hyacinthacine C5, the dithioketal moiety of pyrrolizidine 43 was hydrolysed to its corresponding ketone, which was then selectively reduced to the corresponding diol 45 in an overall 31% yield. Subsequent debenzylation revealed the final product as (−)-hyacinthacine C5. Turning their attention to the related epimers, Yu and colleagues initially protected the free hydroxy moiety in 43 using MOMCl, followed by hydrolysis of the dithioketal to give the corresponding ketone in 61% yield. A stereoselective reduction of the ketone with NaBH4 afforded two separable diastereoisomers favouring the desired alcohol 47 in a 5:1 ratio with an overall high yield (96%). Global deprotection of the MOM and the O-benzyl groups afforded (−)-7-epi-hyacinthacine C5 49. Having established the synthetic pathway, Yu and co-workers successfully synthesized (−)-6-epi-hyacinthacine C5 52 by subjecting 44 to similar methodology. With the three products in hand, Yu et al. reported that none of the 1H and 13C NMR spectroscopic data matched with those reported for the natural isolate labelled (+)-hyacinthacine C5.117 This led to the conclusion that natural (+)-hyacinthacine C5 was in fact, a different isomer. Regardless, all synthetic isomers of this study were assayed as potential glycosidase inhibitors and found that (−)-6-epi-hyacinthacine C5 52 is a weak inhibitor against the α-glucosidases of rat intestinal maltase (IC50 = 58.5 µM) and rice (IC50 = 64.2 µM).
Scheme 2.4: A total synthesis of the proposed structure of (−)-hyacinthacine C₅, (−)-6-epi-hyacinthacine C₅ 52 and (−)-7-epi-hyacinthacine C₅ 49 by Yu et al.¹²₉

Reagents and conditions: (a) TMEDA, 39, THF, -30 °C; (b) CHCl₃, 28% (41), 27% (42) (two steps); (c) Zn, HOAc, 98% (43), 98%, (44).

Reagents and conditions: (d) (i) NBS, AgNO₃, MeCN/H₂O (4:1), (ii) NaBH₄, MeOH, 31% (two steps); (e) H₂, 10% Pd/C, HCl, MeOH, 93%.

Reagents and conditions: (f) NaH, MOMCl, THF, 76% (46), 71% (50); (g) (i) NBS, AgNO₃, MeCN/H₂O (4:1); (ii) NaBH₄, MeOH ; (iii) HCl, MeOH, reflux; (h) H₂, 10% Pd/C, HCl, MeOH, 41% ((−)-7-epi-hyacinthacine C₅ (49) over four steps), 50% ((−)-6-epi-hyacinthacine C₅ (52) over four steps).
1.10.5. Tamayo et al. 2011

At the same time as Yu et al. and for similar reasons, Tamayo and co-workers also published related work detailing a synthetic approach towards (+)-hyacinthacine C$_5$ along with its C$_6$, C$_7$-diepimer. Their synthesis began with the N-Cbz protected pyrrolidine 53, a 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) derivative (Scheme 1.5). In this synthesis, the primary alcohol of 53 was initially oxidised its corresponding aldehyde 54, which was further treated in situ with 1-triphenylphosphoranylidene-2-propanone to afford the α,β-unsaturated ketone 55. A non-selective dihydroxylation of 55 with OsO$_4$ and NMO returned a 1.4:1 separable mixture of diols 56 and 57, respectively. Individually, both diols 56 and 57 were subject to reductive amination to form their respective iminium intermediates 58 and 59. Prolonged exposure to catalytic hydrogenation conditions over Pd/C resulted in a stereospecific hydrogenation occurring anti to the sterically encumbering CH$_2$OTBDPS group of both pyrrolizidines. More specifically, both pyrrolizidines 60 and 61 were synthesized with the same C$_5$-stereochemistry. Individual global deprotections of pyrrolizidines 60 and 61, which included a desilylation and hydrogenolysis of the benzyl protecting groups, revealed both (+)-hyacinthacine C$_5$ and (+)-6,7-di-epi-hyacinthacine C$_5$ 64, respectively. Arriving to a similar conclusion made by Yu et al., Tamayo and colleagues also found that neither of their spectroscopic data for hyacinthacine C$_5$ and its C$_6$, C$_7$-diepimer 64 matched that of the natural isolate further suggesting it to be a different isomer. The final products of this study were not assessed for their glycosidase inhibitory activities.
Scheme 2.5: The total synthesis of the proposed structure of (±)-hyacinthacine C₅ and (±)-6,7-di-epi-hyacinthacine C₅ 64 by Tamayo et al.¹³₀

Reagents and conditions: (a) TPAP, CH₂Cl₂, 4 Å MS, 95%; (b) Ph₃PCHCOMe, PhMe, reflux, 91%; (c) OsO₄, NMO, acetone/H₂O (8:1), 37% (56), 28% (57); (d-e) H₂, 10% Pd/C, MeOH, 65% (60), 56% (61); (f) TBAF, THF, 94% (62), 76% (63); (g) H₂, 10% Pd/C, MeOH, 73% (±)-hyacinthacine C₅, 98% ((±)-6,7-di-epi-hyacinthacine C₅ (64).
1.10.6. Fischer et al. 2014

By 2014, synthetic work towards the hyacinthacine C-type analogues had revealed several structural inconsistencies among their natural isolates. For this reason, and to also expand the scope of information pertaining to these compounds, Fischer and co-workers investigated the usefulness of asymmetric 1,3-dipolar cycloadditions to prepare optically active nitrone templates towards making various polyhydroxylated pyrrolizidine analogues.\(^{142}\) One of the iminosugars prepared by Fisher and co-workers can be considered an unnatural hyacinthacine C-type alkaloid shown in Scheme 1.6. To obtain this analogue, Fischer et al. began their synthesis through the preparation of the mannose-derived nitrone \(65\) (Scheme 1.6). The cyclic nitrone \(65\) underwent a 1,3-dipolar cycloaddition with vinyl acetate and afforded a 9:1 separable mixture of isoxazolidines, favouring the formation of \(66\) for steric reasons. The acetate moiety of \(66\) then underwent an \(S_N1\)-like reaction with silylketene acetal \(67\), which exclusively produced the 2,3a-\(\text{trans}\)-isoxazolidine \(68\) in a 69% yield. In a two-step procedure, the methyl ester of isoxazolidine \(68\) was initially reduced with LiAlH\(_4\), then subsequently protected with tert-butyl(diphenyl)silyl chloride to give isoxazolidine \(69\) in good yield (84% over two steps). The dimethoxy ketal moiety in compound \(69\) was then hydrolysed to the ketone to give product \(70\), which was subject to \(N-O\) bond reduction and then reductive amination by hydrogenation over Pd/C to afford the pyrroizidine \(71\) as a single isomer. A global deprotection including the removal of the silyl and the isopropylidene moieties returned the hexahydroxylated pyrroizidine \(73\) in 76% yield over two steps.
Scheme 2.6: A total synthesis of an unnatural hyacinthacine C_{2/3} analogue 73 by Fischer et al.\textsuperscript{142}

In the same year, Vankar and co-workers reported their synthetic work towards the synthesis of homoanalogues of hyacinthacine C_{5}.\textsuperscript{143} Their 2014 report details starting from the arabinose-based cyclic nitrore 74 which underwent a 1,3-dipolar cycloaddition reaction with the D-mannitol-aldehyde derived dipolarophile 75 (Scheme 1.7). The desired cycloadduct 76 was obtained in a 9:1 ratio in a combined yield of 88%. Compound 76 was then subject to a Zn/HOAc mediated N-O cleavage which produced the cyclic amine 77 which then underwent selective benzylation at the secondary amine to give 78 in 85% over two steps. The ester moiety of 78 was then reduced to its corresponding alcohol to afford
diol 79. A non-selective O-benzylation of both hydroxy groups afforded 80, which upon removal of the cyclohexylidene acetal, resulted in the formation of the diol 81. The diol moiety of compound 81 was then oxidatively cleaved with sodium periodate to give aldehyde 82 which was then treated with MeMgI, returning a selective 10:1.2 mixture of diastereomers 83a and 83b. The diastereomeric mixture was then oxidised to the respective ketone product 84, which after a reductive amination followed by a benzyl ether deprotection, afforded the crude hyacinthacine C-type analogue 86. To purify 86, Vankar et al. peracetylated the crude mixture, followed by its deprotection. After washing the residue with chloroform, the purified 86 was obtained.
Scheme 2.7: A total synthesis of the hyacinthacine C-type related analogue 86 by Vankar et al.\textsuperscript{143}


In continuation with synthetic efforts towards synthesizing different unnatural analogues of bicyclic iminosugars, Vankar et al. reported in 2016, a new strategy for accessing a large number of complex hyacinthacine C\textsubscript{2}/C\textsubscript{3} related products.\textsuperscript{144} Vankar and co-workers’ synthesis of the unnatural hyacinthacine C-type analogues can be summarised in Scheme 1.8. Their synthetic work began with the oxidation of pyrrolidine 87 to its corresponding aldehyde using a CrO\textsubscript{3}-pyrridine-Ac\textsubscript{2}O reagent system. The aldehyde formed was then...
treated with allylzinc bromide to afford compound 88 which underwent O-benzylation to the product 89. The olefin moiety of compound 89 was then subject to a nonselective dihydroxylation with OsO₄ and NMO which returned an inseparable mixture of diols. A selective protection of the primary alcohol in each diol resulted in the corresponding silyl ethers 90 and 91, which were readily separable. To establish the methodology, Vankar and co-workers quantitatively mesylated the free hydroxy group in compound 90. Without purification, the mesylated 90 then underwent a N-Boc deprotection which under basic conditions furnished pyrrolizidine 92. A global deprotection, followed by peracetylation as a means of purification afforded the pentaacetylate 94. After a comprehensive structural analysis, the pentaacetylate 94 was deprotected using aqueous ammonia in methanol which gave the final pentahydroxylated hyacinthacine C₂/C₃-type analogue 96. Having established this synthetic route, Vankar and co-workers then employed a similar reaction pathway to diastereomer 91 which successfully converted into compound 97. Vankar et al. also readily obtained two more epimers, 107 and 108, from subjecting pyrrolidine 98 to the same sequence of reactions. The only notable difference is that the hydroxy group of olefin 99 was protected as the acetate 100 instead of the benzyl ether. Of the four hyacinthacine C₂/₃ products synthesized, none matched the putative structure for hyacinthacine C₃, but they certainly provided a better understanding towards the access to these complex iminosugars. Additionally, the biological activities the final products were evaluated as potential glycosidase inhibitors against a panel of eight commercially available enzymes. The hyacinthacine analogues 96 and 107 displayed selective activity against jack bean α-mannosidase (IC₅₀ = 81.2 μM and IC₅₀ = 95 μM, respectively) with analogue 107 also displaying moderate activity against coffee bean α-galactosidase (IC₅₀ = 120 μM). Final remarks from the authors suggested that given these activities, hyacinthacine analogues 96 and 107 are potential anti-diabetic and anti-cancer leads, respectively.
Scheme 2.8: A stereodivergent synthesis of hyacinthacine C₂/C₃ related analogues 96, 107, 97 and 108 by Vankar et al. ¹⁴⁴

Reagents and conditions: (a) (i) CrO₃, pyridine, Ac₂O, CH₂Cl₂, 0 °C; (ii) AllyBr, Zn, satd NH₄Cl, 85% (88 over two steps), 78% (99 over two steps); (b) BnBr, NaH, TBAI (cat.), THF, 90% (89); (c) Ac₂O, Et₃N, DMAP, CH₂Cl₂, 87% (100); (d) (i) OsO₄, NMO, acetone/H₂O/I-BuOH (5:1:1); (ii) TBDPSCI, Et₃N, DMAP, 50% (90 over two steps), 38% (91 over two steps), 63% (101 over two steps), 26% (102 over two steps); (e) (i) Et₃N, MsCl, DMAP; (ii) TFA; (iii) Et₃N, THF, 70 °C, 45% (92 over three steps), 40% (93 over three steps), 46% (103 over three steps), 47% (104 over three steps); (f) (i) H₂, Pd(OH)₂/C, MeOH; (ii) Ac₂O, pyridine, 80% (94 over two steps), 80% (95 over two steps), 85% (105 over two steps), 85% (106 over two steps); (g) aq. NH₃/MeOH (1:3), 89% (96), 78% (97), 89% (107), 90% (108).
1.10.9. Goti et al. 2017

A recent addition towards the synthesis of the hyacinthacine C-type analogues was reported in 2017 by Goti and co-workers through their synthesis of the proposed structure of (−)-hyacinthacine C5 and its C5 epimer 120. Their goal was to implement a straightforward and convenient synthesis towards these complex iminosugar motifs. To access both these structures, their convergent synthesis began with the preparation of both the arabinose-derived cyclic nitrone 109 and the racemic 3-methyl-substituted benzyloxyallene 110 (Scheme 1.9). The lithiated benzyloxyallene 110 was then added to nitrone 109 to form intermediate 111, which upon standing at room temperature for 24 h, cyclized to afford a 1:1 separable mixture of 1,2-oxazines 112 and 113. Both oxazines were then individually subjected to hydroboration which delivered respective alcohols 114 and 115. Goti and co-workers were initially focused on establishing the synthetic pathway towards the proposed structure of (−)-hyacinthacine C5 and so their attention turned to alcohol 114 which was protected to give the penta-benzylated product 116. Next, a reductive amination of 116 with samarium diiodide in THF cleaved the N-O bond to give the amino-alcohol, which was subsequently converted to pyrrolizidine 118 via mesylation of the primary hydroxy group, then spontaneous cyclization. Finally, all benzyl ether groups were removed via hydrogenolysis to afford the purported (−)-hyacinthacine C5.

Using a similar method, 5-epi-hyacinthacine C5 120 was synthesized after alcohol 115 underwent a reductive amination followed by bis-mesylation of the hydroxy groups which concurrently cyclized to give pyrrolizidine 119. After a LiAlH4 reduction of the sulfonyl group on 119, the corresponding alcohol was subject to hydrogenolysis to give the globally deprotected pyrrolizidine, 5-epi-hyacinthacine C5 120.
Scheme 2.9: The total synthesis of the proposed structure of (+)-hyacinthacine C₅ and 5-epi-hyacinthacine C₅ 120 by Goti et al.³¹

Reagents and conditions: (a) 110, THF, -78 °C; (b) CH₂Cl₂, 38% (112 over two steps), 36% (113 over two steps); (c) BH₃·THF, THF, -30 °C, then H₂O₂, NaOH, 57% (114), 41% (115); (d) NaH, BnBr, TBAI (cat.), THF, 39%; (e) (i) SmI₂, THF; (ii) Et₂N, MsCl, CH₂Cl₂, 73% (two steps); (f) H₂, 10% Pd/C, HCl, MeOH, quant.; (g) SmI₂, THF, 96%; (h) Et₂N, MsCl, CH₂Cl₂, 80%; (i) (i) LiAlH₄, THF, reflux, 74%; (ii) H₂, 10% Pd/C, HCl, MeOH, 69%.
As per the previous reports by Yu et al.\textsuperscript{129} and Tamayo et al.,\textsuperscript{130} Goti and co-workers also found that the configuration of the putative structure of hyacinthacine C\textsubscript{5} was incorrect. Neither structure synthesized in this report matched the spectroscopic data of the natural isolate. Despite being unable to resolve the true structure of hyacinthacine C\textsubscript{5}, Goti \textit{et al.} revised the originally reported NOE enhancements for the natural isolate and after finding similar NOE correlations in their synthetic products, were able to suggest an alternative configuration which could be appropriately labelled as 1-\textit{epi}-hyacinthacine C\textsubscript{4} (Figure 1.20).

\textbf{Figure 2.20:} The NOE signals reported for the putative hyacinthacine C\textsubscript{5} (left) along with the revised NOE signals proposed by Goti \textit{et al.}\textsuperscript{117, 131}

\textbf{1.11. Project aims:}

The history and synthetic development towards the hyacinthacine alkaloids is certainly rich and exciting. They have been isolated from the under-explored aqueous fractions of various plants and contain an inherent glycosidase activity, making this class of compound a suitable lead for the treatment of diabetes, cancer and bacterial infection. Additionally, these novel compound motifs are highly complex and in this present day would not be conceivably produced by technologies such as combinatorial chemistry. Their configurational differences prove vital for their selectivity and potency against inhibiting various glycosidases, and so has led to many synthetic approaches towards accessing this class of natural product. Structurally, the hyacinthacine C-type analogues can be considered the most diverse group of hyacinthacine, as they contain at least six possible stereogenic centres and are analogous to glucose. Owing to their complexity, mistakes
have been made when assigning their structures as proved in some cases by total synthesis. More recently, this class of compound has been the focus of an increasing number of synthetic attempts in hope to elucidate the mistakes and correct the structures currently labelled as hyacinthacine C₁, C₃, C₄ and C₅. In addition to this, synthetic access to this class of compound allows researchers to probe the structure activity relationship for glycosidase inhibition, and design related analogues that can be marketed as treatment for the illnesses previously mentioned.

After reviewing the entirety of the synthetic approaches towards the hyacinthacine C-type analogues several observations can be made.

1. All pathways begin with a cyclic pyrrolidine derivative. Of these, just over half favour the use of a sugar derived cyclic nitroine.
2. Although some syntheses are divergent the pathways discussed generally allow no more than four hyacinthacine C-type analogues to be synthesized.
3. All nine publications raise awareness of the hyacinthacine C-type analogues and their inherent glycosidase inhibitory activity but only two have been able to assess the final products as glycosidase inhibitors.
4. Natural hyacinthacines C₁, C₃, C₄ and C₅ have insecure structures and the true structures have not yet been revealed.

Starting this Ph.D. work in 2015, the aims of this project were to:

1. Develop a novel synthetic strategy to prepare hyacinthacine C-type analogues in an efficient and flexible manner
2. Elucidate and correct the structure for either hyacinthacine C₁, C₄ or C₅
3. Generate a moderate number of hyacinthacine C-type configurational analogues and use the data generated from glycosidase inhibition studies to determine a general structure-activity relationship.

Overall, these goals were achieved and although they will be discussed in greater detail in the remaining Chapters, this work can be briefly summarised in the following paragraph.

Eleven configurationally different hyacinthacine C-type analogues were successfully synthesized using a common but unique pathway. All final products were individually assessed as potential glycosidase inhibitors, providing an interesting structure-activity relationship. The highlight of this body of work was the elucidation of the correct structure
for both natural hyacinthacine C₁ and C₅, which had been effectively a mystery for the past 20 and 10 years, respectively. In light of this, all final hyacinthacine C-type analogues synthesized in this work are named in accordance to the newly corrected structures for hyacinthacine C₁ and C₅.
Chapter 2: Synthesis of unnatural (+)-7-epi-hyacinthacine C₅ and (−)-6-epi-hyacinthacine C₅.

The crux of this Ph.D. study is the successful synthesis and correction of the structures for the natural isolates labelled as hyacinthacine C₁⁰⁵ and C₅¹¹⁷ and will be discussed later. This Chapter describes the strategy employed towards the synthesis of unnatural (+)-7-epi-hyacinthacine C₅ ¹⁵⁵ and (−)-6-epi-hyacinthacine C₅ ¹⁵⁶. Accessing both these structures proved integral towards establishing the synthetic methodology and foundations that would later result in the correction of the aforementioned natural products. Herein, both successful and unsuccessful synthetic attempts towards the hyacinthacine C-type analogues are presented and will allow the reader to understand the logic that governed the methodology which resulted in accessing the remaining nine hyacinthacine C-type analogues.

2.1. The Petasis borono-Mannich reaction

The chiral anti-1,2-amino alcohol moiety is a common structural feature of many bioactive azasugars including the hyacinthacine alkaloids A₂, A₃, A₅, B₃, B₄, C₃ and C₅, as highlighted in Figure 2.1.

![Figure 2.1](image_url)

**Figure 2.1**: Selected natural hyacinthacine alkaloids containing an anti-amino diol motif (blue).
In recent years, the Pyne research group has demonstrated synthetic methods to prepare chiral anti-1,2-amino alcohols include: aminolysis of chiral vinyl epoxides, the borono-Mannich reaction (Petasis reaction) and the borono-Mannich reaction via cyclic N-acyliminium ions (Figure 2.2 adapted from Pyne et al. review\textsuperscript{145}).

![Diagram](image_url)

**Figure 2.2:** An adapted figure from the review of Pyne et al. displaying the three reaction types studied often used to prepare the 1,2 anti-amino alcohols.\textsuperscript{145}

Of these three synthetic routes, the Pyne research group has explored the efficiency of synthesizing bicyclic polyhydroxylated iminosugars containing this motif from a suitably prepared borono-Mannich (Petasis) product (Scheme 2.1).\textsuperscript{89, 92, 126-127, 145-146} The successful application of the Petasis borono-Mannich acid reaction in natural product synthesis is largely because the resultant anti-1,2-amino alcohol is often obtained in high yields and almost exclusively as a single diastereomer and enantiomer.\textsuperscript{147} Another desirable facet of this reaction is the simple experimental procedure that generally involves mixing an aryl or vinyl boronic acid, a primary or secondary amine and a chiral α-hydroxy-aldehyde under relatively mild conditions.\textsuperscript{147} Previous Pyne group members, Davis, Machan and Ritthiwigrom, demonstrated that the anti-1,2-amino alcohol \textsuperscript{121} was the product of the multi-component Petasis borono-Mannich acid reaction between (E)-β-styrenylboronic acid, allylamine and L-xylose. More importantly, this Petasis product was a viable precursor for the synthesis of natural products: (−)-uniflorine A, (+)-castanospermine, (+)-casuarine and (+)-australine (Scheme 2.1A).\textsuperscript{89, 146, 148} Of particular interest to this body of
work, both Au\textsuperscript{126} and Savaspun\textsuperscript{127} have shown that hyacinthacine B\textsubscript{3} and B\textsubscript{7} could be synthesized from the appropriate \textit{anti}-1,2-amino alcohol \textsuperscript{124}; also a product of a Petasis borono-Mannich acid reaction between \textsuperscript{122, 123} and \((E)\)-\(\beta\)-styrenylboronic acid (Scheme 2.1B). From these examples, particularly the latter, it was therefore reasonable to propose that the hyacinthacine alkaloids shown in Figure 2.1, could be synthesized in a similar manner.

\textbf{Scheme 2.1:} Natural products resulting from the Petasis reaction in the Pyne research group. \textsuperscript{89, 92, 126-127, 145-146}

\textbf{A)}

\textbf{B)}

2.2. Rationale for synthesizing hyacinthacine C\textsubscript{5}-type analogues

Discussed earlier in Chapter 1, there is a correlation between the degree of oxidation in the hyacinthacine alkaloids and the prevalence for misassigned structures. For example, all the synthetic attempts towards the A-type hyacinthacine alkaloids have confirmed so far that
the natural isolates are correct in their stereoconfigurational assignment. Synthetic attempts towards the hyacinthacine B-type suggest that only one is incorrect: hyacinthacine B7.\textsuperscript{126} The hyacinthacine C-type (containing the highest relative degree of oxidation) have four insecure structures, where only hyacinthacine C2 has been confirmed to be the correct stereoconfiguration.\textsuperscript{105, 117, 128-131} We felt particularly drawn to synthesizing the hyacinthacine C-type analogues due to the higher number of relative mistakes associated with their structures. Additionally, synthesizing analogues would help increase our understanding of the SARs and inherent glycosidase inhibition that these iminosugars possess.

For each of the naturally occurring hyacinthacine C-type alkaloids, it becomes apparent that each isolate contains the same absolute configuration at stereocentres C2, C3 and C7a (Figure 2.3). The logical outcome of this would be that these stereocentres are the result of a similar biosynthetic pathway. If these stereocentres are the correct absolute configuration, then the stereoconfiguration of C1 is less ambiguous since the orientation can be spectroscopically cross-examined against both C7a and C2. At this point, it seemed reasonable that the most likely mistake would occur at the assignment of stereocentres C5, C6 or C7 (Figure 2.3). Concurrent to our investigation, synthetic work towards hyacinthacine C5 and various analogues was gaining considerable attention. Since their first synthetic attempt in 2009,\textsuperscript{128} there has been an additional eight total syntheses detailing access to related pyrrolizidine structures.\textsuperscript{129-131, 139-140, 142-144} Of these, the putative structure for hyacinthacine C5, along with various diastereomers were synthesized in 2011\textsuperscript{129-130} and were sought after due to their inherent biological activities against various glycosidases. The syntheses of hyacinthacine C5 have shown that the initially proposed structure was incorrect since the \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopic data of the synthetic structures did not match the natural isolate.\textsuperscript{117} Until recently, Goti and co-workers re-interpreted the originally reported NOE enhancements for the natural isolate and proposed an alternative configuration based on this interpretation.\textsuperscript{131} More specifically, their interpretations suggested that the true structure for hyacinthacine C5 is actually the configuration for 1-\textit{epi}-hyacinthacine C4 (Chapter 1, Figure 1.20).\textsuperscript{131} However, before this revision, the only other measure for determining the correct configuration of hyacinthacine C5 was to compare the \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopic data of previously synthesized
hyacinthacine C₅ analogues with that of the natural isolates. After searching the literature, it was evident that no one had yet synthesized the true structure for hyacinthacine C₅.

![Figure 2.3: General structure for hyacinthacine C-type analogues.](image)

Prior to Goti et al.,¹³¹ there were no reported attempts to synthesize hyacinthacine C₅ alkaloids containing both an (R)-C7a nitrogen bridgehead proton and a (R)-C5 methyl. For this reason, we initially focused on installing this stereochemical relationship in our final hyacinthacine C₅ related products. In addition to this, we were particularly interested in manipulating the stereocentres at the C6 and C7 whilst simultaneously retaining the original stereochemistry proposed in hyacinthacine C₅ for stereocentres C7a, C1, C2 and C3. As discussed earlier, a close inspection of the putative structure for hyacinthacine C₅ reveals an anti-configuration between the C7a-N and the C1 carbinol and so our synthetic strategy was designed around the Petasis borono-Mannich acid reaction to implement this key motif.

Shown in Scheme 2.2, our initial retrosynthetic analysis suggested manipulating the C6 and C7 hydroxy group stereochemistry at later stages in the synthesis. The final hyacinthacine C₅-type analogues would be the result obtained from syn-dihydroxylation across the alkene in pyrrolizidine 125. The diastereofacial selectivity would be influenced by the concave nature of the pyrrolizidine core, as well as possible steric hindrance by the (R)-C5 methyl. However, a non-selective dihydroxylation might still afford two syn-diol diastereomers which would lead to two possible hyacinthacine C₅-type analogues. Pyrrolizidine 125, would be obtained by subjecting pyrrolidine 126 to ring closing metathesis (RCM) conditions using either Grubbs’ I- or II-generation catalysts. Pyrrolidine 126 would be synthesised by N-alkylation of the amino-diol 127 using a steric-directed, regioselective O-mesylation, then spontaneous cyclisation through the nitrogen. These
reactions ultimately rely on synthesizing the stereochemically pure *anti*-amino-diol 127; this is a product derived from the Petasis borono-Mannich acid reaction between 3,5-di-\(O\)-benzyl-\(\alpha,\beta\)-\(L\)-xylofuranose, 3-(\(R\))-amino-1-butene-hydrochloride and \((E)\)-\(\beta\)-styrenylboronic acid.

**Scheme 2.2:** Retrosynthetic analysis for the synthesis of hyacinthacine C\(_5\)-type analogues.

2.3. Synthesis of 3,5-di-\(O\)-benzyl-\(\alpha,\beta\)-\(L\)-xylofuranose

Within the Pyne research group, the *anti*-amino-diol 127 had been previously synthesized but not reported in the literature.\(^{149}\) To synthesize this product, 3,5-di-\(O\)-benzyl-\(\alpha,\beta\)-\(L\)-xylofuranose was required. This protected sugar can be readily obtained from the commercially available \(L\)-\(\text{--}\)-xylose by using a modified three-step procedure employed by Terashima and Matsuda\(^{150}\) as well as Baker and Schaue.\(^{151}\) The inherent stereochemistry of \(L\)-\(\text{--}\)-xylose was envisioned to produce the stereochemical configuration on the A-ring of the final hyacinthacine C-type products. More specifically the hydroxy groups at C3, C4 and C6 (Scheme 2.3) should account for the stereochemistry at C1, C2 and C3 in the final product, respectively. To prepare this sugar, step one of the synthesis involved dissolving \(L\)-\(\text{--}\)-xylose in acetone followed by the addition of two equivalents of copper(II) sulphate and a catalytic amount of sulfuric acid. After stirring the reaction for 24 h, a TLC analysis showed a near quantitative conversion to the corresponding bis-
acetonide 128. Stirring a solution of 128 in 0.25 M solution of hydrochloric acid for 45 min returned the mono-acetonide 129 in quantitative yield.

The second step involved the conversion of acetonide 129 to its respective bis-benzyl ether 130. This reaction involved dissolving compound 129 in THF, followed by heating at reflux for 30 min after the addition NaH, BnBr and a catalytic amount of the transfer catalyst, tetrabutylammonium iodide (TBAI). After working up the reaction, the crude mixture was purified by column chromatography and product 130 was obtained in overall good yield (88%).

The last step towards the preparation of 3,5-di-O-benzyl-α,β-L-xylofuranose involved an acid hydrolysis of the isopropylidene ketal from bis-benzylether 130. This involved stirring 130 in a solution of acetic acid (30% v/v) followed by the addition of a few drops of 5 M HCl. The reaction was heated at 45 °C for 3 h or until the TLC confirmed the full consumption of the starting material. After work up, the crude mixture was purified by column chromatography to return a waxy solid which by 1H NMR analysis was determined to be a 3:1 mixture of anomers of 3,5-di-O-benzyl-α,β-L-xylofuranose.

Although the products at each step had been well documented, 1H and 13C NMR spectroscopic data was still obtained for each product which were consistent with those reported in the literature. 150

Scheme 2.3: Synthesis of 3,5-di-O-benzyl-α,β-L-xylofuranose.
2.4. The synthesis of anti-amino-diol 127

During the final stages of his Ph.D. candidature, Kongdech Savaspun attempted to develop a facile route towards the synthesis of hyacinthacine B from the anti-amino-diol 127. Although unsuccessful, we hypothesized this intermediate to be more suited in our total synthesis for accessing hyacinthacine C-type analogues. The anti-amino-diol 127 proves integral to our synthetic plan as it accounts for five of the seven stereocentres required for the final hyacinthacine C-type analogues. Moreover, it incorporates the nitrogen such that it adopts the anti-configuration between the C7a-N and the C1 carbinol. Finally, this product delivers two suitably placed alkenes for the desired RCM reaction highlighted in our retrosynthetic analysis (Scheme 2.2). Fortunately, optimised Petasis reaction conditions had been explored. Following the procedure described by Savaspun, the multicomponent reaction between 3,5-di-O-benzyl-α,β-L-xylofuranose, 3-(R)-amino-1-butene-hydrochloride and (E)-β-styrenylboronic acid in a solution of ethanol under basic conditions, took place over 3 d. Scheme 2.4 shows the proposed mechanism for this reaction where the resultant product is dependent on the equilibrium interconversion between 3,5-di-O-benzyl-α,β-L-xylofuranose (preferred form) and the respective ring-opened aldehyde. When the aldehyde is formed, the base (Et$_3$N)-activated 3-(R)-amino-1-butene can react with the aldehyde to form the iminium ion intermediate A. A key component of this intermediate is the availability of an α-hydroxy group. Provided this is available, it is believed that the boron reagent can coordinate to this hydroxy group, and subsequently deliver the desired reagent. In this example, prior to delivery of the (E)-β-styrenyl unit, the iminium ion boron-complex B adopts a conformation that minimizes the 1,3-allylic strain between the substituents at the α-position and on the N atom of the iminium ion. This involves the smallest α-substituent (proton) on the stereogenic carbon being eclipsed with the NH of the iminium moiety. As drawn here, the (E)-β-styrenyl group is then transferred to the bottom face of the iminium ion to give the desired product 127. After 3 d and in comparable yield to Savaspun (~65%), the purified product was subject to $^1$H and $^{13}$C NMR spectroscopic studies and was concluded to be the anti-amino-diol 127 previously characterised by Savaspun. Although extensively characterised, key $^1$H NMR resonances of 127 appeared at δH 6.48 (1H, d, $J = 16.0$ Hz) 6.10 (1H, dd, $J = 16.0, 8.7$ Hz), 5.72 (1H, ddd, $J = 17.3, 10.2, 7.2$ Hz) and 5.09 (1H, d, $J =
10.2 Hz) to 4.98 (1H, d, $J = 16.8$ Hz), and are representative of the olefinic moieties at H7, H6, H2’ and H3’ _cis/trans_, respectively (Figure 2.4A).

**Scheme 2.4:** The mechanism of the Petasis borono-Mannich reaction and synthesis of _anti_-amino-diol 127.

It was also serendipitously found that the crystalline _anti_-amino-diol 127 HCl salt could form after dissolving 127 in EtOAc and washing the organic layer with 1N HCl. An X-ray crystallographic analysis returned the ORTEP plot displayed in Figure 2.4B, and unambiguously confirmed the spectroscopically determined absolute configuration of the _anti_-amino diol 127.
2.5. The synthesis of RCM product 132

Following similar methodology to Savaspun, the *anti*-amino-diol 127 was converted to the pyrrolidine 131 using well established mesylation-cyclisation conditions. Details of this synthesis involved treating the *anti*-amino-diol 127 with 1.05 equivalents of methanesulfonyl chloride in CH₂Cl₂ at 0 °C for 1.5 h. A regioselective mesylation was anticipated to occur at the C2 hydroxy group since this is less sterically hindered than the alternative C4 hydroxy group (Scheme 2.5). Once mesylated, a base-induced cyclisation occurred and presumably furnished the pyrrolidine 131 (and consequently formed the A-ring of the desired product). The ¹H and ¹³C NMR spectroscopic data of 131 were consistent with the same product made by Savaspun. Unfortunately, due to the overlap of key proton resonances for H3, H4 and H5, the absolute configuration was unable to be
confidently assigned for this compound. Regardless, Savaspun attempted to synthesize the pyrrolizidine core 132 by employing RCM conditions.\textsuperscript{149} Savaspun’s approach involved deactivating the tertiary amine of pyrrolidine 131 with titanium isopropoxide to form a Ti-N complex.\textsuperscript{154} This complex was then subject to several different RCM conditions involving either Grubbs’ II or Hoveyda Grubbs’ II catalyst (Table 2.1, entries 1-3).\textsuperscript{149} Unfortunately, Savaspun abandoned this pathway after each attempt resulted in formation of the undesired pyrrole 133. It was concluded that the RCM product 132 was initially formed but was prone to aromatization resulting from elimination of H7a from the titanium complex. To avoid this problem, we employed an unexplored set of reaction conditions which instead of making the Lewis acid complex at N, involved generating the relatively mild p-toluene sulfonylic acid salt of the tertiary amine 131 (Table 2.1, entry 4).\textsuperscript{155-156} With our weakly deactivated tertiary amine 131 in hand, the compound was heated at reflux in CH\textsubscript{2}Cl\textsubscript{2} under an argon atmosphere in the presence of a catalytic amount of Hoveyda Grubbs’ II generation ruthenium catalyst over the course of 3 d. To avoid formation of the pyrrole 133, careful preparation was required to ensure no water or oxygen entered the reaction environment during this time. This stringent set of conditions afforded the desired RCM product 132 in 65% yield (Scheme 2.5).

\textbf{Scheme 2.5:} Synthesis of RCM product 133.
Table 2.1: Results of Savaspuns trialled conditions for RCM of 131 (entries 1-3) and the successful conditions (entry 4) employed to generate RCM 132 from this Ph.D. work.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Entry</th>
<th>Reagents used</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Time. (h)</th>
<th>Yield of 132</th>
<th>Yield of 133</th>
</tr>
</thead>
<tbody>
<tr>
<td>Savaspun et al. 149</td>
<td>1</td>
<td>10 mol% Grubbs’ II cat., 0.2 eq. Ti(OiPr)₄, under N₂</td>
<td>CH₂Cl₂</td>
<td>45</td>
<td>24</td>
<td>0%</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10 mol% Hoveyda Grubbs’ II cat., 1.0 eq. Ti(OiPr)₄, under Ar</td>
<td>CH₂Cl₂</td>
<td>40</td>
<td>20</td>
<td>0%</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10 mol% Grubbs’ II cat., 0.3 eq. Ti(OiPr)₄, under Ar</td>
<td>Toluene</td>
<td>60</td>
<td>20</td>
<td>0%</td>
<td>17%</td>
</tr>
<tr>
<td>This work</td>
<td>4</td>
<td>20 mol% Hoveyda Grubbs’ II cat., 1.0 eq. pTsOH, under Ar</td>
<td>CH₂Cl₂</td>
<td>50</td>
<td>72</td>
<td>65%</td>
<td>Trace amounts</td>
</tr>
</tbody>
</table>

In addition to the mass spectrometric data (a LRESIMS [M+Na]⁺ ion peak at m/z 388 [M+Na]⁺ for 132 and a LRESIMS [M+Na]⁺ ion peak at m/z 386 for 133), the RCM product 132 can be largely differentiated from the corresponding pyrrole 133 based on appearance of the H1” resonance in the ¹H NMR spectrum (Figure 2.5). When compared, the ¹H NMR resonance of the methyl protons, H1” in alkene 132, should appear as a doublet and would be expected to resonate below δH 2.0. After analysing the spectra from the RCM product 132 generated by our conditions, the resonance labelled H1” appeared as a doublet (J = 5.3 Hz) at δH 1.26 (Figure 2.5A). In contrast, the ¹H NMR resonance for H1” in the pyrrole 133 appeared as a singlet and resonated at δH 2.11 (Figure 2.5B). This implied the loss of a proton H5 and consequently the destruction of this stereocentre. Although we successfully obtained the desired RCM product 132, we were unable to obtain reliable NOESY data that would confirm the relative stereochemistry of the A-ring of the final product. The ¹H NMR spectrum displayed in Figure 2.5A shows that the key proton resonance peaks for H7a and H3 were overlapping with those of H5 and H1’, respectively, and so would have proved redundant for a clear NOESY extrapolation. More importantly, we also noted the products tendency to degrade to the previously reported pyrrole 133. Upon standing at room temperature for 1 h (in an NMR tube) degradation was noted. Figure 2.5A shows a zoomed in region of the ¹H NMR spectrum between δH 6.50 – 5.50 and displays characteristic aromatic peaks of pyrrole 133. Due to the limited amount of
product 132, in conjunction with its instability, the purified product was taken through to the next step in our planned pathway.

![NMR spectra](image)

**Figure 2.5:** A) $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 132 and B) $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 133.$^{149}$

### 2.6. Attempted cis-dihydroxylation of 132

At this stage, the degradation of alkene 132 to the corresponding pyrrole 133 suggested the product to be air sensitive and was suspected to have a low tolerance towards oxidative reaction conditions. Since our aim was to install hydroxy groups at C6 and C7 using Upjohn cis-dihydroxylation conditions,$^{157}$ we anticipated difficulty in obtaining the desired product. This dichotomy influenced the decision to try only pilot reactions with little exploration into optimising reaction conditions. More specifically, alkene 132 was dissolved in a 3:1 solution of acetone/water followed by the addition of 2 eq. of $N$-
methylmorpholine-N-oxide along with a catalytic amount of potassium osmate dihydrate (Scheme 2.6). Unsurprisingly, these conditions only encouraged faster degradation of 132 to the corresponding pyrrole 133. This was determined by the substantial increase in $R_f$ of the new product spot after analysing the reaction mixture by TLC. This $R_f$ was characteristic of the pyrrole 133, and not evident of the diol, as this would be considerably more polar than the starting alkene 132. Regardless, purification of the reaction was achieved and resulted in obtaining the pyrrole 133 which matched the spectroscopic data of the pyrrole reported by Savaspun (Figure 2.5B).149

**Scheme 2.6:** Attempted *cis*-dihydroxylation of 132.

![Scheme 2.6: Attempted *cis*-dihydroxylation of 132.](image)

Although two steps (dihydroxylation, then global deprotection) remained towards the final product, it was evident that the spontaneous degradation of the RCM product 132 to the pyrrole 133 would be difficult and inefficient to work with (even under inert conditions). Although compound 132 was successfully synthesized, the strict reaction conditions were not easily reproduced and often required lengthy preparation periods. Not many conditions were trialled since initial evidence suggested that formation of pyrrole 133 would most likely accelerate under oxidative conditions. For this reason, our retrosynthetic pathway was revised and focused on forming the B-ring of the hyacinthacine C5-type analogues first.

**2.7. Revised retrosynthetic strategy towards hyacinthacine C5-type analogues**

The original retrosynthetic path was reviewed to consider implementing the *syn*-C6, C7-diol on the B-ring of the pyrrolizidine before establishing the A-ring. This new
retrosynthetic pathway can be summarised in Scheme 2.7. The furnished pyrrolizidine that would lead to the hyacinthacine C₅-type analogues would be synthesised by N-alkylation of the amino-diol 134 implementing a similar procedure used to obtain pyrrolidine 131 (Scheme 2.5). Prior to this, the hydroxy groups at both C6 and C7 would be installed using UpJohn cis-dihydroxylation conditions, and then suitably protected to give 134. Although unsuccessful in our earlier attempt, this was hypothesized to work on the basis of similar substrates and reaction conditions employed within the Pyne group. The alkene in compound 135 would be obtained using Grubbs’ I- or II-generation catalyst and RCM conditions. For the RCM reaction to efficiently synthesize, the anti-amino-diol 127 would require protection at the secondary amine to give substrate 136. To ensure selectivity and efficiency in the proposed reaction scheme, suitable hydroxy or amine protecting groups at various stages would also be considered.

Scheme 2.7: Revised retrosynthetic analysis for the synthesis of hyacinthacine C₅-type analogues.

2.8. N-Protection of anti-amino-diol 127

It has been well established that unprotected primary and secondary amines interfere with Ru-catalysed olefin-metathesis reactions. More specifically, their catalyst deactivating affect and thus induced impairment of the RCM reaction is caused by coordination and thus stabilization of the Ru-catalyst. Towards the end of his Ph.D. candidature, Savaspun
briefly explored conditions to convert the anti-amino-diol 127 to its corresponding oxazolidinone 137 (Scheme 2.8).\textsuperscript{149} We envisioned the usefulness of this derivative since it simultaneously protected the nitrogen as well as the hydroxy group at C4 of 127. Interestingly, the conditions he employed resulted in the formation two products, namely the oxazoline-2-one 137 and the oxazepin-2-one 138 (Scheme 2.8, Table 2.2). This result was not necessarily detrimental to the overall synthetic plan since this protecting group would be removed at a later stage in the synthesis. However, the main concern with this outcome was the number of isomeric products that would result at each stage until deprotection. Even if the isomers would separate effectively, characterising both compounds at each stage seemed inefficient. Fortunately, a literature search found a report by Yahara and colleagues which detailed a one-step conversion between a related 7-membered oxazepinone to its 5-membered oxazolidinone analogue after simply stirring a solution of the oxazepinone in MeOH for 24 h.\textsuperscript{159} In light of Savaspuns result, we considered this might overcome selectivity issues if the procedure established by Yahara and co-workers was applied to the mixture of the oxazolidinone and oxazepinone. The anti-amino-diol 127 was therefore subject to standard oxazolidinone conditions (CH\textsubscript{2}Cl\textsubscript{2}, triphosgene, Et\textsubscript{3}N, 1 h) which returned a mixture of the oxazolidin-2-one 137 and the oxazepin-2-one 138 (37% yield, 1:2, respectively, Scheme 2.8, Table 2.2). The mixture of was left in HPLC grade MeOH for 20 h, but no conversion of the oxazepin-2-one 138 to its 5-membered counterpart 137 was observed. This was confirmed by \textsuperscript{1}H NMR and TLC analysis. However, after piloting the catalytic (0.1 eq.) addition of the non-nucleophilic base DBU, followed by 24 h stirring under N\textsubscript{2} conditions, \textsuperscript{1}H NMR and TLC analysis confirmed the complete conversion of 138 to 137 (Scheme 2.9).

**Scheme 2.8:** Synthesis of the oxazolidinone 137 and the oxazepinone 138.
Table 2.2: Savaspun’s reaction conditions (entry 1-3) and our reaction attempt (entry 4) towards synthesizing oxazolidinone 137.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Entry</th>
<th>Reagents used</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Time. (h)</th>
<th>Yield of 137</th>
<th>Yield of 138</th>
</tr>
</thead>
<tbody>
<tr>
<td>Savaspun et al. (^{149})</td>
<td>1</td>
<td>1.5 eq. 1,1’ carbonyldiimidazole</td>
<td>CH₂Cl₂</td>
<td>rt (19)</td>
<td>2.5</td>
<td>11%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.5 eq. Triphosgene, 1.5 eq Et₃N</td>
<td>CH₂Cl₂</td>
<td>rt (18)</td>
<td>1</td>
<td>10%</td>
<td>52%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.33 eq. Triphosgene, 2.0 eq Et₃N</td>
<td>THF</td>
<td>rt (18)</td>
<td>24</td>
<td>53%</td>
<td>19%</td>
</tr>
<tr>
<td>This work</td>
<td>4</td>
<td>0.5 eq. Triphosgene, 2.0 eq Et₃N</td>
<td>CH₂Cl₂</td>
<td>0- rt (18)</td>
<td>1</td>
<td>12%</td>
<td>25%</td>
</tr>
</tbody>
</table>

Further investigations found a strong correlation between increasing the relative molar amount of DBU to compound 138, and a faster facile migration of the carbonyl and conversion to the desired product 137. Therefore, future attempts to synthesise the oxazolidinone involved treatment of a mixture of 137 and 138 with 1.5 eq. DBU in MeOH for 4 h so as to afford clean conversion to the desired 5-membered product 137 in an overall yield of 37%. In addition to the identical match of the \(^1\)H NMR spectrum with Savaspun’s product, the observed 7.1 Hz vicinal coupling constant \(J_{4,5}\), in the \(^1\)H NMR spectrum of 137 was consistent with the 4,5-cis relative configuration\(^{160-162}\).

Despite the clean conversion of oxazepinone 138 to oxazolidinone 137, a collective yield similar to Savaspun’s finding was unable to be replicated. After a number of attempts, only a maximum yield of 37% could be achieved for the synthesis of oxazolidinone 137 (Table 2.2, entry 4). In a bid to improve the yield, different nitrogen protecting groups were considered. Initial trials explored synthesising the N-Fmoc derivative. To obtain this, the anti-amino-diol 127 was dissolved in MeCN, followed by the addition of 2 eq. of Et₃N and 2 eq. of Fmoc chloride and sonicated at 35 °C over 24 h.\(^{163}\) However, as confirmed by TLC and \(^1\)H NMR analysis, only starting material was recovered. The Boc protecting group was then next considered. N-Boc protection of the anti-amino-diol 127 was trialled by dissolving 127, Boc anhydride and Et₃N in MeCN.\(^{163}\) The mixture was stirred at room temperature for 24 h but only the starting anti-amino-diol 127 was recovered. These results suggested the secondary amine 127 to be a poor nucleophile, most likely due to the
terically hindered nature of the N atom. To overcome this issue, increasing the susceptibility of the Boc anhydride carbonyl group was considered. A literature search identified that using iron chloride as a Lewis acid could promote activation of the carbonyl group of Boc anhydride. Shown in Figure 2.6, the oxygen of either carbonyl would coordinate to the FeCl$_3$ which would increase the susceptibility towards mild nucleophiles such as the secondary amine in the anti-amino-diol 127.$^{164}$

![Figure 2.6: Lewis acid promoted Boc protection for mild nucleophiles.](image)

Therefore, after dissolving both Boc anhydride and FeCl$_3$ in 1:1 MeCN/H$_2$O, the anti-amino-diol 127 was added to the reaction mixture and stirred at room temperature for 24 h.$^{164}$ Unfortunately, these conditions did not work and only returned starting material 127. To overcome the steric hinderance of the amino diol, increasing the temperature of the reaction was explored. This involved dissolving the anti-amino-diol 127 in 1,2-dichloroethane, along with the addition of 2 eq. of $N,N$-diisopropylethyl amine and 2 eq. of di-tert-butyl-dicarbonate and the solution was heated at reflux for 2 d. Fortunately, the $N$-Boc diol 139 was obtained in 62% yield with 23% recovered starting material 127 (Scheme 2.9).
Scheme 2.9: Synthesis of compound 139.

With successful protection of the anti-amino-diol 127 as both the oxazolidine-2-one 137 and the N-Boc diol 139, both were considered for ring closing metathesis in the next step of the synthesis.

2.9. The synthesis of RCM products 140 and 141

Choosing either Grubbs’ I or II catalyst is generally decided based on sensitivity of the substrate and the catalyst. Both catalysts have been thoroughly documented and so understanding their different kinetics can also influence which is used. Shown in Figure 2.7, Grubbs’ I catalyst has been found to readily dissociate from its phosphine ligand, PCy3. Upon dissociation, the Ru-species can interact with a number of dienes before it is quenched again with the free PCy3 ligand. To stop intermolecular reactions and ensure an efficient turnover, RCM reactions involving Grubbs’ I catalyst are often performed using dilute conditions. In contrast, Grubbs’ II catalyst undergoes a relatively inefficient dissociation from its PCy3 ligand (Figure 2.7). However, once dissociated, the Ru-species can undergo a four orders-of-magnitude higher rate of reaction relative to its counter catalyst because re-coordination of the PCy3 ligand is less favourable.
Considering that both catalysts were capable of returning the desired RCM products, Grubbs’ II generation catalyst was ultimately chosen since Grubbs’ I catalyst is generally more reactive, and so is prone to side reactions. With a suitable amount of the oxazolidinone 137 and the N-Boc alcohol 139 in hand, separate Ru-catalysed RCM reactions using 5 mol % Grubbs’ II generation catalyst were employed. The most common conditions used in the Pyne research group for similar substrates was to heat the diene with a 10% loading Grubbs’ I or II generation catalyst in CH$_2$Cl$_2$ at reflux until the full consumption of the starting material$^{89,127,146}$ For this reason, similar conditions were used, trialling first the oxazolidinone 137 (Scheme 2.10). After 4 h, a TLC analysis showed full consumption of the starting material as well as only one new spot on TLC with a significantly lower $R_f$. A $^1$H NMR analysis of the crude reaction product indicated a clean conversion to what was later deduced to be the RCM product 140. Careful monitoring by TLC analysis proved important as the alkene group in 140 was prone to migration under
extended reaction times and would give an inseparable mixture of olefin-containing species as well as lower the yield of the desired product. Using similar conditions, the N-Boc alcohol 139 also afforded the RCM derivative 141 in comparable yields (70%) (Scheme 2.11).

Shown in Figure 2.8, The $^1$H NMR spectrum of 140 displayed two, nearly overlapping, characteristic cis-alkene resonances for H6 and H7 at $\delta_H$ 5.79 (1H, d, $J = 6.6$ Hz) and 5.81 (1H, d, $J = 6.6$ Hz), respectively. In conjunction with this, the olefinic proton resonances from the starting diene 137 had disappeared. To eliminate the possibility that the pyrrole had formed, the characteristic H1′′ methyl group was examined and found to resonate as a defined doublet at $\delta_H$ 1.39 (3H, $J = 6.8$ Hz). In addition to this, the $^{13}$C NMR spectrum of 140 only displayed two olefinic-like resonances at $\delta_C$ 137.4 (C7) and 123.8 (C6). These alkene $^1$H and $^{13}$C NMR assignments were based upon gCOSY and gHSQC experiments. The HRESIMS of 140 showed a characteristic [M+Na]$^+$ ion peak at $m/z$ 432.1787, which also established that the desired product had been formed.

**Scheme 2.10:** Synthesis of RCM product 140.
Figure 2.8: $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 140.

Similar resonances were also observed for the $N$-Boc pyrrolidine 141. Unfortunately, the $^1$H NMR analysis became difficult due to the presence of Boc-induced rotamers and consequent broadening of peaks (Figure 2.9). To confirm that this was not the corresponding pyrrole derivative, the most reliable evidence was obtained from a the HRESIMS of 141 which showed a characteristic [M+Na]$^+$ ion peak at $m/z$ 506.2542. Although high-temperature $^1$H NMR studies can be used to improve the definition of the rotamer resonances, it was hopeful that subjecting the product to further chemical manipulation might instead improve the resolution of the $^1$H NMR spectrum. For this reason, the RCM $N$-Boc diol 141 was also continued through to the next step of the synthetic pathway.

Scheme 2.11: Synthesis of RCM product 141.

Figure 2.9: $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 141.
2.10. Dihydroxylation of 140 and 141

Although the initial attempts towards synthesis of the syn-diol at C6 and C7 in 132 were unsuccessful, this did not deter trying the same conditions on the newly formed RCM substrates 140 and 141. In fact, we hypothesized that the reaction would work well for both 140 and 141 using standard Upjohn cis-dihydroxylation conditions. This rationale was largely influenced from previous co-workers results within the Pyne group. More specifically, implementing these conditions on similar substrates containing an N-carbonyl seemed to return high-yielding, high diastereoselective syn-diol products. Although this is a non-selective dihydroxylation, the high diastereoselectivity of akin substrates seems to be influenced by the size of the protecting group attached to the secondary amine. Considering the mechanism shown in Figure 2.10, the first step in this cycle is the sterically influenced cycloaddition between the osmium(VIII) tetroxide and the alkene 140 to form the osmate ester A. In the presence of water, the osmate ester is hydrolysed to the corresponding diol 142. Since both oxygen atoms were added in a concerted manner, their relative stereochemistry remains syn. Interestingly, without the addition of NMO, the reaction would require a molar equivalent of the expensive, and toxic OsO₄. Here, only a catalytic amount is required because the relatively safe and inexpensive NMO can readily oxidize the Os(VI) back to the reactive Os(VIII).

Figure 2.10: Upjohn cis-dihydroxylation mechanism.
Therefore, the RCM product 140 was subject to Upjohn Os(VIII)-catalyzed cis-dihydroxylation reaction conditions\textsuperscript{157} which resulted in two diastereomeric diols 142 (major product) and 143 (minor product) in 78\% yield (dr = 3:1 from the crude reaction mixture) (Scheme 2.12). Only small amounts of pure compounds 142 and 143 could be separated by flash column chromatography (FCC) and so were subject to $^1$H and $^{13}$C NMR analysis and other spectroscopic studies for characterisation. Although 2D NMR spectroscopic data was obtained for both products, evidence towards their absolute configuration will be discussed in Section 2.11.

Scheme 2.12: Synthesis of compounds 142 and 143.

![Scheme 2.12](image)

Interestingly, applying similar dihydroxylation conditions to the N-Boc alkene 141 only returned a single diastereomer 144 in high yield (95\%) (Scheme 2.13). Fortunately, the $^1$H NMR spectrum of compound 144 displayed clear resonance peaks. The C3, C4 stereochemistry of compound 144 was determined to be 3$S$ and 4$R$, respectively after a 2D ROESY experiment displayed clear correlations between the (5$R$)-methyl with the protons resonances labelled H3 and H4 (Figure 2.11). Although the dihydroxylation was successful for 141, the pathway was discontinued on the basis that it would be difficult to selectively protect the newly formed diol at C3, C4 in 144 without impacting on the hydroxy groups at C1’ and C3’. Comparatively, compounds 142 and 143 proved superior as it was envisioned that forming the acetonide at C6, C7 would not impact on C2’. If both the hydroxy group in the alkyl chain were available, such as with the tetraol 144, the acetonide would form at the C3, C4 hydroxy groups, as well as potentially form the 6-membered-C1’ and C3’ acetal 145. Although further investigation is required, these results demonstrated how the N-protecting groups on 5-membered pyrrolidines can sterically influence the selectivity of the Upjohn Os(VIII)-catalyzed cis-dihydroxylation reaction.
Scheme 2.13: Synthesis of compound 144.

Figure 2.11: 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 144 (hydroxy and benzyl protons not shown).

At this point in the course of the project, a colleague within the Pyne research group found that N-Boc products could be converted to the corresponding oxazolidinones provided there is an α-hydroxy group present.¹⁷⁵ This motif was recognized in our N-Boc substrate 139 and so following a similar procedure, a base-induced (1.5 eq. NaH, THF) conversion of 139 to the oxazolidinone 137 was employed and proved successful (85%) with no observable formation of the oxazepinone 139. A comparison of the ¹H and ¹³C NMR spectroscopic data was consistent with our initial oxazolidione 137 product. After comparing both pathways to access the oxazolidinone 137 (triphosgene vs Boc anhydride), we concluded that first synthesizing the N-Boc derivative of amino-diol 127, followed by
base mediate conversion to 137 proved the better yielding (55% over two steps, compared to 37% after one step).

2.11. The synthesis of acetonides 146 and 147

Although syn-dihydroxylation of the RCM product 140 was not completely selective, the inseparable mixture of diastereomers 142 and 143 still proved important as there was the potential to synthesize two hyacinthacine C₅-type analogues. With the inseparable mixture of diols 142 and 143 in hand, it was decided that conversion to their respective C6, C7 acetonides would differentiate the characteristics of both diastereomers and allow for their efficient separation by column chromatography. Having previous experience with the formation of the acetonide 6 from L-xylose, the same reaction conditions were applied to the mixture of diols 142 and 143 (Scheme 2.14). This resulted in a clean conversion to acetonides 146 and 147, respectively and also accentuated the Rᵣ difference between the resultant diastereomers allowing for easier separation by column chromatography. The major diastereomer 146 obtained was the result from syn-dihydroxylation to the alkene face of 142 that was anti to the C5 methyl substituent due to steric directing effects. An extensive ROESY NMR analysis of 146 found key correlations between H₁ˮ–H₈a, H₁ˮ–3a, H₈a–H₃a, H₈–H₃b and H₃b–H₄ (Figure 2.12 and Figure 2.14) which were consistent with the assigned configurations. In comparison, 1D NOE difference studies on 147 showed NOE enhancements between H₈–H₈a, H₈–H₃a, H₈a–H₃a, H₃a–H₃b and H₃b–H₄ (Figure 2.13 and Figure 2.14). These were consistent with the structure assigned to 147.

Scheme 2.14: Synthesis of the acetonides 146 and 147.
Figure 2.12: 2D ROESY NMR spectrum (500 MHz, CDCl$_3$) of 146.

Figure 2.13: Selected 1D-NOE irradiation spectrums (500 MHz, CDCl$_3$) of A) H8a and B) H3a of 147.
Figure 2.14: 2D ROESY and 1D NOE difference NMR spectrum key observed correlations projected onto DFT minimized molecular models [B3LYP/6-31G(d)] of acetonides 146 and 147, respectively (C4 alkyl chain not shown).

2.12. Attempts to hydrolyse and cyclize oxazolidinones 146 and 147

Before hydrolysing the oxazolidinone group in acetonides 146 and 147, individual samples were subject to mesylation of the hydroxy group at C3’. It was anticipated that mesylation of the only free hydroxy groups in these compounds would require less stringent molar equivalents of methane sulfonyl chloride (often difficult to measure an exact equivalent on smaller scales). In addition to this, mesylation of the only hydroxy group, followed by base hydrolysis of the oxazolidinone, may result in a one pot deprotection and consequent cyclization of intermediate 148 (Scheme 2.15). As anticipated, mesylation of both acetonides 146 and 147 worked reasonably well and afforded near quantitative amounts of the respective mesylates 148 and 150 (Scheme 2.15). Both compounds were then subject to standard microwave base-induced hydrolysis conditions, which involved dissolving the mesylate in ethanol, followed by the addition of a few drops of NaOH solution, then microwave heating (200 W, 100 °C) for 1 h. After this time, no observable product was obtained, rather, both reactions returned a complex mixture as indicated from the many spots observed by TLC analysis as well as the indistinguishable 1H NMR spectra. Although a theoretically good idea, the poor results from this approach influenced the decision to first perform the microwave assisted base hydrolysis of the oxazolidinones 146 and 147,
followed by mesylation-cyclisation on the respective amino diols 152 and 153. This approach resulted in a good yield of both amino diols 152 and 153, respectively (Scheme 2.16). These were then individually subject to similar mesylation-cyclisation conditions initially used to convert the anti-amino-diol 127 to the pyrrolidine 131.

Scheme 2.15: Unsuccessful pathway to access pyrrolizidine core A) 149 and B) 151.
**Scheme 2.16:** Hydrolysis of oxazolidinones 146 and 147 to afford amino diols 152 and 153, respectively.

A) \[ \begin{align*} \text{BnO} & \quad \text{BnO} \\ \text{H} & \quad \text{H} \\ \text{O} & \quad \text{O} \\ \text{N} & \quad \text{N} \\ \text{OH} & \quad \text{OH} \end{align*} \] 146

\[ \text{NaOH, EtOH} \quad \text{MW, 110 °C, 1 h} \]

\[ \begin{align*} \text{H} & \quad \text{H} \\ \text{O} & \quad \text{O} \\ \text{N} & \quad \text{NH} \\ \text{OH} & \quad \text{OH} \end{align*} \] 152 86%

B) \[ \begin{align*} \text{BnO} & \quad \text{BnO} \\ \text{H} & \quad \text{H} \\ \text{O} & \quad \text{O} \\ \text{N} & \quad \text{N} \\ \text{OH} & \quad \text{OH} \end{align*} \] 147

\[ \text{NaOH, EtOH} \quad \text{MW, 110 °C, 1 h} \]

\[ \begin{align*} \text{H} & \quad \text{H} \\ \text{O} & \quad \text{O} \\ \text{N} & \quad \text{NH} \\ \text{OH} & \quad \text{OH} \end{align*} \] 153 87%

**2.13. Synthesis of pyrrolizidines 149 and 151**

To furnish the pyrrolizidine core found in the hyacinthacine C5-type analogues, precedent work with similar amino alcohols have employed treatment with a stoichiometric amount of MsCl under basic conditions (Et3N).127-128, 130-131, 144 Having already had experience with this reaction to obtain pyrrolidine 131 from *anti* amino diol 127, this method was employed for both amino diols 152 and 153 (Scheme 2.17). The mesylation-cyclization of 152 afforded a 30% yield of pyrrolizidine 149 as well as a 32% yield of the N,O-dimesylated adduct 154 (Scheme 2.17). In an attempt to improve the yield of the desired product, Appel cyclization reaction conditions176 were used and fortunately returned the same pyrrolizidine 149 with an improved yield of 87% (Scheme 2.17A). Interestingly, both 149 and 154 crystallized after purification by column chromatography (Figure 2.15).

The large colourless crystals obtained for 149 were subject to single crystal X-ray structural analysis which unambiguously confirmed the absolute configuration initially proposed for 149 (Figure 2.16). In addition to this, the crystal structure established that mesylation occurs preferably at the hydroxy group of C3’ as opposed to C1’, which would otherwise could have returned the 4-membered bicyclic N-heterocycle (azetidine).
Comparatively, mesylation, then heat-induced cyclization of the $O$-mesylate of 153 proved extremely regioselective, returning product 151 in high yield (87%) (Scheme 2.17B). Since no crystal structure was obtained for this product, the pyrrolizidine was subject to extensive 1D and 2D NMR spectroscopic experiments. More specifically, to confirm the newly formed stereochemistry on the A-ring of the pyrrolizidine 151, 1D NOE difference studies were run where NOE enhancements were observed between H8b–H3a, H7–H8a, H1''–H6, H1'–H7, H8a–H8b and H4–H3a (Figure 2.16). These were consistent with the structure assigned to 151.

Scheme 2.17: Synthesis of pyrrolizidines 149 and 151.
Figure 2.15: (left) Crystals of 149 and the ORTEP plot of the crystal structure for a C_{26}H_{33}NO_5 molecule of 149 with labelling of selected atoms. Anisotropic displacement ellipsoids display 30% probability levels. Hydrogen atoms are drawn as circles with small radii. (Right) Crystals of 154 and the ORTEP plot of the crystal structure for a C_{28}H_{39}NO_{10}S_2 molecule of 154 with labelling of selected atoms. Anisotropic displacement ellipsoids display 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
Figure 2.16: Selected 1D-NOE irradiation spectrums (500 MHz, CDCl₃) of A) H8b, B) H8a and C) H1” of 151.

This contrast in the formation of both pyrrolizidine 149 and 151 was intriguing, and the differing yields from each reaction pathway can be attributed to steric factors. More specifically, mesylation, then cyclisation of the amino diol 152 returned nearly equal amounts of the bis-mesylate 154 and the pyrrolizidine 149. Confirmed by the crystal structure for the bis-mesylate 154, regioselectivity of the mesylation of the hydroxy group at C3’ suggests that this hydroxy group is less sterically hindered than the hydroxy group.
at C1’. To form the pyrrolizidine 149, an intramolecular $S_N2$ displacement (shown earlier in Scheme 2.15) then takes place. When the $^1H$ NMR spectrum for 154 was obtained, the only certainty was that two mesylates resided in the compound; it was difficult to determine the bis-mesylation regioisomer obtained. However, prior to obtaining a crystal structure there were a few $^1H$ NMR spectrum characteristics that were supportive of mesylation at the nitrogen and the hydroxy group at C3’. Shown in Figure 2.17B, two mesylate peaks with different $^1H$ NMR chemical shifts were present. Although it was not definitive, it was proposed that the mesylate shift at $\delta_H 2.98$ was characteristic of $N$-mesylation and the other at $\delta_H 3.06$ would more likely resonate with a de-shielding effect induced if the mesylate group was attached to a hydroxy group. Comparison of the $^1H$ NMR spectra for both the parent amino-diol 152 (Figure 2.17A) and the bis-mesylate 154 (Figure 2.17B), we noted significant differences in chemical shifts for proton resonances labelled H4 ($\delta_H 3.08$ and 4.38, respectively), H6 ($\delta_H 3.05$ and 3.95, respectively) and H1$''$ ($\delta_H 1.05$ and 1.36, respectively). This de-shielding effect was convincing enough to hypothesize that the secondary amine of amino-diol 152 had undergone mesylation. After a gCOSY analysis, we also identified that the H3’ resonance had shifted from $\delta_H 4.06$ in 152, to $\delta_H 5.00$ in 154. In a similar manner, the resonance identified as H1’ in both structures 152 and 154, had changed chemical shift slightly from $\delta_H 3.74$ to $\delta_H 3.90$, respectively. This comparison supported the claim that both the nitrogen and the hydroxy group at H3’ had undergone mesylation.
Obtaining both the pyrrolizidine 149 and the bis-mesylate 154 led to the rationalization that C3′ O-mesylation and N-mesylation occurs at comparable rates. If O-mesylation at C3′ occurs first, then the molecule will undergo a spontaneous intramolecular cyclization to give 149. If N-mesylation occurs, then cyclisation is prevented and will eventually result in product 154 provided a second mesylation occurs. In contrast, reversing the orientation of the acetonide at C6a and C3a, such as for compound 153, O-mesylation at C3′ is preferred. However, heat was required to furnish pyrrolizidine 151. Since the only difference between both amino diols 152 and 153 was the orientation of the acetonide, it is proposed that this moiety sterically hinders the secondary amine in compound 153 whereas the secondary amine in compound 152 is more susceptible to mesylation. Interestingly, employing Appel cyclisation conditions resulted in exclusive formation of pyrrolizidine 149 (Scheme 2.18). Since no nitrogen bound triphenylphosphine was observed, it is assumed that the in situ formed triphenylphosphine bromide is too sterically encumbering to bind to the secondary amine and so instead binds selectively to the hydroxy group at C3′. Although retention of stereochemistry can occur through C3′ inversion via a
bromide anion, our crystal structure and improved yield of 149 displays the preference for intramolecular cyclization via the $S_N2$ N-alkylation pathway (Scheme 2.18).

Scheme 2.18: Appel cyclization of 152 to give pyrrolizidine 149.

2.14. Global deprotection of pyrrolizidines 149 and 151

After successfully furnishing the pyrrolizidine core, individual samples of 149 and 151 were then globally deprotected under hydrogenolysis conditions over PdCl$_2$ to return novel hyacinthacine C$_5$-type compounds (+)-7-epi-hyacinthacine C$_5$ 155 [[$\alpha$]$_{D}^{25}$ +5.4 (c 1.00, H$_2$O)] and (−)-6-epi-hyacinthacine C$_5$ 156 [[$\alpha$]$_{D}^{25}$ -7.2 (c 1.00, H$_2$O)] (Scheme 2.19) in good to high yields (87% and 98%, respectively), following purification and neutralization by basic ion-exchange chromatography.
**Scheme 2.19:** Global deprotection of pyrrolizidines 149 and 151 to give (+)-7-epi-hyacinthacine C₅ 155 and (‒)-6-epi-hyacinthacine C₅ 156, respectively.

All spectroscopic experiments used to determine the final structures have been included in the appendix. After confidently identifying and assigning each proton resonance through a gCOSY analysis, the relative configurations of each compound was extensively studied. For 155, an extensive ROESY NMR analysis found key correlations between H9–H3, H9–H6, H9–H7, H7–H6, H7–H1 and H2–H8 (Figure 2.18) which were consistent with the assigned configurations. Similarly, compound 156 was subject to extensive ROESY NMR analysis, where key correlations between H9–H3, H6–H5, H6–H7, and H7a–H7 were observed (Figure 2.19). The ¹H and ¹³C NMR spectroscopic data of both synthetic 155 and 156 were also compared with the natural isolates labelled hyacinthacine C₁, C₄ and C₅ (Table 2.3 and Table 2.4). When compared, both synthetic 155 and 156 did not spectroscopically match with any of the questionable natural products. Additionally, a Scifinder Scholar™ search could not identify these structures in the literature and so it was concluded that (+)-7-epi-hyacinthacine C₅ 155 and (‒)-6-epi-hyacinthacine C₅ 156 are novel, unnatural hyacinthacine C₅-type analogues.
Figure 2.18: 2D ROESY NMR spectrum (500 MHz, CDCl$_3$) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of (+)-7-epi-hyacinthacine C$_5$ 155 (hydroxy protons not shown).

Figure 2.19: 2D ROESY NMR spectrum (500 MHz, CDCl$_3$) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of (−)-6-epi-hyacinthacine C$_5$ 156 (hydroxy protons not shown).
Table 2.3: Comparison of the literature $^1$H NMR spectroscopic data of natural (+)-hyacinthacine C$_1$$^{105}$ (400 MHz, D$_2$O), natural (+)-hyacinthacine C$_4$$^{117}$ (500 MHz, D$_2$O) and natural (+)-hyacinthacine C$_5$$^{117}$ (500 MHz, D$_2$O) with synthetic 155 and synthetic 156 (500 MHz, D$_2$O).

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Table 2.4: Comparison of the literature $^{13}$C NMR spectroscopic data of natural (+)-hyacinthacine C$_1$$^{105}$ (100 MHz, D$_2$O), natural (+)-hyacinthacine C$_4$$^{117}$ (125 MHz, D$_2$O) and natural (+)-hyacinthacine C$_5$$^{117}$ (125 MHz, D$_2$O) with synthetic 155 and synthetic 156 (125 MHz, D$_2$O).

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2.15. Chapter 2: Final remarks

The synthetic strategy employed towards the synthesis of hyacinthacine Cs-type analogues in this Chapter has been described. If the successful pathway alone were presented, the reader would question the number of seemingly “unnecessary” steps. Hopefully, the reader
will agree that our pathway must first furnish the B-ring of the pyrrolizidine prior to the A-ring to avoid formation of the pyrrole 133. Although not explored, the author notes that it would be worth subjecting RCM intermediate 132 to hydrogenation conditions, as this may provide a short facile route to a number of hyacinthacine A-type alkaloid analogues. In conclusion, this was the first known synthesis of the unnatural (+)-7-epi-hyacinthacine C₅ 155 and (−)-6-epi-hyacinthacine C₅ 156. Establishing this pathway was important, and the reader will see how it was the basis for accessing the remaining nine hyacinthacine C₅-type analogues. Although the hyacinthacine alkaloids synthesised in this Chapter did not match with any of the ambiguous structures for hyacinthacine C₁, C₄ or C₅, they were still assessed as glycosidase inhibitors and their biological activities will be discussed in Chapter 6.
Chapter 3 : Synthesis of unnatural (−)-5,6-di-epi-hyacinthacine C₅

The methodology towards accessing hyacinthacine C₅-type analogues was established through the synthesis of unnatural (+)-7-epi-hyacinthacine C₅ 155 and (−)-6-epi-hyacinthacine C₅ 156. As per the nature of total synthetic pathways, the reader can assume that some of the foundational compounds (3,5-di-O-benzyl-α,β-L-xylofuranose, the anti-1,2-amino diol 127 the RCM products and the C₆, C₇-diols) have been synthesized a large number of times to accommodate divergent pathways throughout the course of this work. Of these, the anti-1,2-amino diol 127 was synthesized many times. One of the reagents required for this reaction is 3-amino-1-butene-hydrochloride, which at later stages in the project became difficult to acquire. Herein, the reader will see how synthesizing this starting amine resulted in the synthesis of the third unnatural hyacinthacine C₅-type analogue (−)-5,6-di-epi-hyacinthacine C₅.

3.1. The Gabriel synthesis of 3-amino-1-butene-hydrochloride

The 3-(R)-amino-1-butene-hydrochloride used in the Petasis borono-Mannich reaction was originally purchased from a company called NetChem™. Unfortunately, during the later years of this work, NetChem™ became unresponsive and so we could not reliably purchase this starting material as a pure enantiomer. Comparatively, other chemical suppliers often listed small amounts of the amine at high costs. As an up-to-date example, the US company, Enamine Building Blocks™, retails 5 g of the enantiomerically pure 3-(R)-amino-1-butene-hydrochloride for AUD $4914; this value far exceeded the original purchase price of this product when the project first started. Even though we were confident that this material would be profitable towards accessing additional hyacinthacine C₅-type analogues, the exponential price increase in combination with the need to find a reliable chemical company, influenced the decision to consider synthesizing this starting product from a cheaper derivative.
After searching the literature, it became apparent that the desired primary amine could be made from implementing a two-step Gabriel synthesis using a suitable alkyl halide.\textsuperscript{178} The difficulty (and partially justified cost) is that the 3-\((S)\)- and 3-\((R)\)-amino-1-butene-hydrochloride are hard to separate as pure enantiomers. Despite this predicament, we ultimately could not justify the current expense of the stereochemically pure amine. Therefore, the decision was made to synthesize racemic 3-amino-1-butene-hydrochloride and at a later point, consider resolving its enantiomers.

Interestingly, synthesizing the racemic 3-amino-1-butene-hydrochloride is relatively simple and reported as high yielding.\textsuperscript{179, 180} More specifically, based on the precedent literature by Pallavicini \textit{et al.} and Wang \textit{et al.}, the related alkyl halide chosen to undergo this transformation was 3-chloro-1-butene, which could be readily purchased.\textsuperscript{179, 180}

3-Chloro-1-butene could be obtained from Sigma Aldrich\textsuperscript{TM} in two packing sizes of 5 mL and 100 mL at $48.00 and $215.00 AUD, respectively. The latter of these was purchased for economic value, especially considering that synthesizing the racemate would diminish the yield of our desired 3-\((R)\)-amino-1-butene-hydrochloride by at least half. Therefore, starting with 5 g of 3-chloro-1-butene, Gabriel’s synthesis and methodology were employed. Shown in Scheme 3.1A, this initially involved dissolving both 3-chloro-1-butene and potassium phthalimide in a solution of DMF, followed by heating at reflux for 3 h. The general mechanistic consideration for this reaction occurs via displacement of the halogen via an SN2 attack of the phthalide to give a racemic mixture of the \(N\)-substituted phthalimide \textsuperscript{157}. When this reaction was performed, colourless needle-like crystals of the \(N\)-substituted phthalimide \textsuperscript{157} were obtained in high yield (80%). The phthalimide of racemate \textsuperscript{157} was then cleaved using hydrazine hydrate along with heating the mixture at 60 °C in ethanol for 3 h. After this time, the reaction mixture had formed a gelatinous white semi-solid which was then subject to an excess amount of concentrated hydrochloric acid to return the desired 3-amino-1-butene-hydrochloride. This product was purified by a series of acid-base extractions, and the HCl salt of this amine was formed by the addition of 4 N HCl/EtOH followed by concentration \textit{in vacuo}. The \(^1\)H and \(^{13}\)C NMR spectroscopic data of the phthalimide intermediate \textsuperscript{157} as well as the 3-amino-1-butene-hydrochloride, matched with those in the literature.\textsuperscript{179}
This method is known as the Ing-Manske procedure and can be described by the mechanism shown in Scheme 3.1B. Hydrazinolysis occurs when a hydrazine unit attacks one of the carbonyls of the phthalimide moiety of 157. This results in a cascade ring opening (Scheme 3.1B, intermediates A and B) which then allows for an intramolecular nucleophilic attack at the remaining carbonyl from the other half of the attached hydrazine unit (Scheme 3.1B, intermediate C). This attack at the second carbonyl liberates the final primary amine, as well as the phthalhydrazide by-product.

**Scheme 3.1:** A) Garbiel synthesis of 3-amino-1-butene-hydrochloride from 3-chloro-1-butene and B) Mechanism for hydrazinolysis via the Ing-Manske procedure.
While methods have been published for the resolution of the 3-amino-1-butene-hydrochloride,\(^{179,180}\) the decision was made to subject the racemic amine to the Petasis reaction which would afford a diastereomeric mixture of the \textit{anti}-1,2-amino-diols 127 and 161 (Scheme 3.2). Considering the basis of diastereomeric resolution, it was envisioned that the amine would be incorporated into a larger stereochemically defined motif which would display different chemical properties that might result in efficient separation of both (1′\textit{S}) and (1′\textit{R})-\textit{anti}-amino-diols 127 and 161, by column chromatography. Additionally, the resultant (1′\textit{S})-diastereomer 161 was valued since it would lead to potential hyacinthacine C\(_5\)-type derivatives containing the (5\textit{S})-methyl and would prove important for gaining insight towards the SARs of glycosidase inhibition. If the envisioned diastereomers would not separate at any point during the synthetic pathway, then other purification methods would be considered.\(^{179,180}\)

3.2. Synthesis of \textit{anti}-amino diols 127 and 161

In a similar manner to the synthesis described in Chapter 2, but included for clarity, a retrosynthetic analysis towards hyacinthacine C\(_5\)-type analogues containing a (5\textit{S})-methyl is shown in Scheme 3.2. The reader will find that this retrosynthetic analysis is quite specific and has been generated based on the reasonable success we experienced with the previously synthesized unnatural (\textit{+})-7-\textit{epi}-hyacinthacine C\(_5\) 155 and (\textit{−})-6-\textit{epi}-hyacinthacine C\(_5\) 156. The intention of this pathway was to synthesize more of the (1′\textit{R})-methyl \textit{anti}-1,2-amino-diol 127 for later divergency as well as explore the possibility of synthesizing (5\textit{S})-methyl hyacinthacine C\(_5\)-type analogues. In hope that diastereomeric resolution via synthesis of the \textit{anti}-1,2-amino-diols 127 and 161 would allow separation of the (1′\textit{S})- and (1′\textit{R})-methyl products, obtaining pure (5\textit{S})-methyl hyacinthacine C-type analogues would demonstrate the flexibility and effectiveness of this synthetic pathway. Additionally, this result would be an interesting contrast for resultant SAR studies, where we could compare the impact of the stereochemistry of C\(_5\) in the final hyacinthacine C-type analogues. The retrosynthetic plan shown in Scheme 3.2 details that the pyrrolizidine core would be derived from acetonide 158. Similarly, \textit{cis}-dihydroxylation would result from UpJohn \textit{cis}-dihydroxylation\(^{157}\) across the alkene of RCM adduct 159. The alkene of 159 would be obtained using Grubbs’ I- or II-generation catalyst under ring-closing
metathesis conditions on the suitably prepared oxazolidinone 160. Oxazolidinone 160 would be formed by subjecting the \textit{anti}-1,2-amino-diol 161 to either \textit{N}-Boc formation then treatment with sodium hydride or by trichlorophosgene under basic conditions. The diastereomeric mixture of \textit{anti}-1,2-amino-diols, 127 and 161, would be derived from the Petasis borono-Mannich reaction between 3,5-di-O-benzyl-\(\alpha,\beta\)-L-xylofuranose, racemic 3-amino-1-butene-hydrochloride and \(\textit{E}\)-\(\beta\)-styrenylboronic acid.

\textbf{Scheme 3.2:} Retrosynthetic analysis for the synthesis of hyacinthacine C5-type analogues containing a \(\text{(S,S)}\)-methyl.

Following this procedure, racemic 3-amino-1-butene-hydrochloride was treated with 3,5-di-O-benzyl-\(\alpha,\beta\)-L-xylofuranose and \(\textit{E}\)-\(\beta\)-styrenylboronic acid using the same conditions we employed for the synthesis of \textit{anti}-1,2-amino-diol 127 (Scheme 3.3). This afforded an approximate 1:2.5 inseparable mixture of \textit{anti}-1,2-amino-diol 127 and 161, respectively as
determined by the relative resonance intensities in the $^1$H NMR spectrum (Figure 3.1). Since this mixture could not be separated into pure diastereomers, the $^1$H NMR resonance peaks for *anti*-1,2-amino-diol 161 were deciphered by the process of elimination through comparison of the previously characterized *anti*-1,2-amino-diol 127. This result indicated some degree of kinetic preference for the reaction of the iminium ion intermediate arising from the (S)-enantiomer 161 of the amine component. Allowing the mixture a longer reaction time in future might afford a 1:1 mixture of *anti*-1,2-amino-diols 127 and 161.

**Scheme 3.3:** Synthesis of *anti*-amino diols 127 and 161.

In hindsight, the poor separation of *anti*-1,2-amino-diols 127 and 161 was not a surprising result. It is well established that compounds containing amines or quaternary ammonium salts often exhibit sever peak tailing and broad overlapping bands on silica gel; they do not follow a regular hydrophobic retention behaviour. This phenomenon was experienced even with the pure (1'R)-*anti*-1,2-amino-diol 127 described in Chapter 2, as the product contained poor TLC peak symmetry and eluted over an excessive number of otherwise reasonably sized collecting tubes during its purification by column chromatography.
3.3. N-protection of the anti-1,2-amino-diols 127 and 161

In Chapter 2, two different protecting groups of the anti-1,2-amino-diol 127 were trialled and results were described. More specifically, this entailed the synthesis of N-Boc 139 and the oxazolidinone 137. Due to the previously described selectivity issues, it was found that oxazolidinone 137 was more amendable for our synthetic approach. Therefore, attempts to subject the mixture of (1′R)- and (1′S)-anti-1,2-amino-diols 127 and 161, respectively to similar conditions were employed. It was envisioned that synthesizing the oxazolidinone of the mixture may lead to better separation of the diastereomers as a result of increasing the molecules rigidity which would accentuate the subtle characteristic differences between both compounds. Considering anti-1,2-amino-diol 127, we concluded that synthesizing the N-Boc product 139, then base induced cyclization to the respective oxazolidinone 137 returned an overall better yield than compared to when the starting anti-1,2-amino-diol 127 was subject to triphosgene under basic conditions. In hope that this would be similar for anti-1,2-amino-diol 161, a pilot reaction of the diastereomeric mixture of anti-1,2-amino-diols 127 and 161 was subject to N-Boc protection using our established conditions. Unfortunately, this seemed to only work moderately well for the minor anti-1,2-amino-diol 127, whilst only returning predominantly starting material for the major anti-1,2-amino-diol 161. Although this reaction was only tried once, these preliminary results suggest it could potentially be used as a method of purification, as diastereomer 127 can be converted to its N-Boc derivative 139. The obvious inefficiency with this method is that anti-1,2-amino-diol 127 does not undergo complete conversion. Attempting to N-
Boc the mixture of anti-1,2-amino-diols 127 and 161 would need to be performed and purified several times before a majority of the minor anti-1,2-amino-diol 127 is removed from the mixture as the N-Boc derivative 139. Our attention then focused on synthesizing oxazolidinones 137 and 160 using triphosgene. Therefore, the diastereomeric mixture of 127 and 161 was subject to triphosgene/Et₃N conditions, where unsurprisingly, an inseparable mixture of two oxazolidinones 137 and 160 and two oxazepinones 138 and 162 were obtained in an overall yield of 43% (Scheme 3.4). Unfortunately, the ratio of these four products was unable to be determined due to the complexity of the ¹H NMR spectrum even after column purification (Figure 3.2). The N-Boc pathway for the mixture of anti-1,2-amino-diols 127 and 161 was never re-visited. This is acknowledged with the explanation that enough product from the triphosgene protection of the mixture of anti-1,2-amino-diols 127 and 161 was obtained and never needed to be synthesized for the remainder of this project.

**Scheme 3.4:** Formation of the oxazolidinones 137 and 160 along with the oxazepinones 138 and 162.
Figure 3.2: $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the inseparable mixture of 137, 160, 138 and 162.

3.4. Oxazepinones 138 and 162 conversion to oxazolidinones 137 and 160, respectively

The inseparable mixture of the four diastereomers 160, 137, 138 and 162 was then subject to our established conditions that would induce a facile migration of the carbonyl group to give exclusively the oxazolidinone products 160 and 137 (Scheme 3.5). This fortuitously resulted in returning a clean conversion of both oxazepinones 138 and 162 to their respective oxazolidinones 137 and 160 (1:2 mixture, Figure 3.3). The reaction mixture still proved inseparable by TLC and column chromatography. Oxazolidinone 160 was characterized by the process of elimination from comparison of the $^1$H and $^{13}$C NMR spectrum of the diastereomeric mixture with the same data used to characterise the pure (S)-methyl diastereomer 137.
Scheme 3.5: Facile carbonyl migration: conversion of oxazepinones 138 and 162 to oxazolidinones 137 and 160.

Figure 3.3: $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the inseparable mixture of 137 and 160.

3.5. Synthesis of compounds 140 and 149

Having experienced reasonable success in our previous RCM reaction with the oxazolidinone 137, it was thought that treating the resultant mixture of oxazolidinones 137 and 160 to similar conditions would return a high yield of both RCM products 140 and
159, respectively. In addition to this, it was hypothesized that increasing the rigidity of the structures via synthesis of the bicyclic core might accentuate the subtle chemical differences between both diastereomers, enough for them to at least separate using standard column chromatography methods. Applying these conditions, both RCM products 140 and 159 were successfully synthesized and fortuitously separated after column chromatography in 27% and 53% yields, respectively (Scheme 3.6). The diastereoselective resolution was therefore successful when the rigidity of the structure was increased. Interestingly, column chromatography proved effective since there was a significant $R_f$ difference (Figure 3.4, $R_f = 0.20$ for 140 and $R_f = 0.45$ for 159) between each RCM product using 1:1 EtOAc/$n$-hexane as the solvent system. Spectroscopic data for the minor isomer matched that of the previously synthesized 140.

To confirm that the major isomer 159 was the desired RCM product, the HRESIMS and $^1$H NMR spectrum were examined. More specifically, the HRESIMS of 159 showed a characteristic [M+Na]$^+$ ion peak at $m/z$ 432.1782. After examining the $^1$H NMR spectrum (Figure 3.5), a proton resonance was found to reside at $\delta_H$ 4.86 (1H, dd, $J = 8.5, 4.8$ Hz) and proved characteristic of H7a. In addition to this, the observed 8.5 Hz vicinal coupling constant $J_{1,7a}$ between resonances labelled H1 and H7a were consistent with the 4,5-cis relative configuration in 159. To eliminate the possibility that the corresponding pyrrole had formed, careful inspection of the resonance labelled H1′′ at $\delta_H$ 1.21 (3H, $J = 6.8$ Hz) revealed that it appeared with the multiplicity of a doublet; this would otherwise appear as a singlet if there was no proton at C5 and the pyrrole had formed.
Scheme 3.6: Ring-closing metathesis of oxazolidinones 137 and 160 to give 140 and 159, respectively.

Figure 3.4: The thin-layer-chromatography plate (solvent: 1:1 EtOAc/n-hexane) showing the efficient separation of 140 and 159.
3.6. Synthesis of compound 163

Having successfully formed and separated the RCM product 159 from its (5R)-isomer 140, we next focused our attention on establishing the syn-diol at C6 and C7. To do this, compound 159 was subject to UpJohn cis-dihydroxylation (Scheme 3.7).\textsuperscript{157} Interestingly, applying these reaction conditions to compound 159 resulted in the formation of a single diastereomer 163. An extensive ROESY NMR analysis shown in Figure 3.6, indicated that the syn-diol at C6, C7 of 163 formed anti to the (5S)-methyl. Key correlations confirming this stereoconfiguration were observed between the resonances labelled H1″–H6, H7–H7a, and H7a–H1. The product was also crystalline (Figure 3.7) and so was examined by X-ray crystallographic analysis. This unambiguously confirmed the absolute configuration deduced by NMR spectroscopy for compound 163.

Scheme 3.7: UpJohn cis-dihydroxylation of 159 to give diol 163.

Figure 3.5: $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 159.
Figure 3.6: 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 163.

Figure 3.7: Crystals of 163 (left) and the ORTEP plot of the crystal structure for C₂₄H₂₉NO₇ molecule of 163 (right) with labelling of selected atoms. Anisotropic displacement ellipsoids display 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
3.7. Synthesis of acetonide 158

Having obtained the pure syn-diol 163, the next step in the synthetic pathway was to protect the C6, C7 diol as the corresponding acetonide. In a similar manner described in Chapter 2, syn-diol 163 was dissolved in acetone followed by the addition of two equivalents of copper(II) sulphate and a catalytic amount of sulfuric acid. After stirring the reaction for 1 h, a TLC analysis showed a near quantitative conversion to the respective acetonide 158 (Scheme 3.8). In addition to the HRESIMS of 158 which showed a characteristic [M+Na]+ ion peak at m/z 506.2182, both the 1H NMR and the 13C NMR spectrum displayed key resonances that correspond to the newly formed acetonide 158. More specifically, through the comparison of the 13C NMR spectroscopic data between the parent syn-diol 163 and the acetonide 158 (Figure 3.8). In this comparison, acetonide 158 displays three additional carbon resonances at δC 112.7, 26.3 and 24.1 which coincide with C2 and both the methyl carbons contingent at C2, respectively. Although subject to extensive 1D and 2D NMR studies, the compound fortuitously crystalized (Figure 3.9) and so the relative configuration was unequivocally confirmed by single-crystal X-ray studies.

Scheme 3.8: Synthesis of acetonide 158.
Figure 3.8: A) $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 163 and B) $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 158 with C2 and C2 contingent methyl resonances for 158 highlighted in red.

Figure 3.9: Crystals of 158 (left) and the ORTEP plot of the crystal structure for C$_{27}$H$_{33}$NO$_7$ molecule 158 (right) with labelling of selected atoms. Anisotropic displacement ellipsoids display 30% probability levels. Hydrogen atoms are drawn as circles with small radii.
3.8. Synthesis of compound 166

With the acetonide 158 in hand, studies towards a one-pot oxazolidinone hydrolysis followed by annulation to give 165 were explored. Despite our unsuccessful attempts reported in Chapter 2, we recognized that acetonide 158 had exhibited differing properties (i.e. formed crystals) compared to the related acetonide 147 (resultant oil). These notably different properties were the result of a single stereocentre difference. Therefore, it was optimistically envisioned that the (8S)-methyl acetonide would react differently relative to the (8R)-methyl containing acetonide 147. More specifically, if 158 was subject to mesylation at C2’, followed by base-induced microwave hydrolysis, then pyrrolizidine 165 might form. Consequently, acetonide 158 underwent mesylation at C2’ using an excess amount of methanesulfonyl chloride and Et3N (Scheme 3.9A). This provided the mesylated product 164 in near quantitative yields which after purification, was then subject to base-induced microwave hydrolysis. Unfortunately, when subject to these conditions, no observable product was formed, rather, a complex mixture of products was obtained. Although unsuccessful, the curiosity towards the feasibility of this one-pot deprotection and subsequent cyclisation was satisfied; our results suggest that the one-pot oxazolidinone hydrolysis then cyclisation is inefficient for this synthetic pathway. Hydrolysis of the oxazolidinone before mesylation was concluded to be the optimal approach towards synthesizing the desired hyacinthacine C-type analogues. Therefore, subjecting the larger bulk of the acetonide 158 to base-mediated microwave hydrolysis of the oxazolidinone returned the desired amino diol 166 in high yield (84%) (Scheme 3.9B).
3.9. Synthesis of pyrrolizidine 165

To furnish the pyrrolizidine core, the amino-diol 166 was reacted with a stoichiometric amount of methanesulfonyl chloride (Scheme 3.10) and returned two equally intense product spots as determined by TLC analysis.

Prior to purification by column chromatography, TLC analysis was undertaken again but with two parallel areas marked with samples of the crude reaction mixture. Shown in Figure 3.10, a similar process to that of preparative TLC was used in order to determine
the products formed in this reaction. More specifically, only one half of the TLC was stained and processed. It was assumed that the observed spot stains were mirrored on the unprocessed half of the TLC plate and so the silica where it was assumed these spots presided, was gently scrapped off and washed with HPLC grade methanol. The methanolic extracts of the spots with $R_f$ values of 0.45 and 0.35 were then filtered and subsequently analysed by LRESIMS and resulted in the ion peaks at $m/z$ 536 and 440, respectively. These were characteristic of the [M+H]$^+$ ion for the single mesylated product 167, as well as the [M+H]$^+$ ion for the pyrrolizidine 165, respectively. After purification, the suspected pyrrolizidine 165 was the subject of extensive spectroscopic analysis and deduced to be the target compound. Shown in Figure 3.11, the ROESY analysis of pyrrolizidine 165 displays clear correlations between H1′′–H3a, H3a–H8b, H8b–H8a, H8a–H7 and H7–H1′, suggesting the desired absolute configuration had formed on both A and B-rings of the pyrrolizidine product.

**Figure 3.10:** The thin-layer-chromatography plate (solvent: 5:95 MeOH/CH$_2$Cl$_2$) showing the different spots for products 167 and 165 according to their respective $R_f$ values. The reaction mixture was spotted on both halves of the TLC plate, but only one side was processed as indicated by the blue shading. The unprocessed half had the silica removed at $R_f$ values of 0.45 and 0.35 and underwent subsequent analysis via LRESIMS.
Figure 3.11: 2D ROESY NMR (500 MHz, CDCl$_3$) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 165.

Comparatively, the suspected singularly mesylated compound was determined to be the mesylated amino-diol 167. In addition to the LRESIMS, examination of the $^1$H NMR spectrum also indicated the formation of this product. Interestingly, it was initially thought that the mesylate chemical shift in the $^1$H NMR spectrum at $\delta_H \sim 2.95$ (3H, s) was reflective of N-mesylation and so prevented annulation. However, on closer inspection of the $^1$H NMR spectrum for both the starting amino-diol 166 and the mesylate adduct 167, strong evidence suggested that the hydroxy group at C3′ had undergone mesylation. More specifically, it was noted that the proton resonances deduced to be H1′′, H6, and H4 remained relatively unshifted between both spectra at $\delta_H \sim 1.00, 3.20$ and 3.09, respectively (Figure 3.12). In our earlier finding, the same proton chemical shifts for the N-mesylate 154 were significantly deshielded in the $^1$H NMR spectrum. It was therefore confidently deduced that N-mesylation was not apparent in 167. The only other sites that mesylation could occur were at the hydroxy groups at C1’ and C3’. From the $^1$H NMR spectrum comparison between the parent amino-diol 166 and the mesylate adduct, convincing evidence was found to support C3’ hydroxy mesylation. More specifically, the resonance labelled as H1’ in 167 remained relatively unshifted at $\delta_H \sim 4.00$ when compared with the $^1$H NMR spectrum of the parent amino-diol 166. Comparatively, the $^1$H NMR chemical shifts for the resonances labelled H3’ in both 166 and 167 were significantly different at
δ_H ~ 4.00 (1H, m) and 5.00 (1H, bs), respectively. This was consistent with the C3’ hydroxy mesylation deshielding effect and true for structure 167. To further strengthen this argument, it was hypothesized that if the proposed structure was in fact mesylate 167, then subjecting this compound to heat in the presence of a non-nucleophilic base should induce cyclization to afford the previously obtained pyrrolizidine 165. Therefore, compound 167 was dissolved in DCE, followed by the stoichiometric addition of Et$_3$N and heating the solution at reflux overnight. After this time, the starting material spot had completely disappeared to quantitatively return a TLC spot characteristic of pyrrolizidine 165. After purification by column chromatography, the resultant product was obtained in a high yield (76%) and confirmed to be the desired pyrrolizidine 165 by $^1$H and $^{13}$C NMR spectroscopic analysis.

**Figure 3.12:** A) $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 166 and B) $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 167.
3.10. Synthesis of (−)-5,6-di-epi-hyacinthacine C₅ 169

With a suitable amount of the pyrrolizidine 165 in hand, the final step in the synthesis was to globally deprotect this compound. The pyrrolizidines 149 and 151 synthesized in Chapter 2 were converted to their corresponding hyacinthacine C₅-type analogues via hydrogenolysis over PdCl₂. After cleaving the O-benzyl ethers, the *in situ* generated HCl eventually deprotects the acetonide and reveals the final products after the hydrochloride salt is neutralized by basic ion-exchange chromatography. However, deprotection of pyrrolizidine 165 occurred over two steps to reveal (−)-5,6-di-epi-hyacinthacine C₅ 169 (Scheme 3.11). This entailed first deprotecting the acetonide moiety of pyrrolizidine 165 followed by hydrogenolysis of both O-benzyl ethers. Although it was likely that a one-pot global deprotection of pyrrolizidine 165 would have resulted using our initial procedure, deprotection of the acetonide first provided the intermediate 168 which was extensively characterized prior to the final deprotection. Considering the stereogenic complexity of the final products, it is of paramount importance that the synthesis of the correct structure and absolute configuration at each stage has been achieved; all final structures and any possible intermediates should be subject to extensive NMR spectroscopic analysis. More specifically, pyrrolizidine 165 was dissolved in an EtOH/H₂O (1:9, respectively) solution followed by the addition of a few drops of HCl. After stirring at room temperature for 4 h, the desired product 168 was obtained in near quantitative yield after neutralizing the HCl salt with aqueous NaHCO₃. Compound 168 was then subject to various spectroscopic experiments. Of these, an extensive ROESY NMR analysis (Figure 3.13) displayed key correlations between H1″−H6, H1″−H1′, H5−H3, H6−H7, H7−H7a, and H1−H3 and therefore confirmed the absolute configuration depicted in pyrrolizidine 168. After confirming the synthesis and absolute configuration of pyrrolizidine 168, the O-benzyl ethers of this compound were then removed by hydrogenolysis over PdCl₂, and the final hyacinthacine C₅-type alkaloid 169 was neutralized by basic ion-exchange chromatography. This returned (−)-5,6-di-epi-hyacinthacine C₅ [[α]₂⁰D⁻6.9 (c 1.00, H₂O)] 169 which was the subject of extensive NMR spectroscopic experiments and found to spectroscopically match (consistent 0.8 – 1.4 ppm difference in the ¹³C NMR Table 3.1) with its enantiomer 49 [[lit.¹²⁹[α]₂⁰D⁺4.3 (c 0.35, H₂O)] reported synthesized by Yu *et al.*
in 2011\textsuperscript{129} (Figure 3.14). In a similar manner, this compound was also assessed for its glycosidase inhibitory potential which will be discussed in Chapter 6.

**Scheme 3.11:** Global deprotection of pyrrolizidine 165 via two steps: acid hydrolysis of the acetonide to give 168, then hydrogenolysis of the O-benzyl ethers to give (−)-5,6-di-epi-hyacinthacine C\textsubscript{5} 169.

![Scheme 3.11](image_url)

**Figure 3.13:** 2D ROESY NMR spectrum (500 MHz, CDCl\textsubscript{3}) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 168.

**Figure 3.14:** Our synthetic (−)-5,6-di-epi-hyacinthacine C\textsubscript{5} 169 and its enantiomer 49 which was synthesized in 2011 by Yu \textit{et al.}\textsuperscript{129}
Table 3.1: Comparison of literature $^1$H NMR (600 MHz, D$_2$O) and $^{13}$C NMR (75 MHz, D$_2$O) data of Yu et al. Lit. (--)7-epi-hyacinthacine C$_5$ 49$^{129}$ with the $^1$H NMR (500 MHz, D$_2$O) and $^{13}$C NMR (125 MHz, D$_2$O) of our synthetic (--)5,6-di-epi-hyacinthacine C$_5$ 169.

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<th>$^{13}$C NMR ($\delta$)</th>
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<td>(--)7-epi-hyacinthacine C$_5$ 49 (Yu et al.)$^{129}$</td>
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<tr>
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<td>3.89, t (8.7)</td>
</tr>
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<td>2.78, dt (4.3, 9.1)</td>
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<tr>
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<td>2.84, dq (6.4, 12.7)</td>
</tr>
<tr>
<td>6</td>
<td>3.85-3.78, m</td>
<td>3.76-3.68, m</td>
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<td>1.20, d (6.2)</td>
</tr>
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3.11. Chapter 3: Final remarks

The total synthesis of our third hyacinthacine C$_5$-type analogue has been described. The thorough spectroscopic analysis of each synthetic intermediate, as well as the X-ray analysis of both crystal structures for 163 and 158 helped us to confidently assign the absolute configuration for the resultant (--)5,6-di-epi-hyacinthacine C$_5$ 169. This product enantiomer, 49, was synthesized in 2011 by Yu et al.$^{129}$ and it was reassuring to find that the $^1$H and $^{13}$C NMR spectroscopic data were a close match for both products. This further validates our synthetic approach towards accessing the pyrrolizidine core via our mesylation-cyclisation method. Although the chief focus of this project was to synthesize hyacinthacine C$_5$-type analogues containing the ($5R$)-methyl, we were pleased to readily obtain the hyacinthacine C-type derivatives containing the ($5S$)-methyl. (--)5,6-Di-epi-hyacinthacine C$_5$ 169 was cross examined with the hyacinthacine C$_5$-type natural products and did not match any of the spectroscopic data reported for hyacinthacines C$_1$, C$_4$ or C$_5$. However, its synthesis provided an important foundation towards accessing the remaining hyacinthacine C$_5$-type analogues described in this body of work. Additionally, the inherent glycosidase inhibitory activity of this product was assessed and provided valuable information towards SAR studies (Chapter 6).
Chapter 4 - Part A: Initial attempts to synthesize hyacinthacine C₅-type analogues with an anti- C₆, C₇ diol

The segment that comprises Chapter 4 is disclosed in two parts. Chapter 4-Part A describes the unsuccessful attempts towards accessing an anti-diol configuration at the C₆, C₇ stereocentres in the final hyacinthacine C₅-type analogues. Although ineffectual, the author notes that these attempts were an important foundation that ultimately influenced the successful pathway towards accessing these compounds. However, this will be discussed in greater detail in Chapter 4-Part B.

4A.1. Rationale for synthesizing hyacinthacine C₅-type compounds containing a C₆, C₇ anti-diol configuration

Up until this point in the Ph.D. work, three hyacinthacine C₅-type analogues had been successfully synthesized. A spectroscopic cross examination of these synthetic analogues with the hyacinthacine C-type natural products C₁, C₄ and C₅ revealed that they did not match and so the true structures of these natural isolates were still yet to be discovered. With seven stereocentres in our final products, it was calculated that 64 possible diastereomers (excluding their enantiomers) could be synthesized. Since only a few hyacinthacine C-type analogues had been synthesized in the literature so far, a ¹H NMR spectrum pattern that related the absolute configuration with specific proton or carbon chemical shifts could not be generated. With reasonable certainty that most of the originally reported stereocentres for the hyacinthacine C-type natural products would be correct, we continued our investigation towards synthesizing closely related diastereomers of the hyacinthacine C₅-type compounds synthesized in Chapters 2 and 3. More specifically, our chief focus was to implement an anti-diol configuration at the hydroxy stereocentres labelled C₆ and C₇ in the final products. Details of this are described in our retrosynthetic analysis shown in Scheme 4A.1. Having been influenced by the success of our previous synthetic pathways described in Chapter 2 and 3, we envisioned that the final pyrrolizidine core containing the C₆, C₇ anti-diol configuration would be obtained by
firstly removing the protecting group 1 (PG₁) of compound 170 or 171, then oxazolidinone hydrolysis followed by cyclization of the resultant amino diol. The key reaction to ensure that the anti-diol configuration at C6, C7 will appear in the final product, would be the selective ring-opening of the epoxide 172. It was envisioned that the ring-opening the epoxides 172-(6S)-methyl and 172-(6R)-methyl could each afford two potential (four in total) C6, C7 anti-diol diastereomers 170 and 171. The acetonide protecting group described in Chapters 2 and 3 would not work for the resultant C6, C7 anti-diols 170 and 171 since they contain an opposite stereoconfiguration and so a different protecting group synthesis [protecting group 2 (PG₂)] would be trialled. The desired epoxide 172 would form across the alkene of either RCM products 173 or 174, as the alkene displayed susceptibility towards syn-dihydroxylation using UpJohn cis-dihydroxylation conditions¹⁵⁷ and so a similar reactivity towards epoxidation was envisioned. For this reason, we believed the alkene in RCM products 173 or 174 would undergo a stereoselective epoxidation that would occur opposite to either the (5R)-methyl (173) or (5S)-methyl (174) based on precedent work within the Pyne research group.¹⁸³
Scheme 4A.1: Retrosynthetic analysis for hyacinthacine C₅-type analogues containing a C6, C7 anti-diol.

4A.2. O-PMB protection of 137 to give 175

At the time this reaction pathway was considered, a substantial amount of the oxazolidinone intermediate 137 was on hand and so this compound became the model study to explore the feasibility of this reaction pathway. With 137 in hand, we considered protecting the compounds only free hydroxy group at C2''' as the O-PMB ether for several reasons. The PMB protecting group presented itself as a suitable candidate for this pathway as it has been thoroughly explored (synthesized and selectively removed) on similar substrates by previous members from the Pyne research group. Its primary purpose in this pathway was to prevent the hydroxy group at C2''' from intramolecularly attacking and cyclising at C6 or C7 during the ring-opening of the epoxide 172. In addition to this, synthesizing the O-PMB ether factors a selective deprotection of this hydroxy group prior to cyclization to afford the pyrrolizidine core. Using this basis, the hydroxy group at C2'''
of oxazolidinone 137 was subject to O-PMB protection by dissolving compound 137 in THF, followed by heating to 30 °C for 18 h after the addition of NaH, 4-methoxybenzyl chloride and a catalytic amount tetrabutylammonium iodide (TBAI) (Scheme 4A.2A). In this example, addition of TBAI is used to displace the chlorine atom in the 4-methoxybenzyl chloride reagent; this forms 4-methoxybenzyl iodide (PMBI) which is far more reactive and can readily react with the present nucleophilic hydroxy group at C2″″. This salt also acts as a phase transfer catalyst in this reaction by solubilizing the alkoxide anion. After a TLC analysis indicated full consumption of the starting material, the crude mixture was purified by column chromatography and the O-PMB protected product 175 was obtained in a good yield of 86%. An LRESIMS experiment of the sample initially confirmed the successfulness of this reaction, as it displayed a characteristic LRESIMS [M+Na]+ ion peak at m/z 656. The 1H NMR spectrum also displayed a characteristic singlet resonance at δH 3.87 (3H) which was identified to be the OCH3 of the newly added PMB group. This evidence and the additional aromatic resonances δH 7.38 – 7.06 (17H, m, ArH) and δH 6.88 (2H, dd, J = 8.2, 4.7 Hz, ArH) suggested the successful synthesis of product 175.

Scheme 4A.2: O-PMB protection of the oxazolidinone 137.

4A.3. Synthesis of compound 176

Compound 175 was then subject to our established RCM reaction conditions (Scheme 4A.3). As anticipated, employing this procedure returned the RCM product 176 in a high yield of 78%. Formation of the respective pyrrole was initially disregarded on the basis of a HRESIMS experiment which found a [M+H]+ ion peak at m/z 530.2540. Additionally, the 1H NMR spectrum showed that the multiplicity of the resonance labelled H1″ at δH
1.24 (3H, J = 6.8 Hz), appeared as a doublet and so was concluded to be the intended RCM product 176.

**Scheme 4A.3: Synthesis of RCM product 176.**

![Scheme 4A.3: Synthesis of RCM product 176.](image)

**Figure 4A.1:** $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 176.

**4A.4. Attempts to synthesize epoxide 179**

The RCM compound 176 was then considered for epoxidation of the alkene at C6, C7. Deciding the appropriate reaction conditions was largely influenced by precedent results reported by a previous group member Thunwadee Ritthiwigrom.\textsuperscript{183} Shown in Scheme 4A.4, Ritthirigrom found that epoxidation of the alkene moiety of 177, using 1,1,1-trifluoroacetone and oxone,\textsuperscript{185-188} returned exclusively the desired β-epoxide 178 in a high yield (81%). The high d.r. was attributed to the steric influence exerted by the C2 α-side chain.

**Scheme 4A.4: Ritthirigrom’s synthesis of epoxide 178.**\textsuperscript{183}
In this example, we noticed that both the starting material 177 and final product 178 from Rithiwigrom’s work were analogous to our RCM intermediate 176 and the desired epoxide 179, respectively. We therefore hypothesized that subjecting 176 to the procedure described by Rithiwigrom would result in a highly stereoselective synthesis of epoxide 179. The envisioned diastereoselectivity can be explained considering the mechanism shown in Scheme 4A.5. More specifically, the dioxirane E is formed in situ from an initial reaction between 1,1,1-trifluoroacetone A and oxone® B to give intermediate D. After the sulfate ion is lost from D, the dioxirane E will then collapse back to give 1,1,1-trifluoroacetone A after it has attacked the alkene at the less sterically hindered face present in the olefin containing species such as the RCM compound 176. For RCM 176, the envisioned stereochemistry proposed in 179 would be due to the potential steric hindrance that the C1 α-side chain might offer. In addition to this, the orientation of the (5R)-methyl group in 176 would also be a determining factor towards this diastereoselectivity as depicted in Scheme 4A.5.

Scheme 4A.5: Mechanism for epoxidation of 176 using 1,1,1-trifluoroacetone A and oxone® B.
Therefore, the RCM product 176 was subject to the epoxidation procedure reported by Ritthiwigrom but no product 179 was observed. Instead, only starting material 176 was recovered along with initial signs of degradation to a complex mixture. Although several attempts to synthesize epoxide 179 are summarised in Table 4A.1, it was clear that only starting material 176 would be recovered each time (Scheme 4A.6). Therefore, an attempt to synthesize epoxide 179 was performed using the peracid mCPBA (Table 4A.1). Known as the Prilezhaev reaction, peracids such as mCPBA contain a high degree of polarisation within their peracid moiety. This results in a strong electrophilic oxygen atom that can add to alkenes. Shown in Scheme 4A.7, the electrophilic hydroxy group in mCPBA would ideally react with 176 by adding the electrophilic oxygen to the C6, C7 alkene to form a cyclic “butterfly” transition state A. This procedure and mechanism is believed to be concerted and ultimately results in the synthesis of the desired epoxide as well as 3-chlorobenzoic acid as a by-product. Unfortunately, this proved unsuccessful even when freshly recrystallized mCPBA was used and only returned a series of complex mixtures as deduced by TLC analysis and $^1$H NMR experiments of the crude reaction mixtures. After several attempts and various reaction conditions (Table 4A.1) we concluded that our substrate 176 would not readily undergo the formation of epoxide 179 and so our retrosynthetic analysis was reconsidered.
Scheme 4A.6: Attempts to synthesize epoxide 179.

Scheme 4A.7: Proposed mechanism of the epoxidation of 176 using mCPBA.

Table 4A.1: Epoxidation reaction conditions and attempts towards synthesizing epoxide 179.
<table>
<thead>
<tr>
<th></th>
<th>Reaction Conditions</th>
<th>Medium</th>
<th>Temp (°C)</th>
<th>Time (h)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Na₂EDTA.2H₂O, CF₃C(O)CH₃, oxone NaHCO₃</td>
<td>MeCN</td>
<td>0°C</td>
<td>1 h</td>
<td>0% + Starting material 176</td>
</tr>
<tr>
<td>2</td>
<td>Na₂EDTA.2H₂O, CF₃C(O)CH₃, oxone NaHCO₃</td>
<td>MeCN</td>
<td>0°C</td>
<td>2 h</td>
<td>Starting material 176 + complex mixture</td>
</tr>
<tr>
<td>3</td>
<td>EDTA, CF₃C(O)CH₃, oxone NaHCO₃</td>
<td>MeCN</td>
<td>0°C</td>
<td>1 h</td>
<td>Starting material 176 + complex mixture</td>
</tr>
<tr>
<td>4</td>
<td>Na₂EDTA.2H₂O, CF₃C(O)CH₃, oxone NaHCO₃</td>
<td>MeCN</td>
<td>45°C</td>
<td>1 h</td>
<td>Starting material 176 + complex mixture</td>
</tr>
<tr>
<td>5</td>
<td>mCPBA</td>
<td>DCM</td>
<td>rt</td>
<td>1 h</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>6</td>
<td>mCPBA, 2,6-di-tert-butyl-4-methylphenol</td>
<td>DCE</td>
<td>60°C</td>
<td>1 h</td>
<td>Complex mixture</td>
</tr>
</tbody>
</table>

**4A.5. Cis-dihydroxylation then cyclic sulfate synthesis of RCM product 176**

Despite our unsuccessful attempts to synthesize the epoxide 179, a review of previous Ph.D. students’ works within our laboratory found that related anti-diol products were not always obtained from ring-opening the respective epoxide. Since 2003, Sharpless cyclic sulfate chemistry has become an integral procedure within the Pyne research group and has undoubtedly led to the synthesis of many natural products that contain anti-diol relationships. Shown in Scheme 4A.8 and considering syn-diol 180 as an example, this procedure operates through the conversion of the syn-diol 180 to the corresponding cyclic sulfate 181. Cyclic sulfate 181 then undergoes a regioselective S_N2 ring-opening of the cyclic sulfate with an oxygen nucleophile like cesium benzoate (Scheme 4A.8). Acid hydrolysis then cleaves the resultant sulfate ester which in this example, reveals the C6, C7 anti-protected-diol product 182.

Having experienced reasonable success with our earlier established syn-dihydroxylation procedure described in Chapters 2 and 3, we envisaged that synthesizing the C6, C7 syn-
diol 180 followed by its conversion to the C6, C7 anti-protected-diol 182 via Sharpless cyclic sulfate chemistry\textsuperscript{190} might prove to be a more reliable approach than epoxidation. We were particularly confident this would work, especially since the literature regarding the synthesis of cyclic sulfites and cyclic sulfates suggests they readily form from syn-1,2-diols and are considered to be a more reactive variant of their related epoxides.\textsuperscript{190}

**Scheme 4A.8**: The mechanism for the formation of cyclic sulfate 181 and its ring-opening with cesium benzoate.

Therefore, the RCM product 176 was initially subject to UpJohn cis-dihydroxylation\textsuperscript{157} reaction conditions and returned two separable products which were spectroscopically deduced to be diol diastereomers 180 (62% yield) and 183 (18% yield) (Scheme 4A.9). The resultant d.r. was expected since a similar result was obtained and described in Chapter 2. Since a larger portion of 180 was acquired, it was decided that this diastereomer would be used as the pilot study for the conversion to its respective C6, C7 anti-protected-diol 182. Therefore, the ROESY NMR analysis of the parent diol 180 is shown in Figure 4A.2 and will be referred to in a later comparison of the resultant products. Key correlations for 180 were observed between H1′′–H6 and H1′′–H7 and so confirm the designated C6, C7 syn-diol configuration for this compound.
Scheme 4A.9: Synthesis of diols 180 and 183.

![Chemical structure of 176, 180, and 183 with reaction conditions](image)

Following our synthetic plan, diol 180 was then converted to the respective cyclic sulfate 181. This was successfully performed by dissolving diol 180 in CH$_2$Cl$_2$, followed by the addition of an excess amount of Et$_3$N and sulfuryl chloride (Scheme 4A.10). After stirring at 0 °C for 1 h, a TLC analysis indicated that the diol had quantitatively converted to a new product. An initial LRESIMS [M+H]$^+$ ion peak at $m/z$ 626 was observed and matched with the cyclic sulfate 181. Upon standing at room temperature over-night, the product had changed from a light-yellow oil colour to an orange brown and so a second TLC analysis indicated the products unfortunate tendency to readily decompose. This was detrimental.
to the yield and so for the remainder of this Ph.D. work, it was decided that the crude mixture of future cyclic sulfate derivatives should be subject to ring-opening as soon as they are synthesized. Despite the loss of the product, the cyclic sulfate 181 was quickly purified by column chromatography and subsequently subject to an S\textsubscript{N}2 ring-opening of the cyclic sulfate.

**Scheme 4A.10:** Synthesis of the cyclic sulfate 181.

4A.6. Ring-opening the cyclic sulfate 181 with cesium benzoate

The cyclic sulfate 181 was then subject to ring-opening reaction conditions using an *in situ* formed benzoate anion as the nucleophile (Scheme 4A.11). More specifically, compound 180 was dissolved in anhydrous DMSO followed by the addition of benzoic acid and cesium carbonate and heated at 40 °C for 3 h. After this time, a solution of THF, H\textsubscript{2}O and H\textsubscript{2}SO\textsubscript{4} (8:3:1) was added to the reaction and the mixture was stirred at 40 °C for 24 h. After a TLC analysis indicated full consumption of the starting material, the reaction mixture was neutralized with NaHCO\textsubscript{3} and extracted using EtOAc. Unfortunately, an initial \textsuperscript{1}H NMR spectrum of the crude reaction mixture indicated a largely complex mixture of products had formed. Regardless, the reaction was purified by column chromatography and a small amount of an inseparable mixture of single benzoate regioisomers 182 and 184 was obtained. Although the TLC analysis indicated a single spot which had a LRESIMS [M+H]\textsuperscript{+} ion peak at *m/z* 668, there were many convoluted \textsuperscript{1}H NMR resonances; this was characteristic of two products. Unfortunately, the resolution was not clear enough to determine the diastereotopic ratio between both regioisomers. Since the mixture remained inseparable, we were persuaded to hydrolyse the benzoate in 182 and 184 to give the corresponding diol diastereomers 185 and 186, respectively. It was envisioned that both
diol products might be easier to separate, and so each resultant diastereomer could then be extensively characterised and also compared with the parent diol 180.

Scheme 4A.11: Ring-opening of cyclic sulfate 181 with in situ formed cesium benzoate, then sulfate hydrolysis to give benzoate regioisomers 182 and 184.

4A.7. Methanolysis of benzoates 182 and 184

The inseparable mixture of benzoate regioisomers 182 and 184 was therefore subject to methanolysis using K$_2$CO$_3$ (1.5 eq.) in methanol which returned a near quantitative mixture of separable diols 185 and 186, respectively (17% and 7% yield, respectively over two steps) (Scheme 4A.12). Although the R$_f$ for both products were considerably close, their successful separation was achieved and so extensive characterisation for both diastereomers was performed. For products 185 and 186, individual HRESIMS experiments found [M+H]$^+$ ion peaks at $m/z$ 564.6401 and 564.6498, respectively. More importantly, the $^1$H NMR spectra of both products were comparatively different from one another and did not match either of the earlier synthesized diol products 180 and 183. At this point, through comparison of the $^1$H NMR spectra in conjunction with the well-established mechanisms of this reaction, it was confidently ascertained that both diols 185 and 186 were diastereomeric C6, C7 anti-diol products. It was rationalized that the benzoate nucleophile would regioselectively ring-open the cyclic sulfate 181 at the relatively less hindered stereocentre C3a. Therefore, our initial suspicion was that the larger yielding of the two diol products was the C6, C7 anti-diol 185. This was confirmed
based on an extensive ROESY analysis, whereby strong resonance correlations were observed between H1”–H6 and H7–H7a (Figure 4A.3). The configuration of the minor diastereomer 186 was deduced in a similar manner with the finding of a key resonance correlation between H1”–H7 (Figure 4A.4).

Scheme 4A.12: Methanolysis of 182 and 183 to give diol products 185 and 186.
Figure 4A.3: 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 185 (PMB ether not shown).

Figure 4A.4: 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 186 (PMB ether not shown).

4A.8. O-Benzyl ether synthesis at C6, C7 of 185 and 186
To ensure that the C3’ hydroxy group undergoes a selective mesylation, both diols 185 and 186 were individually subject to C6, C7 hydroxy group protection. Since the acetonide is only suited for synthesizing cyclic syn-diols, it was decided that both these hydroxy groups would undergo the conversion to their respective O-benzyl ethers which would later be removed in the final hydrogenolysis deprotection step. We envisioned that this would then allow for selective O-PMB deprotection, followed by subsequent oxazolidinone hydrolysis then annulation to give the desired pyrrolizidine core. Using our already established O-benzyl ether synthetic procedure, (NaH, TBAI, BnBr, THF), both diols 185 and 186 were individually subject to this methodology (Scheme 4A.13). When attempted, only 185 was successfully protected and purified by column chromatography to give 187. Unfortunately, the other diastereomer 186 proved relatively inert to these reaction conditions (mostly starting material recovered). Although the desired product 188 may have been synthesized in small amounts, its recovery by column chromatography proved inefficient and the synthesis of 188 was unable to be confirmed. An attempt to synthesize 188 was only performed once and so further investigation is required to determine the seemingly inert starting material 186. Therefore, only 187 was available for an extensive ROESY NMR analysis. However, the significant closeness and overlap of key proton resonances (H7 overlapping with H6 and H2’ overlapping with H5) made these stereochemical analyses difficult for 187 (Figure 4A.5). Since this was only a protective step, there was no reason to doubt the stereochemical assignments deduced in Section 4A.7. For this reason, compound 187 was taken through to the next stage of the synthesis which entailed the selective hydrolysis of the O-PMB ether at C2’.

Scheme 4A.13: Synthesis of compound 187 and attempted synthesis of compound 188.
4A.9. O-PMB deprotection of 187

It was unfortunate that diol 186 seemed inert to the O-benzyl ether conversion of the hydroxy groups at both C6 and C7. However, this procedure and set of conditions for the O-benzyl ether synthesis of diol 186 was only performed once and so these preliminary results may be unreliable. Further attempts could not be explored as this material was difficult to generate since it was the minor isomer of a relatively low yielding ring-opening of the cyclic sulfate 181. If the C6, C7 hydroxy benzylation of 186 was successful, the inefficiency of generating this material proved impractical. In contrast, the benzyl-ethers at C6, C7 readily formed to give 187 and so this product was subject to O-PMB ether
hydrolysis. Following a similar procedure reported by a previous group member, Karl Lindsay, compound 187 was treated with DDQ (2 eq.) in a CH₂Cl₂ and H₂O (20:1) mixture. The reaction was stirred at 30 °C for 4 h and the full consumption of the starting material after this time was determined by TLC analysis. Unfortunately, the ¹H NMR spectrum and the TLC analysis of the crude reaction mixture displayed a complex combination of products. Ideally, the desired product 189 would have formed via a single electron transfer (SET) resulting in complexes B-D (Scheme 4A.15). Complex D would then aromatize to generate complex E which would decompose to give the desired alcohol 189 and the p-anisaldehyde by-product. However, the substrate 187 seemed to have undergone several different chemical transformations that were not extensively explored. Due to the small scale that this reaction was performed on (30 mg of compound 187), O-PMB deprotection was never re-considered and so this pathway was discontinued. In the interest of time and reaction economy, this reaction pathway ultimately proved to be an unjustified expense. For these reasons, access to the hyacinthacine C-type analogues was reconsidered and will be discussed in detail in Chapter 4-Part B.

**Scheme 4A.14:** Attempted O-PMB hydrolysis of compound 187.

![Scheme 4A.14](image)

**Scheme 4A.15:** Mechanism for DDQ assisted O-PMB hydrolysis of 187.
4A.10. Chapter 4-Part A: Final remarks

The initial attempt to access the hyacinthacine C₅-type analogues containing a C₆, C₇ anti-diol configuration has been described in this Chapter. Although it might not be apparent at first, exploring the feasibility of this pathway consumed a large portion of time and material allocated for the Ph.D. work described. The poor yield of the ring-opening of the cyclic sulfate 181 with the in situ formed benzoate anion delayed the progress of the pathway significantly and so this resulted in a lack of experimental conditions explored. The difficulty to synthesize more of the benzoate regioisomers 182 and 184 in conjunction with the seemingly difficult O-PMB removal of the diastereomer 187, diminished any enthusiasm to repeat this reaction series. These factors in conjunction with the short time remaining for this Ph.D. work led to a revision of the described pathway. This decision
proved successful and resulted in the synthesis of four C6, C7, anti-diol hyacinthacine C₅-type analogues. These will be discussed in detail in Chapter 4-Part B.
Chapter 4 -Part B : Synthesis of putative (+)-hyacinthacine C₅, (+)-5-epi-hyacinthacine C₅, the corrected structure of natural hyacinthacine C₅ and (+)-6,7-di-epi-hyacinthacine C₅

The unsuccessful attempts towards synthesizing hyacinthacine C₅-type analogues containing a C6, C7 anti-diol configuration were described in Chapter 4-Part A. The pathway was discontinued largely based on its uneconomical yields, the unreactivity of key intermediates and the limited remaining timeframe for this work. However, these results helped influence the new revised pathway which ultimately led to the synthesis of our desired products. More specifically, it was found that the C6, C7 anti-diol configuration could be implemented after the pyrrolizidine core had been formed. Herein, the synthesis of the putative structure of hyacinthacine C₅, the corrected structure of hyacinthacine C₅, and two analogues that had been previously synthesized in the literature are described. These results in addition to the successful pathways described in Chapters 2 and 3 were summarised as a manuscript and accepted for publication in the ACS Journal of Organic Chemistry. Full NMR spectroscopic experiment data are available in the related Supporting Information for this article.

4B.1. Revised synthetic strategy towards hyacinthacine C₅-type analogues containing a C6, C7 anti-diol configuration

Chapter 4-Part A concluded that the C6, C7 alkene of RCM compound 176 was not susceptible to epoxidation. However, when 176 was subject to UpJohn cis-dihydroxylation parameters, the diol(s) 180 and 183 readily formed. Diol 180 was then converted to the respective cyclic sulfate 181, which then underwent a S₈2 ring-opening of the cyclic sulfate moiety with benzoate and returned two benzoate regioisomers 182 and 184. Unfortunately, this was a low yielding reaction and so in conjunction with other contributing factors, this reaction pathway was discontinued. In contrast, Chapters 2 and 3 demonstrated that the pyrrolizidine core containing the C6, C7 syn-diol was readily
accessible. It was therefore decided that the pyrrolizidine core would need to be synthesized first prior to establishing the C6, C7 *anti*-dil. After reviewing the successful synthetic pathway described in Chapters 2 and 3, an opportunity to perform our established C6, C7 *anti*-dil chemistry using pyrrolizidines 165, 149 and 151 was recognized and is shown in Scheme 4B.1. More specifically, pyrrolizidines 165, 149 and 151 contain two different hydroxy protecting groups within the compound and are specific to either the A or B ring of the bicyclic compound. On the A-ring of the pyrrolizidine, the hydroxy groups at C6 and C7 were protected as their respective O-benzyl ethers. On the B-ring of the pyrrolizidine, the *syn*-dil moiety at C8b and C3a was protected as the respective acetonide. The only remaining hydroxy group that is unprotected is found at C8. We therefore envisioned protecting this hydroxy group at C8 to give 190, followed by a selective deprotection of the acetonide under acidic conditions. Then, employing our procedure to synthesize the cyclic sulfate 191, followed by an S_N2 ring-opening of the cyclic sulfate moiety with benzoate would likely result in the synthesis of potentially six benzoate regioisomers. Methanolysis of the C6 or C7 benzoate group would return four diols, 192, 193, 194 and 195 which after hydrogenolysis and purification by ion-exchange chromatography would give their respective hyacinthacine C5-type analogues.
Scheme 4B.16: Revised Synthetic plan towards the C6, C7 anti-diol containing hyacinthacine C5-type analogues.

At the time this reaction pathway was considered, pyrrolizidine 165 was on hand and so became the model study for this work. This led to the successful synthesis of the proposed structure for (+)-hyacinthacine C5 and (+)-5-epi-hyacinthacine C5. However, for clarity, the results and procedures employed on each pyrrolizidine analogue 165, 149 and 151 will be presented at their appropriate segments. It is also noted that employing this synthetic pathway for pyrrolizidines 149 and 151 ultimately lead to the same hyacinthacine C5-type products. Both pyrrolizidines were still subject to the same procedures and have been included since they resulted in noteworthy diastereotopic ratios of the desired C6, C7 anti-diol related hyacinthacine C5-type analogues.
4B.2. *O*-Benzyl ether synthesis at C8 of pyrrolizidines 165, 149 and 151

The pyrrolizidines 165, 149 and 151 were considered for the protection of their remaining free hydroxy group at C8 due to foreseen selectivity issues during the formation of their respective cyclic sulfate. The decision was made to convert the hydroxy group to its *O*-benzyl ether since we were familiar with this procedure and had previously obtained reasonably reliable results and overall high yields. Therefore, individual samples of pyrrolizidine 165, 149 and 151 were subject to *O*-benzyl protection using NaH, TBAI, BnBr, THF, and were successful after returning products 196, 197 and 198, respectively in yields of 87%, 89% and 78%, respectively (Scheme 4B.2). After purification, each product was extensively characterized but will be discussed in further detail in the next Section 4B.3.

Scheme 4B.17: Synthesis of 196, 197 and 198.
4B.3. Acid hydrolysis of the acetonide from pyrrolizidines 196, 197 and 198

With the protected pyrrolizidines 196, 197 and 198 in hand, each was individually subject to acid hydrolysis of the acetonide. This was performed in a similar manner to the acetonide hydrolysis of pyrrolizidine 165 described in Chapter 3 which entailed dissolving the compound in a solution of EtOH/H₂O (9:1, respectively), followed by the addition of several drops of 5 M HCl (Scheme 4B.3). The reaction was then stirred at room temperature until full consumption of the starting material was confirmed by TLC analysis. After neutralizing the HCl salt with aqueous NaHCO₃ a generally high yield was obtained for each resultant diol, 199, 200 and 201. In addition to this, the full consumption of the starting material was easily confirmed by the R_f change since each new product became significantly more polar.

Scheme 4B.18: Acid hydrolysis of the acetonide from pyrrolizidines 196, 197 and 198.
All three pyrrolizidine diols were extensively characterized. Although we were confident their relative configurations assigned in both Chapters 2 and 3 had not changed, their characterisation is included for a later comparison and proof of the respective resultant C6, C7 anti-diol products formation. More specifically, syn-diol 199 was subject to an extensive ROESY NMR analysis where key correlations between H1''–H6, H1''–H7a (weak), H6–H7 and H5–H3 are shown below (Figure 4B.1).

**Figure 4B.6:** 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 199 (C1 O-benzyl ether not shown).

Syn-diols 200 and 201 were also extensively characterized through NOE experiments to confirm their relative configurations. These results can be found in the Supporting Information accompanying the resultant ACS Journal of Organic Chemistry publication, but the for the purpose of this work, selected NOE enhancements for 200 are included and display key correlations between H7–H1'', H7–H6, H6–H3, and H6– H1'' (Figure 4B.2).
Figure 4B.7: Top: Selected 1D-NOE spectra (500 MHz, CDCl₃) of 200 of A) H7 and B) H6 and their respective key observed correlations projected onto Bottom: a DFT minimized molecular model [B3LYP/6-31G(d)] of 200 (C1 O-benzyl ether not shown).

Similarly, 201 was also subject to extensive NOE experiments, but for brevity only the enhancements for H6 and H7 are included and display key positive correlations between H7–H6, H7–H7a, H7–H5 and H6–H5 (Figure 4B.3) as expected for this structure.
Figure 4B.8: Top: Selected 1D-NOE spectra (500 MHz, CDCl$_3$) of 201 of A) H7 and B) H6 and their respective key observed correlations projected onto Bottom: a DFT minimized molecular model [B3LYP/6-31G(d)] of 201 (C1 O-benzyl ether not shown).

4B.4. Synthesis of cyclic sulfates 202, 203 and 204, then ring-opening with in situ formed cesium benzoate

In three separate reactions, the diols 199, 200 and 201 were each dissolved in CH$_2$Cl$_2$, followed by the addition of an excess amount of Et$_3$N (2.5 eq.) and sulfuryl chloride (1.5 eq.) (Scheme 4B.4). These reaction conditions converted 199, 200 and 201 to the corresponding cyclic sulfates 202, 203 and 204, respectively. Described in Chapter 4A, our previous experience with the fast degrading nature of the cyclic sulfate 181 influenced the decision to use the crude reaction mixture in the next step of the synthetic pathway without further purification. Indication that the cyclic sulfate had formed was inferred by
the LRESIMS [M+H]^+ ion peak at m/z 552 and TLC analysis to indicate full consumption each starting diol and formation of their respective cyclic sulfate products. Each cyclic sulfate then underwent subsequent S_N2 ring-opening with CsOBz (prepared in situ from Cs_2CO_3 and BzOH) and each individual result is discussed below.

**Scheme 4B.19:** Synthesis of cyclic sulfates 202, 203 and 204.

**4B.4.1. Ring opening cyclic sulfate 202**

Ring opening the cyclic sulfate 202 with the in situ generated CsOBz returned regioisomers 205 and 206 in isolatable yields of 61% and 12%, respectively (Scheme 4B.5). The regio- and stereochemistry of 205 was determined through observed correlations from both gCOSY and ROESY NMR analysis. More specifically, it was observed that the resonance for the proton sharing the stereogenic carbon with the newly added benzoate proved the most deshielded and characteristically appeared slightly above δ_H 5.0 ppm. Analysis of the gCOSY of 205 found a strong correlation between H1'' (δ_H 1.24 ppm) and H5 (δ_H 3.21 ppm), which was subsequently found to correlate with H6 (δ_H 3.94 ppm) (Figure 4B.4). The H6 correlation with the lone triplet resonance at δ_H 5.13 ppm (otherwise H7) clearly indicated that the benzoate group was at C7 as opposed to C6. With the regiochemistry
established, our focus turned to confirming the stereoconfiguration of the benzoate at C7. Consulting the ROESY experiment for 205, we noted a relatively weaker correlation between H6–H7 than that observed between H6–H7 in the parent diol 199. Additionally, the H1′−H6 relationship showed to be far more intense and so collectively suggested successful inversion at C7 (Figure 4B.5). Similarly, the gCOSY analysis of compound 206 identified H5 to strongly correlate with the doublet resonance at δH 5.31 ppm, which was logically assigned H6 (Figure 4B.6). Although the ROESY analysis showed a weak correlation between H6–H7, it undoubtedly found that the correlation between H5–H6 was of stronger intensity (Figure 4B.7). Here, the diastereoselectivity of the synthesized regioisomers 205 and 206 is a result of steric hindrance induced by the C4 methyl stereochemistry from the cyclic sulfate 202. These results suggest that the large benzoate nucleophile attacks the less sterically hindered carbon at C7 resulting in the dominant product 205. The reaction is not completely selective since the C6 benzoate regioisomer 206 was also obtained.

**Scheme 4B.20:** Ring-opening of cyclic sulfate 202 with *in situ* formed cesium benzoate, then sulfate hydrolysis to give benzoate regioisomers 205 and 206.
Figure 4B.9: gCOSY NMR spectrum (500 MHz, CDCl$_3$) of 205 and a DFT minimized molecular model [B3LYP/6-31G(d)] of 205 (O-benzyl ethers not shown).

Figure 4B.10: 2D ROESY NMR spectrum (500 MHz, CDCl$_3$) with the H1"--H6 correlation projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 205 (C1, C2 and C1’ O-benzyl ethers not shown).
Figure 4B.11: gCOSY NMR spectrum (500 MHz, CDCl₃) of 206 and a DFT minimized molecular model [B3LYP/6-31G(d)] of 206 (O-benzyl ethers not shown).

Figure 4B.12: 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 206 (C1, C2 and C1’ O-benzyl ethers not shown).
4B.4.2. Ring opening of cyclic sulfate 203

Ring-opening of 203 gave an inseparable mixture of two pyrrolizidines, 207 and 208, in a 49% yield (Scheme 4B.6). We were confident both these regioisomers had formed on the basis of LRESIMS [M+H]^+ ion peak at m/z 594 of the purified product, as well as the appearance of characteristic resonance peaks for two regioisomers displayed in the \(^1\)H NMR spectra of the purified products. Unfortunately, the ratio between both diastereomers was difficult to ascertain by \(^1\)H NMR analysis and so the mixture was taken through to the next step in hope that the resultant C6, C7 diols would separate.

Scheme 4B.21: Ring-opening of cyclic sulfate 203 with in situ formed cesium benzoate, then sulfate hydrolysis to give benzoate regioisomers 207 and 208.

4B.4.3. Ring opening of cyclic sulfate 204

Ring opening the cyclic sulfate 204 with in situ formed CsOBz returned a 2:1 mixture of separable benzoate pyrrolizidines regioisomers 209 and 210, in yields of 39% and 23%, respectively (Scheme 4B.7). Due to their ease of separation, compounds 209 and 210 were the attention of gCOSY and extensive NOE analysis. It was observed that the proton sharing the stereogenic carbon with the newly added benzoate proved the most deshielded and characteristically appeared at δ_H > 5.0 ppm. More specifically, the gCOSY analysis of 209 identified a strong correlation between H1′′ (δ_H 1.32 ppm) and H5 (δ_H 3.42–3.30 ppm) which was subsequently found to correlate with the most deshielded proton at δ_H 5.04 ppm, namely H6 (Figure 4B.8). After the remaining protons were identified in a similar manner, NOE experiments of 209 identify key correlations between H6–H1′′, H6–H3, H7a–H7, and H7a–H5 (Figure 4B.8). Using a similar method of reasoning, 210 was identified to have resulted from S_N2 attack of the benzoate anion at the C8b position of the cyclic sulfate.
204. Subsequent NOE experiments included in this Chapter display key correlations between H1″−H3, H5–H6, H7–H1, H7a–H2, and H1′–H2 (Figure 4B.9). The regioselectivity of the products formed is similar to the results obtained for the ring opening of cyclic sulfate 202. More specifically in cyclic sulfate 204, the benzoate nucleophile slightly favours attack at C8b. However, because the C4 methyl of the cyclic sulfate 204 is on the concave face of the molecule, the regioisomeric ratio is relatively closer to equal amounts for each regio-isomer 209 and 210 obtained.

**Scheme 4B.22:** Ring-opening of cyclic sulfate 204 with *in situ* formed cesium benzoate, then sulfate hydrolysis to give benzoate regioisomers 209 and 210.
Figure 4B.13: Top: gCOSY NMR spectra (500 MHz, CDCl$_3$) of 209 and Bottom: Selected 1D-NOE spectra (500 MHz, CDCl$_3$) of 209 of A) H6 and B) H7a and their respective key observed correlations projected a DFT minimized molecular model [B3LYP/6-31G(d)] of 209 (C1, C2 and C1’ O-benzyl ethers not shown).
Figure 4B.14: Top: gCOSY NMR spectra (500 MHz, CDCl$_3$) of 210 and Bottom: Selected 1D-NOE spectra (500 MHz, CDCl$_3$) of 210 of A) H6 and B) H7 and their respective key observed correlations projected a DFT minimized molecular model [B3LYP/6-31G(d)] of 210 (C1, C2 and C1' O-benzyl ether not shown).

4B.5. Methanolysis of the benzoates 205, 206, 209, 210 and the inseparable mixture of 207 and 208

The individual benzoate regio-isomers 205, 206, 209, 210 and the inseparable mixture of 207 and 208 were next subject to methanolysis of the benzoate group. This was performed
by using the same procedure described in Chapter 4A, which involved dissolving each compound in methanol, followed by the addition of K$_2$CO$_3$.

**4B.5.1. Methanolsysis of benzoates 205 and 206**

Methanolsysis of the benzoate regio-isomers 205 and 206 returned diols 211 (79% yield) and 212 (90% yield), respectively (Scheme 4B.8).

**Scheme 4B.23:** Methanolsysis of 205 and 206 to give diol products 211 and 212, respectively.

The HRESIMS of 211 and 212 displayed [M+H]$^+$ ion peaks at m/z 490.2617 and 490.2613, respectively. An initial comparison of the $^1$H NMR spectra of 211 and 212 with that of the parent syn-diol 199 clearly indicated that these were not the same product. This, in combination with an extensive NMR analysis, suggested that the single-stereocentre at either C6 or C7 for compounds 211 and 212, respectively had undergone inversion. Interestingly, diol 211 was found to be the enantiomer of the pyrrolizidine 45 reported by Yu et al. in 2011 (Figure 4B.10) and so it was reassuring to find an identical match in both the $^1$H and $^{13}$C NMR ($\Delta \delta_H = 0.01–0.03$ ppm, $\Delta \delta_C = 0.1–0.2$ ppm) spectroscopic data when compared with this reported literature compound (Table 4B.1). In addition to this, compound 211 fortuitously crystallized and so allowed the unequivocal confirmation of its stereochemistry by single-crystal X-ray analysis (Figure 4B.11).
Figure 4B.15: Our synthetic 211 and its enantiomer 45 which was synthesized in 2011 by Yu et al.\textsuperscript{129}

Figure 4B.16: Crystals of 211 (left) and the ORTEP plot of the crystal structure of the C\textsubscript{30}H\textsubscript{35}NO\textsubscript{5} molecule of 211 with labelling of selected atoms, showing only one set of sites for the disorder in the benzyl groups. Anisotropic displacement ellipsoids display 30\% probability levels. Hydrogen atoms are drawn as circles with small radii.
Table 4B.2: Comparison of literature $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (75 MHz, CDCl$_3$) data of Yu et al. Lit. 45$^{129}$ with the $^1$H NMR (500 MHz, CDCl$_3$) and $^{13}$C NMR (125 MHz, CDCl$_3$) of our synthetic 211.

<table>
<thead>
<tr>
<th>position</th>
<th>$^1$H NMR ($\delta$, $J$ in Hz)</th>
<th>$^{13}$C NMR ($\delta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 (Yu et al.)$^{129}$</td>
<td>211</td>
<td>45 (Yu et al.)$^{129}$</td>
</tr>
<tr>
<td>1</td>
<td>4.08, t (3.3)</td>
<td>4.07, t (3.6)</td>
</tr>
<tr>
<td>2</td>
<td>4.12, t (3.4)</td>
<td>4.10, t (3.6)</td>
</tr>
<tr>
<td>3</td>
<td>3.27-3.22, m</td>
<td>3.23, dt (4.5, 9.8)</td>
</tr>
<tr>
<td>5</td>
<td>2.99-2.91, m</td>
<td>2.93, dq (6.4, 12.6)</td>
</tr>
<tr>
<td>6</td>
<td>3.67, t (8.0)</td>
<td>3.68, t (8.1)</td>
</tr>
<tr>
<td>7</td>
<td>3.98, t (7.5)</td>
<td>3.95, t (7.5)</td>
</tr>
<tr>
<td>7a</td>
<td>3.41, dd (3.9, 7.4)</td>
<td>3.48-3.40, m</td>
</tr>
<tr>
<td>1’</td>
<td>3.47, d (6.5)</td>
<td>3.48-3.40, m</td>
</tr>
<tr>
<td>1’’</td>
<td>1.20, d (6.2)</td>
<td>1.17, d (6.2)</td>
</tr>
</tbody>
</table>

Focusing our attention to the related diol 212, a ROESY analysis displayed a key correlation between H5–H6 which corroborated with the fact that the earlier benzoate inversion had successfully occurred at C6 (Figure 4B.12).

Figure 4B.17: 2D ROESY NMR spectrum (500 MHz, CDCl$_3$) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 212.
4B.5.2. Methanolysis of benzoates 209 and 210

Individual base-mediate hydrolysis of the benzoate group in pyrrolizidines 209 and 210 returned their respective diols 213 (90%) and 214 (83%) (Scheme 4B.9). The HRESIMS of 213 and 214 found [M+H]⁺ ion peaks at \( m/z \) 490.2608 and 490.2615, respectively.

Scheme 4B.24: Methanolysis of benzoates 209 and 210 to give diol products 213 and 214, respectively.

After conducting NMR spectroscopic experiments for both products, it was noted that the \(^1\)H and \(^{13}\)C NMR spectra of these pyrrolizidines were different to that of their parent pyrrolizidines 200 and 201, and so clearly indicated successful configurational inversion at either C6 or C7, respectively.

For diol 213, a ROESY NMR analysis indicated the successful inversion at C6, as key correlations between H1″−H6 and H7−H7a were identified (Figure 4B.13). Comparatively, a ROESY NMR analysis for diol 214 displayed a distinct correlation between H5−H6 indicating a \( \text{syn} \)-relationship between the protons, suggesting that the C6 hydroxy group is on same face as the (5\( \text{R} \))-methyl group and has not inverted (Figure 4B.14). This evidence therefore suggested the inversion at C7 since it is the only other site that the benzoate could have attacked.
Figure 4B.18: 2D ROESY NMR spectrum (500 MHz, CDCl$_3$) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 213.

Figure 4B.19: 2D ROESY NMR spectrum (500 MHz, CDCl$_3$) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 214.
4B.5.3. Methanolysis of the inseparable mixture of benzoates 207 and 208

The inseparable mixture of benzoates 207 and 208 was also subject to methanolysis which resulted in a clean hydrolysis of their benzoate moieties to give the separable pyrrolizidine diols 213 and 214 in a 1:4 ratio. The $^1$H and $^{13}$C NMR spectra of both products matched the respective diols obtained from hydrolysis of benzoates 209 and 210 described above. This led us to believe their respective benzoate pyrrolizidines, 207 and 208, were originally synthesized in an approximately 1:4 ratio. Here, the ring-opening of cyclic sulfate 203 seems to return a contrasting regio-selectivity to our prior results. More specifically, the regiochemical outcome of the ring-opening reaction of the cyclic sulfate 203 with CsOBz can be best described by considering the two possible reactive conformations 203A and 203B of cyclic sulfate 203, and the preference for 1,2-diaxial-like ring opening as shown in Scheme 4B.10. In this example, attack predominates at C3a of conformer 203A resulting in 208 as the major regioisomer. When considering conformation 203B, attack at C8b is less likely to occur (to give 207) due to unfavourable steric interactions between the nucleophile (−OBz) and the pseudoaxial C4 methyl group and the pseudoaxial proton H8a.

**Scheme 4B.25:** Proposed reactive conformers of 203 to give the major benzoate 208 along with the minor benzoate 207.
4B.6. Hydrogenolysis of pyrrolizidines 211, 212, 213 and 214

Confident that we had successfully synthesized all four diastereomers 211, 212, 213 and 214, the final step in the synthesis was to hydrolyse the O-benzyl ethers from each of these compounds. To do this, each pyrrolizidine was individually subject to hydrogenolysis over PdCl$_2$ and stirred until LRESIMS confirmed full consumption of the starting material. All final products were then neutralized by basic ion-exchange chromatography and returned the hyacinthacine C$_5$-type related alkaloids as described.

4B.6.1. Hydrogenation of pyrrolizidines 211 and 212

Debenzylation of pyrrolizidines 211 and 212 returned the putative structure of hyacinthacine C$_5$ [[α]$^D_{25}$+9.5 (c 1.00, H$_2$O)] along with its (+)-5-epi-hyacinthacine C$_5$ 215 [[α]$^D_{25}$+10.3 (c 1.00, H$_2$O)], respectively (Scheme 4B.11). Since we were confident in their structures and absolute configurations, it was unsurprising to find that the $^1$H and $^{13}$C NMR spectroscopic data of our synthetic purported hyacinthacine C$_5$ and 215 matched their literature reported structures. More specifically, a close spectroscopic match was noted for our synthetic purported hyacinthacine C$_5$ and the previously synthesized proposed structure of (+)-hyacinthacine C$_5$ (Table 4B.2: Δ δ$_H$ = 0.01−0.04 ppm, Δ δ$_C$ = 0.6−0.7 ppm) [lit.$^{130}$ [α]$^D_{30}$+8.0 (c 1.00, H$_2$O)]. Synthetic 215 also proved to be a close spectroscopic match with Tamayo and coworkers synthetic hyacinthacine C$_5$-type analogue 64 (lit.$^{130}$ [α]$^D_{30}$+1.5 (c 1.00, H$_2$O)) An internal reference was not included in the NMR sample of 215 since it would be difficult to remove prior to glycosidase testing. Therefore, the $^1$H NMR spectrum of 215 was referenced to the D$_2$O solvent residual peak at 4.79 ppm and the $^1$H NMR spectrum resonance peaks were consistently close with those of 64 (Table 4B.3: Δ δ$_H$ = 0.02−0.03 ppm). The $^{13}$C NMR of 215 was referenced to internal MeOH, but the chemical shifts shown in Table 4B.3 were consistently shifted 2.5−2.6 ppm when compared with the $^{13}$C NMR of 64. Comparatively, the $^1$H and $^{13}$C NMR chemical shifts reported for 64 are expressed in reference to the internal standard sodium 3-(trimethylsilyl)propionate (TPS) in D$_2$O.$^{130}$ This satisfactorily explains the slight NMR spectroscopic differences observed between synthetic 215 and 64.
Scheme 4B.26: Hydrogenolysis of 211 and 212 to give the putative structure of hyacinthacine C₅ and hyacinthacine C₅-type analogue 215, respectively.

Table 4B.3: Comparison of the literature ¹H NMR (500 MHz, D₂O) and ¹³C NMR (125 MHz, D₂O) spectroscopic data of Tamayo et al. Lit. (+)-hyacinthacine C₅ with the ¹H NMR (500 MHz, D₂O) and ¹³C NMR (125 MHz, D₂O) data of our synthetic purported compound for (+)-hyacinthacine C₅.

<table>
<thead>
<tr>
<th>position</th>
<th>¹H NMR (δ, J in Hz)</th>
<th>¹³C NMR (δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+)-hyacinthacine C₅ (Tamayo et al.)¹³⁰</td>
<td>The purported (+)-hyacinthacine C₅ from this work</td>
</tr>
<tr>
<td>1</td>
<td>3.99, t (6.9)</td>
<td>4.19, t (6.9)</td>
</tr>
<tr>
<td>2</td>
<td>2.97-2.93, m</td>
<td>3.96, t (7.0)</td>
</tr>
<tr>
<td>3</td>
<td>3.74-3.66, m</td>
<td>2.96-2.87, m</td>
</tr>
<tr>
<td>4</td>
<td>4.15, t (6.9)</td>
<td>4.11, t (7.1)</td>
</tr>
<tr>
<td>5</td>
<td>3.08, t</td>
<td>3.05, t (6.8)</td>
</tr>
<tr>
<td>6</td>
<td>3.72-3.66, m</td>
<td>3.74-3.61, m</td>
</tr>
<tr>
<td>7a</td>
<td>1.24, d (6.3)</td>
<td>1.20, d (6.2)</td>
</tr>
</tbody>
</table>
Table 4B.4: Comparison of the literature $^1$H NMR (500 MHz, D$_2$O) and $^{13}$C NMR (125 MHz, D$_2$O) spectroscopic data of Tamayo et al. Lit. 64$^{130}$ with the $^1$H NMR (500 MHz, D$_2$O) and $^{13}$C NMR (125 MHz, D$_2$O) data of our synthetic 215.

<table>
<thead>
<tr>
<th>position</th>
<th>$^1$H NMR ($\delta$, J in Hz)</th>
<th>$^{13}$C NMR ($\delta$)</th>
<th>$^1$H NMR ($\delta$)</th>
<th>$^{13}$C NMR ($\delta$)</th>
<th>$^{13}$C NMR ($\delta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64 (Tamayo et al.)$^{130}$</td>
<td>(+)-5-epi-hyacinthacine C$_5$ 215</td>
<td>4.31, t (7.5)</td>
<td>4.28, t (7.5)</td>
<td>73.1</td>
<td>75.6</td>
</tr>
<tr>
<td>64 (Tamayo et al.)$^{130}$</td>
<td>(+)-5-epi-hyacinthacine C$_5$ 215</td>
<td>3.99, t (7.5)</td>
<td>3.96, t (8.4)</td>
<td>78.7</td>
<td>81.2</td>
</tr>
<tr>
<td>215</td>
<td>2.81, m</td>
<td>2.78, dd (4.6, 9.3)</td>
<td>70.6</td>
<td>73.1</td>
<td></td>
</tr>
<tr>
<td>215</td>
<td>3.16, m</td>
<td>3.14, q (4.9, 6.1)</td>
<td>62.7</td>
<td>65.2</td>
<td></td>
</tr>
<tr>
<td>215</td>
<td>4.15, m</td>
<td>4.13, dd (2.4, 4.2)</td>
<td>79.7</td>
<td>82.2</td>
<td></td>
</tr>
<tr>
<td>215</td>
<td>4.24, m</td>
<td>4.21, dd (2.4, 4.7)</td>
<td>74.3</td>
<td>76.8</td>
<td></td>
</tr>
<tr>
<td>215</td>
<td>3.49, m</td>
<td>3.46, dd (4.6, 7.2)</td>
<td>69.1</td>
<td>71.6</td>
<td></td>
</tr>
<tr>
<td>215</td>
<td>3.71, dd (4.7)</td>
<td>3.68, dd (4.6, 12.0)</td>
<td>61.6</td>
<td>64.2</td>
<td></td>
</tr>
<tr>
<td>215</td>
<td>3.80, dd (4.0, 11.9)</td>
<td>3.78, dd (4.0, 12.3)</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>215</td>
<td>1.19, d (6.7)</td>
<td>1.17, d (6.9)</td>
<td>14.0</td>
<td>16.5</td>
<td></td>
</tr>
</tbody>
</table>

4B.6.2. Hydrogenation of pyrrolizidines 213 and 214

Debenzylation of 213 and 214 returned hyacinthacine C$_5$-type compounds (+)-6,7-di-epi-hyacinthacine C$_5$ 216 [[$\alpha$]$^D_{25}^{25}$+5.2 (c 1.00, H$_2$O)] and 217 [[$\alpha$]$^D_{25}^{25}$+6.8 (c 1.00, H$_2$O)] (Scheme 4B.12). To our fortune and as hypothesized by Reissig and Goti et al.,$^{131}$ the $^1$H and $^{13}$C NMR spectroscopic data of our synthetic 216 were found to be essentially identical with those of the isolate labelled hyacinthacine C$_5$ [lit.$^{117}$ [$\alpha$]$^D_{25}$+1.5 (c 0.22, H$_2$O)] in 2007 (Table 4B.4). An internal standard was not included in the NMR sample of 216 since it would be difficult to remove prior to glycosidase testing. Therefore, the $^1$H NMR chemical shifts of 216 were referenced to the D$_2$O solvent residual peak at 4.79 ppm and the $^1$H NMR spectrum resonance peaks were consistently close with those of the isolate labelled hyacinthacine C$_5$ (Table 4B.4: $\Delta \delta_H = 0.11$–0.15 ppm). The $^{13}$C NMR of 216 was referenced to internal MeOH, but the chemical shifts shown in Table 4B.4 were consistently shifted 2.9–3.0 ppm when compared with the $^{13}$C NMR of the isolate labelled hyacinthacine C$_5$. Comparatively, the $^1$H and $^{13}$C NMR chemical shifts reported for the natural isolate labelled hyacinthacine C$_5$ are expressed in reference to the internal standard sodium 3-(trimethylsilyl)propionate (TPS) in D$_2$O.$^{117}$
Thus, the correct structure of natural hyacinthacine C₅ has the opposite configuration at the three contiguous stereogenic centres, C5, C6, and C7, to that of the originally proposed structure. The authors on the isolation of this natural product had determined the correct relative configurations of the individual A and B-rings of hyacinthacine C₅ but had incorrectly assigned the absolute configuration of the A-ring. Confirmation of these assignments were made through detailed NOESY studies which unequivocally showed 216 to be the correct structure (included in the Appendix for this work).

Scheme 4B.27: Hydrogenolysis of 213 and 214 to give the corrected structure of hyacinthacine C₅ 216 and hyacinthacine C₅-type analogue 217, respectively.
Table 4B.5: Comparison of literature $^1$H NMR (500 MHz, D$_2$O) and $^{13}$C NMR (125 MHz, D$_2$O) spectroscopic data of Kato et al. Lit. (+)-hyacinthacine C$_5$\textsuperscript{117} with the $^1$H NMR (500 MHz, D$_2$O) and $^{13}$C NMR (125 MHz, D$_2$O) data of our synthetic \textit{216}.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{position} & \textbf{$^1$H NMR ($\delta$, $J$ in Hz)} & \textbf{Corrected structure of natural (+)-hyacinthacine C$_5$ \textit{216}} & \textbf{$^1$C NMR ($\delta$)} & \textbf{$^1$C NMR ($\delta$)} \\
\hline
1 & 4.01, t (7.6) & 4.14, apparent q (7.7) & 78.2 & 75.2 & 3.0 \\
2 & 3.80, t (7.6) & 3.94, t (7.8) & 81.0 & 78.1 & 2.9 \\
3 & 3.01, m & 3.14, td (4.0, 9.1) & 65.1 & 62.1 & 3.0 \\
5 & 2.81, dq (6.9, 7.6) & 2.95, dq (6.6, 7.4) & 61.4 & 58.4 & 3.0 \\
6 & 3.60, t (7.6) & 3.74, t (7.7) & 81.7 & 78.8 & 2.9 \\
7 & 3.99, t (7.6) & 4.14, apparent q (7.7) & 77.8 & 74.8 & 3.0 \\
7a & 3.24, t (7.6) & 3.38, t (7.6) & 69.2 & 66.3 & 2.9 \\
8 & 3.55, dd (4.1, 12.0) & 3.66, dd (4.7, 9.5) & 65.7 & 62.8 & 2.9 \\
& 3.50, dd (5.3, 12.0) & & & & \\
9 & 1.13, d (6.9) & 1.27, d (6.7) & 15.7 & 12.8 & 2.9 \\
\hline
\end{tabular}
\end{table}

However, when comparing the NMR spectroscopic data of our synthetic hyacinthacine C$_5$-type analogue \textit{217} \textbf{[}$[\alpha]_D^{25}$+6.8 (c 1.00, H$_2$O)\textbf{]} and its HCl salt \textit{217}.HCl \textbf{[}$[\alpha]_D^{25}$-4.5 (c 1.00, H$_2$O)\textbf{]} with those reported for the same compound \textit{120} \textbf{[}lit.\textsuperscript{131} \textbf{[}$[\alpha]_D^{25}$+0.75 (c 0.48, H$_2$O)\textbf{]}], prepared by Reissig and Goti \textit{et al.} we noted significant spectroscopic differences (Table 4B.5 and Table 4B.6). In the $^1$H NMR spectrum of compound \textit{217}, the chemical shifts of H3, H5, and H7a appeared upfield in comparison to those for \textit{120} (Table 4B.5). Additionally, the chemical shifts of the aforementioned protons in \textit{217}.HCl appeared significantly downfield when compared with compound \textit{120} (Table 4B.5). Comparison of the $^{13}$C NMR spectroscopic data of both \textit{217} and \textit{217}.HCl with those published for \textit{120} seems to more closely aligned with \textit{217}.HCl (Table 4B.6). However, it was concluded that Reissig and Goti \textit{et al.} did not make this salt, as there was still significant differences between both spectra (especially for the carbons identified in the product as C2, C3, C5, C7a, and C8). Despite both having been proposed to contain the same stereochemistry and absolute configuration, we could not conclude that our synthetic \textit{217} was the same structure as \textit{120} reported by Reissig and Goti \textit{et al.}
Table 4B.6: Comparison of literature \(^1\)H NMR (400 MHz, D\(_2\)O) spectroscopic data of Reissig and Goti et al. Lit. 120\(^{131}\) with the \(^1\)H NMR (500 MHz, D\(_2\)O) data of our synthetic 217 and 217·HCl.

<table>
<thead>
<tr>
<th>position</th>
<th>(^1)H NMR ((\delta J) in Hz)</th>
<th>(^{13})C NMR ((\delta))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.99, t (8.6) 4.07-3.97, m 4.24, t (8.7)</td>
<td>76.7 78.0 75.6</td>
</tr>
<tr>
<td>2</td>
<td>3.80, dd (8.4, 9.6) 3.90, t (8.1) 3.99, dd (8.5, 10.2)</td>
<td>75.3 77.9 73.6</td>
</tr>
<tr>
<td>3</td>
<td>3.41, dt (3.6, 9.6) 3.33, dt (4.9, 9.0) 3.78-3.70, m</td>
<td>63.4 62.2 65.2</td>
</tr>
<tr>
<td>4</td>
<td>3.64-3.56, m 3.41, dt (5.7, 11.7) 4.06, qd (2.9, 7.0)</td>
<td>60.8 58.1 64.0</td>
</tr>
<tr>
<td>5</td>
<td>3.95, t (3.0) 4.07-3.97, m 4.15, d (2.9)</td>
<td>79.1 79.7 or 78.6 79.4</td>
</tr>
<tr>
<td>6</td>
<td>4.15, t (2.4) 4.18, t (4.0) 4.40, s</td>
<td>76.8 79.7 or 78.6 76.50</td>
</tr>
<tr>
<td>7</td>
<td>3.28, dd (1.0, 8.4) 3.07, dd (3.7, 8.1) 3.78-3.70, m</td>
<td>76.8 79.7 or 78.6 76.50</td>
</tr>
<tr>
<td>7a</td>
<td>3.64-3.56, m 3.67, t (4.0) 3.81, t (2.6)</td>
<td>7a 74.1 72.1 76.46</td>
</tr>
<tr>
<td>8</td>
<td>1.21, d (6.8) 1.24, d (7.1) 1.46, d (7.0)</td>
<td>59.1 62.9 56.5</td>
</tr>
<tr>
<td>9</td>
<td>9.5 10.7 9.2</td>
<td>9.5 10.7 9.2</td>
</tr>
</tbody>
</table>

Table 4B.7: Comparison of literature \(^{13}\)C NMR (100 MHz, D\(_2\)O) spectroscopic data of Reissig and Goti et al. Lit. 120\(^{131}\) with the \(^{13}\)C NMR (125 MHz, D\(_2\)O) data of our synthetic 217 and 217·HCl.

<table>
<thead>
<tr>
<th>position</th>
<th>(^1)H NMR ((\delta J) in Hz)</th>
<th>(^{13})C NMR ((\delta))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.99, t (8.6) 4.07-3.97, m 4.24, t (8.7)</td>
<td>76.7 78.0 75.6</td>
</tr>
<tr>
<td>2</td>
<td>3.80, dd (8.4, 9.6) 3.90, t (8.1) 3.99, dd (8.5, 10.2)</td>
<td>75.3 77.9 73.6</td>
</tr>
<tr>
<td>3</td>
<td>3.41, dt (3.6, 9.6) 3.33, dt (4.9, 9.0) 3.78-3.70, m</td>
<td>63.4 62.2 65.2</td>
</tr>
<tr>
<td>4</td>
<td>3.64-3.56, m 3.41, dt (5.7, 11.7) 4.06, qd (2.9, 7.0)</td>
<td>60.8 58.1 64.0</td>
</tr>
<tr>
<td>5</td>
<td>3.95, t (3.0) 4.07-3.97, m 4.15, d (2.9)</td>
<td>79.1 79.7 or 78.6 79.4</td>
</tr>
<tr>
<td>6</td>
<td>4.15, t (2.4) 4.18, t (4.0) 4.40, s</td>
<td>76.8 79.7 or 78.6 76.50</td>
</tr>
<tr>
<td>7</td>
<td>3.28, dd (1.0, 8.4) 3.07, dd (3.7, 8.1) 3.78-3.70, m</td>
<td>76.8 79.7 or 78.6 76.50</td>
</tr>
<tr>
<td>7a</td>
<td>3.64-3.56, m 3.67, t (4.0) 3.81, t (2.6)</td>
<td>7a 74.1 72.1 76.46</td>
</tr>
<tr>
<td>8</td>
<td>1.21, d (6.8) 1.24, d (7.1) 1.46, d (7.0)</td>
<td>59.1 62.9 56.5</td>
</tr>
<tr>
<td>9</td>
<td>9.5 10.7 9.2</td>
<td>9.5 10.7 9.2</td>
</tr>
</tbody>
</table>

Since there is disagreement in the NMR spectroscopic data between compound 217 and its literature reported compound 120\(^{131}\) by Reissig and Goti et al. the structural elucidation of compound 217 is detailed below. All NOE enhancements for 217 can be found in the Appendix for this work but for brevity, only key correlations for compound 217 are shown in Figure 4B.15. These correlations are observed between H7–H9, H7–H1, H5–H6, and H5–H7a; this suggested we had assigned the correct stereochemistry for the A-ring of 217.
**Figure 4B.20:** Top: Selected 1D-NOE spectra (500 MHz, CDCl₃) of 217 of A) H7 and B) H5 and their respective key observed correlations projected onto Bottom: a DFT minimized molecular model [B3LYP/6-31G(d)] of 217.

4B.7. Peracetylation of 217

To further support the absolute configuration assigned to this structure, synthetic 217 was peracetylated to the pentaacetate 218. This procedure was based on precedent literature from previous members of the Pyne research group and involved dissolving 217 in a solution of pyridine and adding acetic anhydride along with 4-dimethylaminopyridine (Scheme 4B.13). The reaction returned a near quantitative yield of the desired pentaacetate product, as indicated by the LRESIMS [M+H]+ ion peak at m/z 430. After column chromatography purification, 218 was subject to extensive NOE NMR studies which
displayed significant correlations between H1–H7, H2–H7a, H5–H6, H2–H8, H6–H7a, and Me–H3 (See Appendix). For brevity, only the 1D NOE enhancements for H6 and H7 have been included in this Chapter (Figure 4B.16). These correlations are consistent with our proposed structures for 218 and indirectly support the absolute configuration of 217.

**Scheme 4B.28:** Synthesis of peracetylate 218.
Figure 4B.21: Top: Selected 1D-NOE spectra (500 MHz, CDCl₃) of 218 of A) H6 and B) H7 and their respective key observed correlations projected onto Bottom: a DFT minimized molecular model [B3LYP/6-31G(d)] of 218.

Throughout the course of this work, the relative configurations assigned for each intermediate as well as each final product have been consistent with our NOE/ROESY NMR analyses. Moreover, the pyrrolizidines obtained in this work have been anticipated as their synthesis has been based on precedent work within the Pyne research group as well as the established mechanism of the O-mesylation then cyclization reaction. This specifically involves an initial O-mesylation of the less-hindered hydroxy of the aminodiols 152, 153 and 166 (described in Chapter 2 and 3), followed by an SN₂-type ring closure to form the desired pyrrolizidine products 165, 149 and 151. Although there is the potential for double inversion at the participating carbinol carbon, this only occurs if the secondary mesylate is displaced by a chloride ion prior to ring-closure. We are confident this did not happen particularly since the synthesis of pyrrolizidine 149 described in Chapter 2 and the
C6, C7 anti-diol 211 described in this Chapter fortuitously crystalized and unequivocally confirmed the stereochemistry and pyrrolizidine structure.

Interestingly, it is noted that Reissig and Goti et al. successfully synthesised the proposed structure of hyacinthacine C₅ but did not employ the same procedure to obtain its C₅ epimer 120. Instead they cyclized the bis-mesylated amino product 117A described earlier in Chapter 1 and so there is the possibility that cyclization could afford the surprisingly stable 4-membered bicyclic N-heterocycle 219 (Scheme 4B.14). This issue has not yet been resolved and so highlights the difficulty often associated with assigning the structures of the hyacinthacine C-type analogues, synthetic or naturally derived. Further investigation is certainly required.

Scheme 4B.29: Proposed synthesis of the alternative 4-membered bicyclic N-heterocycle 220 that Reissig and Goti et al. may have unintentionally synthesized.

Regardless, our synthetic purported compound for hyacinthacine C₅, (+)-5-epi-hyacinthacine C₅ 215 and (+)-6,7-di-epi-hyacinthacine C₅ 217 were cross examined with the hyacinthacine C-type natural products labelled hyacinthacines C₁ 105 or C₄ 117 but no NMR spectroscopic match was apparent with any of the natural isolates. All final products
synthesized in this Chapter were subsequently assessed for their glycosidase inhibitory potential and these are reported in Chapter 6.

4B.8. Chapter 4-Part B: Final remarks

Chapter 4B has disclosed the successful synthetic approach that resulted in four of the eleven hyacinthacine C-type analogues synthesized in this Ph.D. work. A key finding was the synthesis of compound 216 and that it spectroscopically matched the NMR spectra obtained for natural isolate labelled hyacinthacine C5. In addition to this, the synthesis of purported compound for hyacinthacine C5 and 215 generated spectroscopic data that matched with those of the same compounds reported in literature, and so confirmed their structures. At this stage, we are confident that the structure for our synthetic 217 is correct since it, along with preceding intermediates have generated NMR spectroscopic evidence consistent with their assigned structures. Further work is required to identify the cause of the inconsistent spectroscopic data between 217 and compound 120 reported by Reissig and Goti et al., especially since these should be identical compounds. Regardless, the newfound discovery of the true structure for natural hyacinthacine C5 led to its publication in the ACS Journal of Organic Chemistry along with the six hyacinthacine C5-type analogues described between Chapters 1-4B. All intermediates and final products that led to the final hyacinthacine C5-type analogues have undergone extensive NMR spectroscopic analysis and the reader will find almost all the 1D and 2D spectroscopic experimental results as part of this manuscripts Supporting Information. Alternatively, a copy of all the spectroscopic evidence of the final products has been included in the Appendix of this thesis.
Chapter 5: Synthesis of (−)-7-epi-hyacinthacine C₁, the corrected structure of natural hyacinthacine C₁, (+)-6,7-di-epi-hyacinthacine C₁ and (+)-7-epi-hyacinthacine C₁

Chapters 2-4B detail both the successful and unsuccessful attempts that ultimately resulted in the synthesis of the natural (+)-hyacinthacine C₅ (as well as six additional hyacinthacine C-type compounds) which allowed correction of its initially proposed structure. These compounds were readily accessible from two epimeric anti-1,2-amino diols 127 and 161. Until this Chapter, the work described largely focused on the chemical manipulation of the B-ring (stereocentres C₅, C₆ and C₇) of the hyacinthacine C-type products through methods of syn-dihydroxylation, SN₂ ring-opening of a cyclic sulfate, and also employing either the (R)- or (S)-α-methylallyl amine for the Petasis borono Mannich reaction. The successful pathway that led to these results was summarised and accepted as a publication in the ACS Journal of Organic Chemistry. Although the work towards elucidating the true structure for hyacinthacine C₅ proved successful, none of the synthesized analogues solved the structural ambiguities between hyacinthacine C₁ and C₄. For this reason, our new objective was to synthesize the proposed compounds for these natural isolates. Herein, the report of the true structure and synthesis of the compound hyacinthacine C₁ is described. In addition to this, our synthetic pathway also accessed another three diastereomers of hyacinthacine C₁/C₄. All final products were assessed as glycosidase inhibitors and will be discussed in Chapter 6. These results were also reported in a manuscript which at the time of writing this Ph.D. thesis, was accepted for publication in the ACS Journal of Natural Products. A comprehensive NMR spectroscopic experimental data set is available in the related Supporting Information for this article.

5.1. Retrosynthetic analysis towards accessing hyacinthacine C₁/C₄-type analogues

The proposed structure for hyacinthacine C₁/C₄ is shown in Figure 5.1. After reviewing the successful pathway that led to the structural elucidation of hyacinthacine C₅, we
recognized that the revised structure of (+)-hyacinthacine $C_5$ compared to hyacinthacine $C_1$ and $C_4$, contained a single opposite stereocentre at C1.

![Corrected structure of natural hyacinthacine $C_5$ and Proposed structures for hyacinthacine $C_1/C_4$]

**Figure 5.1**: The corrected structure for hyacinthacine $C_5$ and the proposed structures for the natural isolates labelled hyacinthacine $C_1$ and $C_4$ with the single stereocentre C1 difference highlighted in red.

We envisioned that using our established chemistry would access this compound. More specifically, the retrosynthetic analysis detailing our initial synthetic approach towards accessing hyacinthacines $C_1/C_4$ is shown below (Scheme 5.1). For our synthetic approach to be successful, we recognized that the stereocentre at C1 would need to be subjected to inversion at some point during the synthesis. After revising our initial pathway described in Chapter 2, we identified that this stereocentre could be potentially inverted if the $N$-Boc diol 139 was converted to the respective cyclic-sulfate 225 then to the oxazolidinone 224.
This idea was influenced by precedent literature reported by Kim et al.\textsuperscript{193} Shown in Scheme 5.2, Kim and co-workers found that the carbonyl of the carbamate in the N-Boc protected 3,4-vicinal diol could induce a hydroxy inversion at either the 3 or the 4 position, provided the respective 5-membered cyclic sulfate \textsuperscript{226} was synthesized. They unequivocally proved that the nucleophilic carbonyl would induce an S\textsubscript{N}2 like attack at either position via the formation of a hemisulfateanion/oxonium intermediate A. An irreversible fragmentation step then leads to the oxazolidinone product \textsuperscript{227} as desired. The major difference between our N-Boc substrate \textsuperscript{139} and the substrates reported by Kim et al. was the cyclic sulfate ring size formed. More specifically, Kim et al. only demonstrated this principle to effectively work for 5-membered cyclic sulfates. However, since it is possible to synthesize various 6-membered cyclic sulfates,\textsuperscript{194} we considered the overarching principle would remain true, and that either the oxazolidinone \textsuperscript{224} or the corresponding oxazepinone would form via this mechanism. Provided oxazolidinone \textsuperscript{224} was successfully synthesized, using similar procedures previously described in Chapters 2 and 3 (RCM reaction, UpJohn cis-dihydroxylation, mesylation then cyclisation) would ensure that the desired final hyacinthacine C\textsubscript{11/4} type analogues are synthesised.
Scheme 5.2: Kim and co-workers N-Boc conversion to the oxazolidinone 227 from the cyclic sulfate 226.193

5.2. Synthesis of cyclic sulfate 225 and its conversion to oxazolidinone 224

Therefore, the N-Boc 139 was synthesized and converted to the corresponding cyclic sulfate 225 via our standard procedure (Et₃N, sulfuryl chloride, CH₂Cl₂). Indication that the cyclic sulfate 225 had formed was inferred by a LRESIMS [M+H]+ ion peak at m/z 650 and TLC analysis confirming full consumption of the starting material. Owing to our previous handling experiences with cyclic sulfate derivatives, the crude compound 225 was subsequently converted to oxazolidinone 224 using the same procedure described by Kim et al.193 This procedure involved dissolving the newly formed cyclic sulfate 225 in a solution of acetonitrile followed by stirring the mixture at 50 °C for 2 h. After this time, a solution of THF, H₂O and H₂SO₄ (8:3:1) was added to the mixture and was stirred at 40 °C for 1 h. After neutralization, a TLC analysis confirmed that a new product spot had formed with an Rf and a LRESIMS [M+H]+ ion peak at m/z 514 similar to our previous oxazolidinone 137 described in Chapter 2. The crude reaction mixture was then purified by column chromatography and returned the oxazolidinone 224 in 39% yield over two steps. In the synthesis of oxazolidinones 137 and 160 described in Chapters 2 and 3, the proton resonances at H4 and H5 were described as having a syn-relationship since they displayed a vicinal coupling constant, J₄,₅, of 7.1 Hz in the ¹H NMR spectrum. This is consistent with the 4,5-cis relative configuration. Unfortunately, we could not find clear resolution for the appropriate resonance for H4 and H5 as they overlapped with other resonances in the spectrum and so the J₄,₅ trans coupling constant could not be reliably determined. Although the success of the inversion was not definitively confirmed, the ¹H
NMR spectrum (Figure 5.2) of the resultant oxazolidinone 224 was clearly different to the $^1$H NMR spectrum of oxazolidinone 137 described in Chapter 2. Synthesis of the respective oxazepinone was disregarded on the basis that the $^1$H NMR spectrum olefinic resonances for H2″′, H1″′, H2′ and H3′ at $\delta_H$ 6.47, 6.02, 5.84 and 5.15 ppm, respectively, were deshielded in a pattern consistent with formation of the oxazolidinone, rather than the oxazepinone (Figure 5.2).

**Scheme 5.3:** Synthesis of N-Boc cyclic sulfate 225 and its thermal fragmentation to oxazolidinone 224.
Figure 5.2: A) $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 137 and B) $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 224.

Unfortunately, this reaction only proved to be a one-time-success. It was speculated that the sulfuryl chloride or the THF solvent stock used as in this reaction may have degraded over time since it is very reactive. Despite careful preparation and ensuring that all reagent and solvent stock containers were always stored under an inert atmosphere, the constant use and exposure throughout the course of this work may have lessened their integrity via unavoidable hydrolysis and or oxidation. This reaction was attempted many times but failed to recreate oxazolidinone 224. Successive attempts only resulted in complex mixtures. With the small amount of the oxazolidinone 224 in hand, the pathway was continued in an attempt to explore the feasibility of this methodology. Although seemingly high yielding reactions followed from the synthesis of the oxazolidinone 224, resultant intermediates were eventually exhausted due to gradual loss of yield and so could not be reliably characterised due to insufficient amounts. It was optimistically thought that we would obtain the final product, but this proved unsuccessful. Therefore, the following work details the results we were able to obtain before this synthetic pathway had to be ultimately reconsidered.
5.3. RCM reaction of oxazolidinone 224

Oxazolidinone 224 was next subjected to our procedure for ring-closing metathesis. This afforded the RCM product 223 in a high yield of 95% (Scheme 5.4). The HRESIMS of 223 displayed the [M+Na]+ ion peak at m/z 432.1790 and so suggested the successful synthesis of 223. In addition to this, the corresponding pyrrole was disregarded on the basis of the 1H NMR spectrum displaying a clear doublet for the methyl resonance identified at δH 1.55 ppm for H1’’ (Figure 5.3). More importantly, the 1H NMR spectrum resonances for H7a and H1 clearly resolved at δH 4.98 and 4.37 ppm, respectively. The J1,7a coupling constant between both these resonances was calculated to be 10.8 Hz and is a higher value than the corresponding J4,5 cis coupling constant for RCM product 140 (Chapter 2). This correlates well with the trans relationship between H1 and H7a in compound 223. In addition to this, the 1H NMR spectrum generated for 223 was different to the RCM product 140 discussed in Chapter 2 and so suggested 223 to be the diastereomer depicted in Scheme 5.4.

Scheme 5.4: Synthesis of RCM product 223.

Figure 5.3: 1H NMR spectrum (500 MHz, CDCl3) of 223.
5.4. Syn-dihydroxylation of RCM product 223

At this stage, only a small amount (15 mg) of the RCM intermediate 223 was available to undergo Upjohn Os(VIII)-catalyzed cis-dihydroxylation. These conditions had been well explored and so employing the same procedure to RCM 223 that was previously described in Chapters 2, 3 and 4, resulted in high conversion to the C6, C7 syn-diol 228 which was obtained in a 72% yield after column chromatography (Scheme 5.5). Due to the small scale of this reaction clear evidence to suggest formation of the minor (6S), (7R)- syn-diol was unable to be determined. If the reaction was completed on a larger scale both diol 228 and its minor diastereomer would have been isolated and characterised. Since it could not be recovered, synthesis of the (6S), (7R)- syn-diol could not be confirmed and for this reason has not been included. Regardless, diol 228 was characterised and was confirmed to be the (6R), (7S)- syn-diol from an extensive analysis of the ROESY NMR for this product. In particular, key proton resonance correlations were observed between H1''–H6, H1''–H7 (weak) and H6–H7 and are shown in Figure 5.4.

Scheme 5.5: UpJohn cis-dihydroxylation of 223 to give diol 228.
Figure 5.4: 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 228.

5.5. The synthesis of acetonide 221 and shortfalls

After column chromatography purification, only 12 mg of diol 228 was obtained. This was converted to its corresponding acetonide 221 by dissolving the syn-diol 228 in acetone, followed by the addition of two equivalents of copper(II) sulfate and a catalytic amount of sulfuric acid (Scheme 5.6).
Scheme 5.6: Synthesis of acetonide 221.

As anticipated, the reaction returned a clean conversion (95% yield after column chromatography) to the respective acetonide 221 as confirmed by HRESIMS, $^1$H and $^{13}$C NMR spectroscopic analysis. More specifically, the HRESIMS of 221 found a [M+H]$^+$ ion peak at $m/z$ 484.5720. The $^{13}$C NMR spectrum best displays the formation of the acetonide 221 and so is shown in Figure 5.5 along with the $^{13}$C NMR spectrum of the parent diol 228. In this comparison, three additional carbon resonances at $\delta$C 112.2, 26.1 and 24.4 ppm for the newly formed acetonide 221 are highlighted and coincide with C2 and both the C2 contingent methyl carbons, respectively.
Figure 5.5: A) $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 228 and B) $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 221 with C2 and C2 contingent methyl resonances for 221 highlighted in red.

Unfortunately, it was at this point that this synthetic pathway was discontinued. The inability to synthesize more of the starting oxazolidinone 224 in combination with the small amount of product at this stage was considered impractical for future reactions and product characterisation.

5.6. Revised synthetic strategy towards hyacinthacine C$_1$/C$_4$-type analogues

These shortcomings led to the revision of our retrosynthetic plan towards synthesizing hyacinthacine C$_1$/C$_4$ type analogues and is shown in Scheme 5.7. More specifically, the pyrrolizidine 230 would be obtained from performing a Swern oxidation at C8 of compounds 149, 151 or 165 then subjecting the representative ketone 229 to diastereoselective reduction by L-selectride®. This would stereoselectively deliver a hydride to the less hindered face of the bicyclic compound and result in an overall inversion at this stereocentre. After this, either global deprotection or similar methodology used to generate the C6, C7 anti-diol (via the corresponding cyclic sulfate), would access a large number of different hyacinthacine C-type diastereomers of which one of these would be
the proposed structure of the natural isolates labelled hyacinthacine C₁/C₄. This approach was certainly considered at the beginning of this work. However, a previous group member, Kongdech Savaspun, found that performing the Swern oxidation, then L-selectride® reduction on similar substrates returned unexpected product mixtures containing very low yields of the desired product as well as various lactam-carboxylic derivatives.¹²⁷ On the other hand, other previous group members have demonstrated reasonable success using this approach on similar substrates.¹⁸⁴, ¹⁹¹ The decision was therefore made to implement this pathway for pyrrolizidine compounds ¹⁴⁹, ¹⁵¹ and ¹⁶⁵. If successful, our established C6, C7 anti-diol chemistry would also be explored in an attempt to synthesize the compound of the putative structure assigned for the natural isolates labelled as hyacinthacine C₁/C₄.

Scheme 5.7: Revised retrosynthetic analysis towards accessing hyacinthacine C₁/C₄-analougues.
5.7. Swern oxidation of pyrrolizidines 149, 151 and 165

5.7.1 Swern oxidation of pyrrolizidine 165

The Swern oxidation was considered for all the synthesized pyrrolizidine substrates 149, 151 and 165 described in Chapters 2, 3 and 4B. Since pyrrolizidine 165 (described in Chapter 3) was on hand at the time this pathway was considered, it was used as the model study. More specifically, pyrrolizidine 165, along with an excess amount of Et₃N (40 eq.), was added to the reaction mixture between oxalyl chloride and dimethyl sulfoxide. The mixture was then stirred at -78 °C for 1 h (Scheme 5.8). After this time, the appearance of a single product spot was observed by TLC analysis. After column chromatography ketone 231 was successfully obtained in an 83% yield.

Scheme 5.8: Swern oxidation of 165 to give ketone 231.

It might seem an unorthodox observation, but it was noted that the reaction mixture contained a foul odour. Interestingly, this was a key indication that the desired ketone 231 had formed. According to the mechanism shown in Scheme 5.9, a by-product of a successful oxidation reaction is the synthesis of dimethyl sulfide. In Scheme 5.9A, an initial reaction between DMSO and oxalyl chloride forms the dimethylchlorosulfonium ion B. Next (Scheme 5.9B), the addition of an alcohol (in this example, substrate 165 is used) at -78 °C leads to the formation of an alkoxy sulfonium ion C via the loss of the chloride and alcohol proton as hydrogen chloride. In the presence of base (Et₃N), deprotonation of this intermediate returns the sulfur ylide D, which undergoes an intramolecular deprotonation via a 5-membered transition state and ultimately fragments into the respective ketone 231 and the odorous by-product, dimethyl sulfide. If the reaction
is not kept near -78 °C, mixed thioacetals can result and prove detrimental to the product yield.

**Scheme 5.9:** Proposed mechanism for the Swern oxidation of 165.

A)

B)

To confidently confirm the desired ketone 231 had formed, the crude reaction was purified via column chromatography and the product was then subjected to extensive spectroscopic experiments. More specifically, the HRESIMS of 231 found a [M+H]+ ion peak at m/z 438.2275 and indicated the loss of the mass of two protons. In addition to this, the gCOSY NMR spectrum of 231 identified that the pyrrolizidine ring only contained protons at H8b, H3a, H7, H8a, H6 and H4, at δH 5.04, 4.56, 3.94, 3.87 (2H), and 3.74 ppm, respectively (Figure 5.6A). Additionally, the 13C NMR spectrum displayed a downfield resonance at δC 211.0 ppm, which was characteristic of the newly formed carbonyl at C8 (Figure 5.6B). The sharp C=O stretching band at 1716 cm⁻¹ in the IR spectrum also supported this newly formed carbonyl moiety.
Having proved the successful oxidation at C8 of pyrrolizidine 165, the same Swern oxidation procedure was performed on pyrrolizidine 151 and also returned a high yield (80%) of the respective ketone product 232 (Scheme 5.10). This was confirmed after the product was subjected to extensive spectroscopic experiments. In addition to the HRESIMS of 232 indicating a characteristic [M+H]^+ ion peak at m/z 438.2300, ketone 232 was confirmed to be successfully formed on the basis of the gCOSY NMR spectroscopic analysis which identified that the pyrrolizidine ring only contained protons at H8b, H3a, H7, H6, H8a, and H4 at δ_H 4.89, 4.54, 4.16, 3.96, 3.69 and 3.74 ppm, respectively (Figure 5.7A). Additionally, the ^13C NMR spectrum of 232 displayed a downfield resonance at δ_C 211.8 ppm, which was characteristic of the newly formed carbonyl at C8 (Figure 5.7B). The sharp C=O band at 1743 cm\(^{-1}\) in the IR spectrum also supported this newly formed carbonyl moiety.
**Scheme 5.10:** Swern oxidation of 151 to give ketone 232.

\[
\begin{align*}
151 & \xrightarrow{\text{Oxalyl chloride,}} \text{DMSO, Et}_3\text{N.} \xrightarrow{\text{CH}_2\text{Cl}_2} 232 \\
\text{Oxyl chloride,} & \text{DMSO, Et}_3\text{N.} \text{CH}_2\text{Cl}_2 & -78 \degree\text{C, 1 h}} \end{align*}
\]

**Figure 5.7:** A) \(^1\text{H NMR spectrum (500 MHz, CDCl}_3\) of 232 and B) \(^{13}\text{C NMR spectrum (125 MHz, CDCl}_3\) of 232.

**5.7.3 Swern oxidation of pyrrolizidine 149**

In a similar manner, the pyrrolizidine 149 was subjected to our established Swern oxidation conditions (Scheme 5.11). Unlike the aforementioned products 231 and 232, the desired ketone 233 proved difficult to purify and seemed prone to degradation on silica medium. The synthesis of 233 was only inferred by an LRESIMS [M+H]^+ ion peak at \(m/z\) 438 which was determined after a sample of the crude reaction mixture was analysed. For this reason, the crude ketone 233 was unable to be completely characterised but was instead subjected to the next step in the reaction pathway.
Scheme 5.11: Swern oxidation of 149 to give ketone 233.

5.8. L-selectride® reduction of ketones 231, 232 and 233

With the successfully synthesized ketones 231, 232 and 233 in hand, the next stage of the synthetic plan was to reduce the ketone moiety at C8 with the sterically hindered reagent L-selectride® in hope that the corresponding C8 alcohols would form. This was based on the mechanism and results described by Zhang et al. in 2009 for their L-selectride® reduction of ketone 234 shown in Scheme 5.12. In this reaction, Zhang and co-workers obtained almost exclusively the endo epimer. They attributed this result to the fact that the bulky hydride would attack the carbonyl predominantly at the less hindered convex face of the ketone 234.

Scheme 5.12: Zhang and co-workers proposed mechanism of L-selectride® reduction of ketone 234.

5.8.1 L-selectride® reduction of ketone 231

We were confident that successful inversion at C8 would occur for our respective ketones, especially since the O-benzyl ether at C7 was on the concave face and would also sterically influence the delivery of the hydride in a similar manner described by Zhang et al. Therefore, with ketone 231 in hand, this was subjected to a reduction with L-selectride® at
-78 °C (Scheme 5.13). After stirring at this temperature for 2 h, the TLC analysis showed a near complete consumption of the starting material and so the reaction was quenched with an aqueous ammonia solution. After column chromatography purification, the product was obtained in a 96% yield. The product was then subjected to various spectral analyses including ¹H and ¹³C NMR spectroscopic experiments as well as other 2D NMR experiments in order to confirm the successful stereoinversion at C8. An initial confirmation that this reduction had been successful was determined by a HRESIMS analysis of the purified sample which found a characteristic [M+H]^+ ion peak for 236 at m/z 440.2446.

Unfortunately, due to the overlap of the H8 resonance with the OCH₂Ph proton resonances in the ¹H NMR spectrum, the ROESY NMR of the newly formed alcohol 236 could not be interpreted to unambiguously confirm that the hydroxy moiety at C8 had inverted. However, the ¹H NMR spectrum of the suspected alcohol 236 (Figure 5.8A) was notably different to the ¹H NMR spectrum of its parent diol 165 (Figure 5.8B), suggesting that ketone 231 had undergone a successful stereoinversion at C8. However, to truly confirm this had been the case, a portion of the alcohol 236 was converted to the C8 O-benzyl ether in hope that it would clearly resolve these proton resonances. Findings from this will be discussed further in section 5.11.

Scheme 5.13: Reduction of 231 with L-selectride® to give alcohol 236.
5.8.2 L-selectride® reduction of ketone 232

Pyrrolizidine ketones 232 and 233 were also individually subjected to reduction with L-selectride®. Both reactions displayed a high degree of selectivity for returning a single diastereomer. More specifically, reduction of ketone 232 returned exclusively the pyrrolizidine alcohol 237 in 66% yield (Scheme 5.14). In addition to the prominent ¹H NMR spectroscopic differences between 237 and the parent C8 epimer 151, an extensive 2D-NOESY analysis was performed to confirm the inversion at C8 in 237. Key positive correlations were observed between H4–H3a, H4–H8a, H1''–H6, H8a–H8b, H7–H8, H8a–H8 and H7–H1' (Figure 5.9), confirming formation of the intended product.

Scheme 5.14: Reduction of 232 with L-selectride® to give alcohol 237.
**Figure 5.9:** 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 237 (C7 and C1’ O-benzyl ethers not shown).

5.8.3 *L*-selectride® reduction of ketone 233

The crude ketone product 233 was also subjected to reduction by *L*-selectride® which successfully afforded the desired alcohol 238 in a 65% yield over two steps (Scheme 5.15). This was confirmed by a comparative analysis of the ¹H NMR spectra between 238 and the parent alcohol 149 suggesting a successful inversion at the stereocentre C8. More convincingly, alcohol 238 was subjected to extensive 1D-NOE difference experiments which confirmed the relative configuration of the newly inverted stereocentre at C8. For brevity, only key positive NOE enhancements for H8 and H8b are shown. More specifically, correlations between H8–H7, H8–H8a and H8b–H3a were observed (Figure 5.10). It is worth noting the relatively weak correlation between H8b–H8a might seem unusual since these protons are depicted on opposite faces of the pyrrolizidine core. However, due to the torsional strain and twist of the pyrrolizidine core, unexpected correlations such as this can be observed, but are usually of weaker intensities (note the stronger intensity of H8b–H3a since they are expected to be on the same side of the pyrrolizidine core).
**Scheme 5.15:** Reduction of 233 with L-selectride® to give alcohol 238.

\[
\begin{align*}
\text{O} & \text{O} \\
\text{H} & \text{O} \\
\text{N} & \text{O} \\
\text{Bn} & \text{Bn} \\
\text{233} & \xrightarrow{\text{L-selectride\textsuperscript{®}, THF, -78 °C}, 1 h; rt, 2 h} \\
\text{O} & \text{O} \\
\text{H} & \text{OH} \\
\text{N} & \text{Bn} \\
\text{238} & \text{65\% (over two steps)}
\end{align*}
\]

**Figure 5.10:** Top: Selected 1D-NOE spectra (500 MHz, CDCl\textsubscript{3}) of 238 of A) H8 and B) H8b and their respective key observed correlations projected onto Bottom: a DFT minimized molecular model \([\text{B3LYP}/6-31G(d)]\) of 238 (C7 and C1' \(O\)-benzyl ethers not shown).
5.9. Synthesis of triols 239, 240 and 241

The relative configurations of alcohols 237 and 238 were confidently assigned. The relative configuration of alcohol 236 was also confirmed through synthesis and characterisation of its respective O-benzyl ether which is discussed later in 5.11. Therefore, each alcohol was subjected to global deprotection. In Chapter 3, a related step-wise deprotection was performed and involved first deprotecting the acetonide moiety of each pyrrolizidine. This established procedure was easily transferable to pyrrolizidines 236, 237 and 238 whereby they were each individually dissolved in EtOH/H₂O (9:1, respectively), followed by the addition of several drops of 5 N HCl (Scheme 5.16). This successfully returned triols 239, 240 and 241 in 79%, 87% and 55% yield, respectively.

Scheme 5.16: Acid hydrolysis of the acetonide moiety from pyrrolizidines 236, 237 and 238 to give the triols 239, 240 and 241, respectively.
5.10. Hydrogenolysis of pyrrolizidines 239, 240 and 241

Individual samples of alcohols 239, 240 and 241 were then debenzylated under hydrogenolysis conditions over PdCl$_2$ to return novel hyacinthacine C$_1$/C$_4$-type compounds (−)-5-epi-hyacinthacine C$_1$ 242 [[$\alpha$]$^D_{25}$−14.1 (c 0.90, H$_2$O)], the corrected structure of (+)-hyacinthacine C$_1$ 243 [[$\alpha$]$^D_{25}$+13.0 (c 0.20, H$_2$O)], and (+)-6,7-di-epi-hyacinthacine C$_1$ 244 [[$\alpha$]$^D_{25}$+13.4 (c 0.20, H$_2$O)], respectively in moderate to high yields of 80%, 52% and 61%, respectively following purification and neutralization by basic ion-exchange chromatography (Scheme 5.17).

Scheme 5.17: Hydrogenolysis of 239, 240 and 241 to give final hyacinthacine C$_1$/C$_4$-type analogues 242, 243 and 244, respectively.

![Scheme 5.17: Hydrogenolysis of 239, 240 and 241 to give final hyacinthacine C$_1$/C$_4$-type analogues 242, 243 and 244, respectively.](image)

Each resultant product was then subjected to extensive spectroscopic analysis. To our delight, a comparative NMR analysis of our synthetic 243 against the spectroscopic data...
reported for the original isolates labelled hyacinthacine C₁ [\([\alpha]_D +14.7 \text{ (c 0.28, H}_2\text{O)}\)]^{105} and hyacinthacine C₄ [\([\alpha]_D -37.9 \text{ (c 0.44, H}_2\text{O)}\)]^{117} revealed that the \(^1\text{H NMR spectrum multiplicities and chemical shifts of the natural isolate labelled hyacinthacine C₁ were a close match with our synthetic compound 243 (Table 5.1). Additionally, a consistent 0.4 – 0.6 ppm difference for the \(^{13}\text{C NMR chemical shifts between both products (Table 5.1) was observed. The NMR sample of 243 contained a small amount of MeOH, since it would be easily removed prior to glycosidase testing. The }^1\text{H NMR spectrum of 243 was therefore referenced to the D}_2\text{O solvent residual peak at 4.79 ppm and the }^{13}\text{C NMR was referenced to internal MeOH. Comparatively, the }^1\text{H and }^{13}\text{C NMR spectrum chemical shifts of the natural (+)-hyacinthacine C₁ are expressed in reference to the internal standard sodium 3-(trimethylsilyl)propionate (TPS) in D}_2\text{O.}^{105} \text{ This may explain the slight NMR spectroscopic differences observed between synthetic 243 and the natural product. Regardless, the specific rotation of synthetic 243 was found to be a close match with this natural product. In conjunction with the fact that the natural isolates hyacinthacine C₄ and C₁ cannot be the same structure, it was satisfactorily concluded that the true structure for hyacinthacine C₁ is in fact, the C₆ epimer 243. We were excited to solve this nearly 20-year old literature mistake and were confidently able to propose that hyacinthacine C₄ is most likely correct in its absolute configurational assignments.

Table 5.1: Comparison of literature \(^1\text{H NMR (500 MHz, D}_2\text{O) and }^{13}\text{C NMR (125 MHz, D}_2\text{O) data of Kato et al.}^{105} \text{ Lit. (+)-hyacinthacine C₁ with the }^1\text{H NMR (500 MHz, D}_2\text{O) and }^{13}\text{C NMR (125 MHz, D}_2\text{O) of our synthetic 243.}

<table>
<thead>
<tr>
<th>(\text{position})</th>
<th>(^1\text{H NMR (} \delta \text{, } J \text{ in Hz)})</th>
<th>(^{13}\text{C NMR (} \delta \text{)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-hyacinthacine C₁ (Kato et al.) (^{105})</td>
<td>243 (+)-hyacinthacine C₁ (Kato et al.) (^{105})</td>
<td>243</td>
</tr>
<tr>
<td>1</td>
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<td>4.18, t (3.8)</td>
</tr>
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<td>4.02, dd (3.7, 9.2)</td>
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<td>3</td>
<td>3.44, dt (4.4, 9.0)</td>
<td>3.47, dt (4.6, 9.2)</td>
</tr>
<tr>
<td>5</td>
<td>3.29, dt (3.9, 7.0)</td>
<td>3.31, dt (3.9, 7.1)</td>
</tr>
<tr>
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<td>3.89, dd (3.9, 4.6)</td>
<td>3.92, t (4.4)</td>
</tr>
<tr>
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<td>4.50, dd (4.6, 8.6)</td>
<td>4.53, dd (4.7, 8.6)</td>
</tr>
<tr>
<td>7a</td>
<td>3.66, dd (3.9, 8.6)</td>
<td>3.68, dd (4.2, 11.1)</td>
</tr>
<tr>
<td>8</td>
<td>3.66, m</td>
<td>3.68, dd (4.2, 11.1)</td>
</tr>
<tr>
<td>9</td>
<td>1.27, d (7.0)</td>
<td>1.29, d (7.2)</td>
</tr>
</tbody>
</table>
The remaining two hyacinthacine C-type analogues (−)-5-epi-hyacinthacine C₁ 242 and (+)-6,7-di-epi-hyacinthacine C₁ 244 were also cross examined with the spectroscopic data reported for the original isolate hyacinthacine C₄, however they did not match the respective chemical shifts. A literature search also concluded these to be novel hyacinthacine C-type analogues as they had not yet been reported to date. The \(^1\)H and \(^{13}\)C NMR spectra along with 2D NMR spectroscopic data for 242, 243 and 244 are included in the Appendix of this Ph.D. work.

5.11. Synthesis of compounds 245 and 246

It was fortunate that the corrected compound for hyacinthacine C₁ was successfully synthesized during this work. With this correction, it is suspected that the putative structure for hyacinthacine C₄ is most likely correct in its assignment. However, the only way to confidently prove this is to synthesize this compound and then compare the obtained spectral data of the synthetic product with the natural isolate. When reviewing the putative structure for hyacinthacine C₄, it was envisioned that we could potentially access this compound if we combined the recently established stereoinversion at C8 with the C6, C7 cyclic sulfate ring-opening procedure described in Chapter 4A. The compounds chosen to undergo this transformation were pyrrolizidines 236 and 238 as they were available in the final months prior to finishing this Ph.D. work. Therefore, both pyrrolizidines were subjected to C8 \(O\)-benzyl ether protection in a similar manner to that described in Chapter 4B, which returned the desired pyrrolizidine analogues 245 and 246 in 54% and 52% yield, respectively (Scheme 5.18). Indication that both pyrrolizidines had undergone a successful \(O\)-benzylation at C8 was inferred as both were found to contain a LRESIMS [M+H]\(^+\) ion peak at \(m/z\) 530.
Scheme 5.18: C8 O-Benzylolation of pyrrolizidines 236 and 238 to give 245 and 246.

It was at this point that the stereochemistry at C8 of pyrrolizidine 245 was confirmed through extensive spectroscopic analysis. More specifically, after purification, a clearly resolved $^1$H NMR spectrum of the product 245 was obtained and subsequently subjected to extensive 1D-NOE difference analysis. For brevity, only the NOE enhancement of H8 is shown in Figure 5.11 and clearly displays the expected positive NOE correlation between H8–H7 and H8–H8a.
Figure 5.11: Top: A selected 1D-NOE spectra (500 MHz, CDCl₃) of 245 of H₈ and its respective key observed correlations projected onto Bottom: a DFT minimized molecular model [B3LYP/6-31G(d)] of 245 (C₈, C₇ and C₁’ O-benzyl ethers not shown).

Indeed, similar evidence and logic was used to check the stereochemistry of pyrrolizidine 246 at the hydroxy group of C₈. Since the parent compound 238 has already been described from a stereochemical point of view, detail for this compound will be discussed after the successful inversion at C₆ or C₇ has been described in Section 5.13.

5.12. Acetonide hydrolysis of 245 and 246

With the pyrrolizidines 245 and 246 in hand, each underwent individual hydrolysis of the acetonide moiety. This was performed in a similar manner to the acetonide hydrolysis of pyrrolizidine 165 described in Chapter 3 which entailed individually dissolving 245 and 246 in a solution of EtOH/H₂O (9:1, respectively), followed by the addition of several drops of 5 N HCl and stirred at room temperature until full consumption of the starting material was confirmed by TLC analysis (Scheme 5.19). The HRESIMS of 247 and 248 displayed the [M+H]+ ion peaks at m/z 490.2615 and 490.2612, respectively, indicating
that both had undergone successful hydrolysis of the acetonide moiety. Additionally, the $^{13}$C NMR spectrum of compounds 247 and 248 clearly indicated the loss of this group when compared with their parent acetonides 245 and 246, respectively (Figure 5.12 and Figure 5.13).

**Scheme 5.19**: Acid hydrolysis of the acetonide moiety from pyrrolizidines 245 and 246.
**Figure 5.12:** A) $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 245 and B) $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 247 with C2 and C2 contingent methyl resonances for 245 highlighted in red.

**Figure 5.13:** A) $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 246 and B) $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 248 with C2 and C2 contingent methyl resonances for 246 highlighted in red.
5.13. Synthesis of Cyclic sulfates 249 and 250 and attempts to ring opening with in situ formed CsOBz

Both diols 247 and 248 were individually converted to their respective cyclic sulfates 249 and 250 (Scheme 5.20), using our previously established methodology described in Chapter 4B.190 Prone to degradation, both cyclic sulfates were immediately treated with Cs₂CO₃ and benzoic acid and were expected to return two potential regioisomers for each of the cyclic sulfates 249 and 250. Unfortunately, the subsequent CsOBz ring-opening of cyclic sulfate 249 degraded the compound into an indeterminable mixture of products (Scheme 5.20). Due to time contraints, this reaction could only be performed as a one-time reaction trial and so the reaction conditions were not optimized nor re-attempted. In contrast, the CsOBz ring-opening of the cyclic sulfate 250 returned in 42% yield, the benzoate regioisomer 251 as confirmed initially by a gCOSY NMR analysis (Scheme 5.20). More specifically it was observed that the proton sharing the stereogenic carbon with the newly added benzoate proved the most deshielded and characteristically appeared at δ_H > 5.0 ppm. In this case, the gCOSY analysis of 251 identified a strong correlation between H1″ (δ_H 1.25 ppm) and H5 (δ_H 3.93 ppm) which was subsequently found to resonate with the most deshielded proton at δ_H 5.30 ppm, which was deduced to be H6 (Figure 5.14).

Scheme 5.20: Synthesis of cyclic sulfates 249 and 250 and the attempted ring opening of cyclic sulfates 249 and 250 with the successful synthesis of benzoate 251.
The benzoate product 251 was then subjected to a comprehensive 2D-NOESY analysis which showed key correlations between protons H1''–H7, H1''–H3, H5–H6, H1–H7a, H1–H2, H7a–H2 and H1'–H2, suggesting the proposed relative configuration of structure 251 to be correct (Figure 5.15). Interestingly, this result was consistent with the regioselectivity observed in the earlier proposed 1,2-diaxial-like ring-opening of the cyclic sulfate of 203 as described in Chapter 4B (Scheme 4B.10). More specifically, for cyclic sulfate 250, attack at C8b requires a different reactive conformation in which the C4 methyl group is pseudo-axial and sterically blocks substitution at C8b. A referee of our publication in the ACS Journal of Natural Products suggested that the preference for nucleophilic substitution of the CsOBz at C3a could be the result of hyperconjugative stabilisation from the C4 methyl group (anti to the leaving group). Both these steric and stereoelectronic effects could be clearly valid, however determining their relative contributions will require further study. It is suspected that the C8b benzoate regioisomer was also formed but could not be obtained due to the insufficient amount of starting material in combination with the generally low yield for this reaction.
Figure 5.14: gCOSY NMR spectrum (500 MHz, CDCl₃) of 251 and a DFT minimized molecular model [B3LYP/6-31G(d)] of 251 (C8, C7 and C1’ O-benzyl ethers not shown).

Figure 5.15: 2D ROESY NMR spectrum (500 MHz, CDCl₃) with key observed correlations projected onto a DFT minimized molecular model [B3LYP/6-31G(d)] of 251 (C8, C7 and C1’ O-benzyl ethers not shown).
5.14. Methanolysis of the benzoate 251

After the Ph.D. stipend had exhausted, attempts to synthesize larger quantities of cyclic sulfates 249 and 250 were never revisited. This work therefore ended with the successful two-step global deprotection of benzoate regioisomer 251. More specifically, the C6 benzoate of pyrrolizidine 251 was subjected to our previously established methanolysis procedure to give the diol product 252 in an 87% yield (Scheme 5.21). The HRESIMS of 252 displayed the [M+H]+ ion peak at \( m/z \) 490.2597, thus confirming the successful de-benzoylation.

Scheme 5.21: Methanolysis of 251 to give diol 252

The \(^1\)H NMR spectrum of the parent C6, C7 syn-diol 248 was then compared with the \(^1\)H NMR spectrum of the newly revealed C6, C7 anti-diol 252, where significant differences between these spectra were observed. From these results, we concluded that they were not the same product and that 252 was the C6, C7 anti-diol. Unfortunately, the relative closeness of the chemical shifts of each proton resonance in the \(^1\)H NMR spectrum of 252 led to difficulties in definitively establishing the C6, C7 anti-diol relationship. However, we were confident that the proposed compound had been synthesized based on the previous spectroscopic investigation of benzoate 251. For this reason, diol 252 was taken through to the next step and subjected to hydrogenolysis.

5.15. Synthesis of (+)-7-epi-hyacinthacine C1 253

The final reaction reported in this Ph.D. work is the debenzylation of alcohol 252 under hydrogenolysis conditions over PdCl\(_2\) (Scheme 5.22). This was completed after 4 h and
confirmed to have successfully formed the desired product as the LRESIMS of 253 found a [M+H]⁺ ion peak at m/z 220. Purification of 253 and neutralization by basic ion-exchange chromatography returned the expected hyacinthacine C₁/C₄-type analogue (+)-7-epi-hyacinthacine C₁ 253 ([α]D²⁵+6.5 (c 0.10, H₂O)) in 75% yield and was confirmed through extensive spectroscopic evidence (included in the Appendix for this Ph.D. work).

**Scheme 5.22:** Hydrogenolysis of 252 to give final hyacinthacine C₁/C₄-type analogue 253.

With this compound, a Scifinder® search indicated that this compound and structure was novel. As with all the final hyacinthacine C₄-type products synthesized in this Ph.D. work, it was also assessed and characterised for its glycosidase inhibitory potential. When spectroscopically compared to hyacinthacine C₄, the ¹H and ¹³C NMR spectroscopic data were notably different (Table 5.2), and so the hypothesis that the structure proposed for the naturally isolated hyacinthacine C₄ is correct, is still applicable.
Table 5.2: Comparison of literature $^1$H NMR (500 MHz, D$_2$O) and $^{13}$C NMR (125 MHz, D$_2$O) data of Kato et al. Lit. (+)–hyacinthacine C$_4$ with the $^1$H NMR (500 MHz, D$_2$O) and $^{13}$C NMR (125 MHz, D$_2$O) of our synthetic 253.

<table>
<thead>
<tr>
<th>position</th>
<th>$^1$H NMR (δ, J in Hz)</th>
<th>$^{13}$C NMR (δ)</th>
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<td>4.11, dd (6.0, 7.4)</td>
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<td>1.12, d (7.0)</td>
</tr>
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</table>

5.16. Chapter 5: Final remarks

The successful synthesis of four novel analogues of the hyacinthacine C$_1$/C$_4$ analogues, 242, 243, 244 and 253, is described in this Chapter. The initial attempts to invert the cyclic sulfate of the N-Boc product 139 seemed promising but only worked once and so resulted in insufficient foundational products towards the synthesis of the hyacinthacine C$_1$/C$_4$-type analogues. Its inclusion in this Chapter helps the reader to understand the basis that ultimately resulted in the successful synthesis of the four hyacinthacine C$_1$/C$_4$-type analogues. Unfortunately, due to the time constraints, the synthesis of hyacinthacine C$_4$ was not achieved despite the inkling that its parent benzoate regiosiomer was synthesized as a minor product from the ring-opening of the cyclic sulfate 250. In addition to this, it was also unfortunate that trying to ring-open the cyclic sulfate 249 resulted in a complex mixture of products and could not be further investigated. Regardless, the key highlight was serendipitously synthesizing compound 243 which turned out to be the true and corrected structure for hyacinthacine C$_1$. This new finding in addition to the other diastereomers synthesized in this study means that there is a strong possibility that the structure for hyacinthacine C$_4$ proposed in 2007$^{117}$ is correct. This certainly warrants further exploration, but more importantly presents itself as a synthetic target for future projects. Obtainment of four novel stereoisomers helped shed further light to the recently
discovered field of hyacinthacine alkaloids, where now only a few structural assignment puzzles remain unsolved. On this respect, the most valuable contribution of this Chapter rests in the found evidence of identity of one of the synthesized pyrrolizidines with natural hyacinthacine C₁, to which identical structure as hyacinthacine C₄ had been assigned. The full characterization of all products leading to this structural correction have been included as part of our current *J. Nat. Prod.* manuscript (Supporting Information). Additionally, a copy of all the spectroscopic evidence of the final products has been included in the Appendix of this Ph. D. thesis.
Chapter 6 : Glycosidase Inhibitory summary

After their successful synthesis and characterisation, natural (+)-hyacinthacine C₅ 216, natural (+)-hyacinthacine C₁ 243, along with all synthesized epimers including the originally proposed structure for hyacinthacine C₅, 155, 156, 169, 215, 217, 242, 244 and 253 were sent to Japan and were assessed for their glycosidase inhibitory activities. The collaborative effort from both Assoc. Prof. Atsushi Kato and his student Masako Hoshino saw our final products tested against a panel of enzymes summarized in Tables 6.1, 6.2 and 6.3. As a measure of their anti-diabetic and anti-obesity potential, our results were compared with the glycosidase inhibitory results from casuarine (Table 6.2). For clarity, the SAR will be discussed in two sections: the hyacinthacine C₅-type analogues then the hyacinthacine C₁/C₄-type analogues synthesized.

6.1. Glycosidase inhibitory potential of the hyacinthacine C₅-type analogues (Table 6.1 and Table 6.2)

The anti-diabetic and anti-obesity characteristics of our synthetic products are ultimately dependant on how well they can inhibit the α-glucosidases. This is because these enzymes are the key enzymes in catalysing the final step in the digestive process of carbohydrates. Inhibiting these enzymes can result in a reduction of postprandial plasma glucose concentrations and ultimately suppress postprandial hyperglycemia. In this glycosidase inhibition study, a panel of enzymes, including key α-glucosidases were chosen. In this section, the glycosidase inhibitory activities of the seven hyacinthacine C₅-type analogues described in Chapters 2, 3 and 4B are discussed and summarised in Tables 6.1 and 6.2. Considering first the C₆, C₇ syn-diol pyrrolizidines 155, 156, and 169, pyrrolizidines 155 and 156 showed weak to moderate inhibition of the α-glucosidase of rat intestinal isomaltase (IC₅₀ values of 20 μM and 42 μM, respectively) and moderate inhibition against the α-glucosidase of rat intestinal sucrase (IC₅₀ values of 13 μM and 9.9 μM, respectively). Compound 155 displayed a weak inhibition of yeast α-glucosidase (IC₅₀ value of 83 μM) and rat intestinal maltase (IC₅₀ value of 61 μM). It also displayed a moderate activity against the amylglucosidases from Aspergillus niger and Rhizopus sp.
(IC$_{50}$ values of 25 μM and 16 μM, respectively). Compound 169 proved relatively inactive among the entire panel of enzymes.

Upon testing the glycosidase inhibition for the C6, C7 anti-diol series (the originally proposed structure for hyacinthacine C$_5$, 216, 217, and 215) results showed that compound 215 proved relatively inactive among the entire panel of enzymes. Compound 217 displayed a weak to moderate inhibition against α-glucosidases of yeast, rice, rat intestinal maltase and human lysosome (IC$_{50}$ values of 123 μM, 99 μM, 21 μM and 15 μM, respectively) and weak inhibition against the amylglucosidase of Aspergillus niger (IC$_{50}$ value of 41 μM). From this series, only the putative hyacinthacine C$_5$ and compound 216 showed inhibition against the α-L-rhamnosidase of Penicillium decumbens (IC$_{50}$ values of 74 μM and 197 μM, respectively), however, the putative hyacinthacine C$_5$ was only selective for this enzyme. Contrastingly, compound 216 proved less selective since it displayed a similar range of activities for yeast and rat intestinal maltase α-glucosidase (IC$_{50}$ values of 195 μM and 108 μM, respectively). Compound 216 is the corrected structure for the natural isolate labelled as hyacinthacine C$_5$ and so it was interesting to compare the glycosidase tests between our synthetic 216 and the 2007 natural isolate.$^{117}$ From the common enzymes tested, results seemed to differ between 216 and the natural isolate. For example, the 2007 natural isolate displayed a moderate activity against the amylglucosidases from Aspergillus niger (IC$_{50}$ value of 57 μM), whereas synthetic 216 displayed an IC$_{50}$ value of 370 μM against this enzyme. The 2007 natural isolate was recorded as being relatively inert against the α-glucosidase of rat intestinal maltase (IC$_{50}$ value in excess of 1000 μM) and also against the β-galactosidase of bovine liver (IC$_{50}$ value of 900 μM). Although we have unequivocally proven that compound 216 is the same structure, this compound was found to be relatively more active against these two enzymes with IC$_{50}$ values of 630 μM and 42 μM, respectively. To account for these differences, we can only speculate that either provision of more sample was required for a more accurate result, or that the natural isolate may have been contaminated with extra-cellular plant matrix that may not have been evident in the NMR analysis; this could lead to an unknowingly impure sample and misleading mass and IC$_{50}$ of product. Moreover, the enzyme purities between our study and the 2007 study by Kato et al.$^{117}$ may have been different, thus affecting functional activities. Further study towards this uncertainty is required.
Table 6.1: Concentration of hyacinthacine C₅-type analogues 216, 155, 156 and 217 giving 50% inhibition of various glycosidases.

<table>
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<th>Corrected structure of natural hyacinthacine C₅</th>
<th>(-)-7-epi hyacinthacine C₅</th>
<th>(-)-6-epi hyacinthacine C₅</th>
<th>(-)-6,7-di-epi hyacinthacine C₅</th>
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<td>o-glucosidase</td>
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<tr>
<td>Yeast</td>
<td>195</td>
<td>83</td>
<td>NI (32.6 %)</td>
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<tr>
<td>Rice</td>
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<td>Rat intestinal maltase</td>
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<td>NT</td>
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<tr>
<td>Rat intestinal sucrase</td>
<td>NT</td>
<td>13</td>
<td>9.9</td>
<td>NT</td>
</tr>
<tr>
<td>ER-9-glucosidase II</td>
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<td>NT</td>
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<td>355</td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Almond</td>
<td>NI (5.0 %)</td>
<td>NI (5.42 %)</td>
<td>NI (39.3 %)</td>
<td>NI (17.3 %)</td>
</tr>
<tr>
<td>Bovine liver</td>
<td>NI (28.1 %)</td>
<td>NI (27.4 %)</td>
<td>NI (25.0 %)</td>
<td>NI (43.3 %)</td>
</tr>
<tr>
<td>Human bryonose</td>
<td>NI (2.6 %)</td>
<td>NT</td>
<td>NT</td>
<td>NI (36.4 %)</td>
</tr>
<tr>
<td>o-galactosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee beans</td>
<td>NI (10.6 %)</td>
<td>NI (14.97 %)</td>
<td>NI (0 %)</td>
<td>NI (32.2 %)</td>
</tr>
<tr>
<td>β-galactosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine liver</td>
<td>571</td>
<td>NI (27.5 %)</td>
<td>NI (37.3 %)</td>
<td>NI (43.0 %)</td>
</tr>
<tr>
<td>o-mannosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jack bean</td>
<td>NT (3.4 %)</td>
<td>NT (5.04 %)</td>
<td>NT (0.315 %)</td>
<td>NI (0.5 %)</td>
</tr>
<tr>
<td>β-mannosidase</td>
<td>Sheep</td>
<td>NI (0 %)</td>
<td>NI (0 %)</td>
<td>NI (0 %)</td>
</tr>
<tr>
<td>o-L-fucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine kidney</td>
<td>NI (1.1 %)</td>
<td>NI (0 %)</td>
<td>NI (0 %)</td>
<td>NI (2.1 %)</td>
</tr>
<tr>
<td>o,a-trehalase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine kidney</td>
<td>119</td>
<td>735</td>
<td>NI (45.6 %)</td>
<td>NI (40.5 %)</td>
</tr>
<tr>
<td>amyloglucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>370</td>
<td>22</td>
<td>574</td>
<td>41</td>
</tr>
<tr>
<td>Rhizopus sp</td>
<td>NT</td>
<td>16</td>
<td>231</td>
<td>NT</td>
</tr>
<tr>
<td>o-L-rhamnosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pecticellum decumbens</td>
<td>197</td>
<td>NI (25.8 %)</td>
<td>NI (7.74 %)</td>
<td>NI (23.4 %)</td>
</tr>
<tr>
<td>β-glucoronidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>NI (9.3 %)</td>
<td>NI (0 %)</td>
<td>NI (5.08 %)</td>
<td>NI (8.2 %)</td>
</tr>
<tr>
<td>Bovine liver</td>
<td>NI (16.8 %)</td>
<td>NI (12.4 %)</td>
<td>NI (23.8 %)</td>
<td>NI (16.0 %)</td>
</tr>
</tbody>
</table>

a "-": Not tested, b"NI": No inhibition (less than 50 % inhibition at 1000 µM), c( ) : Inhibition % at 1000 µM
Table 6.2: Concentration of hyacinthacine $C_5$-type analogues the putative structure for hyacinthacine $C_5$, 169, 215 and Casuarine giving 50% inhibition of various glycosidases.

<table>
<thead>
<tr>
<th>enzyme</th>
<th>Originally proposed structure of (+)-hyacinthacine $C_5$</th>
<th>$\text{IC}_{50}$ ($\mu$M)</th>
<th>$\text{IC}_{50}$ ($\mu$M)</th>
<th>$\text{IC}_{50}$ ($\mu$M)</th>
<th>$\text{IC}_{50}$ ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-glucosidase</td>
<td>Yeast</td>
<td>635</td>
<td>NI (49.2 %)</td>
<td>NI (31.4 %)</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>NI(29.4 %)</td>
<td>NI (41.2 %)</td>
<td>527</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>Rat intestinal maltase</td>
<td>NI (31.4 %)</td>
<td>960</td>
<td>NI (38.1 %)</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Rat intestinal isomaltase</td>
<td>NT</td>
<td>NI (48.1 %)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Rat intestinal isomaltose</td>
<td>NT</td>
<td>NI (44.6 %)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>ER $\alpha$-glucosidase II</td>
<td>NI (0 %)</td>
<td>NT</td>
<td>NI (19.6 %)</td>
<td>39.6</td>
</tr>
<tr>
<td>Human lysosome</td>
<td></td>
<td>804</td>
<td>NT</td>
<td>823</td>
<td>3.7</td>
</tr>
<tr>
<td>$\beta$-glucosidase</td>
<td>Almond</td>
<td>NI (2.6 %)</td>
<td>NI (8.03 %)</td>
<td>NI (3.4 %)</td>
<td>NI (14.5 %)</td>
</tr>
<tr>
<td></td>
<td>Bovine liver</td>
<td>NI (33.4 %)</td>
<td>NI (16.9 %)</td>
<td>NI (7.4 %)</td>
<td>NI (4 %)</td>
</tr>
<tr>
<td>Human lysosome</td>
<td></td>
<td>NI (0 %)</td>
<td>NT</td>
<td>NI (35.7 %)</td>
<td>NI (43.0 %)</td>
</tr>
<tr>
<td>$\alpha$-galactosidase</td>
<td>Coffee beans</td>
<td>NI (13.1 %)</td>
<td>NI (5.59 %)</td>
<td>NI (27.7 %)</td>
<td>NI (8.5 %)</td>
</tr>
<tr>
<td>$\beta$-galactosidase</td>
<td>Bovine liver</td>
<td>986</td>
<td>NI (34.2 %)</td>
<td>1070</td>
<td>NI (27.3 %)</td>
</tr>
<tr>
<td>$\alpha$-mannosidase</td>
<td>Jack bean</td>
<td>NI (1.1 %)</td>
<td>NI (14.2 %)</td>
<td>NI (3.2 %)</td>
<td>NI (1.4 %)</td>
</tr>
<tr>
<td>$\beta$-mannosidase</td>
<td>Seal</td>
<td>NI (0 %)</td>
<td>NI (0 %)</td>
<td>NI (0 %)</td>
<td>NI (1.6 %)</td>
</tr>
<tr>
<td>$\alpha$-fucosidase</td>
<td>Bovine kidney</td>
<td>NI (9.7 %)</td>
<td>NI (0 %)</td>
<td>NT (7.5 %)</td>
<td>NI (4.1 %)</td>
</tr>
<tr>
<td>$\alpha$-trehalase</td>
<td>Porcine kidney</td>
<td>NI (6.4 %)</td>
<td>NI (5.47 %)</td>
<td>NI (5.7 %)</td>
<td>168</td>
</tr>
<tr>
<td>amylglucosidase</td>
<td>Aspergillus niger</td>
<td>423</td>
<td>NI (40.7 %)</td>
<td>NI (9.9 %)</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Rhizopus sp</td>
<td>NT</td>
<td>NI (46.0 %)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>$\alpha$-D-mannosidase</td>
<td>Penicillium decumbens</td>
<td>74</td>
<td>NI (26.8 %)</td>
<td>NI (29.2 %)</td>
<td>NI (18.8 %)</td>
</tr>
<tr>
<td>$\beta$-glucosidase</td>
<td>E. coli</td>
<td>NI (8.6 %)</td>
<td>NI (5.08 %)</td>
<td>NI (22.2 %)</td>
<td>NI (0.4 %)</td>
</tr>
<tr>
<td></td>
<td>Bovine liver</td>
<td>NI (17.8 %)</td>
<td>NI (18.2 %)</td>
<td>NI (22.7 %)</td>
<td>NI (0 %)</td>
</tr>
</tbody>
</table>

$^a$NI : No inhibition (less than 50 % inhibition at 1000 $\mu$M), $^b$( ) : Inhibition % at 1000 $\mu$M, $^c""""$ : Not tested
6.2. Glycosidase inhibitory potential of the hyacinthacine C₁/C₄-type analogues (Table 6.3)

In this section, the glycosidase inhibitory activities of the four hyacinthacine C₁/C₄-type analogues, 242, 243, 244 and 253 described in Chapter 5 are discussed and are summarised in Table 6.3. From these four analogues, analogue 244 was found to be selective for the amyloglucosidase of Aspergillus niger and displayed a weak inhibition with an IC₅₀ value of 95.5 µM. The corrected structure for hyacinthacine C₁ 243 displayed a weak to moderate inhibition for the α-glucosidase of rice, rat intestinal maltase and human lysosome (IC₅₀ values of 33.7, 78.2 and 55 µM, respectively). These results were then compared with glycosidase inhibitory results of the naturally occurring hyacinthacine C₁. Interestingly, a notable difference was found between the natural isolate and our synthetic 243. In 1999, the natural isolate was found to be relatively inert against the α-glucosidase of rice and rat intestinal maltase (IC₅₀ values for both enzymes were in excess of 1000 µM), but selective and active against the amyloglucosidase of Aspergillus niger (IC₅₀ value of 84 µM). Comparatively, synthetic 243 was found to display the aforementioned α-glucosidase activities but proved inactive against the amyloglucosidase of Aspergillus niger (IC₅₀ value in excess of 1000 µM). These differences may have been the result of not enough sample being provided for a reliable glycosidase inhibition assessment, or maybe extra-cellular plant matrix that may not have been evident in the NMR analysis was apparent in the natural isolate sample. Regardless, there are too many variables, and this certainly warrants further investigation since we have unequivocally proven our synthetic 243 to be the same structure as the natural isolate labelled hyacinthacine C₁ from 1999. In contrast with compounds 243 and 244, compounds 242 and 253 were relatively inactive against all glycosidases tested.
Table 6.3: Concentration of hyacinthacine C₁/C₄-type analogues 242, 243, 244 and 253 giving 50% inhibition of various glycosidases.

<table>
<thead>
<tr>
<th>enzyme</th>
<th>(-)-5-gpi-hyacinthacine C₁</th>
<th>Corrected structure for hyacinthacine C₁</th>
<th>(+)-6,7-dia-gpi-hyacinthacine C₄</th>
<th>(+)-7-gpi-hyacinthacine C₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀(µM)</td>
<td>IC₅₀(µM)</td>
<td>IC₅₀(µM)</td>
<td>IC₅₀(µM)</td>
</tr>
<tr>
<td>a-glucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>NI (42.5%)</td>
<td>NI (26.5%)</td>
<td>NI (50.2%)</td>
<td>NI (2.3%)</td>
</tr>
<tr>
<td>Rice</td>
<td>NI (0%)</td>
<td>33.7</td>
<td>NI (22.8%)</td>
<td>NI (1.6%)</td>
</tr>
<tr>
<td>Rat intestinal maltase</td>
<td>NI (3.1%)</td>
<td>78.2</td>
<td>870</td>
<td>NI (20.8%)</td>
</tr>
<tr>
<td>Rat intestinal isomaltase</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Rat intestinal sucrase</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>ER α-glucosidase II</td>
<td>NI (5.2%)</td>
<td>NI (46.4%)</td>
<td>NI (8.4%)</td>
<td>NI (0%)</td>
</tr>
<tr>
<td>Human lysosome</td>
<td>NI (2.9%)</td>
<td>55</td>
<td>916</td>
<td>NI (6.2%)</td>
</tr>
<tr>
<td>β-glucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Almond</td>
<td>NI (7.9%)</td>
<td>NI (42.6%)</td>
<td>NI (6.7%)</td>
<td>NI (3.2%)</td>
</tr>
<tr>
<td>Bovine liver</td>
<td>NI (20.4%)</td>
<td>NI (40.1%)</td>
<td>NI (30.1%)</td>
<td>NI (3.1%)</td>
</tr>
<tr>
<td>Human lysosome</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>a-galactosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee beans</td>
<td>NI (3.5%)</td>
<td>459</td>
<td>487</td>
<td>NI (24.4%)</td>
</tr>
<tr>
<td>β-galactosidase</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bovine liver</td>
<td>NI (32.4%)</td>
<td>892</td>
<td>NI (35.4%)</td>
<td>NI (6.2%)</td>
</tr>
<tr>
<td>α-mannosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jack bean</td>
<td>NI (7.3%)</td>
<td>NI (2.4%)</td>
<td>NI (3.6%)</td>
<td>NI (5.3%)</td>
</tr>
<tr>
<td>β-mannosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stool</td>
<td>NI (0.2%)</td>
<td>NI (4.1%)</td>
<td>NI (9.3%)</td>
<td>NI (0.8%)</td>
</tr>
<tr>
<td>α-L-fucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine kidney</td>
<td>NI (7.8%)</td>
<td>NI (5.8%)</td>
<td>NI (0%)</td>
<td>NI (1.8%)</td>
</tr>
<tr>
<td>α-D-glucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine kidney</td>
<td>NI (0%)</td>
<td>NI (2.1%)</td>
<td>NI (16.3%)</td>
<td>NI (0%)</td>
</tr>
<tr>
<td>amylglucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>NI (0%)</td>
<td>NI (47.8%)</td>
<td>95.5</td>
<td>605</td>
</tr>
<tr>
<td>Rhizopus sp</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>α-L-rhamnosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium decumbens</td>
<td>NI (0.7%)</td>
<td>NI (0%)</td>
<td>NI (5.3%)</td>
<td>NI (3.0%)</td>
</tr>
<tr>
<td>β-glucoronidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>NI (21.4%)</td>
<td>NI (9.0%)</td>
<td>NI (0%)</td>
<td>NI (3.9%)</td>
</tr>
<tr>
<td>Bovine liver</td>
<td>NI (24.4%)</td>
<td>NI (35.2%)</td>
<td>NI (2.3%)</td>
<td>NI (6.1%)</td>
</tr>
</tbody>
</table>

aNI : No inhibition (less than 50 % inhibition at 1000 µM), b( ) : Inhibition % at 1000 µM, c"-" : Not tested
6.3. Assessing the anti-diabetic and anti-obesity potential of our 11 hyacinthacine C-type analogues

To gain a better understanding of the structure-activity-relationship (SAR), all hyacinthacine C-type analogues synthesized in this Ph.D. were collectively compared.

6.3.1. SAR Effects of the stereochemistry of the C5 methyl group

From our results of all the enzyme inhibition results, we noted correlation between the orientation of the C5 methyl group and the IC$_{50}$ against each glycosidase tested. More specifically, if the hyacinthacine C-type alkaloid contains a (5S)-methyl, the IC$_{50}$ values increase with consequently a reduction in the selectivity and potency of the compound as a glycosidase inhibitor. Comparatively, for a decrease in the IC$_{50}$, the hyacinthacine C-type compounds containing an (5R)-methyl proved to be more suitable candidates for potential as glycosidase inhibitors.

6.3.2. SAR Effects of the stereocentres at C1, C2 and C3

Our observed glycosidase activities seemed to be influenced largely by the C1, C2 and C3 stereocentres which are originally sourced from the starting sugar L-xylose. This trend can be best explained through comparison of the synthetic hyacinthacine C-type analogues described in this work, with the structurally analogous casuarine (one of the most powerful glucosidase inhibitors) shown in Table 6.2. More specifically, the glycosidase activities of our synthetic hyacinthacine C-type analogues were generally less potent than casuarine. Interestingly, it has been proven by crystal structure determination and computational methods, that casuarine perfectly mimics the stereochemical arrangement of glucose, with the A-ring overlapping the C3 to C6 portion of glucose.

In this Ph.D. work, the hyacinthacine C-type analogues discussed in section 6.1 contain an A-ring configuration to that of L-xylose. Inverting the configuration at C1 as operated in Chapter 5, leads to another related carbohydrate, namely L-lyxose. Both L-xylose and L-lyxose are non-natural sugars. Since glucosidases are functionally deputed to recognize
glucose, it can be therefore generalized that iminosugars containing any configurational deviation from glucose (such as L-xylose and L-lyxose), decreases the glucose nature of the iminosugar, and so will diminish glucosidase inhibition activity.

When the hyacinthacine C-type analogues described in section 6.1 were compared with the hyacinthacine C-type analogues described in section 6.2, it was observed that the potency and selectivity of the latter were relatively weak. Therefore, inverting the C1 stereocentre decreases the potency and selectivity of the iminosugar as a glycosidase inhibitor. More specifically, hyacinthacine C5-type compounds 155, 156, 169, 215, 216, 217 and the putative structure of hyacinthacine C5 contain identical configurations with that of casuarine at C7a, C1, C2 and C3.132 The relatively diminished glycosidase activity discussed in section 6.2 for compounds 242, 243, 244 and 253 can be compared with a worse mimicry of casuarine (and ultimately glucose), since only configurations at C7a, C2 and C3 were preserved. Similarly, inverting C1 of casuarine gives rise to an allose related configuration and is responsible for a similar diminished glycosidase inhibition activity.197-198

6.3.3. SAR Effects of the C5 methyl group

When all hyacinthacine C-type analogues synthesized in this study were compared with casuarine, it was observed that containing a methyl substituent at C5 resulted in a decrease in glycosidase inhibition properties.132 This behaviour appears to be general for related pyrrolizidines reported in literature. For example, a comparison of 7-deoxycasuarine198 and its 5-methyl substituted homologue199 shows a one order magnitude loss of activity towards the amyloglucosidase of A. niger (IC50 value of 4.5 µM and 39 µM, respectively). In another example, australine200 and its 5-methyl substituted homologue, hyacinthacine B4113 display a similar loss of activity towards the amyloglucosidase of A. niger (IC50 value of 5.8 µM and 89 µM, respectively). It is therefore reasonable to suggest that in general, this moiety sterically prevents the pyrrolizidine from effectively binding to the glycosidase active site.
Chapter 7: Conclusions

The initial aims for this project were to:

1. Develop a novel synthetic strategy to prepare hyacinthacine C-type analogues in an efficient and flexible manner
2. Elucidate and correct the structure for either hyacinthacine C_1, C_4 or C_5.
3. Generate a moderate number of hyacinthacine C-type configurational analogues and use the data generated from glycosidase inhibition studies to determine a general structure-activity relationship.

Chapters 2 and 3 report the synthetic strategy employed towards the synthesis of the hyacinthacine C-type analogues. These Chapters detail the successful synthesis of (+)-7-epi-hyacinthacine C_5 155, (–)-6-epi-hyacinthacine C_5 156 and (+)-5,6-di-epi-hyacinthacine C_5 169. The spectroscopic data for these compounds did not match any of the spectroscopic data reported for hyacinthacines C_1, C_4 or C_5. However, these results led us to consider implementing a C_6, C_7 anti-diol configuration in the final hyacinthacine C-type analogues.

Chapter 4A describes our unsuccessful attempts towards generating the C_6, C_7 anti-diol within the final hyacinthacine C-type analogues. These results led to the conclusion that installing the C_6, C_7 anti-diol is most effective after the pyrrolizidine core has been formed and can be achieved via synthesis of the corresponding cyclic sulfate then subsequent SN2 ring-opening of the cyclic sulfate moiety with a benzoate nucleophile. This approach is discussed in Chapter 4B and details the successful synthesis of four hyacinthacine C-type products which include: the corrected structure of the natural (+)-hyacinthacine C_5 216, (+)-6,7-di-epi-hyacinthacine C_5 217, the originally proposed structure for (+)-hyacinthacine C_5 and (+)-5-epi-hyacinthacine C_5 215. Our synthetic product 216 displayed essentially identical $^1$H and $^{13}$C NMR spectroscopic data for the natural isolate labelled hyacinthacine C_5. Thus, the correct structure of hyacinthacine C_5 has the opposite configuration at the three contiguous stereogenic centres, C_5, C_6, and C_7, to that of the originally proposed structure (Figure 7.1).
Unfortunately, we could not conclude that our synthetic hyacinthacine C₅-type analogue 217 was the same as the compound 120 reported by Reissig and Goti et al.¹³¹ More specifically, there was a significant spectroscopic difference between the ¹H and ¹³C NMR spectroscopic data for both products. The parent crystalline compound 149 that led to the synthesis of compound 217, was unequivocally confirmed to be correct by X-ray crystallographic analysis and so in addition to the extensive spectroscopic analysis of 217, we were confident that we had synthesized the correct structure. After reviewing the pathway reported by Reissig and Goti et al.,¹³¹ we were able to propose that they may have unexpectedly synthesized and isolated the 4-membered bicyclic N-heterocycle 220 rather than 120 (Scheme 7.1).

Figure 7.1: DFT minimized molecular models [B3LYP/6-31G(d)] of the putative structure of hyacinthacine C₅ and its corrected structure 216 (hydroxy protons not shown).
Scheme 7.1: The unconfirmed but potential synthesis of $N$-heterocycle 220 by Reissig and Goti et al.\textsuperscript{131}

The remaining four hyacinthacine C-type analogues synthesized in this work are detailed in Chapter 5. These include (−)-5-epi-hyacinthacine C\textsubscript{1} 242, the corrected structure of hyacinthacine C\textsubscript{1} 243, (+)-6,7-di-epi-hyacinthacine C\textsubscript{1} 244 and (+)-7-epi-hyacinthacine C\textsubscript{1} 253. Our synthetic product 243 displayed essentially identical $^1$H and $^{13}$C NMR spectroscopic for the natural isolate labelled hyacinthacine C\textsubscript{1}.\textsuperscript{105} Thus, the correct structure of hyacinthacine C\textsubscript{1} has the opposite configuration at C6 to that of the originally proposed structure (Figure 7.2). It is suspected that the torsional strain and twist of the pyrrolizidine core may have resulted in either an observed (or absence of) several NOESY correlations for this natural isolate thus resulting in the incorrect proposed stereochemistry.
Figure 7.2: DFT minimized molecular models [B3LYP/6-31G(d)] of the putative structure of hyacinthacine C₁ and its corrected structure 243 (hydroxy protons not shown).

The successful synthesis of all eleven hyacinthacine C-type analogues in this work is summarised in Scheme 7.2.
Scheme 7.2: A summary of the synthesis of the natural and unnatural hyacinthacine C-type iminosugars reported in this Ph.D. work.
All eleven hyacinthacine C-type analogues synthesized in this work were each assessed for their glycosidase inhibitory activities. The hyacinthacine C-type analogues described in Chapters 2-4B were derived from L-xylose. Inverting the stereocentre at C1 as described for the hyacinthacine C-type analogues synthesized in Chapter 5, led to another related carbohydrate, namely L-lyxose. In comparison to the structurally analogous casuarine (one of the most powerful glucosidase inhibitors), the synthetic hyacinthacine C-type analogues synthesize in this Ph.D. work were generally less potent than casuarine. The SAR for these compounds suggest that the diminished glycosidase activity was a result of steric hindrance induced by the C5-methyl (regardless of orientation), as well as configurational deviations away from glucose (such as L-xylose and L-lyxose). Both these factors seem to decrease the glucose nature of the iminosugar, and so will diminish glucosidase inhibition activity. However, both (−)-6-epi-hyacinthacine C₅ 156 and (+)-7-epi-hyacinthacine C₅ 155 displayed noteworthy inhibition against the α-glucosidase of rat intestinal sucrase (IC₅₀ values of 13 μM and 9.9 μM, respectively) and so can be used as a foundation towards investigating suitable drug candidates for anti-diabetic and anti-obesity medications.

The work described in this thesis is believed to satisfactorily meet the aforementioned aims initially proposed in 2015. Although we were unable to synthesize the putative structure for hyacinthacine C₁/C₄, the correction of the structure of hyacinthacine C₁ means that there is a strong possibility that the structure for the 2007 natural isolate labelled as hyacinthacine C₄ is correctly assigned.¹¹⁷ This certainly warrants further exploration.
Chapter 8 : Experimental

General Methods

Silica gel 60 230–400 Mesh was used for flash column chromatography (FCC) purifications. LRESIMS data was acquired on a single quadrupole, (MeOH as solvent) mass spectrometer. HRESIMS were determined on a quadrupole time–of–flight mass spectrometer. FTIR data were determined on neat samples. The optical rotation was measured using a 2 mL cell at room temperature. An average of 40 measurements was used to calculate the specific rotation. $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded in deuterochloroform (CDCl$_3$), deuterium oxide (D$_2$O) or deuterated methanol (CD$_3$OD) solutions. All signals obtained in CDCl$_3$ were relative to the tetramethylsilane (TMS) signal for $^1$H NMR and the CDCl$_3$ signal for $^{13}$C NMR, referenced at 0.00 ppm and 77.16 ppm, respectively. All signals which were recorded in CD$_3$OD were relative to the CD$_2$HOD signal for $^1$H NMR and the CD$_3$OD for $^{13}$C NMR, referenced at 3.31 and 49.00 ppm, respectively. All signals which were recorded in D$_2$O were relative to the D$_2$O signal for $^1$H NMR, referenced at 4.49 ppm. For $^{13}$C NMR the referencing of peaks is relative to internal MeOH ($\delta$ 49.50 ppm). NMR assignments were based upon gCOSY, APT, gHSQC, and NOESY or ROESY experiments. In some cases, $^{13}$C NMR signals that were absent in the standard $^{13}$C NMR spectrum were identified using gHSQC experiments. Pyrrolizidine compounds are named using systematic nomenclature. The NMR assignments of those compounds are based on the numbering system of the hyacinthacine alkaloids and not the systematic name. The three–dimensional structures generated were prepared with ChemDraw 16.0 and Avogadro: an open–source molecular builder and visualization tool. Version 1.2.0. The Density Functional Theory (DFT) used to minimize the 3D structures was calculated with B3LYP/6–31G(d) using the Gaussian 09 basis set. ORTEP representations shown for the crystal structures were generated in accordance with CrysAlis PRO 1.171.38.46; cell refinement: CrysAlis PRO 1.171.38.46; data reduction: CrysAlis PRO 1.171.38.46, CrysAlis PRO 1.171.38.43; cell refinement: CrysAlis PRO 1.171.38.43; data reduction: CrysAlis PRO 1.171.38.43. Program(s) used to solve the structure: SIR92; program(s) used to refine structure:
Chapter 2

(-)-1,2-O-Isopropylidene-α-L-xylofuranose (129)\textsuperscript{149-151, 216}

In this order, concentrated H\textsubscript{2}SO\textsubscript{4} (0.10 mL, 1.9 mmol), CuSO\textsubscript{4} (2.1 g, 13.4 mmol) and L–xylose (1.0 g, 6.7 mmol) were added to anhydrous acetone (20 mL). The mixture was vigorously stirred under a nitrogen atmosphere at rt for 28 h, causing the solution to turn yellow. TLC analysis indicated full consumption of the starting material. The CuSO\textsubscript{4} was removed via vacuum filtration through a celite pad. The filter cake was washed with additional acetone (3 x 5 mL) and the combined filtrate and washings were basified by the addition of concentrated NH\textsubscript{3} solution (0.4 mL). The precipitated (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} was filtered and the solvent was concentrated \textit{in vacuo} to give a yellow syrup. After treatment with HCl (11 mL, 0.24 M) and continual stirring at rt for 45 min, TLC analysis confirmed the formation of the desired product. The solution was neutralised with NaHCO\textsubscript{3} to pH 7–8, followed by concentration \textit{in vacuo}. The resultant residue was then dissolved in chloroform, dried (MgSO\textsubscript{4}), filtered and concentrated \textit{in vacuo} to give the desired acetonide 129 as a pale–yellow oil (1.3 g, quant.) which was used for the next step without any purification. The \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopic data of this product were consistent with those published.\textsuperscript{149-151, 216}

\textbf{R}_{f}(\text{EtOAc}) = 0.40

\textbf{LRESIMS} m/z 191 (100%) [M+H]\textsuperscript{+}

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 5.99 (1H, d, \textit{J} = 3.5 Hz, H1), 4.53 (1H, d, \textit{J} = 3.5 Hz, H2), 4.33 (1H, s, H3), 4.22 – 4.11 (3H, m, H4, H5), 4.09 – 4.04 (1H, m, OH), 3.84 (1H, bs, OH), 1.49 (3H, s, Ac–CH\textsubscript{3}), 1.32 (3H, s, Ac–CH\textsubscript{3}).
**General method for O-benzylation**

(−)-3,5-Di-O-benzyl-1,2-O-isopropylidene-α-L-xylofuranose (130)\(^{149-151,216}\)

A solution of the acetonide 129 (0.50 g, 2.6 mmol) in THF (3 mL) was slowly added to a cooled (0 °C) and stirred suspension of sodium hydride (0.38 g, 9.5 mmol) (60% dispersion in mineral oil) in anhydrous THF (15 mL) under a nitrogen atmosphere. The reaction mixture was heated at reflux for 5 min and then cooled to rt. Benzyl bromide (0.75 mL, 6.31 mmol) and TBAI (0.15 g, 0.39 mmol) were added to the mixture, which was heated at reflux for 15 min. By this time, TLC analysis had confirmed full consumption of the starting material. The reaction mixture was cooled to 0 °C, and quenched dropwise with water (10 mL). The mixture was extracted with Et\(_2\)O (3 x 10 mL) and the combined extracts were dried (MgSO\(_4\)), filtered and concentrated in vacuo. The resultant brown oil was purified by FCC (gradient of 3:20 EtOAc/\(n\)-hexane to 1:5 EtOAc/\(n\)-hexane) to afford the bis–benzylated acetonide 130, as a pale–yellow oil (0.83 g, 86%). The \(^1\)H and \(^{13}\)C NMR spectroscopic data of this product were consistent with those published.\(^{149-151,216}\)

\(\text{R}_f(1:5\ \text{EtOAc}/n\)-hexane\) = 0.30

**LRESIMS** \(m/z\ 371 (100\%) \ [M+H]^+\)

\(^1\)H NMR (500 MHz, CDCl\(_3\)) δ 7.41 – 7.17 (10H, m, ArH), 5.93 (1H, d, \(J = 3.7\) Hz, H1), 4.68 – 4.54 (3H, m, H2, H3\(^3\)), 4.49 (2H, 2 x d, \(J = 12.0, 9.9\) Hz, H5\(^5\)), 4.41 (1H, td, \(J = 6.1, 3.2\) Hz, H4), 3.96 (1H, d, \(J = 3.2\) Hz, H3), 3.77 (2H, qd, \(J = 9.9, 6.1\) Hz, H5\(^5\)), 1.47 (3H, s, AcCH\(_3\)), 1.30 (3H, s, AcCH\(_3\)).

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) δ 138.2 (ArC), 137.7 (ArC), 128.5 (2 x ArCH), 128.5 (2 x ArCH), 128.0 (2 x ArCH), 127.9 (2 x ArCH), 127.7 (2 x ArCH), 111.7 (AcC), 105.2 (C1), 82.4 (C2), 81.8 (C3), 79.3 (C4), 73.6 (C5\(^5\)), 72.0 (C3\(^3\)), 67.7 (C5), 26.9 (AcCH\(_3\)), 26.4 (AcCH\(_3\)). [Ac = acetonide].
(+)-3,4-Di-\textit{O}-benzyl-\textit{\alpha},\textit{\beta}-\textit{L}-xylofuranose (\textit{a} and \textit{b})\textsuperscript{149-151, 216}

To a solution of 130, (0.880 g, 2.38 mmol) in AcOH/H\textsubscript{2}O (20 mL, 30% v/v) was added 5 M HCl (1 mL). TLC analysis confirmed full consumption of the starting material after heating the reaction to 45 °C for 3 h by which this time the solution was neutralised by the addition of NaHCO\textsubscript{3} (0.5 g or until pH 7). The solution was then evaporated under reduced pressure to 20 mL and extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 15 mL), the combined extracts were dried (MgSO\textsubscript{4}), filtered and concentrated \textit{in vacuo}. FCC (1:5 EtOAc/n-hexane) resulted in a mixture of anomers (3:1, \textit{a}:\textit{b}, respectively) as a clear oil that solidified to a waxy solid overnight (0.52 g, 83%). The \textit{\textsuperscript{1}H} and \textit{\textsuperscript{13}C} NMR spectroscopic data of this product were consistent with those published.\textsuperscript{149-151, 216}

\textit{R}_{f}(1:5 \text{EtOAc/n-hexane}) = 0.20

\textbf{LRESIMS} \textit{m/z} 331 (100\%) [M+H]\textsuperscript{+}

\textit{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}) \textit{\delta} 7.37 – 7.21 (20H, m, ArH), 5.45 (1H, d, \textit{J} = 4.2 Hz H1a), 5.11 (1H, d, \textit{J} = 3.3 Hz, H1b), 4.69 – 4.43 (9H, m, H3\textsuperscript{\textit{\alpha}}, b, H5\textsuperscript{\textit{\alpha}}, a, b, H4a), 4.41 (1H, tt, \textit{J} = 5.1, 3.3 Hz, H4b), 4.20 (1H, d, \textit{J} = 2.6 Hz, H2b), 4.17 – 4.10 (2H, m, H2a, H3b), 4.01 – 3.91 (2H, m, OH\textsubscript{a}, H3a), 3.75 (1H, q, \textit{J} = 5.1Hz, H4a ), 3.86 – 3.62 (4H, m, H5a, H5b), 3.21 (1H, s, OH), 2.03 (1H, s, OH).

\textit{\textsuperscript{13}C NMR} (125 MHz, CDCl\textsubscript{3}) \textit{\delta} 137.9 (ArCa), 137.9 (ArCb), 137.6 (ArCa), 137.4 (ArCb), 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.8, 127.6 (20 x ArCH), 103.5 (C1b), 96.0 (C1a), 83.6 (C3a), 82.9 (C3b), 79.9 (C4b), 79.3 (C2b), 77.5 (C4a), 75.6 (C2a), 73.7 (C5b), 73.6 (C5a), 72.8 (C3b), 72.0 (C3a), 69.1 (C5a), 69.0 (C5b).
General Method for the Petasis (Borono-Mannich) reaction

(2S,3S,4R,5R,E)-1,3-Bis(benzyloxy)-5-(((R)-but-3-en-2-yl)amino)-7-phenylhept-6-ene-2,4-diol (127)\textsuperscript{49}

Triethylamine (434 µL, 2.83 mmol) was added to a solution of (R)–\(\alpha\)–methylallyl amine·HCl (0.220 g, 3.12 mmol) in EtOH (50 mL) and the solution was stirred at rt for 20 min. (+)–3,4–Di–\(\delta\)–benzyl–\(\alpha\),\(\beta\)–L–xylofuranose (0.930 g, 2.83 mmol) was added to the reaction mixture, followed by the addition of (E)–2–phenylvinylboronic acid (0.460 g, 3.12 mmol). TLC analysis confirmed complete consumption of the starting material after stirring at rt for 3 d, whereby the mixture was concentrated \textit{in vacuo}, affording a brown viscous oil. The crude product was dissolved in EtOAc (50 mL) and washed with 0.5 M NaOH (2 x 40 mL) solution. The organic fraction was dried (MgSO\(_4\)), filtered, concentrated \textit{in vacuo} and purified by FCC (3:97 MeOH/CH\(_2\)Cl\(_2\)) affording the desired product 127 as a brown oil (0.900 g, 65%). To prepare the crystalline hydrochloride salt of 127, a portion of 127 (0.100 g) was dissolved in CH\(_2\)Cl\(_2\) (5 mL) and washed with 1N HCl (3 x 10 mL). The CH\(_2\)Cl\(_2\) solution was concentrated \textit{in vacuo} to give 127·HCl as white needle–like crystals.

127

\(R_f\)0.20 (3:97 MeOH/CH\(_2\)Cl\(_2\)).

\textbf{LRESIMS} \textit{m/z} 488 (100%) [M+H]\(^+\). \textbf{\(^1\)H NMR (500 MHz, CDCl\(_3\))} \(\delta\) 7.37 – 7.20 (15H, m, ArH), 6.48 (1H, d, \(J = 16.0\) Hz, H7), 6.10 (1H, dd, \(J = 16.0, 8.7\) Hz, H6), 5.72 (1H, ddd, \(J = 17.3, 10.2, 7.2\) Hz, H2\(\prime\)), 5.09 (1H, d, \(J = 10.2\) Hz, H3\(\prime\)\textit{cis}), 4.98 (1H, d, \(J = 16.8\) Hz, H3\(\prime\)\textit{trans}), 4.64 (2H, s, OCH\(_2\)Ph), 4.51 – 4.42 (2H, m, OCH\(_2\)Ph), 4.05 (1H, dd, \(J = 8.3, 5.5\) Hz, H2), 3.97 (1H, t, \(J = 4.6\) Hz, H4), 3.69 – 3.49 (4H, m, H3, H1, H5), 3.30 (1H, dq, \(J = 12.6, 6.2\) Hz, H1\(\prime\)), 1.10 (3H, d, \(J = 6.4\) Hz, H1\(\prime\)\textit{`).
\[ ^{13}C \text{ NMR (125 MHz, CDCl}_3 \delta 142.0 (C^2'), 138.3 (ArC), 138.2 (ArC), 136.8 (ArC), 133.6 (C7), 128.8 (ArCH), 128.3 (ArCH), 128.0 (C6, ArCH), 128.0 (ArCH), 127.0 (ArCH), 126.7 (ArCH), 114.9 (C^3'), 80.1 (C3), 74.5 (OCH}_2\text{Ph), 73.6 (OCH}_2\text{Ph), 72.4 (C4), 71.5 (C1), 70.3 (C2), 60.6 (C5), 53.0 (C1'), 20.3 (C1''). \]

**127.HCl**

Rf 0.15 (5:97 MeOH/CH\(_2\)Cl\(_2\)).

MP: 165 – 170 °C.

\[ ^1\text{H NMR (500 MHz, CDCl}_3 \delta 7.47 - 7.07 (15H, m, ArH), 6.69 (1H, d, J = 16.0 Hz, H7), 6.43 (1H, dd, J = 16.0, 9.9 Hz, H6), 6.03 (1H, ddd, J = 17.5, 10.4, 7.4 Hz, H2'), 5.32 - 5.20 (2H, m, H3'), 4.80 (1H, d, J = 10.9 Hz, OCH}_2\text{Ph), 4.68 - 4.57 (1H, m, H5), 4.55 - 4.29 (3H, m, OCH}_2\text{Ph, H4), 4.20 (1H, dd, J = 9.9, 3.4 Hz), 4.10 (2H, dd, J = 6.3, 4.3 Hz), 3.81 - 3.68 (2H, m, H3, H1'), 3.63 (1H, dd, J = 6.8, 1.9 Hz, H2), 3.53 (1H, dd, J = 9.4, 6.5 Hz, H1), 3.42 (1H, dd, J = 9.4, 6.0 Hz, H1), 1.46 (3H, d, J = 6.8 Hz, H1''). \]

\[ ^{13}C \text{ NMR (125 MHz, CDCl}_3 \delta 138.7 (C^2'), 137.9 (ArC), 137.6 (ArC), 135.2 (ArC), 134.7 (C7), 128.9 (ArCH), 128.8 (ArCH), 128.4 (ArCH), 127.9 (ArCH), 127.69 (ArCH), 127.68 (ArCH), 127.19 (ArCH), 120.18 (C^3'), 78.8 (C3), 74.8 (OCH}_2\text{Ph), 73.2 (OCH}_2\text{Ph), 70.9 (C4), 69.9 (C1), 68.6 (C2), 62.1(C5), 54.9 (C1'), 16.7 (C1''). \]

**\((2R,3R,4R,5R)-4-(Benzyloxy)-5-(((benzyloxy)methyl)-1-((R)-but-3-en-2-yl)-2-((E)-styryl)pyrrolidin-3-ol (131)\)**

Triethylamine (47 \(\mu\)L, 0.34 mmol) was slowly added to a solution of 127 (166 mg, 0.340 mmol) in anhydrous CH\(_2\)Cl\(_2\) (5 mL) at 0 °C under a N\(_2\) atmosphere. Whilst stirring, a 0.13 M solution of methanesulfonyl chloride in anhydrous CH\(_2\)Cl\(_2\) (2.62 mL, 0.340 mmol) was slowly added to the reaction mixture. The reaction mixture was heated at 40 °C and stirred for 10 h or until TLC analysis confirmed full consumption of the starting material. The
reaction was quenched through the addition of a sat. NaHCO₃ solution (4 mL) and extracted with CH₂Cl₂ (3 x 5 mL). The combined organic extracts were dried over MgSO₄, gravity filtered and concentrated in vacuo to give an orange oil. Purification by FCC (5% MeOH/CH₂Cl₂) gave 131 as a yellow oil (101 mg, 63%).

\[ R_f(5\text{%} \text{MeOH/CH}_2\text{Cl}_2) = 0.45 \]

LRESIMS m/z 470 [M+H]+.

\(^1\)H NMR (500 MHz, CDCl₃) δ 7.42 – 7.14 (15H, m, ArH), 6.58 (1H, dd, J = 16.0, 9.7 Hz, H1‴), 6.48 (1H, d, J = 16.0 Hz, H2‴), 5.80 (1H, ddd, J = 17.4, 10.3, 7.6 Hz, H2′), 5.06 (1H, d, J = 17.4 Hz, H3′) trans, 4.94 (1H, d, J = 10.3 Hz, H3′) cis, 4.63 – 4.46 (4H, m, OCH₂Ph), 4.01 – 3.89 (3H, m, H2, H3, H4), 3.57 (1H, dd, J = 9.7, 3.4 Hz, H1‴), 3.53 – 3.43 (2H, m, H1′, H1‴), 3.33 (1H, s, H5), 1.15 (3H, d, J = 6.3 Hz, H1″).

\(^13\)C NMR (125 MHz, CDCl₃) δ 142.8 (C2′), 138.5 (ArC), 137.8 (ArC), 137.4 (ArC), 131.3 (C2‴), 130.3 (C1‴), 128.7 (ArCH), 128.5 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.8 (2 x ArCH), 127.7 (ArCH), 127.3 (ArCH), 126.4 (ArCH), 114.5 (C3′), 88.6 (C4), 77.9 (C3), 73.6 (OCH₂Ph), 72.8 (C2), 71.7 (OCH₂Ph), 71.5 (C1‴), 66.2 (C5), 56.9 (C1′), 19.6 (C1″).

(1R,2R,3R,5R,7aR)-2-(Benzyloxy)-3-((benzyloxy)methyl)-5-methyl-2,3,5,7a-tetrahydro-1H-pyrrolizin-1-ol (132)

To a solution of 131 (120 mg, 0.255 mmol) in anhydrous CH₂Cl₂ (20 mL) under N₂ conditions was added p–toluenesulfonic acid monohydrate (73 mg, 0.384 mmol). The reaction mixture was held at reflux for 30 min or until TLC confirmed the full consumption of the starting material. After cooling to room temperature, the reaction mixture was concentrated in vacuo to give a dark brown oil. The oil was dissolved in anhydrous CH₂Cl₂ (20 mL) and purged with a balloon of argon gas. Hoveda Grubb’s II generation ruthenium catalyst (11 mg, 0.051 mmol) was added to the solution and purged with another balloon of argon gas. The reaction mixture was heated at reflux for 3 days under an argon gas...
atmosphere. After TLC confirmed the full consumption of the starting material, the reaction mixture was washed with Na$_2$CO$_3$ (3 x 5 mL), dried over MgSO$_4$ followed by concentration in vacuo to give a black oil. Purification by FCC (2% MeOH/CH$_2$Cl$_2$) gave the title compound 132 as a black oil (61 mg, 65%).

$R_f(2\%$ MeOH/CH$_2$Cl$_2) = 0.27$

$[\alpha]_D^{25} = -27.0 (c$ 0.75, CHCl$_3$).

**IR** $\delta_{max}$ (cm$^{-1}$): 3390, 2980, 2862, 1110, 896, 733 cm$^{-1}$

**LRESIMS** m/z 366 (100%) [M+H$^+$].

**HRESIMS** found 366.2075, calcd for C$_{23}$H$_{28}$NO$_3$, 366.2069 [M+H$^+$].

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.42 – 7.20 (10H, m, ArH), 5.84 (1H, d, $J = 5.9$ Hz, H7), 5.69 (1H, d, $J = 5.9$ Hz, H6), 4.73 – 4.49 (4H, m, OCH$_2$Ph), 4.37 – 4.27 (2H, m, H7a, H5), 3.98 (1H, s, H1), 3.88 (1H, t, $J = 4.3$ Hz, H2), 3.67 – 3.49 (3H, m, H3, H1'), 1.26 (3H, d, $J = 5.3$ Hz, H1 '').

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 137.7 (ArC), 136.1 (ArC), 133.8 (C6), 128.47 (ArCH), 128.4 (ArCH), 127.9 (ArCH), 127.78 (ArCH), 127.75 (ArCH), 127.6 (ArCH), 127.4 (C7), 89.1 (C2), 80.7 (C1), 73.8 (OCH$_2$Ph), 73.5 (C1''), 72.5 (OCH$_2$Ph), 64.2 (C5 or C7a), 64.0 (C5 or C7a), 62.1 (C3), 18.0 (C1').

$^{1H,2R,3R}$-2-(Benzyloxy)-3-((benzyloxy)methyl)-5-methyl-2,3-dihydro-1H-pyrrolizin-1-ol (133)

Compound 132 (55 mg, 0.15 mmol) was dissolved in acetone/water (3:1, 12 mL,) and the solution was cooled to 0 °C. N–methylmorpholine–N–oxide (35 mg, 0.30 mmol) and potassium osmate dihydrate (4 mg, 0.011 mmol) were added and the solution was stirred vigorously at 35 °C for 48 h. The reaction was concentrated in vacuo, affording a black viscous oil which was diluted with H$_2$O (20 mL) and extracted with EtOAc (3 x 15 mL). The combined extracts were dried (MgSO$_4$), filtered and concentrated in vacuo to afford a
black oil which was subsequently purified by FCC (5:95 MeOH/CH₂Cl₂). This gave pure compound 133 (37 mg, 68%) as a black oil and recovered starting material 132 (8 mg, 15%).

R_f(5%MeOH/CH₂Cl₂) = 0.70

LRESIMS m/z 386 [M+Na]^+

^1H NMR (500 MHz, CDCl₃) δ 7.37 – 7.23 (9H, m, ArH), 7.14 – 7.08 (1H, m, ArH), 6.04 (1H, d, J = 2.9 Hz, H7), 5.97 (1H, bs, H6), 4.92 – 4.84 (1H, m, H1), 4.73 (1H, d, J = 12.0 Hz, OCH₂Ph), 4.61 (1H, d, J = 12.0 Hz, OCH₃Ph), 4.47 (1H, d, J = 12.1 Hz, OCH₂Ph), 4.41 (1H, d, J = 12.1 Hz, OCH₃Ph), 4.35 (1H, s, H2), 4.26 (1H, bs, H3), 3.71 (2H, bs, H1’), 3.22 (1H, d, J = 10.4 Hz, OH), 2.11 (3H, s, H1”).

^13C NMR (125 MHz, CDCl₃) δ 137.7 (ArC), 136.8 (ArC), 136.7 (C7a), 128.7 (2 x ArC), 128.6 (2 x ArCH), 128.2 (ArCH), 128.0 (ArCH), 127.9 (4 x ArCH), 123.5 (C5), 111.5 (C6), 101.6 (C7), 93.2 (C2), 73.6 (OCH₂Ph), 71.8 (OCH₃Ph), 71.0 (C1), 69.4 (C8), 62.7 (C3), 11.9 (C9).

(4R,5R)-5-(((1R,2S)-1,3-Bis(benzyloxy)-2-hydroxypropyl)-3-((R)-but-3-en-2-yl)-4-((E)-styryl)oxazolidin-2-one (137) and (4R,5R,6S,7S)-6-(benzyloxy)-7-((benzyloxy)methyl)-3-((R)-but-3-en-2-yl)-5-hydroxy-4-((E)-styryl)-1,3-oxazepan-2-one (138)

Procedure A: Compound 127 (540 mg, 1.11 mmol) was dissolved in anhydrous CH₂Cl₂ (40 mL) and cooled to 0 °C under an atmosphere of nitrogen. Triethylamine (310 µL, 2.22 mmol) was added and after 15 min triphosgene (164 mg, 0.555 mmol) was added portionwise and the solution was allowed to warm to rt. After stirring for 1 h, TLC analysis confirmed full consumption of the starting material. The reaction was diluted with H₂O (15 mL) and extracted with CH₂Cl₂ (3 x 15 mL). The combined extracts were dried (MgSO₄), filtered and concentrated in vacuo to afford a brown oil which whereby purification by FCC (gradient of 1:5 EtOAc/n-hexane to 1:1 EtOAc/n-hexane) produced
an inseparable mixture of isomers (37%, 1:2) consisting of the oxazolidinone and the oxazepinone respectively. Pure samples of 137 (12 mg) and 138 (20 mg) could be obtained for analytical purposes.

Procedure B: To solution of 139 (500 mg, 0.851 mmol) in dry THF (15 mL) at 0 °C was added NaH (85.0 mg, 2.13 mmol, 60% in mineral oil). The mixture was heated at 65 °C for 45 min, or until full consumption of 139 by TLC analysis. The reaction mixture was cooled to 0 °C, and quenched by the slow addition of water (10 mL). The solution was extracted with EtOAc (3 x 20 mL). The combined extracts were dried (MgSO₄), filtered and concentrated in vacuo. Purification by FCC (1:1 EtOAc/n-hexane) returned the oxazolidinone 137 as a pale–yellow oil (372 mg, 85%).

137:

Rf 0.65 (1:1 EtOAc/n-hexane).

LRESIMS m/z 536 (100%) [M+Na]⁺.

1H NMR (500 MHz, CDCl₃) δ 7.39 – 7.19 (13H, m, ArH), 7.18 – 7.00 (2H, m, ArH), 6.56 (1H, d, J = 15.8 Hz, H2‴), 6.12 (1H, dd, J = 15.8, 10.9 Hz, H1‴), 5.99 – 5.89 (1H, m, H2′), 5.25 – 5.09 (2H, m, H3′), 4.87 – 4.76 (2H, m, H5, OCH₂Ph), 4.56 (1H, d, J = 11.2 Hz, OCH₂Ph), 4.47– 4.39 (2H, m, H4, H1′), 4.32 (2H, s, OCH₂Ph), 3.96 (1H, s, H2‴), 3.79 (1H, dd, J = 7.0, 2.3 Hz, H1‴), 3.53 – 3.39 (2H, m, H3‴), 2.37 (1H, s, OH), 1.30 (3H, d, J = 6.5 Hz, H1‴). 13C NMR (125 MHz, CDCl₃) δ 156.9 (C2), 137.93 (ArC), 137.89 (ArC), 136.9 (C2′), 135.6 (ArC), 135.4 (C2‴), 129.2 (2 x ArCH), 128.9 (ArCH), 128.7 (4 x ArCH), 128.5 (2 x ArCH), 127.9 (ArCH), 127.2 (ArCH), 127.3 (2 x ArCH), 126.7 (2 x ArCH), 124.7 (C1‴), 117.1 (C3′), 77.8 (C5), 76.5 (C1‴), 74.2 (OCH₂Ph), 73.1 (OCH₂Ph), 70.6 (C3‴), 68.9 (C2‴), 60.0 (C4), 51.7 (C1′), 18.4 (C1″).

138:

Rf 0.65 (1:1 EtOAc/n-hexane).

LRESIMS m/z 536 (100%) [M+Na]⁺.

1H NMR (500 MHz, CDCl₃) δ 7.35 – 7.16 (13H, m, ArH), 7.12 – 7.06 (2H, m, ArH), 6.69 (1H, dd, J = 15.9, 9.0 Hz, H1‴), 6.43 (1H, d, J = 15.9 Hz, H2‴), 6.06 (1H, ddd, J = 17.4, 10.5, 5.1 Hz, H2′), 5.34 (1H, d, J = 10.0 Hz, H3″cis), 5.31 (1H, d, J = 16.2 Hz, H3″trans), 4.79 (1H, td, J = 6.8, 1.8 Hz, H1′), 4.62 (1H, d, J = 11.8 Hz, OCH₂Ph), 4.53 – 4.50 (3H,
m, H7 and 2 x OCH₂Ph), 4.40 (1 H, d, J = 11.2 Hz, OCH₂Ph), 4.07 – 4.04 (1H, m, H5), 4.01 (1 H, dd, J = 9.0, 4.3 Hz, H4), 3.83 (1H, dd, J = 9.3, 6.0 Hz, H1‴‴), 3.78 (1H, d, J = 9.3 Hz, H1‴‴), 3.75 – 3.73 (1H, m, H6), 1.84 (1H, d, J = 5.8 Hz, OH), 1.28 (3H, d, J = 6.8 Hz, H1″).

¹³C NMR (125 MHz, CDCl₃) δ 158.4 (C2), 139.2 (C2′), 138.0 (ArC), 137.6 (ArC), 136.5 (ArC), 131.6 (C2‴′), 128.7 (ArCH), 128.6 (2 x ArCH), 128.3 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 127.3 (C1‴′), 126.8 (ArCH), 118.1 (C3′), 78.2 (C6), 74.8 (C7), 73.6 (OCH₂Ph), 72.9 (OCH₂Ph), 69.0 (C5), 68.4 (C1‴′), 61.6 (C4), 55.6 (C1′), 16.1 (C1″).

tert-Butyl ((3R,4R,5S,6S,E)-5,7-Bis(benzyloxy)-4,6-dihydroxy-1-phenylhept-1-en-3-yl)((R)-but-3-en-2-yl)carbamate (139).

To a solution of 127 (1.10 g, 2.25 mmol) in dry 1,2-dichloroethane (10 mL) was added dry N,N–diisopropylethylamine (784 µL, 4.50 mmol) and di–tert–butyl–dicarbonate (0.980 g, 4.50 mmol). The reaction mixture was stirred at 70 °C for 48 h. CH₂Cl₂ (15 mL) was added and the solution was washed with 1 M HCl (2 x 20 mL), and brine. The organic fraction was dried (MgSO₄), filtered and concentrated in vacuo. Purification by FCC (1:4 EtOAc/n-hexane, then to 5:95 MeOH/CH₂Cl₂) gave 139 (0.820 g, 62%) and recovered starting material 127 (0.250 g, 23%) as pale–yellow and brown oils, respectively.

Rf 0.30 (3:7 EtOAc/n-hexane).

[α]₂⁵° +46.5 (c 1.00, CHCl₃).

IR νₘₐₓ (cm⁻¹): 3383, 3029, 2976, 2869, 1680, 1253, 1094 cm⁻¹

LRESIMS m/z 588 (100%) [M+H]⁺.

HRESIMS found 588.3311, calcd for C₃₆H₄₆NO₆, 588.3325 [M+H]⁺.

¹H NMR (500 MHz, CDCl₃) δ 7.40 – 7.19 (15H, m, ArH), 6.59 (1H, dd, J = 16.1, 7.0 Hz, H6), 6.49 (1H, d, J = 16.1 Hz, H7), 5.97 (1H, ddd, J = 17.0, 10.7, 4.9 Hz, H2′), 5.06 (1H,
t, \( J = 13.8 \) Hz, H3’), 4.74 (1H, d, \( J = 11.2 \) Hz, OCH2Ph), 4.67 (1H, d, \( J = 11.2 \) Hz, OCH2Ph), 4.51 (1H, d, \( J = 11.8 \) Hz, OCH2Ph), 4.46 (1H, d, \( J = 11.8 \) Hz, OCH2Ph), 4.40 – 4.27 (2H, m, H1’, H5), 4.09 (2H, s, H4, H2), 3.65 (1H, bs, H3), 3.56 (2H, app. q, \( J = 9.5, 8.2 \) Hz, H1AB), 1.47 (9H, s, (CH3)3C), 1.26 (2H, d, \( J = 6.7 \) Hz, H1’’).

\( ^{13} \)C NMR (125 MHz, CDCl3) \( \delta \) 139.8 (C2’), 138.2 (CO), 138.1 (ArC), 137.0 (ArC), 133.25 (C7), 128.5 (ArCH), 128.4 (ArCH), 128.4 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 126.6 (C6), 115.4 (C3’), 80.8 (CH3)3C, 78.5 (C3), 74.9 (C4 or C2), 74.5 (OCH2Ph), 73.4 (OCH2Ph), 71.4 (C1), 70.8 (C4 or C2), 59.6 (C1’), 54.5 (C5), 28.6 (CH3)3C, 18.2 (C1’’).

**General method for Ring-closing Metathesis**

\( (1R,5R,7aR)-1-((1R,2S)-1,3-Bis(benzyloxy)-2-hydroxypropyl)-5-methyl-5,7a-dihydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (140) \)**

![140](image)

A solution of 137 (162 mg, 0.316 mmol) in anhydrous CH2Cl2 (10 mL) was treated with Grubbs’ II ruthenium catalyst (14 mg, 0.016 mmol). The mixture was heated at reflux for 4 h under a nitrogen atmosphere, by which time, TLC analysis confirmed full consumption of the starting material. The solution was concentrated in vacuo to afford a black oil. Purification by FCC (gradient of 1:1 EtOAc/n-hexane to 4:1 EtOAc/n-hexane) afforded the title compound 140 as a pale–brown gum (110 mg, 85%).

\( R_f 0.19 \) (1:1 EtOAc/n-hexane).

**LRESIMS** \( m/z \) 432 (100%) [M+Na]+.

\( ^1 \)H NMR (500 MHz, CDCl3) \( \delta \) 7.40 – 7.19 (10H, m, ArH), 5.81 (1H, d, \( J = 6.6 \) Hz, H7), 5.79 (1H, d, \( J = 6.6 \) Hz, H6), 5.04 (1H, s, H1), 4.86 (1H, dd, \( J = 8.5, 4.8 \) Hz, H7a), 4.68 (1H, d, \( J = 11.2 \) Hz, OCH2Ph), 4.54 (1H, d, \( J = 11.2 \) Hz, OCH2Ph), 4.50 (1H, d, \( J = 11.8 \) Hz, OCH2Ph), 4.40 – 4.27 (2H, m, H1’, H5), 4.09 (2H, s, H4, H2), 3.65 (1H, bs, H3), 3.56 (2H, app. q, \( J = 9.5, 8.2 \) Hz, H1AB), 1.47 (9H, s, (CH3)3C), 1.26 (2H, d, \( J = 6.7 \) Hz, H1’’).
OCH₂Ph), 4.45 (1H, d, J = 11.8 Hz, OCH₂Ph), 4.26 (1H, app. t, J = 6.3 Hz, H5), 3.95 (1H, dd, J = 6.1, 3.7 Hz, H2'), 3.65 – 3.56 (3H, m, H3', H1'), 2.51 (1H, d, J = 5.7, OH), 1.39 (3H, d, J = 6.8, H1'').

13C NMR (125 MHz, CDCl₃) δ 156.5 (C3), 137.9 (ArC), 137.5 (ArC), 137.4 (C6), 128.5 (2 x ArCH), 128.4 (2 x ArCH), 128.2 (2 x ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.8 (2 x ArCH), 123.8 (C7), 77.0 (C1'), 75.9 (C7a), 73.9 (OCH₂Ph), 73.4 (OCH₂Ph), 71.1 (C3'), 69.3 (C2'), 68.6 (C1), 60.0 (C5), 16.1 (C1'').

Tert-butyl (2R,SR)-2-((1R,2S,3S)-2,4-bis(benzyloxy)-1,3-dihydroxybutyl)-5-methyl-2,5-dihydro-1H-pyrrole-1-carboxylate (141)

Compound 141 was synthesized from 139 (305 mg, 0.519 mmol), using the general method for ring–closing metathesis. The crude black oil was purified by FCC (5%MeOH/CH₂Cl₂) to give 141 as a grey oil (175 mg, 70%).

Rf (2:5 EtOAc/n-hexane) = 0.64
[α]D²⁵ +73.5 (c 0.27, CHCl₃).
IR νmax (cm⁻¹): 3300, 2956, 2847, 1823, 1590, 1009 cm⁻¹
LRESIMS m/z 506 (100%) [M+Na]⁺
HRESIMS found 506.2542, calcd for C₂₈H₃⁷NO₆Na, 506.2519 [M+Na]⁺.
1H NMR (500 MHz, CDCl₃) δ 7.4 – 7.2 (10H, m, ArH), 5.9 – 5.6 (2H, m, H3, H4), 4.7 (2H, bs, OCH₂Ph), 4.6 – 4.4 (3H, m, OCH₂Ph, H5), 4.1 (2H, bs, H3', H1''), 3.8 – 3.5 (3H, m, H2', H4'), 1.5 (9H, s, (CH₃)₃C), 1.3 – 1.2 (3H, m, H1'').
13C NMR (125 MHz, CDCl₃) δ 139.0 (ArC), 138.8 (CO) 137.2 (ArC), 134.2 (C4), 128.6 (ArCH), 128.2 (ArCH), 128.0 (ArCH), 125.1 (C3), 79.9 (C2'), 74.7 (OCH₂Ph), 73.6 (OCH₂Ph), 72.5 (C3' or C1'), 71.7 (C4'), 71.5 (C3' or C1'), 60.8 (C5), 28.7 (CH₃)₃C, 21.2 (C1'').
General method for cis-dihydroxylation

(1R,5R,6S,7R,7aR)-1-((1R,2S)-1,3-Bis(benzyloxy)-2-hydroxypropyl)-6,7-dihydroxy-5-methyltetrahydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (142) and (1R,5R,6R,7S,7aR)-1-((1R,2S)-1,3-bis(benzyloxy)-2-hydroxypropyl)-6,7-dihydroxy-5-methyltetrahydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (143).

Compound 140 (177 mg, 0.432 mmol) was dissolved in acetone/water (3:1, 12 mL,) and the solution was cooled to 0°C. N–methylmorpholine–N–oxide (103 mg, 0.879 mmol) and potassium osmate dihydrate (9.6 mg, 0.026 mmol) were added and the solution was stirred vigorously at 35°C for 48 h. The reaction was concentrated in vacuo, affording a black viscous oil which was diluted with H2O (20 mL) and extracted with EtOAc (3 x 15 mL). The combined extracts were dried (MgSO4), filtered and concentrated in vacuo to afford a black oil which was subsequently purified by FCC (gradient of 5:95 MeOH/CH2Cl2 to 1:9 MeOH/CH2Cl2). This gave pure samples of the title compounds 142 (10 mg, 5%), and 143 (7 mg, 4%), as colourless oils, and a 3.5:1 mixture of 142 and 143 (132 mg, 69%).

142:

Rf 0.10 (5:95 MeOH/CH2Cl2).
[α]D –4.1 (c 1.00, CHCl3).
IR νmax (cm⁻¹): 3394, 2940, 1454, 1375, 1259, 1212, 1154, 1071, 1028, 866 cm⁻¹
LRESIMS m/z 466 (100%) [M+Na]+.
HRESIMS found 466.1858, calcd for C24H29NO7Na, 466.1842 [M+Na]+.

1H NMR (500 MHz, CDCl3) δ 7.41 – 7.21 (10H, m, ArH), 4.93 – 4.81 (2H, m, H1, OCH2Ph), 4.62 (1H, d, J = 11.3 Hz, OCH2Ph), 4.43 (1H, d, J = 11.5 Hz, OCH2Ph), 4.35 (1H, d, J = 11.5 Hz, OCH2Ph), 4.08 (1H, t, J = 8.4, H7a) 4.05– 3.98 (2H, m, H6, H2’), 3.89 (1H, dd, J = 8.0, 1.8 Hz, H1’a), 3.78 (1H, d, J = 4.4 Hz, H7), 3.60 – 3.51 (2H, m, H6, H3’), 3.38 (1H, dd, J = 9.0, 7.3 Hz, H3’), 2.86 – 2.70 (2H, m, 2 x OH), 1.46 (3H, d, J = 8.9 Hz, H1’a).
13C NMR (125 MHz, CDCl₃) δ 156.7 (C3), 137.5 (ArC), 137.2 (ArC), 128.9 (ArCH), 128.8 (ArCH), 128.7 (ArCH), 128.5 (ArCH), 128.5 (ArCH), 128.3 (ArCH), 79.6 (C7), 75.6 (C1'), 74.6 (C1), 74.5 (OCH₂Ph), 73.8 (OCH₂Ph), 70.7 (C3'), 68.4 (C6, C2'), 65.1 (C7a), 59.7 (C5), 15.4 (C1'').

143:

Rf 0.08 (5:95 MeOH/CH₂Cl₂).

[α]D²⁵ +24.9 (c 1.00, CHCl₃).

LRESIMS m/z 466 (100%) [M+Na]⁺.

1H NMR (500 MHz, CDCl₃) δ 7.42 – 7.29 (8H, m, ArH), 7.29 – 7.15 (2H, m, ArH), 4.99 – 4.86 (2H, m, H1, OCH₂Ph), 4.56 (1H, d, J = 11.2 Hz, OCH₂Ph), 4.42 (1H, d, J = 11.4 Hz, OCH₂Ph), 4.38 (1H, d, J = 9.0 Hz, H1'), 4.33 (1H, d, J = 11.4 Hz, OCH₂Ph), 4.28 (1H, ddd, J = 10.7, 9.6, 3.8 Hz, H6), 4.04 (1H, d, J = 2.1 Hz, H7), 3.92 – 3.84 (2H, m, H7a, OH), 3.82 – 3.70 (2H, m, H5, H2'), 3.49 (1H, dd, J = 8.6, 5.0 Hz, H3'), 3.23 (1H, t, J = 8.5 Hz, H3'), 2.75 (1H, d, J = 10.5 Hz, OH), 2.72 (1H, d, J = 9.2 Hz, OH), 1.48 (3H, d, J = 7.0 Hz, H1'').

13C NMR (125 MHz, CDCl₃) δ 156.7 (C3), 137.6 (ArC), 136.8 (ArC), 129.0 (ArCH), 128.9 (ArCH), 128.7 (ArCH), 128.7 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 77.1 (C1), 75.2 (C1'), 74.9 (C6), 74.9 (OCH₂Ph), 73.9 (OCH₂Ph), 70.8 (C7), 70.8 (C3'), 68.7 (C2'), 62.5 (C7a), 54.5 (C5), 11.9 (C1''').

tert-Butyl (2R,3S,4R,5R)-2-((1R,2S,3S)-2,4-bis(benzyloxy)-1,3-dihydroxybutyl)-3,4-dihydroxy-5-methylpyrrolidine-1-carboxylate (144)

Compound 144 was synthesized from 141 (20.0 mg, 0.0414 mmol) using the general method for cis–dihydroxylation. The product 144 was purified by FCC (5:95 MeOH/CH₂Cl₂) to give the title compound as a clear oil (20 mg, 95%).

Rf (5:95 MeOH/CH₂Cl₂) = 0.23
[α]$_D$$^25$ +25.5 (c 1.50, CHCl$_3$).

IR $\delta_{\text{max}}$ (cm$^{-1}$): 3431, 2908, 2793, 1609, 1101, 1065, 1031, 949 cm$^{-1}$

LRESIMS $m/z$ 540 (100%) [M+Na]$^+$

HRESIMS found 540.2597, calcd for C$_{28}$H$_{39}$NO$_8$Na, 540.2573 [M+Na]$^+$.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.42 – 7.19 (10H, m, ArH), 4.72 (2H, s, OCH$_2$Ph), 4.57 (1H, d, $J$ = 11.7 Hz, OCH$_2$Ph), 4.51 (1H, d, $J$ = 11.7 Hz, OCH$_2$Ph), 4.45 (1H, t, $J$ = 4.7 Hz, H3), 4.27 – 4.19 (1H, m, H2'), 4.14 (1H, bs, H3'), 3.95 – 3.76 (3H, m, H4, H2, H5), 3.76 – 3.61 (3H, m, H4', H1'), 1.45 (9H, s, (CH$_3$)$_3$C), 1.22 (3H, d, $J$ = 6.8 Hz, H1 '').

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 138.7 (ArC), 138.6 (CO) 137.0 (ArC), 129.0 (ArCH), 128.9 (ArCH), 128.7 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 128.3 (ArCH), 128.24 (ArCH), 128.22 (ArCH), 128.0 (ArCH), 80.0 (C1'), 77.4 (C4), 74.7 (OCH$_2$Ph), 73.8 (OCH$_2$Ph), 71.4 (C4'), 70.80 (C3'), 70.79 (C2'), 70.78 (C3), 65.8 (C2), 59.8 (C5), 28.8 (CH$_3$)$_3$C), 18.7 (C1 ''.)

General method for acetonide protection of cis-diols

(3aS,3bR,4R,8R,8aR)-4-((1R,2S)-1,3-Bis(benzyloxy)-2-hydroxypropyl)-2,2,8-trimethyltetrahydro-4H,6H-[1,3]dioxolo[4',5':3,4]pyrrolo[1,2-c]oxazol-6-one (146) and (3aR,3bR,4R,8R,8aS)-4-((1R,2S)-1,3-bis(benzyloxy)-2-hydroxypropyl)-2,2,8-trimethyltetrahydro-4H,6H-[1,3]dioxolo[4',5':3,4]pyrrolo[1,2-c]oxazol-6-one (147).

To anhydrous acetone (2 mL) was added in order, concentrated H$_2$SO$_4$ (1.6 $\mu$L, 0.032 mmol), anhydrous CuSO$_4$ (36 mg, 0.23 mmol) and a solution of compound 142 (51 mg, 0.11 mmol) in acetone (1 mL). After vigorous stirring at rt for 1 h under a nitrogen atmosphere, TLC analysis confirmed full consumption of the starting diol. The reaction mixture was vacuum filtrated through a celite pad, and the pad was washed with additional acetone (3 x 2 mL). The filtrate was basified with 30% NH$_3$ solution (10.5 $\mu$L) and the (NH$_4$)$_2$SO$_4$ precipitate was separated from the solution by gravity filtration. The filtrate was concentrated in vacuo and purified by FCC (2:98 MeOH/diethyl ether) to afford
compound 146 as a clear oil (52 mg, 99%). Using this exact same method, compound 143 (40 mg, 0.088 mmol), was also treated under similar conditions, using acetone (2 mL), H$_2$SO$_4$ (1.3 µL, 0.026 mmol), anhydrous CuSO$_4$ (29 mg, 0.18 mmol), additional acetone (3 x 2 mL) and concentrated NH$_3$ (8.4 µL). Purification by FCC (2:98 MeOH/diethyl ether), afforded compound 147 as a clear oil (42 mg, 99%). This method was also used on a mixture of the two isomers 142 and 143. More specifically, to dry acetone (5 mL) was added in order, concentrated H$_2$SO$_4$ (4 µL, 0.076 mmol), anhydrous CuSO$_4$ (95 mg, 0.596 mmol) and a solution of 3.5:1 respective mixture of 142 and 143 (132 mg, 0.298 mmol) in acetone (2 mL). Using the same method as above, the title compounds 146 (102 mg, 71%) and 147 were separated (34 mg, 24%) as colourless oils after purification by FCC (2:98 MeOH/diethyl ether).

146:

R$_f$ 0.60 (2:98 MeOH/diethyl ether).

[α]$^\infty_{D}$ +43.8 ($c$ 1.00, CHCl$_3$).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3301, 2890, 1454, 1372, 1210, 1152, 733 cm$^{-1}$.

LRESIMS m/z 506 (100%) [M+Na]$^+$.

HRESIMS found 506.2180, calcd for C$_{27}$H$_{33}$NO$_7$Na, 506.2155 [M+Na]$^+$.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.42 – 7.19 (10H, m, ArH), 4.90 (1H, dd, $J$ = 8.4, 6.1 Hz, H4), 4.73 (2H, q, $J$ = 11.1 Hz, OCH$_2$Ph), 4.59 (1H, app. t, $J$ = 8.7 Hz, H3a), 4.50 (2H, q, $J$ = 11.8 Hz, OCH$_2$Ph), 4.19 – 4.06 (3H, m, H3b, H8a, H2'), 3.89 (1H, dd, $J$ = 5.9, 2.9 Hz, H1'), 3.60 (2H,qd, $J$ = 9.6, 6.2 Hz, H3'), 3.52 (1H,qd, $J$ = 7.0, 4.4 Hz, H8), 2.52 (1H, d, $J$ = 7.1 Hz, OH), 1.54 – 1.46 (6H, m, C2–CH$_3$, H1”), 1.26 (3H, s, C2–CH$_3$).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 156.2 (C6), 138.1 (ArC), 137.7 (ArC), 128.7 (ArCH), 128.7 (ArCH), 128.5 (ArCH), 128.3 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 114.8 (C2), 88.3 (C8a), 77.8 (C3a), 77.5 (C1’), 75.2 (C4), 74.0 (OCH$_2$Ph), 73.5 (OCH$_2$Ph), 71.0 (C3’), 69.2 (C2’), 67.5 (C3b), 59.4 (C8), 27.6 (C2–CH$_3$), 25.5 (C2–CH$_3$), 15.0 (C1”).

147:

R$_f$ 0.17 (2:98 MeOH/diethyl ether).

[α]$^\infty_{D}$ +52.7 ($c$ 1.00, CHCl$_3$).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3239, 2916, 1454, 1380, 1246, 1208, 753 cm$^{-1}$.
LRESIMS *m/z* 506 (100%) [M+Na]^+. 

**^1H NMR (500 MHz, CDCl₃)** δ 7.42 – 7.21 (10H, m, ArH), 4.98 (1H, t, *J* = 8.0 Hz, H₄), 4.92 (1H, d, *J* = 11.4 Hz, OCH₂Ph), 4.66 (1H, dd, *J* = 5.5, 3.7 Hz, H₃a ), 4.60 – 4.47 (3H, m, H₈a, OCH₂Ph), 4.41 (2H, m, OCH₂Ph, H¹'), 3.88 (1H, d, *J* = 5.8 Hz, H²'), 3.68 (1H, dd, *J* = 7.1, 3.5 Hz, H₃b), 3.57 (1H, dd, *J* = 9.4, 7.0 Hz, H³'), 3.41 (1H, dd, *J* = 9.4, 5.6 Hz, H³'), 3.33 (1H, dt, *J* = 11.1, 5.6 Hz, H₈), 2.43 (1H, d, *J* = 7.4 Hz, OH), 1.69 (3H, d, *J* = 7.0 Hz, H₁''), 1.48 (3H, s, C₂–CH₃), 1.21 (3H, s, C₂–CH₃).

**^13C NMR (125 MHz, CDCl₃)** δ 154.5 (C₆), 138.2 (ArC), 137.3 (ArC), 128.9 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.8 (ArCH), 95.0 (C₂), 83.3 (C₈a), 80.5 (C₃a), 78.2 (C₄), 75.7 (C¹'), 74.2 (OCH₂Ph), 73.6 (OCH₂Ph), 71.6 (C₃'), 70.6 (C²'), 64.6 (C₃b), 58.7 (C₈), 26.1 (C₂–CH₃), 24.2 (C₂–CH₃), 10.3 (C¹'').

(1S,2S)-1,3-Bis(benzyloxy)-1-((3aS,3bR,4R,8R,8aR)-2,2,8-trimethyl-6-oxotetrahydro-4H,6H-[1,3]dioxolo[4',5':3,4]pyrrolo[1,2-c]oxazol-4-yl)propan-2-yl methanesulfonate (148)

![Chemical Structure](image)

Triethylamine (20.0 µL, 0.145 mmol) was slowly added to a solution of 146, (70 mg, 0.145 mmol) in anhydrous CH₂Cl₂ (10 mL) at 0 °C under a N₂ atmosphere. Whilst stirring, a 0.13M solution of methanesulfonyl chloride in anhydrous CH₂Cl₂ (5.58 mL, 0.725 mmol) was slowly added to the reaction mixture. After stirring at 0 °C for 3 h, TLC analysis confirmed full consumption of the starting material and so the reaction was quenched through the addition of sat. NaHCO₃ solution (15 mL) and extracted with CH₂Cl₂ (3 x 15 mL) to give a pale–yellow oil. Purification of the residue by FCC (5%MeOH/CH₂Cl₂) gave 148 as a pale–yellow oil (81 mg, 99%).

**Rf** (5%MeOH/CH₂Cl₂) = 0.63

[*α*]²⁵D −64.1 (c 1.00, CHCl₃)

**IR** ν<sub>max</sub> (cm⁻¹): 3024, 2932, 2856, 1743, 1508, 1098, 1043, 961 cm⁻¹.
LRESIMS m/z 584 [M+Na]+.

HRESIMS found 584.2040, calcd for C_{28}H_{35}NO_9SNa, 584.1930 [M+Na]+.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.43 – 7.21 (10H, m, ArH), 5.15 (1H, ddd, J = 8.1, 4.4, 2.4 Hz, H2’), 4.88 (1H, dd, J = 8.9, 2.0 Hz, H4), 4.78 (1H, d, J = 11.2 Hz, OCH$_2$Ph), 4.59 (1H, d, J = 11.2 Hz, OCH$_2$Ph), 4.52 (2H, s, OCH$_2$Ph), 4.46 (1H, t, J = 5.8 Hz, H3a), 4.29 (1H, dd, J = 9.0, 4.8 Hz, H3b), 4.00 – 3.87 (4H, m, H8a, H1’, H3’), 3.51 (1H, qd, J = 7.1, 4.0 Hz, H8), 3.05 (3H, s, OMs), 1.50 (3H, s, C2–CH$_3$), 1.35 (3H, d, J = 6.9 Hz, H1’), 1.26 (3H, s, C2–CH$_3$).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 155.0 (C6), 137.4 (ArC), 136.3 (ArC), 128.8 (ArCH), 128.62 (ArCH), 128.64 (ArCH), 128.3 (ArCH), 128.0 (ArCH), 127.8 (ArCH), 114.1 (C2), 88.9 (C1’), 79.1 (C2’), 77.3 (C3a), 77.1 (C8a), 73.4 (OCH$_2$Ph), 72.6 (OCH$_2$Ph), 72.5 (C4), 68.7 (C3’), 67.1 (C3b), 58.4 (C8), 38.4 (OMs), 27.5 (C2–CH$_3$), 25.4 (C2–CH$_3$), 14.8 (C1’).

(1S,2S)-1,3-Bis(benzyloxy)-1-((3aR,3bR,4R,8R,8aS)-2,2,8-trimethyl-6-oxotetrahydro-4H,6H-[1,3]dioxolo[4’,5’:3,4]pyrrolo[1,2-c]oxazol-4-yl)propan-2-yl methanesulfonate (150)

Triethylamine (49 µL, 0.35 mmol) was slowly added to a solution of 147, (32 mg, 0.066 mmol) in anhydrous CH$_2$Cl$_2$ (8 mL) at 0 ºC under a N$_2$ atmosphere. Whilst stirring, a 0.13M solution of methanesulfonyl chloride in anhydrous CH$_2$Cl$_2$ (2.69 mL, 0.351 mmol) was slowly added to the reaction mixture. After stirring at 0 ºC for 3 h, TLC analysis confirmed full consumption of the starting material and so the reaction was quenched through the addition of sat. NaHCO$_3$ solution (15 mL) and extracted with CH$_2$Cl$_2$ (3 x 15 mL) to give a pale–yellow oil. Purification of the residue by FCC (5%MeOH/CH$_2$Cl$_2$) gave 150 as a pale–yellow oil (31 mg, 83%).

$R_f$(5%MeOH/CH$_2$Cl$_2$) = 0.73

$[\alpha]_D^{25}$ +33.8 (c 1.00, CHCl$_3$)
IR $\nu_{\text{max}}$ (cm$^{-1}$): 3024, 2953, 2850, 1758, 1512, 1102, 967 cm$^{-1}$.

LRESIMS $m/z$ 584 [M+Na]$^+$. 

HRESIMS found 584.1986, calcd for C$_{28}$H$_{35}$NO$_9$SNa, 584.1930 [M+Na]$^+$. 

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.42 – 7.29 (10H, m, ArH), 5.04 (1H, d, $J$ = 7.0 Hz, H2'), 5.01 – 4.92 (2H, m, H4, OCH$_2$Ph), 4.85 (1H, t, $J$ = 4.5 Hz, H8b), 4.66 (1H, d, $J$ = 11.7 Hz, OCH$_2$Ph), 4.62 (1H, t, $J$ = 4.5 Hz, H8a), 4.51 – 4.44 (3H, m, H1', OCH$_2$Ph), 3.91 – 3.83 (2H, m, H3', H3b), 3.43 – 3.32 (2H, m, H3', H8), 3.01 (3H, s, OMs), 1.77 (3H, d, $J$ = 7.1 Hz, H1''), 1.59 (3H, s, C2–CH$_3$), 1.31 (3H, s, C2–CH$_3$).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 158.3 (C6), 137.6 (ArC), 137.2 (ArC), 129.2 (ArCH), 128.8 (ArCH), 128.6 (ArCH), 128.3 (ArCH), 128.0 (ArCH), 112.5 (C2), 83.6 (C8a), 80.42 (C8b or C2'), 80.41 (C8b or C2'), 77.6 (C4), 75.2 (C1'), 74.4 (OCH$_2$Ph), 73.5 (OCH$_2$Ph), 69.9 (C3'), 64.0 (C3b), 58.4 (C8), 39.1 (OMs), 26.2 (C2–CH$_3$), 24.2 (C2–CH$_3$), 10.2 (C1 '').

**General method for oxazolidinone hydrolysis**

(1R,2S,3S)-2,4-Bis(benzyloxy)-1-((3aS,4S,6R,6aR)-2,2,6-trimethyltetrahydro-4H-[1,3]dioxolo[4,5-c]pyrrol-4-yl)butane-1,3-diol (152).

A solution of NaOH (46.0 mg, 1.15 mmol) in H$_2$O (3 drops) was added to a solution of oxazolidinone 146 (185 mg, 0.383 mmol) in EtOH (3 mL) in a microwave reaction tube. The tube was capped and the solution was stirred and irradiated in a CEM microwave reactor for 1 h (the temperature control was set at 110 °C, and the maximum applied power at 200 W). After cooling to rt, the reaction mixture was concentrated in vacuo, diluted with a solution of saturated NaCl (5 mL) and extracted with EtOAc (3 x 10 mL). The combined extracts were concentrated in vacuo to give a brown oil. Purification by FCC (5:95 MeOH/CH$_2$Cl$_2$) gave the target compound 152 as a pale–yellow oil (151 mg, 86%).

$R_f$ 0.27 (5:95 MeOH/CH$_2$Cl$_2$).

$[\alpha]^{25}_D$ –65.4 (c 1.00, CHCl$_3$).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3344, 3063, 2984, 1453, 1208, 1064, 607 cm$^{-1}$.
LRESIMS m/z 458 (100%) [M+H]+.

HRESIMS found 458.2554, calcd for C_{26}H_{36}NO_{6}, 458.2543 [M+H]+.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.41 – 7.24 (10H, m, ArH), 4.70 (2H, app. q, \(J = 11.3\) Hz, OCH\(_2\)Ph), 4.61 (1H, dd, \(J = 7.0, 4.7\) Hz, H3a), 4.52 (2H, app. q, \(J = 11.5\) Hz, OCH\(_2\)Ph), 4.06 (1H, dd, \(J = 8.8, 5.0\) Hz, H3'), 4.01 (1H, dd, \(J = 7.0, 5.2\) Hz, H6a), 3.74 (1H, dd, \(J = 7.0, 5.5\) Hz, H1'), 3.70 – 3.60 (3H, m, H4', H2'), 3.08 (2H, bs, OH), 1.48 (3H, s, C2–CH\(_3\)), 1.28 (3H, s, C2–CH\(_3\)), 1.04 (3H, d, \(J = 6.6\) Hz, H1').

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 137.9 (ArC), 137.9 (ArC), 128.6 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.9 (2 x ArCH), 114.0 (C2), 86.7 (C6a), 83.0 (C3a), 80.1 (C2'), 74.3 (OCH\(_2\)Ph), 73.4 (OCH\(_2\)Ph), 72.3 (C1'), 70.9 (C4'), 70.1 (C3'), 66.5 (C4), 59.5 (C6), 27.4 (C2–CH\(_3\)), 25.2 (C2–CH\(_3\)), 18.7 (C1').

\((1R,2S,3S)-2,4\)-Bis(benzyloxy)-1-((3a\(R\),4\(S\),6\(R\),6a\(S\))-2,2,6-trimethyltetrahydro-4\(H\)-[1,3]dioxolo[4,5-\(c\)]pyrrol-4-yl)butane-1,3-diol (153).

Compound 153 was synthesized from the acetonide 147 (205 mg, 0.414 mmol) using the general method for oxazolidinone hydrolysis. The crude product was purified by FCC (5:95 MeOH/CH\(_2\)Cl\(_2\)) to give the desired product 147 as a pale–yellow oil (165 mg, 87%).

\(R_f\) 0.22 (5:95 MeOH/CH\(_2\)Cl\(_2\)).

\([\alpha]^{25}_D = -24.9\) (c 1.00, CHCl\(_3\)).

IR \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3423, 3065, 2989, 1453, 1230, 1067 cm\(^{-1}\).

LRESIMS m/z 458 (100%) [M+H]+.

HRESIMS found 458.2540, calcd for C_{26}H_{36}NO_{6}, 458.2543 [M+H]+.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.41 – 7.20 (10H, m, ArH), 4.75 – 4.70 (2H, m, H3a, OCH\(_2\)Ph), 4.63 (1H, d, \(J = 11.4\) Hz, OCH\(_2\)Ph), 4.52 (2H, s, OCH\(_2\)Ph), 4.42 (1H, t, \(J = 4.8\) Hz, H6a), 4.06 – 3.98 (2H, m, H1', H3'), 3.73 (1H, dd, \(J = 9.9, 4.2\) Hz, H4'), 3.67 (1H, dd, 239
$J = 6.1, 3.9$ Hz, H2'), 3.61 (1H, dd, $J = 9.8, 4.6$ Hz, H4'), 2.77 – 2.65 (2H, m, H6, H4), 1.28 (3H, s, C2–CH3), 1.25 (3H, s, C2–CH3), 1.15 (d, $J = 6.6$ Hz, H1″).

$^{13}$C NMR (125 MHz, CDCl3) δ 138.3 (ArC), 138.2 (ArC), 128.7 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 110.8 (C2), 82.7 (C6a), 82.6 (C3a), 82.6 (C2'), 74.1 (OCH2Ph), 73.8 (OCH2Ph), 71.0 (C4'), 70.8 (C3'), 70.2 (C1'), 64.5 (C6), 58.1 (C4), 25.7 (C2–CH3), 24.1 (C2–CH3), 13.2 (C1″).

(2S,3S,4R)-1,3-Bis(benzyloxy)-4-hydroxy-4-((3aS,4S,6aR)-2,2,6-trimethyl-5-(methylsulfonyl)tetrahydro-4H-[1,3]dioxolo[4,5-c]pyrrol-4-yl)butan-2-yl methanesulfonate (154) and (3aR,4R,6R,7R,8R,8aS,8bS)-7-(benzyloxy)-6-((benzyloxy)methyl)-2,2,4-trimethylhexahydro-4H-[1,3]dioxolo[4,5-a]pyrrolizin-8-ol (149).

### Procedure A

Triethylamine (16 µL, 0.12 mmol) was slowly added to a solution of 152 (53.0 mg, 0.116 mmol) in anhydrous CH2Cl2 (8 mL) at 0 °C under a N2 atmosphere. Whilst stirring, a 0.13M solution of methanesulfonyl chloride in anhydrous CH2Cl2 (0.893 mL, 0.116 mmol) was slowly added to the reaction mixture. After stirring at 0 °C for 1.5 h, TLC analysis confirmed full consumption of the starting material and so the reaction was quenched through the addition of a sat. NaHCO3 solution (2 mL) and extracted with CH2Cl2 (3 x 5 mL). The combined organic extracts were dried over MgSO4, gravity filtered from inorganic salts and concentrated in vacuo to give a yellow oil. Purification by FCC (gradient of CH2Cl2 to 5%MeOH/CH2Cl2) gave the two title compounds, 154 as colourless crystals (44 mg, 32%) and 149 as a yellow oil (15 mg, 30%).

### Procedure B

To a solution of the amino–diol 152 (297 mg, 0.650 mmol) in dry CH2Cl2 (20 mL) at 0 °C was added Et3N (136 µL, 0.975 mmol), PPh3 (256 mg, 0.975 mmol) and CBr4 (323 mg, 0.975 mmol). The mixture was stirred at 0 °C for 5 min then allowed to warm to room temperature for 30 min by which time TLC analysis confirmed full consumption of the starting material. The mixture was poured into water (15 mL) and...
subsequently extracted with CH₂Cl₂ (3 x 20 mL). The combined extracts were dried (MgSO₄), filtered and concentrated \textit{in vacuo} to give a yellow oil. The residue was purified by FCC (3:5 EtOAc/n-hexane) to give 149 as a pale–yellow oil (248 mg, 87%). After this, compound 149 was dissolved in ethanol and was subjected to slow evaporation at room temperature over 48 h, resulting in the formation of large colourless crystals.

154:
\[ \text{R} \f 0.38 \text{ (5\% MeOH/CH}_2\text{Cl}_2) \]
\[ [\alpha]_{D}^{25} -2.7 \text{ (c 1.00, CHCl}_3) \]
\[ \text{M.P: 75 \textendash} 80 \text{oC} \]
\[ \text{IR } \nu_{\text{max}} \text{ (cm}^{-1}): 3484, 3400, 3064, 2958, 1333, 1153, 913, 622 \text{ cm}^{-1} \]
\[ \text{LRESIMS } m/z 636 [\text{M+Na}]^+ \]
\[ \text{HRESIMS } \text{found 636.1913, calcd for C}_{28}\text{H}_{39}\text{NO}_{10}\text{S}_2\text{Na, 636.1913 [M+Na]}^+ \]
\[ \text{^1H NMR (500 MHz, CDCl}_3) \delta 7.45 - 7.23 \text{ (10H, m, ArH), 4.98 (2H, d, J = 4.2 Hz, H5, H3'), 4.78 (2H, dd, J = 44.9, 10.7 Hz, OCH}_2\text{Ph), 4.53 (2H, dd, J = 25.1, 11.7 Hz, OCH}_2\text{Ph), 4.38 (1H, d, J = 4.8 Hz, H4), 4.14 (1H, s, H1), 4.10 - 3.90 \text{ (2H, m, H3, H1')}, 3.85 (3H, dd, J = 16.9, 7.3 Hz, H4', H2'), 3.00 (6H, d, J = 19.3 Hz, 2 x OMs), 2.78 (1H, d, J = 7.2 Hz, OH), 1.49 (3H, s, CH}_3, 1.36 (3H, d, J = 6.8 Hz, H1''), 1.28 (3H, s, CH}_3) \]
13\text{C NMR (125 MHz, CDCl}_3) \delta 137.7 (ArC), 137.5 (ArC), 128.7 (2 x ArCH), 128.7 (2 x ArCH), 128.5 (2 x ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.0 (2 x ArCH), 111.9 (C7), 86.3 (C4), 81.8 (C5), 79.5 (C3'), 76.7 (C1), 74.3 (OCH}_2\text{Ph), 73.7 (OCH}_2\text{Ph), 71.1 (C2'), 68.8 (C3 or C1'), 68.5 (C4'), 63.6 (C3 or C1'), 38.7 (OMs), 36.8 (OMs), 26.6 (CH}_3, 24.4 (CH}_3, 21.8 (C1'').

149:
\[ \text{R} \f 0.25 \text{ (5.95 MeOH/CH}_2\text{Cl}_2) \]
\[ [\alpha]_{D}^{25} +15.6 \text{ (c 1.00, CHCl}_3) \]
\[ \text{M.P: 92 \textendash} 94 \text{oC} \]
\[ \text{IR } \nu_{\text{max}} \text{ (cm}^{-1}): 3397, 2983, 1520, 1493, 1120, 1066, 866 \text{ cm}^{-1} \]
\[ \text{LRESIMS } m/z 440 (100\%) [\text{M+H}]^+ \]
\[ \text{HRESIMS } \text{found 440.2445, calcd for C}_{26}\text{H}_{34}\text{NO}_5, 440.2437 [\text{M+H}]^+ \]
**1H NMR (500 MHz, CDCl₃)** δ 7.44 – 7.16 (10H, m, ArH), 4.68 (1H, d, J = 12.0 Hz, OCH₂Ph), 4.63 (1H, dd, J = 6.8, 3.5 Hz, H8b), 4.58 (1H, d, J = 11.9 Hz, OCH₂Ph), 4.49 (1H, d, J = 11.9 Hz, OCH₂Ph), 4.45 (1H, d, J = 12.0 Hz, OCH₂Ph), 4.28 (1H, s, H8), 4.17 (1H, t, J = 7.3 Hz, H3a), 3.83 (1H, s, H7), 3.63 (1H, d, J = 3.4 Hz, H8a), 3.45 (1H, dd, J = 9.2, 2.8 Hz, H1’), 3.35 (1H, dd, J = 9.2, 4.0 Hz, H1’), 3.31 – 3.25 (2H, m, H4, H6), 1.49 (3H, s, C2–CH₃), 1.28 (6H, s, C2–CH₃, H1’’).

**13C NMR (125 MHz, CDCl₃)** δ 137.7 (ArC), 137.2 (ArC), 128.5 (ArC), 128.0 (ArC), 128.0 (ArC), 127.8 (ArC), 127.4 (ArC), 114.1 (C2), 88.4 (C7), 85.7 (C3a), 83.8 (C8b), 78.3 (C8a), 76.3 (C8), 73.8 (OCH₂Ph), 73.1 (C1’), 71.5 (OCH₂Ph), 63.7 (C4 or C6), 63.5 (C4 or C6), 27.8 (C2–CH₃), 25.6 (C2–CH₃), 14.9 (C1’’).

(3aS,4R,6R,7R,8R,8aS,8bR)-7-(Benzzyloxy)-6-((benzzyloxy)methyl)-2,2,4-trimethylhexahydro-4H-[1,3]dioxolo[4,5-a]pyrrolizin-8-ol (151).

Triethylamine (7.7 µL, 0.055 mmol) was slowly added to a solution of 153 (23 mg, 0.0503 mmol) in anhydrous DCE (8 mL) at 0 °C under a N₂ atmosphere. Whilst stirring, a 0.13M solution of methanesulfonyl chloride in anhydrous CH₂Cl₂ (423 µL, 0.0553 mmol) was slowly added dropwise to the reaction mixture. After stirring at 0 °C for 30 min, the reaction mixture was quenched through the addition of a sat. NaHCO₃ solution (2 mL) and extracted with CH₂Cl₂ (3 x 5 mL) followed by concentration in vacuo. The crude brown oil was dissolved in DCE followed by the addition of triethylamine (7.7 µL, 0.0553 mmol). The reaction mixture with was heated at 80 °C for 20 h. TLC analysis confirmed full consumption of the starting material and so the reaction was quenched through the addition of NaHCO₃ solution (10 mL) and extracted with CH₂Cl₂ (3 x 10 mL). The combined extracts were dried (MgSO₄), filtered and concentrated in vacuo to give a yellow oil. Purification by FCC (5:95 MeOH/CH₂Cl₂) gave pyrrolizidine 151 as a clear film (19 mg, 87%).

R₉ 0.38 (3:97 MeOH/CH₂Cl₂).
$[\alpha]^2_{D} + 28.7 (c 1.00, CHCl_3)$.

**IR** $\delta_{\text{max}}$ (cm$^{-1}$): 3390, 3033, 2983, 1544, 1502, 1065, 864 cm$^{-1}$.

**LRESIMS** $m/z$ 462 (100%) [M+Na]$^+$.  

**HRESIMS** found 462.2247, calcd for C$_{26}$H$_{33}$NO$_5$Na, 462.2256 [M+Na]$^+$.  

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.38 – 7.22 (10H, m, ArH), 4.68 (1H, d, $J = 11.9$ Hz, OCH$_2$Ph), 4.63 (1H, t, $J = 5.5$ Hz, H8b), 4.57 – 4.49 (4H, m, OCH$_2$Ph, H8, H3a), 4.46 (1H, d, $J = 12.1$ Hz, OCH$_2$Ph), 4.02 (1H, dd, $J = 6.4$, 4.1 Hz, H7), 3.60 (1H, dt, $J = 6.8$, 3.6 Hz, H6), 3.52 (1H, dd, $J = 10.1$, 4.1 Hz, H1'), 3.47 (1H, dd, $J = 10.1$, 3.2 Hz, H1'), 3.24 (1H, dd, $J = 5.0$, 3.6 Hz, H8a), 3.06 (1H, dt, $J = 7.0$, 4.1 Hz, H4), 1.48 (3H, s, C2–CH$_3$), 1.33 – 1.27 (6H, m, C2–CH$_3$, H1').

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 138.2 (ArC), 138.2 (ArC), 128.4 (ArCH), 128.3 (ArCH), 128.0 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 127.5 (ArCH), 112.0 (C2), 91.2 (C7), 85.1 (C3a or C8), 80.2 (C8b), 74.7 (C3a or C8), 74.4 (C8a), 73.4 (OCH$_2$Ph), 72.0 (OCH$_2$Ph), 69.8 (C1'), 62.9 (C6), 57.6 (C4), 26.1 (C2–CH$_3$), 24.0 (C2–CH$_3$), 12.0 (C1').

**General method for O-Benzyl and acetonide deprotection**

$(1R,2R,3R,5R,6R,7S,7aR)-3$-(Hydroxymethyl)-5-methylhexahydro-$1H$-pyrrolizine-$1,2,6,7$-tetraol [(+)-$7$-epi-hyacinthacine C$_5$] (155).

To a nitrogen purged solution of compound 149 (15 mg, 0.034 mmol) in MeOH (3 mL) was added PdCl$_2$ (9.0 mg, 0.051 mmol). A balloon of H$_2$ was attached to the system, and the reaction mixture was stirred at rt for 24 h. After LRESIMS analysis confirmed full consumption of the starting material, the mixture was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo to give a yellow film. An Amberlyst A–26 resin (0.750 g) basic ion–exchange chromatography column, was prepared by mixing the resin with a 15% aqueous ammonia solution (v/v) and allowing it to stand for 15 min followed by washing the column with distilled water until a pH close to 7 was achieved. The compound was dissolved in distilled
water (2 mL) and held on the column for 15 min before its elution with water (3 x 3 mL). Concentration in vacuo provided (+)-7-epi-hyacinthacine C₅ 155 (6.6 mg, 88%) as a colourless film.

[α]D²⁵ +5.4 (c 1.00, H₂O).  
**IR** νmax (cm⁻¹): 3362, 1637, 1074, 1055 cm⁻¹.  
**LRESIMS** m/z 242 (100%) [M+Na]⁺.  
**¹H NMR** (500 MHz, D₂O) δ 4.15 (1H, dd, J = 5.0, 2.7 Hz, H7), 3.94 (1H, t, J = 7.3 Hz, H2), 3.90 – 3.78 (2H, m, H1, H6), 3.71 – 3.59 (2H, m, H8), 3.21 – 3.13 (2H, m, H7a, H5), 3.10 (1H, dt, J = 7.2, 5.0 Hz, H3), 1.26 (3H, d, J = 7.0 Hz, H9).

**¹³C NMR** (125 MHz, D₂O) δ 78.5 (C1 or C6), 77.9 (C2), 75.9 (C6 or C1), 73.6 (C7), 72.6 (C7a), 62.9 (C8), 62.2 (C3), 58.8 (C5), 13.2 (C9).

(1R,2R,3R,5R,6S,7R,7aR)-3-(Hydroxymethyl)-5-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol [(-)-6-epi-hyacinthacine C₅] (156).

(−)-6-Epi-hyacinthacine C₅ 156 was synthesized by the general method for O-benzyl deprotection from 151 (10.0 mg, 0.0227 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo returning a yellow film. The compound was isolated through basic ion-exchange chromatography followed by concentration in vacuo providing the title compound 156 as a colourless film (4.8 mg, 98%).

[α]D²⁵ −7.2 (c 1.00, H₂O).  
**IR** νmax (cm⁻¹): 3300, 2909, 1420, 1098, 1055 cm⁻¹.  
**LRESIMS** m/z 220 (100%) [M+H]⁺.  
**HRESIMS** found 220.1211, calcd for C₉H₁₈NO₅, 220.1185 [M+H]⁺.
$^1$H NMR (500 MHz, D$_2$O) $\delta$ 4.39 – 4.28 (2H, m, H1, H7), 4.03 (1H, t, $J = 4.5$ Hz, H6), 3.91 (1H, t, $J = 8.3$ Hz, H2), 3.67 (2H, d, $J = 4.8$ Hz, H8), 3.38 (1H, quint, $J = 4.8$ Hz, H3), 3.31 (1H, t, $J = 7.6$ Hz, H7a), 3.24 (1H, dt, $J = 7.0$, 5.4 Hz, H5), 1.25 (3H, d, $J = 7.1$ Hz, H9).

$^{13}$C NMR (125 MHz, D$_2$O) $\delta$ 78.5 (C2), 74.8 (C6), 74.4 (C1), 70.8 (C7), 67.5 (C7a), 63.1 (C8), 62.2 (C3), 57.2 (C5), 10.9 (C9).
Chapter 3

2-(But-3-en-2-yl)isoindoline-1,3-dione (157)\(^{180}\)

![Structure](structure.png)

To anhydrous DMF (10 mL) under an N\(_2\) atmosphere was added 3–chloro–1–butene (3.32 mL, 0.0330 mmol) and phthalimide potassium salt (6.72 g, 0.0363 mmol). The solution was heated at reflux for 3 h and poured hot onto 200 g of ice. The reaction mixture was extracted with chloroform (3 x 20 mL). The combined extracts were washed with 1 N KOH (20 mL), water (20 mL), 0.5 N HCl (20 mL) and water (20 mL). The organic layer was dried (MgSO\(_4\)), filtered and concentrated \textit{in vacuo} to give brown needle like crystals. The product was recrystallised from 95% EtOH to give colourless needle shaped crystals 157 (6.64 g, quant.) which were used for the next step without further purification. The \(^1\)H and \(^{13}\)C NMR spectroscopic data of this product were consistent with those published.\(^{180}\)

\textbf{LRESIMS} \(m/z\) 202 (100%) [M+H]\(^+\).

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.83 (2H, ddt, \(J = 10.1, 7.7, 3.8\) Hz, ArH), 7.71 (2H, dd, \(J = 5.6, 3.1\) Hz, ArH), 6.20 (1H, ddd, \(J = 17.0, 10.3, 6.6\) Hz, H3'), 5.24 (1H, d, \(J = 17.2\) Hz, H4'\textit{trans}), 5.17 (1H, d, \(J = 10.3\) Hz, H4'\textit{cis}), 4.94 (1H, quint, \(J = 7.0\) Hz, H2'), 1.59 (3H, d, \(J = 7.1\) Hz, CH3).

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 167.8 (CO), 136.7 (ArC), 133.8 (ArC), 131.9 (ArC), 123.1 (C3'), 123.0 (ArC), 116.2 (Cl'), 48.8 (C2'), 18.1 (C1').

\((R,S)-\alpha\)-Methylallyl amine hydrochloride\(^{180}\)

![Structure](structure.png)

To a solution of 157 (5.00 g, 0.0248 mmol) in 95% EtOH (5 mL) at 0 °C was added hydrazine hydrate (3.08 mL, 0.0496 mmol as a 50–60% solution). The reaction mixture was heated at 60 °C for 3 h whereby it formed a white gelatinous solid. The solid was
cooled to 0 °C and treated with conc. HCl (12 mL, 0.124 mmol) until the solid turned to a less viscous white paste. The paste was concentrated in vacuo and dissolved in water (10 mL). The phthalimide by–product was removed by washing the aqueous layer once with diethyl ether which was discarded. The aqueous layer was made alkaline with KOH. The product was extracted with diethyl ether (4 x 50 mL). The combined extracts were acidified with 4 N HCl/EtOH followed by concentration in vacuo to give a brown crystalline product which was recrystallised from EtOH/diethyl ether (1:2) to give small brown crystals (2.53 g, 95%).

**LRESIMS** m/z 94 (100%) [M+Na]^+.

\[ ^1H \text{ NMR (500 MHz, CDCl}_3 \] δ 8.45 (3H, s, NH), 6.01 (1H, ddd, \( J = 17.1, 10.5, 6.4 \) Hz, H2), 5.46 (1H, d, \( J = 17.2 \) Hz, H1trans), 5.33 (1H, d, \( J = 10.5 \) Hz, H1cis), 4.04 – 3.92 (1H, m, H3), 1.53 (3H, d, \( J = 6.5 \) Hz, CH3).

\[ ^13C \text{ NMR (125 MHz, CDCl}_3 \] δ 134.8 (C2), 119.1 (C1), 49.8 (C3), 18.8 (CH3).

(2S,3S,4R,5R,E)-1,3-Bis(benzyloxy)-5-(((S)-but-3-en-2-yl)amino)-7-phenylhept-6-ene-2,4-diol (161) and (2S,3S,4R,5R,E)-1,3-bis(benzyloxy)-5-(((R)-but-3-en-2-yl)amino)-7-phenylhept-6-ene-2,4-diol (127)

[Chemical Structure Image]

Compound 161 was synthesized by the general method for the Petasis reaction using (+)-3,4-Di–O–benzyl–α,β–L–xylofuranose (2.00 g, 6.06 mmol), Et3N (845 µM, 6.06 mmol), (E)–2–phenylvinylboronic acid (896 mg, 6.06 mmol) and (R,S)–α–methylallyl amine-HCl (652 mg, 6.06 mmol) instead of the enantiomerically pure (R)–amine. The mixture was purified by FCC (5:95 MeOH/CH2Cl2) to give inseperable diastereomers as a brown oil (2.00 g, 65%, as a 2:1 mixture of 1’S 161 and 1’R 127 epimers). \(^1H\) and \(^{13}C\) NMR spectroscopic data of the formed 1’R epimer matched the corresponding data of compound 127 described above.

\( R_f \) 0.20 (3:97 MeOH/CH2Cl2).
confirmed full consumption of the starting material, leading to the subsequent portionwise and the solution was allowed to warm to rt. A 2 : 1 mixture of (137, 162). 

\[(\text{benzyloxy)methyl)}\]

\((\text{benzyloxy})\text{oxazolidin-2-one} \) and \((\text{benzyloxy)methyl)}\)-3-(but-3-en-2-yl)-5-hydroxy-4-((E)-styryl)-1,3-oxazepan-2-one \((138, 162)\).

A 2 : 1 mixture of \(127\) and \(161\) (50.0 mg, 0.102 mmol) were dissolved in anhydrous CH\(_2\)Cl\(_2\) (15 mL) and cooled to 0 °C under an atmosphere of nitrogen. Triethylamine (28 µL, 0.204 mmol) was added and after 15 min triphosgene (15.1 mg, 0.0510 mmol) was added portionwise and the solution was allowed to warm to rt. After stirring for 1 h, TLC analysis confirmed full consumption of the starting material, leading to the subsequent in vacuo
The reaction mixture was concentrated in vacuo via FCC (gradient of 1:5 EtOAc/n-hexane to 1:1 EtOAc/n-hexane) produced an inseparable mixture of the four title isomers consisting of the oxazolidinones **137**, **160** and the oxazepinones **138**, **162** as confirmed by $^1$H NMR analysis and LRESIMS (43%).

$R_f$ 0.65 (1:1 EtOAc/n-hexane).

**LRESIMS** $m/z$ 536 (100%) [M+Na]$^+$.  

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.39 – 7.02 (m), 6.73 – 6.61 [m, H1” (138 and 162)], 6.60 – 6.51 [m, H2” (137 and 160)], 6.41 [d, $J$ = 15.9 Hz, H2” (138)], 6.34 [d, $J$ = 15.8 Hz, H2” (162)], 6.17 – 6.03 {m, H1” [137 and 160, H2’ (138)]}, 5.93 [ddd, $J$ = 17.4, 10.6, 4.7 Hz, H2’ (137 and 162)], 5.83 [ddd, $J$ = 17.1, 10.3, 6.5 Hz, H2’ (160)], 5.30 – 5.21 (m), 5.21 – 5.07 (m), 5.07 – 5.00 (m), 4.89 – 4.71 (m), 4.61 – 4.43 (m), 4.37 – 4.25 (m), 4.11 – 4.03 (m), 4.02 – 3.95 (m), 3.82 [dd, $J$ = 9.5, 6.1 Hz, H1” (162)], 3.79 – 3.71 (m), 3.51 – 3.42 (m), 1.38 [d, $J$ = 7.1 Hz, H1” (160)], 1.34 [d, $J$ = 6.9 Hz, H1” (162)], 1.29 [d, $J$ = 7.0 Hz, H1” (137)], 1.27 [d, $J$ = 6.3 Hz, H1” (138)].

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 158.6 [C2 (162)], 158.5 [C2 (138)], 157.0 [C2 (137)], 156.9 [C2 (160)], 139.2 [C2’ (138)], 138.3 [C2’ (162)], 138.2, 137.88, 137.86, 137.8, 137.7, 137.5, 137.5, 136.8, 136.6, 136.4, 135.7, 135.5, 135.42, 135.39, 131.8, 131.2, 128.8, 128.6, 128.5, 128.4, 128.38, 128.36, 128.2, 127.91, 127.87, 127.85, 127.7, 127.6, 127.59, 127.56, 127.3, 126.70, 126.67, 126.7, 126.57, 124.55 [C1” (137)], 124.4 [C1” (160)], 117.3, 117.2 [C3’ (137)], 116.1, 116.0 [C3’ (160)], 78.6, 78.2, 77.8 [C5 (137)], 77.4, 76.5 [C1”” (160)], 76.3 [C1”” (137)], 74.8, 74.7, 74.1 [OCH$_2$Ph (137)], 74.0 [OCH$_2$Ph (160)], 73.4, 73.1 [OCH$_2$Ph (160)], 73.1 [OCH$_2$Ph (137)], 72.6, 72.5, 70.7 [C3”” (160)], 70.6 [C3”” (137)], 68.9 [C2”” (160)], 68.8 [C2”” (137)], 68.4, 67.9, 62.1 [C4 (162)], 61.6 [C4 (138)], 60.1 [C4 (160)], 60.0 [C4 (137)], 55.6 [C1’ (138)], 55.3 [C1’ (162)], 51.8 [C1’ (160)], 51.7 [C1’ (137)], 18.4 [C1” (137)], 17.2 [C1” (160)], 16.7 [C1” (162)], 16.0 [C1” (138)].

Conversion of 138 and 162 to 137 and 160, respectively. DBU (11.1 µL, 0.0745 mmol) was added to a solution of the above mixture of oxazolidinones **137**, **160** and oxazepinones **138**, **162** (25.5 mg, 0.0497 mmol) in MeOH (3 mL) under a nitrogen atmosphere. After 4 h of stirring, TLC analysis confirmed full consumption of the starting materials. The reaction mixture was concentrated *in vacuo* followed by dissolution in CH$_2$Cl$_2$ (5 mL). The
solution was washed with a saturated solution of NH₄Cl (2 x 3 mL), dried (MgSO₄), filtered and concentrated in vacuo. This afforded an inseparable mixture (1:2.5) of 137 and its C1’ epimer 160 (25.3 mg, 99%). No further purification was required based on ¹H NMR and TLC analysis.

Rᵣ (1:1 EtOAc/n-hexane) = 0.65

LRESIMS m/z 536 (100%) [M+Na]⁺.

¹H NMR (500 MHz, CDCl₃) δ 7.38 – 7.18 (m, ArH), 7.17 – 7.04 (m, ArH), 6.61 – 6.52 (m), 6.18 – 6.05 (m), 5.95 [ddd, J = 16.8, 10.4, 5.8 Hz, H2’ (137)], 5.84 [ddd, J = 17.0, 10.3, 6.4 Hz, H2’ (160)], 5.24 – 5.16 (m), 5.14 – 5.07 (m), 5.06 – 4.99 (m), 4.89 – 4.81 (m), 4.80 – 4.71 (m), 4.62 – 4.53 (m), 4.53 – 4.35 (m), 4.35 – 4.23 (m), 4.03 – 3.92 (m), 3.80 – 3.72 (m), 3.55 – 3.43 (m), 1.38 [d, J = 7.1 Hz, H1’ (160)], 1.29 [d, J = 7.0 Hz, H1” (137)].

¹³C NMR (125 MHz, CDCl₃) δ 157.0 [C2 (137)], 156.9 [C2 (160)], 138.4 [C2’ (160)], 138.0, 137.9, 137.0 [C2’ (137)], 135.7, 135.6 [C2” (160)], 135.5 [C2” (160)], 128.9, 128.6, 128.5, 128.02, 127.97, 127.9, 127.8, 127.72, 127.71, 126.79, 126.77, 124.9 [C2” (137)], 124.7 [C1” (160)], 117.2 [C3’ (137)], 116.0 [C3’ (160)], 77.8, 77.4, 76.7, 76.5, 74.2 [OCH₂Ph (137)], 74.1 [OCH₂Ph (160)], 73.20 [OCH₂Ph (160)], 73.17 [OCH₂Ph (137)], 70.84 [C3” (160)], 70.76 [C3” (137)], 69.0 [C2” (160)], 68.9 [C2” (137)], 60.2 [C4 (160)], 60.0 [C4 (137)], 51.92 [C1’ (160)], 51.75 [C1’ (137)], 18.53 [C1” (137)], 17.4 [C1” (160)].
The title compounds 140 and 159 were synthesized from the above mixture of 137 and its C1’ epimer 160 (486 mg, 0.946 mmol), using the general method for ring–closing metathesis. The crude black oil was purified by FCC (1:1 EtOAc/n-hexane) to give pure samples of compounds 140 (105 mg, 27%) and 159 (205 mg, 53%) as grey oils. $^1$H and $^{13}$C NMR spectroscopic data of the formed product matched the corresponding data of compound 140 described above.

159:

Rt (1:1 EtOAc/n-hexane) = 0.45  
$[\alpha]^{25}_D +58.1$ (c 1.00, CHCl$_3$)  
IR $\tilde{\nu}_{\text{max}}$ (cm$^{-1}$): 3340, 2937, 2870, 1643, 1414, 1329, 1210, 1073, 957 cm$^{-1}$  
LRESIMS m/z 432 (100%) [M+Na]$^+$.

HRESIMS found 432.1782, calcd for C$_{24}$H$_{27}$NO$_3$Na, 432.1787 [M+Na]$^+$.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.39 – 7.22 (10H, m, ArH), 5.97 (1H, dt, $J = 6.1$, 2.2 Hz, H7), 5.86 (1H, dt, $J = 6.0$, 1.6 Hz, H6), 5.02 (1H, t, $J = 7.8$ Hz, H1), 4.79 (2H, app. t, $J = 9.1$ Hz, H7a and OCH$_3$Ph), 4.68 (1H, dt, $J = 8.8$, 5.5, 4.5, 2.7 Hz, H5), 4.50 (2H, d, $J = 2.9$ Hz, OCH$_3$Ph), 4.42 (1H, d, $J = 11.8$ Hz, OCH$_2$Ph), 3.88 (1H, q, $J = 8.0$, 7.3 Hz, H2’), 3.68 (1H, dd, $J = 7.5$, 2.2 Hz, H1’), 3.56 (1H, dd, $J = 9.2$, 6.1 Hz, H3’), 3.47 (1H, dd, $J = 9.2$, 6.8 Hz, H3’), 2.49 (1H, d, $J = 8.0$ Hz, OH), 1.22 (3H, d, $J = 6.8$ Hz, H1’).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 162.5 (C3), 137.8 (ArC), 137.7 (ArC), 137.6 (C7), 128.5 (2 x ArCH), 128.4 (2 x ArCH), 128.1 (2 x ArCH), 127.94 (ArCH), 127.92 (ArCH), 127.82 (2 x ArCH), 124.81 (C6), 78.6 (C1), 77.2 (C1’), 74.4 (OCH$_2$Ph), 73.4 (OCH$_2$Ph), 70.6 (C3’), 69.2 (C2’), 65.9 (C7a), 62.7 (C5), 19.6 (C1’).
Compound 163 was synthesized from 159 (151 mg, 0.369 mmol) using the general method for cis–dihydroxylation. The product 163 was purified by FCC (5:95 MeOH/CH₂Cl₂) to give small white crystals (160 mg, 98%).

\[ \text{R} = (5:95 \text{MeOH/CH}_2\text{Cl}_2) = 0.32 \]
\[ [\alpha]_D^{25} +36.2 \ (c \ 1.00, \text{CHCl}_3) \]

**M.P.:** 145 – 150 °C

**IR \( \nu_{\text{max}} \) (cm\(^{-1}\)):** 3429, 2921, 2858, 1709, 1293, 1126, 1028, 696 cm\(^{-1}\)

**LRESIMS** \( m/z \) 466 (100%) [M+Na]⁺.

**HRESIMS** found 466.1863, calcd for C\(_{24}\)H\(_{29}\)NO\(_7\)Na, 466.1842 [M+Na]⁺.

**\(^1\)H NMR** (500 MHz, CDCl\(_3\)) \( \delta \) 7.44 – 7.16 (10H, m, ArH), 4.91 (2H, app. dd, \( J = 9.4, 6.5 \) Hz, H1 and OCH\(_2\)Ph), 4.63 (1H, d, \( J = 11.2 \) Hz, OCH\(_2\)Ph), 4.46 (1H, d, \( J = 11.5 \) Hz, OCH\(_2\)Ph), 4.37 (1H, d, \( J = 11.5 \) Hz, OCH\(_2\)Ph), 4.33 (1H, dd, \( J = 7.9, 2.0 \) Hz, H7a), 3.96 (1H, s, H7), 3.90 – 3.80 (3H, m, H6, H1’, H2’), 3.64 (1H, quint, \( J = 6.7 \) Hz, H5), 3.57 – 3.49 (2H, m, H3’ and OH), 3.37 (1H, dd, \( J = 9.1, 6.7 \) Hz, H3’), 2.69 (1H, s, OH), 2.61 (1H, d, \( J = 8.0 \) Hz, OH), 1.35 (3H, d, \( J = 6.6 \) Hz, H1”).

**\(^{13}\)C NMR** (125 MHz, CDCl\(_3\)) \( \delta \) 161.6 (C3), 137.2 (ArC), 137.2 (ArC), 128.8 (ArCH), 128.7 (ArCH), 128.6 (ArCH), 128.31 (ArCH), 128.27 (ArCH), 128.1 (ArCH), 81.3 (C6, C1’, C2’), 76.8 (C1), 75.62 (C7a), 74.56 (OCH\(_2\)Ph), 73.7 (OCH\(_2\)Ph), 71.8 (C7), 70.9 (C3’), 69.3 (C6, C1’, C2’), 62.8 (C6, C1’, C2’), 58.7 (C5), 19.5 (Me).
(3aR,3bR,4R,8S,8aS)-4-((1R,2S)-1,3-Bis(benzyloxy)-2-hydroxypropyl)-2,2,8-trimethyltetrahydro-4H,6H-[1,3]dioxolo[4',5':3,4]pyrrolo[1,2-c]oxazol-6-one (158)

Compound 158 was synthesized from 163 (141 mg, 0.317 mmol) by the general method for acetonide protection of cis-diols. Purification by FCC (5:95 MeOH/CH₂Cl₂) returned product 158 as large colourless crystals (150 mg, 98%).

\[ \text{Rf} (5:95 \text{ MeOH/CH₂Cl₂}) = 0.56 \]
\[ [\alpha]_D^{25} +26.05 \text{ (c 1.00, CHCl}_3) \]

M.P: 70 – 75 °C

IR \( \nu_{\text{max}} \text{ (cm}^{-1}) \): 3394, 2936, 2860, 1756, 1261, 1045, 740, 698 cm\(^{-1}\)

LRESIMS \( m/z \ 506 \text{ (100%) [M+Na]}^+ \).

HRESIMS found 506.2211, calcd for C\(_{27}\)H\(_{33}\)NO\(_7\)Na, 506.2155 [M+Na]\(^+\).

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.43 – 7.20 (10H, m, ArH), 5.03 (1H, dd, \( J = 8.8, 7.0 \) Hz, H4), 4.94 (1H, d, \( J = 11.4 \) Hz, OCH\(_2\)Ph), 4.68 (1H, dd, \( J = 5.2, 3.6 \) Hz, H3a), 4.59 (1H, d, \( J = 11.4 \) Hz, OCH\(_2\)Ph), 4.51 (1H, d, \( J = 11.7 \) Hz, OCH\(_2\)Ph), 4.46 – 4.38 (3H, m, OCH\(_2\)Ph, H8a, H1'), 4.22 (1H, q, \( J = 7.4 \) Hz, H8), 3.96 (1H, dd, \( J = 7.1, 3.5 \) Hz, H3b), 3.89 (1H, q, \( J = 6.8 \) Hz, H2'), 3.58 (1H, dd, \( J = 9.4, 6.9 \) Hz, H3'), 3.43 (1H, dd, \( J = 9.5, 5.6 \) Hz, H3'), 2.34 (1H, d, \( J = 7.7 \) Hz, OH), 1.46 (3H, s, C2–CH\(_3\)), 1.20 – 1.11 (6H, m, C2–CH\(_3\), H1').

\(^13\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 160.8 (C6), 137.9 (ArC), 137.8 (ArC), 128.7 (ArCH), 128.44 (ArCH), 128.35 (ArCH), 127.94 (ArCH), 127.86 (ArCH), 127.7 (ArCH), 112.7 (C2), 87.8 (C8a or C1'), 80.7 (C3a), 79.2 (C4), 75.5 (C8a or C1'), 74.0 (OCH\(_2\)Ph), 73.4 (OCH\(_2\)Ph), 71.2 (C3'), 70.5 (C2'), 62.4 (C3b), 59.7 (C8), 26.3 (C2–CH\(_3\)), 24.1 (C2–CH\(_3\)), 17.7 (C1').
(1S,2S)-1,3-Bis(benzyloxy)-1-((3aR,3bR,4R,8S,8aS)-2,2,8-trimethyl-6-oxotetrahydro-4H,6H-[1,3]dioxolo[4',5':3,4]pyrrolo[1,2-c]oxazol-4-yl)propan-2-yl methanesulfonate (164)

Triethylamine (53 µL, 0.38 mmol) was slowly added to a solution of 158, (37 mg, 0.077 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL) at 0 °C under a N$_2$ atmosphere. Whilst stirring, a 0.13M solution of methanesulfonyl chloride in anhydrous CH$_2$Cl$_2$ (2.9 mL, 0.38 mmol) was slowly added to the reaction mixture. After stirring at 0 °C for 1 h, TLC analysis confirmed full consumption of the starting material and so the reaction was quenched through the addition of sat. NaHCO$_3$ solution (15 mL) and extracted with CH$_2$Cl$_2$ (3 x 15 mL) to give a pale–yellow oil. Purification of the residue by FCC (5%MeOH/CH$_2$Cl$_2$) gave 164 as a pale–yellow oil (43 mg, 99%).

$\text{R}_f$ (5%MeOH/CH$_2$Cl$_2$) = 0.76

$[\alpha]^{25}_D +16.7$ (c 0.75, CHCl$_3$)

$\text{IR } \Delta \nu_{\text{max}} ($cm$^{-1}$): 3067, 2942, 2852, 1741, 1550, 1098, 982 cm$^{-1}$.

LRESIMS $m/z$ 584 (100%) [M+Na]$^+$. HRESIMS found 584.1943, calcd for C$_{28}$H$_{35}$NO$_9$Na, 584.1930 [M+Na]$^+$. 

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.49 – 7.25 (10H, m, ArH), 5.04 – 4.90 (3H, m, H2’, H4, OCH$_2$Ph), 4.83 (1H, dd, $J = 5.3$, 3.7 Hz, H3a), 4.64 (1H, d, $J = 11.6$ Hz, OCH$_2$Ph), 4.55 (1H, dd, $J = 8.7$, 2.2 Hz, H1’), 4.52 – 4.47 (3H, m, OCH$_2$Ph, H8a), 4.22 (1H, q, $J = 7.3$ Hz, H8), 4.09 (1H, dd, $J = 7.2$, 3.7 Hz, H3b), 3.85 (1H, dd, $J = 10.9$, 7.5 Hz, H3’), 3.42 (1H, dd, $J = 11.0$, 3.6 Hz, H3’), 3.01 (3H, s, OMs), 1.46 (3H, s, C2–CH$_3$), 1.29 (3H, s, C2–CH$_3$), 1.22 (3H, d, $J = 7.4$ Hz, H1’’).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 160.2 (C6), 137.3 (ArC), 136.9 (ArC), 129.0 (ArCH), 128.6 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.8 (ArCH), 112.6 (C2), 87.9 (C8a), 80.7 (C3a), 80.1 (C4), 78.5 (C2’), 74.8 (C1’), 74.3 (OCH$_2$Ph), 73.3 (OCH$_2$Ph), 69.4 (C3’), 61.8 (C3b), 59.6 (C8), 38.8 (OMs), 26.3 (C2–CH$_3$), 24.0 (C2–CH$_3$), 17.4 (C1’’).
(1R,2S,3S)-2,4-Bis(benzyloxy)-1-((3aR,4S,6S,6aS)-2,2,6-trimethyltetrahydro-4H-[1,3]dioxolo[4,5-c]pyrrol-4-yl)butane-1,3-diol (166)

Compound 166 was synthesized from 158 (113 mg, 0.234 mmol) by the general method for oxozlididnone hydrolysis. The product was purified by FCC (5:95 MeOH/CH2Cl2) to give 166 as a pale–brown solid (90 mg, 84%).

\[ \text{Rf (5:95 MeOH/CH2Cl2)} = 0.38 \]
\[ [\alpha]^2_{25} = 2.6 \text{ (c 1.00, CHCl3)} \]
\[ \text{IR } \nu_{\text{max}} (\text{cm}^{-1}): 3412, 3030, 2967, 1498, 1352, 1072, 1051, 647 \text{ cm}^{-1} \]
\[ \text{LRESIMS } m/z 458 (100\%) [M+H]^+. \]
\[ \text{HRESIMS found 458.2530, calcd for C}_{26}\text{H}_{36}\text{NO}_6, 458.2543 [M+H]^+. \]

\[ ^1\text{H NMR (500 MHz, CDCl3)} \delta 7.43 – 7.18 \text{ (10H, m, ArH), 4.76 (1H, t, } J = 4.0 \text{ Hz, H3a), 4.72 (1H, d, } J = 11.5 \text{ Hz, OCH}_2\text{Ph), 4.66 (1H, d, } J = 11.5 \text{ Hz, OCH}_2\text{Ph), 4.52 (2H, s, OCH}_2\text{Ph), 4.36 (1H, d, } J = 5.4 \text{ Hz, H6a), 4.08 – 3.99 \text{ (2H, m, H1’}, \text{ H3’), 3.74 – 3.68 (2H, m, H4’}, \text{ H2’), 3.58 (1H, dd, } J = 9.8, 4.8 \text{ Hz, H4’), 3.28 (1H, q, } J = 7.4 \text{ Hz, H6), 3.09 (1H, dd, } J = 9.0, 4.0 \text{ Hz, H4), 2.32 (2H, bs, 2 x OH), 1.28 (6H, s, 2 x C2–CH}_3	ext{), 1.03 (3H, d, } J = 7.4 \text{ Hz, H1’).} \]

\[ ^{13}\text{C NMR (125 MHz, CDCl3)} \delta 138.0 \text{ (ArC), 137.9 (ArC), 128.6 (ArCH), 128.5 (ArCH), 128.0 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 110.9 (C2), 86.5 (C6a), 82.4 (C2’), 81.8 (C3a), 74.0 (OCH}_2\text{Ph), 73.7 (OCH}_2\text{Ph), 70.7 (C4’), 69.8 (C1’ and C3’), 61.8 (C4), 59.1 (C6), 25.8 (C2–CH}_3	ext{), 23.8 (C2–CH}_3\text{), 16.6 (C1’).} \]
(3aS,4S,6R,7R,8R,8aS,8bR)-7-(Benzyloxy)-6-((benzyloxy)methyl)-2,2,4-trimethylhexahydro-4H-[1,3]dioxolo[4,5-a]pyrrolizin-8-ol (165)

Triethylamine (44 µL, 0.32 mmol) was slowly added to a solution of 166 (145.7 mg, 0.319 mmol) in anhydrous DCE (15 mL) at 0 °C under a nitrogen atmosphere. Whilst stirring, a 0.13M solution of methanesulfonyl chloride in anhydrous CH₂Cl₂ (2.45 mL, 0.319 mmol) was slowly added dropwise to the reaction mixture. After stirring at 0 °C for 30 min, the reaction mixture was quenched through the addition of a sat. NaHCO₃ solution (2 mL) and extracted with CH₂Cl₂ (3 x 5 mL) followed by concentration in vacuo. The crude brown oil was dissolved in DCE followed by the addition of triethylamine (44 µL, 0.319 mmol). The reaction mixture with was heated at 80 °C for 20 h. TLC analysis confirmed full consumption of the starting material and so the reaction was quenched through the addition of NaHCO₃ solution (10 mL) and extracted with CH₂Cl₂ (3 x 10 mL). The combined extracts were dried (MgSO₄), filtered and concentrated in vacuo to give a yellow oil. Purification by FCC (5:95 MeOH/CH₂Cl₂) gave the title compound 165 as a pale–yellow oil (106 mg, 76%).

**Rf**(5:95 MeOH/CH₂Cl₂) = 0.35

[α]D25° +29.9 (c 1.00, CHCl₃)

**IR υmax (cm⁻¹):** 3358, 2981, 2862, 1702, 1643, 1211, 1089, 1023, 683 cm⁻¹

**LRESIMS** m/z 440 (100%) [M+H]+.

**HRESIMS** found 440.2432, calcd for C₂₆H₃₄NO₅, 440.2437 [M+H]+.

**¹H NMR (500 MHz, CDCl₃) δ** 7.48 – 7.17 (10H, m, ArH), 4.77 (1H, t, J = 6.0 Hz, H8b), 4.72 (1H, d, J = 12.0 Hz, OCH₂Ph), 4.67 (1H, t, J = 6.0 Hz, H8), 4.62 – 4.54 (2H, m, 2 x OCH₂Ph), 4.49 (1H, d, J = 12.1 Hz, OCH₂Ph), 4.35 (1H, dd, J = 6.1, 3.6 Hz, H3a), 3.91 (1H, t, J = 6.5 Hz, H7), 3.54 – 3.46 (3H, m, H1’, H8a), 3.34 – 3.24 (2H, m, H4, H6), 1.51 (3H, s, C2–CH₃), 1.31 (3H, s, C2–CH₃), 1.08 (3H, d, J = 6.7 Hz, H1’).

**¹³C NMR (125 MHz, CDCl₃) δ** 138.5 (ArC), 138.1 (ArC), 128.4 (ArCH), 128.4 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 113.1 (C2), 88.7 (C7), 88.0 (C3a), 80.0
(C8b), 76.0 (C8), 73.4 (OCH₂Ph), 72.4 (OCH₂Ph), 72.0 (C1'), 71.4 (C8a), 67.2 (C6), 64.3 (C4), 26.9 (C2–CH₃), 24.5 (C2–CH₃), 19.0 (C1”).

**General method for hydrolysis of an acetonide**

(1R,2S,3S,5R,6R,7R,7aR)-6-(Benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2,7-triol (168)

To a solution of 165 (20 mg, 0.05 mmol) in EtOH/H₂O (1/9, 5 mL), was added 5 N HCl (0.5 mL). The mixture was stirred at room temperature for 4 h by which time TLC analysis confirmed the consumption of starting material. The reaction mixture was neutralized by the addition of a saturated solution of NaHCO₃ until a pH of 7 was achieved. The solution was evaporated to 5 mL under reduced pressure and extracted with EtOAc (3 x 15 mL), the combined extracts were washed with saturated NaCl (15 mL), dried (MgSO₄), filtered and concentrated *in vacuo* to give a brown oil. Purification by FCC (5:95 MeOH/CH₂Cl₂) gave the title compound 168 as a pale–yellow oil (16 mg, 89%).

R₉(5:95 MeOH/CH₂Cl₂) = 0.14  
[α]D²⁵ +13.0 (c 1.00, CHCl₃)

**IR δmax (cm⁻¹):** 3357, 2918, 2863, 1653, 1539, 1120, 1078, 698 cm⁻¹

**LRESIMS m/z 400 (100%) [M+H]+.**

**HRESIMS** found 400.2126, calcd for C₂₃H₃₀NO₅, 400.2124 [M+H]+.

**¹H NMR (500 MHz, CDCl₃) δ** 7.43 – 7.18 (10H, m, ArH), 4.69 – 4.59 (2H, m, OCH₂Ph), 4.57 – 4.46 (3H, m, OCH₂Ph, H1), 4.06 (1H, d, J = 4.3 Hz, H7), 3.96 (1H, d, J = 2.5 Hz, H2), 3.73 (1H, t, J = 3.5 Hz, H7a), 3.66 (1H, dd, J = 9.2, 3.8 Hz, H6), 3.59 (1H, dd, J = 9.7, 3.6 Hz, H1’), 3.51 (1H, dd, J = 9.6, 3.7 Hz, H1’), 3.21 (1H, q, J = 3.5 Hz, H3), 2.78 (1H, dt, J = 12.4, 6.2 Hz, H5), 1.22 (3H, d, J = 6.1 Hz, H1”).
\(^{13}\text{C} \text{NMR (125 MHz, CDCl}_3\) \(\delta\) 137.3 (ArC), 137.0 (ArC), 128.8 (ArCH), 128.7 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.2 (ArCH), 88.5 (C2), 79.5 (C6), 74.9 (C1), 74.7 (C7a), 74.0 (OCH}_2\text{Ph}, 73.0 (C1'), 72.5 (OCH}_2\text{Ph}, 71.8 (C7), 70.4 (C3), 66.3 (C5), 19.0 (C1")}.

\((1R,2R,3R,5S,6S,7R,7aR)-3-(\text{Hydroxymethyl})-5\text{-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol [(–)-5,6-di-epi-hyacinthacine C}_5\) (169)

\((-\text{-5,6-Di-epi-hyacinthacine C}_5\) 169 was synthesized by the general method of \(O\text{-benzyl deprotection from} \) 168 (15 mg, 0.037 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated \textit{in vacuo} returning a yellow film. The title compound 169 was isolated through basic ion–exchange chromatography followed by concentration \textit{in vacuo}, as a colourless film (8 mg, 99%).

\([\alpha]_{D}^{25}=-6.9 (c 1.00, \text{H}_2\text{O})\)

\text{IR } \nu_{\text{max}} (\text{cm}^{-1}): 3332, 2981, 1623, 1578, 1478, 1065, 699 \text{ cm}^{-1}

\text{LRESIMS } m/z 220 (100\%) [M+H]^+.

\text{HRESIMS} \text{ found 220.1176, calcld for C}_{9}\text{H}_{18}\text{NO}_{5}, 220.1185 [M+H]^+.

\text{H NMR (500 MHz, D}_2\text{O) } \delta 4.33 (1H, t, J = 7.4 \text{ Hz, H1}), 4.13 (1H, t, J = 4.1 \text{ Hz, H7}), 3.89 (1H, t, J = 8.7 \text{ Hz, H2}), 3.76 – 3.68 (2H, m, H6 and H8), 3.65 (1H, dt, J = 11.9, 4.7 \text{ Hz, H8}), 3.26 (1H, dt, J = 5.7, 2.8 \text{ Hz, H7a}), 2.84 (1H, dq, J = 12.7, 6.4 \text{ Hz, H5}), 2.78 (1H, dt, J = 9.1, 4.3 \text{ Hz, H3}), 1.20 (3H, d, J = 6.2 \text{ Hz, H9}).

\text{C NMR (125 MHz, D}_2\text{O) } \delta 79.1 (C6), 78.0 (C2), 73.0 (C1), 70.8 (C3), 69.8 (C7), 67.8 (C7a), 63.2 (C5), 61.5 (C8), 17.5 (C9).
To a solution of compound 137 (425 mg, 0.828 mmol) in THF (25 mL) at 0 °C, was slowly added sodium hydride (50 mg, 1.24 mmol 60% dispersion in mineral oil). The reaction mixture was heated at reflux under a nitrogen atmosphere for 5 min, and cooled to rt. 4-Methoxybenzyl chloride (225 µL, 1.66 mmol) and TBAI (46 mg, 0.124 mmol) were added and the mixture was heated at reflux for 18 h. By this time, TLC analysis had confirmed full consumption of the starting material. The reaction mixture was cooled to 0 °C, and quenched by the dropwise addition of water (10 mL). The mixture was extracted with diethyl ether (3 x 15 mL) and the combined extracts were dried (MgSO₄), filtered and concentrated in vacuo. The resultant orange oil was purified by FCC (1:1 diethyl ether/n-hexane) to afford product 175 as a yellow oil (451 mg, 86%).

**Rf** (1:1 diethyl ether/n-hexane) = 0.17

[α]D²⁵ +48.3 (c 0.96, CHCl₃).

**IR ** νmax (cm⁻¹): 3027, 2950, 1618, 1540, 1245, 1108, 1083, 832 cm⁻¹

**LRESIMS** m/z 656 (100%) [M+Na]+

**HRESIMS** found 656.3102, calcd for C₄₀H₄₃NO₆Na, 656.2988 [M+Na]⁺.

**¹H NMR** (500 MHz, CDCl₃) δ 7.38 – 7.06 (17H, m, ArH), 6.88 (2H, dd, J = 8.2, 4.7 Hz, ArH), 6.10 (1H, d, J = 15.9 Hz, H2′′′′), 5.99 (1H, dd, J = 15.9, 9.5 Hz, H1′′′′), 5.83 (1H, ddd, J = 17.1, 10.4, 5.7 Hz, H2′), 5.20 (1H, d, J = 10.4 Hz, H3′cis), 5.10 (1H, d, J = 17.1 Hz, H3′trans), 4.78 – 4.52 (4H, m, OCH₂Ph, H5), 4.44 – 4.28 (4H, m, OCH₂Ph, OCH₂Ar, H1′), 3.87 – 3.73 (5H, m, H4, H1′′′′, OCH₃), 3.71 – 3.60 (3H, m, H3′′′′, H2′′′′), 1.20 (3H, d, J = 6.9 Hz, H1′).
\[ \text{\( ^{13}C\) NMR (125 MHz, CDCl}_3 \text{\( \delta \)} 159.7 (ArC), 157.0 (C2), 138.3 (ArC), 138.2 (ArC), 137.0 (C2'), 135.7 (ArC), 135.2 (C2''), 130.6 (ArCH), 130.3 (ArCH), 128.9 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.0 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 127.5 (ArCH), 126.7 (ArCH), 125.1 (C1'''), 116.9 (C3'), 114.1 (ArCH), 77.4 (C5), 76.6 (C2'''), 74.5 (C4), 73.9 (OCH}_2\text{Ph}, 73.1 (OCH}_2\text{Ph}, 71.6 (OCH}_2\text{Ar}, 69.4 (C3'''), 59.8 (C1'''), 55.4 (OCH}_3, 51.6 (C1'), 18.4 (C1''). \]

\((1R,5R,7aR)-1-((1S,2S)-1,3-Bis(benzyloxy)-2-((4-methoxybenzyl)oxy)propyl)-5-methyl-5,7a-dihydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (176)\)

\[
\text{\( \begin{array}{c}
\text{BnO} \\
\text{H} \\
\text{H} \\
\text{1} \\
\text{6} \\
\text{7} \\
\text{8} \\
\text{OPMB}
\end{array} \)}
\]

Compound 176 was synthesized from 175 (330 mg, 0.521 mmol), using the general method for ring–closing metathesis. The crude black oil was purified by FCC (3:7 EtOAc/n-hexane) to give 176 as a grey oil (215 mg, 78%).

\[ R_f (3:7 \text{EtOAc/n-hexane}) = 0.32 \]
\[ [\alpha]D^{25} +19.9 \text{ (c 1.00, CHCl}_3) \].

\[ \text{IR } \nu_{\text{max}} (\text{cm}^{-1}): 2980, 2824, 1767, 1698, 1513, 1454, 1375, 1222, 1103, 1083, 831 \text{ cm}^{-1} \]

\[ \text{LRESIMS } m/z 552 (100\%) [M+Na]^+ \]

\[ \text{HRESIMS } \text{found 552.2375, calcd for C}_{32}\text{H}_{35}\text{NO}_6\text{Na 552.2362 [M+Na]^+}. \]

\[ \text{\( ^1H\) NMR (500 MHz, CDCl}_3 \text{\( \delta \)} 7.40 – 7.14 (12H, m, ArH), 6.84 (2H, d, \text{J} = 8.6 \text{ Hz}, \text{ArH}), 5.67 (1H, dt, \text{J} = 6.1, 1.9 \text{ Hz}, \text{H7}), 5.57 (1H, dt, \text{J} = 6.1, 1.9 \text{ Hz}, \text{H6}), 4.89 – 4.82 (1H, m, \text{H7a}), 4.77 (1H, dd, \text{J} = 8.8, 2.4 \text{ Hz}, \text{H1}), 4.71 (1H, d, \text{J} = 11.5 \text{ Hz}, \text{OCH}_2\text{Ph}), 4.59 (1H, d, \text{J} = 11.3 \text{ Hz}, \text{OCH}_2\text{Ph}), 4.54 – 4.41 (4H, m, \text{OCH}_2\text{Ph}, \text{OCH}_2\text{Ar}), 4.20 (1H, dqd, \text{J} = 8.5, 6.3, 3.2 \text{ Hz}, \text{H5}), 3.90 – 3.82 (2H, m, \text{H3'}, \text{H2'}), 3.79 (3H, s, \text{OCH}_3), 3.73 (1H, dd, \text{J} = 11.0, 6.4 \text{ Hz}, \text{H3'}), 3.46 (1H, dd, \text{J} = 5.3, 2.5 \text{ Hz}, \text{H1'}), 1.24 (3H, d, \text{J} = 6.8 \text{ Hz}, \text{H1''}). \]

\[ \text{\( ^{13}C\) NMR (125 MHz, CDCl}_3 \text{\( \delta \)} 159.4 (ArC), 157.5 (C3), 138.4 (ArC), 137.7 (ArC), 136.8 (C7), 130.4 (ArCH), 129.8 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 123.4 (C6), 113.8 (ArCH), 77.8 (C1'), 76.9 (C2'), 75.6 (C1), 73.4 \]

260
Compounds 180 and 183 were synthesized from 176 (202 mg, 0.381 mmol) using the general method for cis–dihydroxylation. Both products were purified by FCC (3:97 MeOH/CH₂Cl₂) to give the title compounds 180 (38 mg, 18%) and 183 (134 mg, 62%) as clear oils.

180:

\( R_f(3.97\text{ MeOH/CH}_2\text{Cl}_2) = 0.49 \)

\[ [\alpha]^{25}_D +3.2 \text{ (c 2.75, CHCl}_3) \].

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3390, 2907, 2827, 1743, 1675, 1510, 1225, 1179, 1171, 1081 cm\(^{-1}\)

LRESIMS \( m/z\) 586 (100%) \([\text{M+Na}]^+\)

HRESIMS found 586.2416, calcd for \( \text{C}_{32}\text{H}_{37}\text{NO}_8\text{Na} \) 586.2417 \([\text{M+Na}]^+\).

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.43 – 7.18 (12H, m, ArH), 6.87 (2H, d, \( J = 8.4 \text{ Hz, ArH} \)), 4.81 – 4.64 (5H, m, OCH\(_2\)Ph, H1), 4.46 – 4.32 (4H, m, OCH\(_2\)Ph, OCH\(_2\)Ar), 4.13 (1H, dd, \( J = 6.6, 3.4 \text{ Hz, H7a} \)), 4.06 – 3.98 (1H, m, H6), 3.84 – 3.60 (8H, m, OCH\(_3\), H5, H7, H3', H2'), 3.15 (1H, dd, \( J = 8.8, 2.1 \text{ Hz, H1'} \)), 2.67 (1H, d, \( J = 10.3 \text{ Hz, OH} \)), 1.43 (3H, d, \( J = 7.0 \text{ Hz, H1''} \)).

\(^13\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 159.7 (ArC), 157.0 (C3), 137.5 (ArCH), 137.1 (ArCH), 130.4 (ArCH), 129.5 (ArCH), 128.8 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 114.0 (ArCH), 76.0 (C7a), 75.8 (C1), 75.4 (C7), 74.6 (C6),
74.4 (OCH$_2$Ph), 73.6 (OCH$_2$Ph), 71.6 (OCH$_2$Ar), 70.6 (C2’ or C5), 68.4 (C3’), 62.9 (C1’), 55.4 (OCH$_3$), 54.7 (C2’ or C5), 11.8 (C1’’).

183:

R$_f$(3:97 MeOH/CH$_2$Cl$_2$) = 0.49

[α]$^D_{25}$ +14.2 (c 1.00, CHCl$_3$).

LRESIMS m/z 564 (100%) [M+H]$^+$

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.36 – 7.17 (12H, m, ArH), 6.85 (2H, d, $J$ = 8.1 Hz, ArH), 4.83 (1H, dd, $J$ = 8.8, 4.3 Hz, H1), 4.73 – 4.62 (2H, m, OCH$_2$Ph), 4.58 (1H, d, $J$ = 11.6 Hz, OCH$_2$Ph), 4.52 – 4.41 (3H, m, OCH$_2$Ph, OCH$_2$Ar), 4.11 – 4.04 (1H, t, $J$ = 7.2 Hz, H7), 3.99 (1H, q, $J$ = 4.6 Hz, H2’), 3.92 (1H, t, $J$ = 8.4 Hz, H7a), 3.78 (4H, s, OCH$_2$Ph, H3’), 3.68 (2H, td, $J$ = 8.8, 6.8, 4.0 Hz, H3’, H6), 3.46 – 3.40 (1H, m, H5), 1.23 (3H, d, $J$ = 6.8 Hz, H1’’).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 159.5 (ArC), 156.6 (C3), 137.9 (ArCH), 137.5 (ArCH), 130.0 (ArCH), 129.8 (ArCH), 128.5 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.8 (ArCH), 113.9 (ArCH), 79.7 (C6), 77.3 (C1’), 76.6 (C2’), 76.3 (C1), 74.2 (OCH$_2$Ph), 73.5 (OCH$_2$Ph), 72.9 (OCH$_2$Ar), 69.5 (C3’), 68.3 (C7), 65.3 (C7a), 58.9 (C5), 55.3 (OCH$_3$), 14.8 (C1’’).

**General method for the synthesis of a cyclic sulfate**

(3aS,3bS,4R,8R,8aR)-4-((1S,2S)-1,3-Bis(benzyloxy)-2-((4-methoxybenzyl)oxy)propyl)-8-methyltetrahydro-4H,6H-[1,3,2]dioxathiolo[4′,5′:3,4]pyrrolo[1,2-c]oxazol-6-one 2,2-dioxide (181)

To a solution of the diol 183 (212 mg, 0.376 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL) at 0 °C under a nitrogen atmosphere was added Et$_3$N (134 µL, 0.96 mmol) and SOCl$_2$ (46 µL, 0.57 mmol) dropwise. After stirring at 0 °C for 1 h, TLC analysis confirmed the consumption of starting material. The reaction was quenched with water (10 mL) and the mixture was
extracted with CH₂Cl₂ (3 x 15 mL). The combined extracts were dried (MgSO₄), filtered and concentrated in vacuo to give 181 as a brown oil (235 mg) which was used for the next step without further purification.

**LRESIMS** m/z 660 (100%) [M+Cl]⁻

(1R,5R,6R,7R,7aR)-1-((1S,2S)-1,3-Bis(benzyloxy)-2-((4-methoxybenzyl)oxy)propyl)-6,7-dihydroxy-5-methyltetrahydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (185) and (1R,5R,6S,7S,7aR)-1-((1S,2S)-1,3-bis(benzyloxy)-2-((4-methoxybenzyl)oxy)propyl)-6,7-dihydroxy-5-methyltetrahydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (186)

To a solution of the cyclic sulfate 181 (235 mg, 0.376 mmol) in dry DMSO (5 mL) was added benzoic acid (184 mg, 1.50 mmol) and cesium carbonate (490 mg, 1.50 mmol). The reaction mixture was stirred under a nitrogen atmosphere at 40 °C for 20 h. After LRESIMS confirmed the consumption of starting material, a mixture of THF (8 mL), distilled water (3 mL) and conc. H₂SO₄ (1 mL) was added to the mixture and stirred at 40 °C for 20 h. The reaction mixture was neutralized with saturated NaHCO₃ until a pH of 7 was achieved, followed by extraction with CH₂Cl₂ (3 x 15 mL). The combined extracts were dried (MgSO₄), filtered and concentrated in vacuo to give a brown oil. The resultant oil was eluted from a silica plug (2:3 EtOAc/n-hexane) to give a light brown oil (63 mg) which was subsequently subjected to O–benzoyl hydrolysis to afford 185 (37 mg, 18% over two steps) and 186 (15 mg, 7% over two steps) which were separable by FCC (5:95 MeOH/CH₂Cl₂).

185:

Rf (5:95 MeOH/CH₂Cl₂) = 0.43  
[α]D²⁵ = -16.1 (c 2.00, CHCl₃).

IR νmax (cm⁻¹): 3412, 2932, 2803, 1756, 1623, 1512, 1256, 1187, 1143, 1009 cm⁻¹
LRESIMS m/z 564 (100%) [M+H]^+.

HRESIMS found 564.2589, caled for C_{32}H_{38}NO_8, 564.2597 [M+H]^+.

^1H NMR (500 MHz, CDCl_3) δ 7.38 – 7.23 (10H, m, ArH), 7.21 (2H, d, J = 8.1 Hz, ArH), 6.85 (2H, d, J = 8.1 Hz, ArH), 4.84 (1H, dd, J = 8.6, 5.8 Hz, H1), 4.71 – 4.57 (4H, m, OCH_2Ph, OCH_2Ar), 4.44 (2H, q, J = 11.3 Hz, OCH_2Ph), 4.00 (1H, t, J = 6.1 Hz, H6), 3.93 (1H, t, J = 5.6 Hz, H7), 3.89 (2H, bs, H2', OH), 3.84 – 3.71 (5H, m, OCH_3, 7a, H3'), 3.71 – 3.58 (3H, m, H5, H3', H1'), 1.31 (3H, d, J = 6.9 Hz, H1'').

^13C NMR (125 MHz, CDCl_3) δ 159.6 (ArC), 157.8 (C3), 157.8 (ArC), 137.4 (ArC), 130.1 (ArCH), 129.5 (ArCH), 128.5 (ArCH), 128.5 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.3 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 114.0 (ArCH), 79.7 (C7), 76.4 (C7a), 76.2 (C2'), 75.2 (C1), 74.1 (C6), 73.5 (OCH_2Ph), 73.2 (OCH_2Ph), 72.5 (OCH_2Ar), 69.1 (C3'), 65.2 (C1'), 55.3 (OCH_3), 55.2 (C5), 11.0 (C1'').

186:

R_f (5:95 MeOH/CH_2Cl_2) = 0.28
[a]_D^{25} +68.4 (c 0.56, CHCl_3).

LRESIMS m/z 564 (100%) [M+H]^+.

^1H NMR (500 MHz, CDCl_3) δ 7.41 – 7.19 (12H, m, ArH), 6.86 (2H, d, J = 8.0 Hz, ArH), 4.82 (1H, dd, J = 8.2, 5.0 Hz, H1), 4.77 – 4.62 (3H, m, OCH_2Ph), 4.51 – 4.42 (3H, m, OCH_2Ph, OCH_2Ar), 4.08 (1H, t, J = 4.6 Hz, H7a), 3.87 (2H, bs, OH, H7), 3.79 (4H, d, J = 4.9 Hz, OCH_3, H6), 3.74 – 3.68 (3H, m, H3', H1'), 3.66 – 3.56 (1H, m, H2'), 3.36 (1H, q, J = 7.8, 7.2 Hz, H5), 1.59 (3H, d, J = 7.0 Hz, H1'').

^13C NMR (125 MHz, CDCl_3) δ 159.5 (ArC), 157.9 (C3), 157.8 (ArC), 136.7 (ArC), 130.1 (ArCH), 129.8 (ArCH), 128.79 (ArCH), 128.76 (ArCH), 128.66 (ArCH), 128.65 (ArCH), 128.5 (ArCH), 128.3 (ArCH), 128.0 (ArCH), 127.98 (ArCH), 127.97 (ArCH), 127.96 (ArCH), 127.8 (ArCH), 114.0 (ArCH), 84.2 (C6), 76.3 (C7), 76.0 (C7a), 75.4 (C2'), 74.2 (OCH_2Ph), 74.1 (C1), 73.5 (OCH_2Ph), 72.1 (OCH_2Ar), 69.4 (C3'), 65.8 (C1'), 62.5 (C5), 55.3 (OCH_3), 15.8 (C1'').
(1R,5R,6R,7R,7aR)-6,7-Bis(benzyloxy)-1-((1S,2S)-1,3-bis(benzyloxy)-2-((4-methoxybenzyl)oxy)propyl)-5-methyltetrahydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (187)

Compound 187 was synthesized from 185 (35 mg, 0.062 mmol) by the general method for O–benzylation. The product 187 was purified by FCC (1:1 EtOAc/n-hexane) to give the target compound as an orange oil (43 mg, 94%).

Rf(1:1 EtOAc/n-hexane) = 0.64
[α]D25 +102.1 (c 1.50, CHCl3).

IR νmax (cm⁻¹): 3059, 2967, 2849, 1798, 1622, 1542, 1212, 1135, 1164 cm⁻¹

LRESIMS m/z 744 (100%) [M+H]+.

HRESIMS found 744.3543, calcd for C₄₆H₅₀NO₈, 744.3536 [M+H]+.

¹H NMR (500 MHz, CDCl3) δ 7.44 – 7.02 (22H, m, ArH), 6.79 (2H, d, J = 8.1 Hz, ArH), 4.91 – 4.81 (2H, m, OCH₂Ph, H1), 4.67 (1H, d, J = 11.7 Hz, OCH₂Ph), 4.48 – 4.16 (9H, m, OCH₂Ph, OCH₂Ar, H7), 3.69 (3H, s, OCH₃), 3.66 – 3.58 (1H, m, H3′), 3.55 (2H, bs, H1′, H2′), 3.49 (2H, d, J = 7.8 Hz, H5, H6), 3.44 – 3.34 (2H, m, H3′, H7a), 1.56 (3H, d, J = 7.2 Hz, H1″).

¹³C NMR (125 MHz, CDCl3) δ 159.6 (ArC), 157.9 (C3), 138.4 (ArC), 138.0 (ArC), 137.4 (ArC), 137.1 (ArC), 130.2 (ArCH), 129.8 (ArCH), 128.8 (ArCH), 128.6 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 127.86 (ArCH), 127.85 (ArCH), 127.67 (ArCH), 127.66 (ArCH), 127.2 (ArCH), 114.0 (ArCH), 87.4 (C1’ or C2’), 80.9 (C1’ or C2’), 76.6 (C1), 76.0 (C7), 75.5 (C6), 74.5 (OCH₂Ph), 73.4 (OCH₂Ph), 71.5 (OCH₂Ar), 70.0 (OCH₂Ph), 69.0 (C3’), 64.1 (C7a), 60.0 (C5), 55.2 (OCH₃), 16.2 (C1″).
Chapter 4B

(3aS,4S,6R,7R,8R,8aS,8bR)-7,8-Bis(benzyloxy)-6-((benzyloxy)methyl)-2,2,4-
trimethylhexahydro-4H-[1,3]dioxolo[4,5-a]pyrrolizine (196)

Compound 196 was synthesized from 165 (115 mg, 0.262 mmol) by the general method
for O–benzylation. The product 196 was purified by FCC (1:4 EtOAc/n-hexane) to give a
yellow oil (122 mg, 87%).

\[ R_f(3:7 \text{ EtOAc/n-hexane}) = 0.56 \]

\[ [\alpha]_D^{25} +19.3 \text{ (c 1.00, CHCl}_3) \]

IR \( \tilde{\nu}_{\text{max}} \text{ (cm}^{-1}): 2987, 1557, 1531, 1278, 1089, 695 \text{ cm}^{-1} \)

LRESIMS \( m/z \ 530 \text{ (100%) [M+H]}^+ \).

HRESIMS found 530.2901, calcd for C\(_{33}\)H\(_{40}\)NO\(_5\), 530.2906 [M+H]\(^+\).

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \ 7.50 – 7.15 \ (15\text{H, ArH}), 4.68 \ (1\text{H, d, } J = 11.8 \text{ Hz, OCH}_2\text{Ph}), 4.61 \ (1\text{H, t, } J = 5.6 \text{ Hz, H8b}), 4.59 – 4.52 \ (5\text{H, m, OCH}_2\text{Ph, H8}), 4.47 \ (1\text{H, d, } J = 12.2 \text{ Hz, OCH}_2\text{Ph}), 4.38 \ (1\text{H, dd, } J = 5.8, 2.6 \text{ Hz, H3a}), 4.02 \ (1\text{H, t, } J = 6.2 \text{ Hz, H7}), 3.60 – 3.48 \ (2\text{H, m, H1’}, \text{ H8a}), 3.47 – 3.36 \ (3\text{H, m, H1’}, \text{ H6, H4}), 1.51 \ (3\text{H, s, C2–CH}_3), 1.31 \ (3\text{H, s, C2–CH}_3), 1.04 \ (3\text{H, d, } J = 7.0 \text{ Hz, H1”}). \)

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \ 138.9 \ (\text{ArC}), 138.8 \ (2 \times \text{ArC}), 128.6 \ (\text{ArCH}), 128.5 \ (\text{ArCH}), 128.5 \ (\text{ArCH}), 128.1 \ (\text{ArCH}), 128.04 \ (\text{ArCH}), 128.00 \ (\text{ArCH}), 127.8 \ (\text{ArCH}), 127.7 \ (\text{ArCH}), 112.7 \ (\text{C2}), 89.2 \ (\text{C3a}), 87.1 \ (\text{C7}), 83.3 \ (\text{C8}), 82.0 \ (\text{C8b}), 73.5 \ (\text{OCH}_2\text{Ph}), 72.6 \ (\text{OCH}_2\text{Ph}), 72.5 \ (\text{OCH}_2\text{Ph}), 72.5 \ (\text{Cl’}), 69.8 \ (\text{C8a}), 66.4 \ (\text{C6}), 62.9 \ (\text{C4}), 27.2 \ (\text{C2–CH}_3), 24.6 \ (\text{C2–CH}_3), 19.6 \ (\text{Cl”}). \)
(3aR,4R,6R,7R,8R,8aS,8bS)-7,8-Bis(benzyloxy)-6-((benzyloxy)methyl)-2,2,4-trimethylhexahydro-4H-[1,3]dioxolo[4,5-α]pyrrolizine (197)

Compound 197 was synthesized from 149 (19 mg, 0.043 mmol) by the general method for O–benzylation. The product was purified by FCC (gradient of 3:20 EtOAc/n-hexane to 1:5 EtOAc/n-hexane) to give the target compound 197 as a clear oil (21 mg, 89%).

Rf (1:4 EtOAc/n-hexane) = 0.50
[α]D25 +85.5 (c 1.00, CHCl3)
IR νmax (cm⁻¹): 2980, 1682, 1374, 1247, 1055 cm⁻¹
LRESIMS m/z 530 (100%) [M+H]+.
HRESIMS found 530.2923, calcd for C₃₃H₄₀NO₅, 530.2906 [M+H]+.

¹H NMR (500 MHz, CDCl₃) δ 7.50 – 7.10 (15H, m, ArH), 4.60 (1H, dd, J = 6.9, 3.7 Hz, H8b) 4.57 – 4.34 (6H, m, OCH₂Ph), 4.21 (1H, t, J = 6.8 Hz, H3a), 4.02 (1H, s, H8), 3.99 (1H, s, H7), 3.66 (1H, t, J = 2.7 Hz, H8a), 3.48 – 3.20 (3H, m, H1’’, H6, H4), 1.50 (3H, s, C2–CH₃), 1.29 (6H, s, C2–CH₃, H1”).

¹³C NMR (125 MHz, CDCl₃) δ 138.39 (ArC), 138.11 (ArC), 137.97 (ArC), 128.41 (ArCH), 128.36 (ArCH), 128.32 (ArCH), 127.69 (ArCH), 127.64 (ArCH), 127.60 (ArCH), 127.57 (ArCH), 127.55 (ArCH), 113.95 (C2), 86.40 (C3a), 85.79 (C7), 84.89 (C8), 83.58 (C8b), 74.44 (C8a), 73.19 (OCH₂Ph), 72.65 (C1’), 71.26 (OCH₂Ph), 71.20 (OCH₂Ph), 62.89 (C4), 62.66 (C6), 27.71 (C2–CH₃), 25.63 (C2–CH₃), 15.36 (C1’”).
(3aS,4R,6R,7R,8R,8aS,8bR)-7,8-Bis(benzyloxy)-6-((benzyloxy)methyl)-2,2,4-
trimethylhexahydro-4H-[1,3]dioxolo[4,5-a]pyrrolizine (198)

Compound 198 was synthesized from 151 (24 mg, 0.055 mmol) by the general method for
O–benzylation. The product was purified by FCC (1:4 EtOAc/n-hexane) to give the target
compound 198 as an orange oil (23 mg, 78%).

\[ R_f(5.95 \text{ MeOH/CH}_2\text{Cl}_2) = 0.50 \]
\[ [\alpha]^{25}_D +5.9 \ (c \ 1.00, \text{CHCl}_3) \]
IR \[ \nu_{\text{max}} (\text{cm}^{-1}) : 2995, 1679, 1443, 1256, 1055, 683 \text{ cm}^{-1} \]
LRESIMS \[ m/z \ 530 (100\%) \ [\text{M+H}]^+. \]
HRESIMS found 530.2930, calcd for C\text{\textsubscript{33}}H\text{\textsubscript{40}}NO\text{\textsubscript{5}}, 530.2906 [M+H]\text{\textsuperscript{+}}.
\[ ^1\text{H NMR (500 MHz, CDCl}_3\text{)} \]
\[ \delta 7.39 - 7.21 \ (15H, \text{m, ArH}), 4.61 \ (1H, d, J = 11.9 \text{ Hz, OCH}_2\text{Ph}), 4.56 - 4.44 \ (7H, \text{m, OCH}_2\text{Ph, H8b, H3a}), 4.39 \ (1H, t, J = 4.1 \text{ Hz, H8}), 4.17 \ (1H, dd, J = 5.1, 4.2 \text{ Hz, H7}), 3.59 \ (1H, dd, J = 5.8, 3.9 \text{ Hz, H6}), 3.49 \ (2H,qd, J = 10.1, 4.2 \text{ Hz, H1'}), 3.31 \ (1H, t, J = 4.0 \text{ Hz, H8a}), 3.02 \ (1H, dt, J = 7.0, 2.8 \text{ Hz, H4}), 1.49 \ (3H, s, C2–
\text{CH}_3), 1.31 \ (6H, app. d, J = 4.9 \text{ Hz, C2–CH}_3 \text{ and H1'}). \]
\[ ^{13}\text{C NMR (125 MHz, CDCl}_3\text{)} \]
\[ \delta 138.8 \ (\text{ArC}), 138.5 \ (\text{ArC}), 138.4 \ (\text{ArC}), 128.3 \ (\text{ArCH}), 128.3 \ (\text{ArCH}), 128.2 \ (\text{ArCH}), 127.9 \ (\text{ArCH}), 127.82 \ (\text{ArCH}), 127.78 \ (\text{ArCH}), 127.6 \ (\text{ArCH}), 127.5 \ (\text{ArCH}), 127.3 \ (\text{ArCH}), 112.0 \ (\text{C2}), 89.3 \ (\text{C7}), 85.5 \ (\text{C3a or C8b}), 82.0 \ (\text{C8}), 80.7 \ (\text{C3a or C8b}), 73.3 \ (\text{OCH}_2\text{Ph}), 72.0 \ (\text{OCH}_2\text{Ph}), 71.9 \ (\text{OCH}_2\text{Ph or C8a}), 71.7 \ (\text{OCH}_2\text{Ph or C8a}), 70.1 \ (\text{C1'}), 62.2 \ (\text{C6}), 57.3 \ (\text{C4}), 26.3 \ (\text{C2–CH}_3), 24.2 \ (\text{C2–CH}_3), 12.1 \ (\text{C1'}). \]
Compound 199 was synthesized from 196 (122 mg, 0.230 mmol) using the general method for hydrolysis of an acetonide. The product was purified by FCC (5:95 MeOH/CH₂Cl₂) to give 199 as a brown oil (95 mg, 85%).

\( \text{R}^{-}(5:95 \text{ MeOH/CH}_2\text{Cl}_2) = 0.26 \)

\([\alpha]_{D}^{25} +3.5 \ (c \ 1.00, \text{CHCl}_3)\)

**IR** \( \nu_{\max} (\text{cm}^{-1}) \): 3397, 2887, 1676, 1592, 1479, 1064, 687 cm\(^{-1}\)

**LRESIMS** \( m/z \) 490 (100%) \([\text{M+H}]^{+}\)

**HRESIMS** found 490.2602, calcd for C\(_{30}\)H\(_{36}\)NO\(_5\), 490.2593 \([\text{M+H}]^{+}\).

**\(^1\)H NMR (500 MHz, CDCl\(_3\))** \( \delta \) 7.48 – 7.07 (15H, m, ArH), 4.61 (1H, d, \( J = 11.6 \text{ Hz, OCH}_2\text{Ph} \)), 4.57 – 4.36 (6H, m, OCH\(_2\)Ph, H1), 4.16 (1H, t, \( J = 4.0\) Hz, H2), 3.95 (1H, t, \( J = 4.3\) Hz, H7), 3.81 (1H, t, \( J = 4.1\) Hz, H7a), 3.69 (1H, dd, \( J = 9.2, 3.9\) Hz, H6), 3.51 (2H, d, \( J = 6.5\) Hz, H1'), 3.26 (1H, q, \( J = 5.8\) Hz, H3), 2.84 (1H, quint, \( J = 6.5\) Hz, H5), 1.25 (3H, d, \( J = 6.2\) Hz, H1').

**\(^{13}\)C NMR (125 MHz, CDCl\(_3\))** \( \delta \) 138.3 (ArC), 138.1 (ArC), 137.4 (ArC), 128.8 (ArCH), 128.7 (ArCH), 128.6 (ArCH), 128.30 (ArCH), 128.28 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.9 (ArCH), 84.3 (C2), 82.1 (C1), 79.7 (C6), 73.5 (OCH\(_2\)Ph), 72.5 (OCH\(_2\)Ph), 72.4 (OCH\(_2\)Ph), 71.9 (C7), 71.1 (C7a), 70.7 (C1'), 69.5 (C3), 65.4 (C5), 18.7 C1'.
(1S,2R,3R,5R,6R,7R,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2-diol (200).

Compound 200 was synthesized from 197 (22 mg, 0.042 mmol) using the general method for hydrolysis of an acetonide. The product was purified by FCC (5:95 MeOH/CH2Cl2) to give the desired product 200 as a pale–yellow oil (20 mg, 98%).

Rf 0.20 (5:95 MeOH/CH2Cl2).
[α]D25 −38.9 (c 1.00, CHCl3).
IR νmax (cm−1): 3324, 3089, 2922, 2853, 1600, 1496, 1095, 608.
LRESIMS m/z 490 (100%) [M+H]+.
HRESIMS found 490.2577, calcd for C30H36NO5, 490.2593 [M+H]+.

1H NMR (500 MHz, CDCl3) δ 7.41 – 7.16 (15H, m, ArH), 4.68 – 4.40 (6H, m, OCH₂Ph), 4.01 (1H, t, J = 3.7 Hz, H2), 3.98 (1H, t, J = 5.0 Hz, H7), 3.91 (1H, t, J = 3.8 Hz, H1), 3.70 (1H, t, J = 6.0 Hz, H6), 3.50 (1H, t, J = 4.3 Hz, H7a), 3.44 (2H, d, J = 6.5 Hz, H1’), 3.32 (1H, td, J = 6.5, 3.6 Hz, H3), 3.22 (1H, quint, J = 6.9 Hz, H5), 3.08 (2H, bs, OH), 1.19 (3H, d, J = 6.9 Hz, H1’).

13C NMR (125 MHz, CDCl3) δ 138.22 (ArC), 137.97 (ArC), 137.94 (ArC), 128.36 (ArCH), 128.29 (ArCH), 127.68 (ArCH), 127.65 (ArCH), 127.62 (ArCH), 127.53 (ArCH), 86.01 (C1), 85.37 (C2), 77.11 (C6), 74.76 (C7a), 73.83 (C7), 73.19 (OCH₂Ph), 72.15 (OCH₂Ph), 71.85 (C1’), 71.53 (OCH₂Ph), 61.52 (C3), 61.08 (C5), 15.18 (C1’).
(1R,2S,3R,5R,6R,7R,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2-diol (201).

Compound 201 was synthesized from 198 (50 mg, 0.094 mmol) using the general method for hydrolysis of an acetonide. The product was purified by FCC (5:95 MeOH/CH₂Cl₂) to give the desired product 201 as a brown oil (39 mg, 85%).

Rₖ 0.26 (5:95 MeOH/CH₂Cl₂).

[α]_D^{25} +24.4 (c 1.00, CHCl₃).

IR ν max (cm⁻¹): 3334, 3063, 2926, 1623, 1453, 1368, 1027, 737.

LRESIMS m/z 490 (100%) [M+H]^+.

HRESIMS found 490.2585, calcd for C₃₀H₃₆NO₅, 490.2593 [M+H]^+.

¹H NMR (500 MHz, CDCl₃) δ 7.37 – 7.21 (15H, m, ArH), 4.60 – 4.43 (6H, m, OCH₂Ph), 4.31 (1H, t, J = 3.3 Hz, H1), 4.24 (1H, dd, J = 7.5, 4.8 Hz, H7), 4.11 (1H, t, J = 3.1 Hz, H2), 3.89 (1H, t, J = 4.7 Hz, H6), 3.78 – 3.70 (2H, m, H7a, H3), 3.42 (2H, dd, J = 7.1, 3.3 Hz, H1'), 3.37 (1H, dd, J = 7.1, 4.6 Hz, H5), 1.25 (3H, d, J = 7.0 Hz, H1 '').

¹³C NMR (125 MHz, CDCl₃) δ 138.2 (ArC), 138.1 (ArC), 137.0 (ArC), 128.6 (ArCH), 128.4 (ArCH), 128.4 (ArCH), 128.10 (ArCH), 128.08 (ArCH), 127.70 (ArCH), 127.69 (ArCH), 127.6 (ArCH), 85.3 (C2), 82.5 (C1), 75.0 (C6), 73.3 (OCH₂Ph), 72.9 (C7), 72.0 (OCH₂Ph), 71.9 (OCH₂Ph), 71.6 (C7a or C3), 71.6 (C1'), 62.4 (C7a or C3), 58.6 (C5), 11.9 (C1 '').
Compound 202 was synthesized from the diol 199 (55 mg, 0.11 mmol) by using the general method for the synthesis of a cyclic sulfate. The resultant crude brown oil 202 (61 mg) was used for the next step without further purification.

**LRESIMS m/z 586 (100%) [M+Cl]⁻.**

Compound 203 was synthesized from the diol 200 (48 mg, 0.098 mmol) by using the general method for the synthesis of a cyclic sulfate. The resultant crude brown oil 203 (54 mg) was used for the next step without further purification.

**LRESIMS m/z 586 (100%) [M+Cl]⁻.**
Compound 204 was synthesized from the diol 201 (40 mg, 0.082 mmol) by using the general method for the synthesis of a cyclic sulfate. The resultant crude brown oil 204 (45 mg) was used for the next step without further purification.

**LRESIMS m/z 586 (100%) [M+Cl]**.

*General method for ring opening of cyclic sulfates with benzoate*

(1S,2S,3S,5R,6R,7R,7aS)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-2-hydroxy-3-methylhexahydro-1H-pyrroloizin-1-yl benzoate (205) and (1R,2R,3S,5R,6R,7R,7aR)-6,7-bis(benzyloxy)-5-((benzyloxy)methyl)-1-hydroxy-3-methylhexahydro-1H-pyrroloizin-2-yl benzoate (206)

To a solution of the cyclic sulfate 202 (61 mg, 0.11 mmol) in dry DMSO (1 mL) was added benzoic acid (67 mg, 0.55 mmol) and cesium carbonate (180 mg, 0.55 mmol). The reaction mixture was stirred under a nitrogen atmosphere at 50 °C for 20 h. After LRESIMS confirmed the consumption of starting material, a mixture of THF (2 mL), distilled water (0.75 mL) and conc. H₂SO₄ (0.25 mL) was added to the mixture and stirred at 60 °C for 20 h. The reaction mixture was neutralized with saturated NaHCO₃ until a pH of 7 was achieved, followed by extraction with CH₂Cl₂ (3 x 15 mL). The combined extracts were dried (MgSO₄), filtered and concentrated *in vacuo* to give a brown oil. Purification by FCC (1:1 diethyl ether/n-hexane) gave 205 (40 mg, 61%) and 206 (8 mg, 12%) as clear films.
205:

R_f (1:1 diethyl ether/n-hexane) = 0.26

[α]^{25}_D = -12.2 (c 1.00, CHCl_3)

IR v_{max} (cm^{-1}): 3367, 3089, 2946, 1687, 1525, 1167, 1038, 598 cm^{-1}

LRESIMS m/z 594 (100%) [M+H]^+.

HRESIMS found 594.2874, calcd for C_{37}H_{40}NO_5, 594.2856 [M+H]^+.

^1H NMR (500 MHz, CDCl_3) δ 8.04 (2H, d, J = 7.7 Hz, o-Bz), 7.60 (1H, t, J = 7.5 Hz, p-Bz), 7.46 (2H, t, J = 7.7 Hz, m-Bz), 7.36 – 7.19 (15H, m, ArH), 5.13 (1H, t, J = 5.7 Hz, H7), 4.63 – 4.47 (6H, m, OCH_2Ph), 4.28 (1H, t, J = 4.0 Hz, H1), 4.14 (1H, t, J = 3.1 Hz, H2), 3.94 (1H, t, J = 6.7 Hz, H6), 3.73 (1H, t, J = 5.3 Hz, H7a), 3.63 (1H, s, OH), 3.52 – 3.45 (2H, q, J = 9.4, 7.4 Hz, H1'), 3.35 (1H, t, J = 9.2, 4.5 Hz, H3), 3.21 (1H, quint, J = 6.5 Hz, H5), 1.24 (3H, d, J = 6.2 Hz, H1').

^13C NMR (125 MHz, CDCl_3) δ 167.8 (CO), 138.4 (ArC), 138.0 (ArC), 137.9 (ArC), 133.6 (ArC), 129.9 (ArCH), 129.4 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.8 (ArCH), 127.73 (ArCH), 127.69 (ArCH), 127.6 (ArCH), 127.6 (ArCH), 87.7 (C1), 86.4 (C2), 85.1 (C7), 82.5 (C6), 73.3 (OCH_2Ph), 71.94 (OCH_2Ph), 71.90 (OCH_2Ph), 71.7 (C1'), 71.5 (C7a), 68.7 (C3), 66.5 (C5), 19.3 (C1')

206:

R_f (1:1 diethyl ether/n-hexane) = 0.38

[α]^{25}_D = +54.7 (c 1.00, CHCl_3)

IR v_{max} (cm^{-1}): 3369, 3097, 2958, 1608, 1539, 1137, 1023, 699 583 cm^{-1}

LRESIMS m/z 594 (100%) [M+H]^+.

^1H NMR (500 MHz, CDCl_3) δ 8.04 (2H, d, J = 7.7 Hz, o–Bz), 7.56 (1H, t, J = 7.5 Hz, p–Bz), 7.43 (2H, t, J = 7.6 Hz, m–Bz), 7.38 – 7.23 (15H, m, ArH), 5.31 (1H, d, J = 4.0 Hz, H6), 4.62 – 4.51 (3H, m, OCH_2Ph), 4.53 – 4.43 (3H, m, OCH_2Ph), 4.30 (1H, d, J = 2.6 Hz, H1), 4.22 (1H, d, J = 2.4 Hz, H2), 4.10 (1H, t, J = 6.1 Hz, H7), 3.98 (1H, dd, J = 4.6, 2.2 Hz, H7a), 3.56 (1H, t, J = 9.0 Hz, H1'), 3.52 – 3.38 (3H, m, OH, H1’, H5), 3.30 (1H, ddd, J = 8.6, 5.9, 2.4 Hz, H3), 1.15 (3H, d, J = 6.5 Hz, H1’).

^13C NMR (125 MHz, CDCl_3) δ 165.9 (CO), 138.4 (ArC), 137.9 (ArC), 137.0 (ArC), 133.1 (ArC), 129.8 (ArCH), 128.6 (ArCH), 128.43 (ArCH), 128.36 (ArCH), 128.3 (ArCH), 274
128.1 (ArCH), 128.1 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 127.6 (ArCH),
84.9 (C2), 83.2 (C6), 82.3 (C1), 75.4 (C7), 73.3 (OCH2Ph), 73.2 (C7a), 72.1 (OCH2Ph),
72.0 (Cl'), 71.7 (OCH2Ph), 69.8 (C3), 62.5 (C5), 16.1 (Cl').

\((1S,2S,3R,5R,6R,7R,7aR)-6,7\text{-bis(benzyloxy)}-5\text{-}((\text{benzyloxy})\text{methyl})-1\text{-hydroxy}-3\text{-methylhexahydro-1H-pyrrolizin-2-yl benzoate (207)}\) and
\((1R,2R,3R,5R,6R,7R,7aS)-6,7\text{-Bis(benzyloxy)}-5\text{-}((\text{benzyloxy})\text{methyl})-2\text{-hydroxy}-3\text{-methylhexahydro-1H-pyrrolizin-1-yl benzoate (208)}\)

Compounds 207 and 208 were synthesized from the cyclic sulfate 203 (61 mg, 0.11 mmol)
using the general method for ring opening of cyclic sulfates with benzoate. Purification by
FCC (1:1 EtOAc/n-hexane) gave an inseparable and complex mixture of 207 and 208 (32
mg, 49%). This mixture was subsequently subjected to \(O\)-benzoyl hydrolysis (see General
method below) to afford 213 (5 mg, 19%) and 214 (20 mg, 75%) which were separable by
FCC (5:95 MeOH/CH2Cl2).

\(R_f(1:1\text{ EtOAc/n-hexane}) = 0.50\)

LRESIMS \(m/z\) 594 (100%) [M+H]⁺.
(1R,2R,3R,5R,6R,7R,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-1-hydroxy-3-methylhexahydro-1H-pyrrolizin-2-yl benzoate (209) and

(1S,2S,3R,5R,6R,7R,7aS)-6,7-bis(benzyloxy)-5-((benzyloxy)methyl)-2-hydroxy-3-methylhexahydro-1H-pyrrolizin-1-yl benzoate (210)

Compounds 209 and 210 were synthesized from the cyclic sulfate 204 (45 mg, 0.082 mmol) using the general method for ring opening of cyclic sulfates with benzoate. Purification by FCC (2:3 EtOAc/n-hexane) gave pure samples of the two title compounds, 209 (11 mg, 23%) and 210 (19 mg, 39%), as clear yellow films.

209:

Rf (2:3 EtOAc/n-hexane) = 0.56
[α]D25 +13.4 (c 1.00, CHCl3)

IR νmax (cm⁻¹): 3340, 3063, 2922, 1707, 1496, 1154, 1027, 583 cm⁻¹

LRESIMS m/z 594 (100%) [M+H]+.

HRESIMS found 594.2877, calcd for C37H40NO6, 594.2856 [M+H]+.

1H NMR (500 MHz, CDCl3) δ 8.04 (2H, d, J = 7.7 Hz, o–Bz), 7.57 (1H, t, J = 7.4 Hz, p–Bz), 7.44 (2H, t, J = 7.7 Hz, m–Bz), 7.38 – 7.20 (15H, m, ArH), 5.04 (1H, dd, J = 7.3, 4.5 Hz, H6), 4.69 – 4.43 (6H, m, OCH2Ph), 4.30 – 4.19 (2H, m, H1, H7), 3.55 (1H, d, J = 6.9 Hz, H3), 3.45 (1H, d, J = 9.4 Hz, H1’), 3.42 – 3.30 (2H, m, H1′, H5), 1.32 (3H, d, J = 7.0 Hz, H1”).

13C NMR (125 MHz, CDCl3) δ 166.7 (CO), 138.3 (ArC), 138.0 (ArC), 137.2 (ArC), 133.2 (ArC), 129.9 (ArCH), 129.8 (ArCH), 128.7 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.3 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.7 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 86.3 (C6), 85.2 (C2), 82.5 (C1), 77.2 (C7), 73.2 (OCH2Ph), 72.2 (C1’), 71.8 (OCH2Ph), 71.7 (OCH2Ph), 71.4 (C7a), 62.5 (C3), 58.4 (C5), 15.3 (C1”).

210:

Rf (2:3 EtOAc/n-hexane) = 0.39
$[\alpha]_D^{25} + 9.0 \ (c \ 1.00, \ CHCl_3)$

**IR** $\nu_{\max} (\text{cm}^{-1})$: 3330, 3112, 2970, 1646, 1453, 1095, 906, 565 cm$^{-1}$

**LRESIMS** $m/z$ 594 (100%) [M+H]$^+$. 

$^1$H NMR (500 MHz, CDCl$ _3 $) $\delta$ 8.02 (2H, d, $J = 7.8$ Hz, o–Bz), 7.57 (1H, t, $J = 7.4$ Hz, p–Bz), 7.43 (2H, t, $J = 7.6$ Hz, m–Bz), 7.37 – 7.22 (15H, m, ArH), 5.16 (1H, d, $J = 3.2$ Hz, H7), 4.63 – 4.42 (7H, m, OCH$_2$Ph, H1), 4.14 (1H, s, H2), 4.02 (1H, d, $J = 3.7$ Hz, H6), 3.84 (1H, t, $J = 7.8$ Hz, H3), 3.66 (1H, d, $J = 3.2$ Hz, H7a), 3.58 (1H, dt, $J = 10.6, 5.1$ Hz, H5), 3.44 (2H, d, $J = 7.8$ Hz, H1’), 1.38 (3H, d, $J = 7.0$ Hz, H1”).

$^{13}$C NMR (125 MHz, CDCl$ _3 $) $\delta$ 166.3 (CO), 138.4 (ArC), 137.9 (ArC), 136.5 (ArC), 133.2 (ArC), 130.0 (ArCH), 129.8 (ArCH), 129.7 (ArCH), 128.6 (ArCH), 128.4 (ArCH), 128.4 (ArCH), 128.3 (ArCH), 128.24 (ArCH), 128.20 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 127.5 (ArCH), 85.6 (C2), 85.3 (C1), 85.1 (C7), 79.5 (C6), 76.4 (C7a), 73.2 (OCH$_2$Ph), 72.7 (C1’), 72.0 (OCH$_2$Ph), 71.6 (OCH$_2$Ph), 63.5 (C3), 61.2 (C5), 11.4 (C1”).

**General method for O-Benzoyl hydrolysis**

(1S,2S,3S,5R,6R,7R,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2-diol (211)

To a solution of 205 (23 mg, 0.039 mmol) in MeOH (5 mL) was added solid K$_2$CO$_3$ (8.2 mg, 0.060 mmol). After stirring at 40 °C for 2 h, all volatiles were evaporated and the residue was dissolved in EtOAc. The solution was washed with water (3 x 5 mL) and brine (5 mL), dried (MgSO$_4$), filtered and concentrated in vacuo to give a brown oil. The crude product was purified by FCC (5:95 MeOH/CH$_2$Cl$_2$) to give the product 211 as colourless crystals (15 mg, 79%).

$R_f$ (5:95 MeOH/CH$_2$Cl$_2$) = 0.28

$[\alpha]_D^{25} + 14.6 \ (c \ 1.00, \ CHCl_3)$

**M.P:** 115 – 120 °C
IR $\nu_{\max}$ (cm$^{-1}$): 3370, 3078, 2968, 2930, 1679, 1502, 1467, 1320, 1089, 687 cm$^{-1}$

LRESIMS $m/z$ 490 [M+H]$^+$.  

HRESIMS found 490.2617, calcd for C$_{30}$H$_{36}$NO$_5$, 490.2593 [M+H]$^+$.  

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.33 – 7.21 (15H, m, ArH), 4.61 – 4.41 (6H, m, OCH$_2$Ph), 4.10 (1H, t, $J = 3.6$ Hz, H2), 4.07 (1H, t, $J = 3.6$ Hz, H1), 3.97 (1H, t, $J = 7.5$ Hz, H7), 3.68 (1H, t, $J = 8.1$ Hz, H6), 3.48 – 3.40 (3H, m, H1’ and H7a), 3.23 (1H, dt, $J = 9.8$, 4.5 Hz, H3), 3.06 (2H, s, OH), 2.93 (1H, dq, $J = 12.6$, 6.4 Hz, H5), 1.17 (3H, d, $J = 6.2$ Hz, H1”).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 138.3 (ArC), 138.01 (ArC), 138.00 (ArC), 128.42 (ArCH), 128.40 (ArCH), 128.3 (ArCH), 127.8 (ArCH), 127.69 (ArCH), 127.67 (ArCH), 127.5 (ArCH), 87.2 (C1), 86.0 (C2), 83.1 (C6), 80.2 (C7), 73.2 (OCH$_2$Ph), 72.3 (C7a), 71.9 (OCH$_2$Ph), 71.6 (OCH$_2$Ph), 71.3 (C1’), 69.2 (C3), 65.3 (C5), 19.0 (C1”).

(1R,2R,3S,5R,6R,7R,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2-diol (212)

Compound 212 was synthesized from 206 (8.0 mg, 0.014 mmol) using the general method for O–benzoyl deprotection. The crude brown oil was purified by FCC (5:95 MeOH/CH$_2$Cl$_2$) to give the product 212 as a clear yellow oil (6.0 mg, 90%).

$R_f$ (5:95 MeOH/CH$_2$Cl$_2$) = 0.15  

$[\alpha]_D^{25}$ $+$17.4 ($c$ 1.00, CHCl$_3$)  

IR $\nu_{\max}$ (cm$^{-1}$): 3387, 3049, 2922, 1643, 1428, 1354, 1067, 697 cm$^{-1}$

LRESIMS $m/z$ 490 [M+H]$^+$.  

HRESIMS found 490.2613, calcd for C$_{30}$H$_{36}$NO$_5$, 490.2593 [M+H]$^+$.  

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.53 – 7.05 (15H, m, ArH), 4.64 – 4.40 (6H, m, OCH$_2$Ph), 4.32 (1H, s, H1), 4.22 – 4.14 (2H, m, H2 and H7), 4.09 (1H, s, H7a), 3.94 (1H, s, H6), 3.61 – 3.55 (2H, m, H1’), 3.37 – 3.32 (1H, m, H3), 3.29 (1H, bs, H5), 1.23 (3H, d, $J = 6.5$ Hz, H1”).

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\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 138.1 (ArC), 137.7 (ArC), 136.7 (ArC), 128.6 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 127.74 (ArCH), 127.70 (ArCH), 127.66 (ArCH), 84.8 (C2), 81.4 (C1), 81.2 (C6), 76.8 (C7a), 73.3 (OCH\(_2\)Ph), 72.9 (C7), 72.4 (OCH\(_2\)Ph), 72.0 (OCH\(_2\)Ph), 70.6 (C1'), 69.3 (C3), 64.2 (C5), 14.0 (H1').

(1\(R\),2\(R\),3\(R\),5\(R\),6\(R\),7\(aR\))-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1\(H\)-pyrrolizine-1,2-diol (213)

\[\text{Compound 213 was synthesized from 209 (11 mg, 0.017 mmol) using the general method for O–benzoyl deprotection. Purification by FCC (5:95 MeOH/CH}_2\text{Cl}_2\text{) returned the product 213 as a brown film (7.5 mg, 90%).}\]

\[\text{R}_f(5:95 \text{ MeOH/CH}_2\text{Cl}_2) = 0.20\]
\[\alpha \text{D}^{25}_2 = -25.4 \text{ (c 1.00, CHCl}_3\text{)}\]
\[\text{IR }\nu \text{max (cm}^{-1})\text{: 3360, 3068, 2937, 1640, 1450, 1029, 681 cm}^{-1}\]
\[\text{LRESIMS } m/z \text{ 490 (100%) [M+H]}^+.\]
\[\text{HRESIMS found 490.2608, calcd for C}_{30}\text{H}_{36}\text{NO}_5, 490.2593 [M+H]^+.}\]
\[\text{iH NMR (500 MHz, CDCl}_3\text{) }\delta \text{ 7.43 – 7.10 (15H, m, ArH), 4.67 – 4.36 (6H, m, OCH}_2\text{Ph), 4.19 (1H, t, }\text{J} = 2.6 \text{ Hz, H1), 4.09 (1H, s, H2), 3.99 (1H, dd, }\text{J} = 7.5, 4.7 \text{ Hz, H7), 3.89 (1H, dd, }\text{J}\text{ = 7.5, 2.8 Hz, H7a), 3.67 (1H, dd, }\text{J} = 7.5, 4.6 \text{ Hz, H6), 3.53 – 3.40 (3H, m, H3, H1')}, \text{ 3.17 (1H, quint, }\text{J} = 6.5, 6.1 \text{ Hz, H5), 2.02 (1H, bs, OH), 1.29 (3H, d, }\text{J} = 6.9 \text{ Hz, H1'}).\]
\[\text{13C NMR (125 MHz, CDCl}_3\text{) }\delta \text{ 138.2 (ArC), 137.9 (ArC), 137.0 (ArC), 128.6 (ArCH), 128.4 (ArCH), 128.4 (ArCH), 128.13 (ArCH), 128.07 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 127.7 (ArCH), 85.0 (C2), 84.4 (C6), 82.3 (C1), 79.4 (C7), 73.3 (OCH}_2\text{Ph), 72.1 (C1'), 71.9 (OCH}_2\text{Ph), 71.8 (OCH}_2\text{Ph), 70.3 (C7a), 62.6 (C3), 60.3 (C5), 14.7 (C1'}).\]
(1S,2S,3R,5R,6R,7R,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2-diol (214)

Compound 214 was synthesized from benzoate 210 (15 mg, 0.025 mmol) using the general method for O–benzoyl deprotection, except that the reaction was stirred at rt for 1 h. The crude brown oil was purified by FCC (5:95 MeOH/CH₂Cl₂) to give the product 214 as a clear brown film (10 mg, 83%).

Rf (5:95 MeOH/CH₂Cl₂) = 0.14
[α]₂⁵^D −1.62 (c 1.00, CHCl₃)
IR νmax (cm⁻¹): 3389, 2890, 1654, 1489, 1054 cm⁻¹
LRESIMS m/z 490 (100%) [M+H]^+.
HRESIMS found 490.2615, calcd for C₃₀H₃₆NO₅, 490.2593 [M+H]^+.
¹H NMR (500 MHz, CDCl₃) δ 7.45 – 7.17 (15H, m, ArH), 4.64 – 4.43 (6H, m, OCH₂Ph), 4.14 – 4.08 (3H, m, H7, H1, H2), 3.81 (1H, dd, J = 3.8, 1.8 Hz, H6), 3.73 (1H, td, J = 6.9, 3.0 Hz, H3), 3.65 (1H, q, J = 5.2, 4.5 Hz, H5), 3.56 (1H, t, J = 3.1 Hz, H7a), 3.44 (2H, d, J = 1.6 Hz, H1’), 1.28 (3H, d, J = 7.0 Hz, H1”).
¹³C NMR (125 MHz, CDCl₃) δ 138.2 (ArC), 137.8 (ArC), 136.9 (ArC), 128.6 (ArCH), 128.5 (ArCH), 128.3 (ArCH), 128.12 (ArCH), 128.10 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 85.8 (C1 or C7 or C2), 85.0 (C1 or C7 or C2), 81.5 (C6), 81.3 (C1 or C7 or C2), 76.8 (C7a), 73.2 (OCH₂Ph), 72.3 (OCH₂Ph), 71.8 (OCH₂Ph), 71.6 (C1’), 62.5 (C3), 60.1 (C5), 11.4 (C1”).
(1R,2R,3R,5S,6S,7S,7aR)-3-(hydroxymethyl)-5-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol [Initially proposed structure of (+)-hyacinthacine C₅]

The compound initially proposed to be (+)-hyacinthacine C₅ was synthesized by the general method of O-benzyl deprotection from 211 (20 mg, 0.04 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated \textit{in vacuo} returning a yellow film. The title compound was isolated through basic ion-exchange chromatography followed by concentration \textit{in vacuo}, as a colourless film (9.0 mg, 99%).

$[\alpha]_D^{25} +9.5 \ (c \ 1.00, \ H_2O)$

**IR $\nu_{\max}$ (cm$^{-1}$):** 3302, 2952, 2926, 1603, 1545, 1212, 1079, 694 cm$^{-1}$

**LRESIMS** $m/z$ 220 [M+H]$^+$.  
**HRESIMS** found 220.1177, calcd for C$_9$H$_{18}$NO$_5$, 220.1185 [M+H]$^+$.  

**$^1$H NMR (500 MHz, D$_2$O) $\delta$** 4.15 (1H, t, $J = 7.0$ Hz, H1), 4.11 (1H, t, $J = 7.1$ Hz, H7), 3.96 (1H, t, $J = 7.0$ Hz, H2), 3.74 – 3.61 (3H, m, H6, H8), 3.05 (1H, t, $J = 6.8$ Hz, H7a), 2.96 – 2.87 (2H, m, H5, H3), 1.20 (3H, d, $J = 6.2$ Hz, H9).

**$^{13}$C NMR (125 MHz, D$_2$O) $\delta$** 82.9 (C6), 80.2 (C1), 79.6 (C7), 79.3 (C2), 71.2 (C7a), 71.1 (C5), 66.0 (C3), 62.9 (C8), 17.8 (C9).

(1R,2R,3R,5S,6R,7R,7aR)-3-(Hydroxymethyl)-5-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol [(+)-5-epi-hyacinthacine C₅] (215)
(+)-6,7-Di-epi-hyacinthacine C₅ 215 was synthesized by the general method of O–benzyl deprotection from 212 (6.0 mg, 0.012 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo returning a yellow film. The title compound 215 was isolated through basic ion–exchange chromatography followed by concentration in vacuo, as a white solid (2.0 mg, 78%).

[α]₂⁵ +10.3 (c 1.00, H₂O)

IR 〈max (cm⁻¹): 3298, 2963, 1593, 1546, 1273, 1086, 699 cm⁻¹

LRESIMS m/z 220 (100%) [M+H]⁺.

HRESIMS found 220.1175, calcld for C₉H₁₈NO₅, 220.1185 [M+H]⁺.

¹H NMR (500 MHz, D₂O) δ 4.28 (1H, t, J = 7.5 Hz, H1), 4.21 (1H, dd, J = 4.7, 2.4 Hz, H7), 4.13 (1H, dd, J = 4.2, 2.4 Hz, H6), 3.96 (1H, t, J = 8.4 Hz, H2), 3.78 (1H, dd, J = 12.3, 4.0 Hz, H8), 3.68 (1H, dd, J = 12.0, 4.6 Hz, H8), 3.46 (1H, dd, J = 7.2, 4.6 Hz, H7a), 3.14 (1H, q, J = 6.1, 4.9, 4.3 Hz, H5), 2.78 (1H, dd, J = 9.3, 4.6 Hz, H3), 1.17 (3H, d, J = 6.9 Hz, H9).

¹³C NMR (125 MHz, D₂O) δ 82.2 (C6), 81.2 (C2), 76.8 (C7), 75.6 (C1), 73.1 (C3), 71.6 (C7a), 65.2 (C5), 64.2 (C8), 16.5 (C9).

(1R,2R,3R,5R,6R,7R,7aR)-3-(Hydroxymethyl)-5-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol [Corrected structure for (+)-hyacinthacine C₅] (216)

The compound for the corrected structure of (+)-hyacinthacine C₅ 216 was synthesized by the general method of O–benzyl deprotection from 213 (7.0 mg, 0.014 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo returning a yellow film. The title compound 216 was isolated through basic ion–exchange chromatography followed by concentration in vacuo, as a colourless film (3.0 mg, 95%).
$[\alpha]_D^{25} + 5.25$ (c 1.00, H$_2$O)

**IR** $\nu_{\text{max}}$ (cm$^{-1}$): 3296, 2928, 1602, 1430, 1072 cm$^{-1}$

**LRESIMS** $m/z$ 220 (100%) [M+H]$^+$.  

**HRESIMS** found 220.1201, calcd for C$_9$H$_{18}$NO$_5$, 220.1185 [M+H]$^+$.  

**$^1$H NMR** (500 MHz, D$_2$O) $\delta$ 4.18 (1H, t, $J = 4.0$ Hz, H7), 4.07 – 3.97 (2H, m, H6, H1), 3.90 (1H, t, $J = 8.1$ Hz, H2), 3.67 (2H, t, $J = 4.0$ Hz, H8), 3.41 (1H, dt, $J = 11.7$, 5.7 Hz, H5), 1.27 (3H, d, $J = 6.7$ Hz, H9).

**$^{13}$C NMR** (125 MHz, D$_2$O) $\delta$ 78.8 (C6), 78.1 (C2), 75.2 (C1), 74.9 (C7), 66.3 (C7a), 62.8 (C8), 62.1 (C3), 58.4 (C5), 12.8 (C9).

(1R,2R,3R,5R,6S,7S,7aR)-3-(Hydroxymethyl)-5-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol [(+)-6,7-di-epi-hyacinthacine C$_5$] (217)

(+)–5-Epi-hyacinthacine C$_5$ 217 was synthesized by the general method of $O$–benzyl deprotection from 214 (10 mg, 0.020 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo returning a green film. The title compound 217 was isolated through basic ion–exchange chromatography followed by concentration in vacuo, as a colourless film (3.4 mg, 75%).

$[\alpha]_D^{25} + 6.8$ (c 1.00, H$_2$O)

**IR** $\nu_{\text{max}}$ (cm$^{-1}$): 3290, 2943, 1593, 1375, 1208, 1079 cm$^{-1}$

**LRESIMS** $m/z$ 220 (100%) [M+H]$^+$.  

**HRESIMS** found 220.1180, calcd for C$_9$H$_{18}$NO$_5$, 220.1185 [M+H]$^+$.  

**$^1$H NMR** (500 MHz, D$_2$O) $\delta$ 4.18 (1H, t, $J = 4.0$ Hz, H7), 4.07 – 3.97 (2H, m, H6, H1), 3.90 (1H, t, $J = 8.1$ Hz, H2), 3.67 (2H, t, $J = 4.0$ Hz, H8), 3.41 (1H, dt, $J = 11.7$, 5.7 Hz,
H5), 3.33 (1H, dt, J = 9.0, 4.9 Hz, H3), 3.07 (1H, dd, J = 8.1, 3.7 Hz, H7a), 1.24 (3H, d, J = 7.1 Hz, H9).

\[ ^{13}C \text{ NMR (125 MHz, D}_2\text{O)} \delta 79.7 (C6 or C7), 78.6 (C6 or C7), 78.0 (C1), 77.9 (C2), 72.1 (C7a), 62.9 (C8), 62.2 (C3), 58.1 (C5), 10.7 (C9). \]

\((1R,2R,3R,5R,6S,7S,7aR)-3-(acetoxymethyl)-5-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetrayl tetraacetate (218)\)

To a solution of 217 (8.0 mg, 0.037 mmol) in pyridine (147 µL, 1.83 mmol) was added acetic anhydride (173 µL, 1.83 mmol) and a crystal of 4–dimethylaminopyridine. The mixture was stirred at rt for 24 h, by which time LRESIMS analysis confirmed full consumption of the starting material. The reaction was quenched through the addition of sat. NaHCO\(_3\) solution (3 mL) and extracted with CH\(_2\)Cl\(_2\) (3 x 5 mL). The combined extracts were dried (MgSO\(_4\)), filtered and concentrated in vacuo to give an orange oil. Purification by FCC (3:97 MeOH/CH\(_2\)Cl\(_2\)) gave the pentaacetylated product 218 as a pale–yellow film (15 mg, 95%).

\[ R_f (3:95 \text{ MeOH/CH}_2\text{Cl}_2) = 0.35 \]

\[ [\alpha]^{25}_D +12.9 \text{ (c 1.00, CHCl}_3\text{)} \]

**IR** \(\nu_{max} (\text{cm}^{-1})\): 2934, 1736, 1432, 1368, 1217, 1033, 886, 603 cm\(^{-1}\)

**LRESIMS** \(m/z\) 430 (100%) [M+H]\(^+\)

**HRESIMS** found 430.1731, calcd for C\(_{19}\)H\(_{28}\)NO\(_{10}\), 430.1713 [M+H]\(^+\).

\[^1H \text{ NMR (500 MHz, CDCl}_3\text{)} \delta 5.36 (1H, t, J = 4.8 Hz, H1), 5.24 (1H, t, J = 4.8 Hz, H2), 5.20 (2H, dd, J = 7.6, 3.8 Hz, H6, H7), 4.04 (2H, qd, J = 11.3, 5.8 Hz, H1\(^\text{a}\)), 3.72 – 3.57 (2H, m, J = 6.1 Hz, H3, H5), 3.41 (1H, t, J = 4.5 Hz, H7a), 2.14 – 2.05 (15H, m, 5 x AcCH\(_3\)), 1.25 (3H, d, J = 7.1 Hz, H1\(^\text{a}\)).\]

\[^{13}C \text{ NMR (125 MHz, CDCl}_3\text{)} \delta 170.8 (CO), 170.2 (CO), 169.9 (CO), 169.7 (CO), 169.6 (CO), 80.4 (C6), 79.5 (C7), 78.6 (C2), 77.6 (C1), 73.1 (C7a), 65.5 (C1\(^\text{a}\)), 60.4 (C3), 58.6 \]
(C5), 20.94 (AcCH₃), 20.92 (AcCH₃), 20.89 (AcCH₃), 20.85 (AcCH₃), 20.8 (AcCH₃), 11.3 (C1”).
Chapter 5

tert-Butyl \((R,E)-1-((4R,5S,6S)-5-(benzyloxy)-6-((benzyloxy)methyl)-2,2-dioxido-1,3,2-dioxathian-4-yl)-3-phenylallyl)((R)-but-3-en-2-yl)carbamate (225)\)

\[
\text{Compound 225 was synthesized from the } N\text{–Boc diol 139 (60 mg, 0.10 mmol) by using the general method for the synthesis of a cyclic sulfate. The resultant crude brown oil 225 (66 mg) was used for the next step without further purification.}
\]

LRESIMS \(m/z\) 684 (100%) [M+Cl].

\((4R,5S)-5-((1R,2S)-1,3\text{bis(benzyloxy)-2-hydroxypropyl)-3-((R)-but-3-en-2-yl)-4-((E)\text{styryl)oxazolidin-2-one (224)\)}}

Cyclic sulfate 225 (66 mg, 0.10 mmol) was dissolved in anhydrous MeCN (10 mL) and heated to 50 °C and stirred for 2 h. After TLC confirmed the consumption of starting material, the reaction mixture was cooled to rt, followed by the addition of a solution of THF (4 mL), distilled water (1.5 mL) and conc. H\(_2\)SO\(_4\) (0.5 mL). The reaction mixture was heated at 50 °C and stirred for 1 h. After TLC confirmed the full consumption of starting material, the reaction mixture was allowed to cool to rt and neutralized with saturated NaHCO\(_3\) until a pH of 7 was achieved. The solution was extracted with CH\(_2\)Cl\(_2\) (3 x 15 mL), dried (MgSO\(_4\)), filtered and concentrated \textit{in vacuo}. Purification by FCC (2:3 EtOAc/n-hexane) returned the oxazolidinone 224 as a pale–yellow oil (20 mg, 39%).
R_f (1:1 EtOAc/n-hexane) = 0.64
[α]_D^{25} +20.2 (c 2.00, CHCl_3).

IR \textit{\nu}_{\text{max}} (\text{cm}^{-1}): 3345, 2998, 2885, 1632, 1456, 1279, 1098, 1020, 965 cm^{-1}

HRESIMS m/z 536 (100%) [M+Na]^+.

LRESIMS m/z 536 (100%) [M+Na]^+.

HREMS found 536.2408, calcd for C_{32}H_{35}NO_5 Na, 536.2413 [M+Na]^+.

\textit{^1}H NMR (500 MHz, CDCl_3) δ 7.40 – 7.22 (15H, m, Ar), 6.47 (1H, d, J = 15.8 Hz, H2’’’), 6.02 (1H, dd, J = 15.8, 9.0 Hz, H1’’’’), 5.84 (1H, ddd, J = 16.8, 10.7, 5.9 Hz, H2’), 5.15 – 5.09 (2H, m, H3’), 4.77 (1H, d, J = 10.9 Hz, OCH_2Ph), 4.64 (1H, d, J = 11.0 Hz, OCH_2Ph), 4.53 (1H, dd, J = 8.9, 5.1 Hz, H4), 4.50 – 4.44 (2H, m, OCH_2Ph), 4.39 – 4.31 (2H, m H5, H1’), 3.91 (1H, t, J = 3.8 Hz, H1’’’’), 3.84 (1H, td, J = 5.8, 3.8 Hz, H2’’’’’), 3.48 (1H, dd, J = 9.7, 5.6 Hz, H3’’’’’), 3.43 (1H, dd, J = 9.7, 5.6 Hz, H3’’’’’), 2.46 (1H, d, J = 6.2 Hz, OH), 1.28 (3H, d, J = 7.0 Hz H1’’).

\textit{^13}C NMR (125 MHz, CDCl_3) δ 156.6 (C2), 137.5 (ArC), 137.4 (ArC), 136.8 (C2’), 135.7 (ArC), 133.9 (C2’’’), 128.7 (ArCH), 128.64 (ArCH), 128.62 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.3 (C1’’’), 128.2 (ArCH), 127.9 (ArCH), 126.6 (ArCH), 117.1 (C3’), 79.9 (C5), 78.9 (C1’’’’), 75.7 (OCH_2Ph), 73.5 (OCH_2Ph), 70.8 (C3’’’’’), 69.4 (C2’’’’’), 58.2 (C7a), 51.7 (C1’), 18.3 (C1’

(1S,5R,7aR)-1-((1R,2S)-1,3-Bis(benzyloxy)-2-hydroxypropyl)-5-methyl-5,7a-dihydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (223)

Compound 223 was synthesized from 224 (20 mg, 0.039 mmol), using the general method for ring-closing metathesis. The crude black oil was purified by FCC (1:1 EtOAc/n-hexane) to give 223 as a grey oil (15 mg, 95%).

R_f (1:1 EtOAc/n-hexane) = 0.64
[α]_D^{25} -6.6 (c 1.37, CHCl_3).

IR \textit{\nu}_{\text{max}} (\text{cm}^{-1}): 3345, 2998, 2885, 1632, 1456, 1279, 1098, 1020, 965 cm^{-1}
LRESIMS \( m/z \) 432 (100\%) [M+Na]⁺.

HRESIMS found 432.1793, calcd for C\(_{24}\)H\(_{27}\)NO\(_5\)Na, 432.1787 [M+Na]⁺.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.39 – 7.22 (10H, m, ArH), 5.83 (1H, d, \( J = 6.1 \) Hz, H6), 5.76 (1H, d, \( J = 6.1 \) Hz, H7), 4.98 (1H, d, \( J = 8.9 \) Hz, H7a), 4.83 (1H, d, \( J = 11.2 \) Hz, OCH\(_2\)Ph), 4.62 (1H, d, \( J = 11.2 \) Hz, OCH\(_3\)Ph), 4.50 – 4.46 (2H, m, OCH\(_2\)Ph), 4.37 (1H, dd, \( J = 9.0 \), 3.5 Hz, H1), 4.30 (1H, t, \( J = 6.2 \) Hz, H5), 3.98 (1H, s, H1'), 3.86 (1H, d, \( J = 6.9 \) Hz, H2'), 3.54 (1H, dd, \( J = 9.4 \), 6.4 Hz, H3'), 3.44 (1H, dd, \( J = 9.4 \), 6.3 Hz, H3'), 2.34 (1H, d, \( J = 7.2 \) Hz, OH), 1.55 (3H, d, \( J = 6.7 \) Hz, H1'').

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 157.0 (C3), 137.55 (ArC), 137.49 (ArC), 136.4 (C7), 128.53 (ArCH), 128.52 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 127.3 (C6), 83.1 (C1), 77.0 (C1'), 75.2 (OCH\(_2\)Ph), 73.5 (OCH\(_2\)Ph), 70.8 (C3'), 70.3 (C2'), 68.2 (C7a), 60.2 (C5), 17.4 (C1'').

\((1S,5R,6R,7S,7aR)-1-((1R,2S)-1,3-Bis(benzyloxy)-2-hydroxypropyl)-6,7-dihydroxy-5-methyltetrahydro-1H,3H-pyrrolo[1,2-c]oxazol-3-one (228)\)

[\( \text{Compound 228 was synthesized from 223 (15 mg, 0.037 mmol) using the general method for cis–dihydroxylation. The product was purified by FCC (5:95 MeOH/CH\(_2\)Cl\(_2\)) to give 228 as a light brown oil (12 mg, 72\%).} \]

\( R_f \) (1:1 EtOAc/n-hexane) = 0.64

[\( [\alpha]_D^{25} \) +9.1 (c 0.40, CHCl\(_3\)).

IR \( \nu_{\text{max}} \) (cm\(^{-1}\)): 3443, 2912, 2829, 1758, 1643, 1509, 1265, 1198, 1086 cm\(^{-1}\)

LRESIMS \( m/z \) 466 (100\%) [M+Na]⁺.

HRESIMS found 466.1834, calcd for C\(_{24}\)H\(_{29}\)NO\(_7\)Na, 466.1842 [M+Na]⁺.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.40 – 7.22 (10H, m, ArH), 4.86 – 4.77 (2H, m, OCH\(_2\)Ph, H1), 4.59 (1H, d, \( J = 11.3 \) Hz, OCH\(_2\)Ph), 4.49 – 4.32 (2H, m, OCH\(_2\)Ph), 4.25 – 4.15 (2H, m, H6, H7a), 4.04 (1H, s, H7), 3.96 (1H, s, H1'), 3.83 (1H, s, H2'), 3.72 – 3.62 (1H, m,
H5), 3.59 – 3.52 (1H, m, H3’), 3.46 – 3.40 (1H, m, H3’), 3.32 (1H, s, OH), 2.84 (1H, s, OH), 2.70 (1H, s, OH), 1.46 (3H, d, J = 6.7 Hz, H1’’).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 156.8 (C3), 137.4 (ArC), 137.2 (ArC), 128.7 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 128.29 (ArCH), 128.25 (ArCH), 128.1 (ArCH), 77.9 (C1), 77.0 (C1’), 75.3 (C6), 75.2 (OCH$_2$Ph), 73.7 (OCH$_2$Ph), 70.9 (C3’), 70.8 (C2’), 70.3 (C7), 60.7 (C7a), 55.0 (C5), 11.5 (C1’’).

(3αS,3βR,4S,8R,8aR)-4-[(1R,2S)-1,3-Bis(benzyloxy)-2-hydroxypropyl]-2,2,8-trimethyltetrahydro-4H,6H-[1,3]dioxolo[4’,5’:3,4]pyrrolo[1,2-c]oxazol-6-one (221)

Compound 221 was synthesized from 228 (12 mg, 0.027 mmol) by the general method for acetonide protection of cis-diols. Purification by FCC (7:15) to give 221 as a pale–yellow oil (13 mg, 95%).

$R_f$ (1:1 EtOAc/n-hexane) = 0.64
$[\alpha]_{D}^{25} +30.1$ (c 0.40, CHCl$_3$).

IR $\nu_{\text{max}}$ (cm$^{-1}$): 2955, 2843, 1798, 1623, 1556, 1190, 1099 cm$^{-1}$

LRESIMS $m/z$ 484 (100%) [M+H]$^+$.  
HRESIMS found 484.2342, calcd for C$_{27}$H$_{34}$NO$_7$, 484.2335 [M+H]$^+$. 

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.39 – 7.24 (10H, m, ArH), 4.83 – 4.77 (2H, m, OCH$_2$Ph, H4), 4.60 (1H, d, J = 11.3 Hz, OCH$_2$Ph), 4.56 (1H, dd, J = 5.5, 4.0 Hz, H8a), 4.53 – 4.46 (3H, m, OCH$_2$Ph, H3a), 4.01 (1H, t, J = 4.4 Hz, H3b), 3.94 – 3.86 (2H, m, H1’, H3’), 3.53 (1H, dd, J = 9.5, 6.3 Hz, H3’), 3.45 – 3.33 (2H, m, H3’, H8), 1.64 (3H, d, J = 7.0 Hz, H1’’), 1.46 (3H, s, C2–CH$_3$), 1.30 (3H, s, C2–CH$_3$).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 157.1 (C4), 137.6 (ArC), 137.6 (ArC), 128.5 (ArCH), 128.4 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 127.9 (ArCH), 112.2 (C2), 83.8 (C8a), 78.8 (C3a), 77.7 (C1’), 75.4 (C4), 75.2 (OCH$_2$Ph), 73.5 (OCH$_2$Ph), 71.0 (C3’), 70.5 (C2’), 62.9 (C3b), 57.2 (C8), 26.1 (C2–CH$_3$), 24.4 (C2–CH$_3$), 10.1 (C1’’).
General method for Swern oxidation

\((3aS,4S,6R,7R,8aR,8bR)-7-(\text{Benzyloxy})-6-(\text{benzyloxy)methyl})-2,2,4\text{-trimethylhexahydro}-8H-[1,3]\text{dioxolo}[4,5-\alpha]\text{pyrrolizin-8-one (231)}\)

\[\text{231}\]

To a cooled (−78 °C), stirred solution of DMSO (93 µL, 1.3 mmol) in CH\(_2\)Cl\(_2\) (5 mL) was added oxalyl chloride (56.0 µL, 0.653 mmol). After stirring for 5 min, a solution of 165 (29 mg, 0.0653 mmol) in CH\(_2\)Cl\(_2\) (2 mL) was added to the mixture followed by the addition of triethylamine (364 µL, 2.61 mmol). After stirring at −78 °C for 1 h, TLC analysis confirmed full consumption of the starting material. The reaction mixture was quenched with the addition of water (5 mL) and extracted with CH\(_2\)Cl\(_2\) (3 x 5 mL). The combined organic extracts were dried (MgSO\(_4\)), filtered and concentrated in vacuo. Purification by FCC (3:7 EtOAc/n-hexane) afforded the ketone 231 as an opaque yellow oil (24 mg, 83%).

\(\text{Rf (3:7 EtOAc/n-hexane)} = 0.25\)

\([\alpha]^\text{D}\)\(_{25}^\circ +22.7\) (c 1.00, CHCl\(_3\))

\(\text{IR } \nu_{\text{max}} (\text{cm}^{-1})\): 3030, 2910, 2875, 1716, 1451, 1380, 1227, 1095, 691 cm\(^{-1}\)

\(\text{LRESIMS } m/z\) 438 (100%) [M+H]\(^+\).

\(\text{HRESIMS } \text{found} 438.2275, \text{calcd for } \text{C}_{26}\text{H}_{32}\text{NO}_5\), 438.2280 [M+H]\(^+\).

\(^1\text{H NMR (500 MHz, CDCl}_3\) \(\delta\) 7.37 – 7.24 (10H, m, ArH), 5.04 – 4.95 (2H, m, OCH\(_2\)Ph, H8b), 4.69 (1H, d, J = 11.8 Hz, OCH\(_2\)Ph), 4.56 – 4.46 (3H, m, OCH\(_2\)Ph, H3a), 3.94 (1H, dd, J = 9.3, 1.3 Hz, H7), 3.89 – 3.83 (2H, m, H8a, H6), 3.79 (1H, q, J = 7.3 Hz, H4), 3.63 (1H, dd, J = 10.2, 2.7 Hz, H1\(^\prime\)), 3.45 (1H, dd, J = 10.3, 4.9 Hz, H1\(^\prime\)), 1.46 (3H, s, C2–CH\(_3\)), 1.25 (3H, s, C2–CH\(_3\)), 1.07 (3H, d, J = 7.3 Hz, H1\(^{\prime\prime}\)).

\(^{13}\text{C NMR (125 MHz, CDCl}_3\) \(\delta\) 211.0 (C8), 138.1 (ArC), 137.8 (ArC), 128.4 (ArCH), 128.3 (ArCH), 128.1 (ArCH), 127.78 (ArCH), 127.76 (ArCH), 127.68 (ArCH), 112.66 (C2), 88.4 (C3a), 84.1 (C8b), 80.3 (C7), 73.3 (OCH\(_2\)Ph), 72.6 (OCH\(_2\)Ph), 70.4 (C1\(^\prime\)), 70.2 (C8a), 63.9 (C4), 63.8 (C6), 26.3 (C2–CH\(_3\)), 23.0 (C2–CH\(_3\)), 19.3 (C1\(^{\prime\prime}\)).
Compound 232 was synthesized from the tricyclic alcohol 151 (35 mg, 0.080 mmol) by using the general method for the Swern oxidation. The product was purified by FCC (2:3 EtOAc/n-hexane) to give the target compound 232 as an opaque yellow oil (28 mg, 80%).

\[ \text{Rf} \text{(2:3 EtOAc/n-hexane) = 0.25} \]
\[ [\alpha]_D^{25} +95.2 \text{ (c 1.00, CHCl}_3] \]
\[ \text{IR} \nu_{\text{max}} \text{ (cm}^{-1}) : 3379, 2980, 2849, 1716, 1612, 1452, 1363, 1212, 1002, 678 \text{ cm}^{-1} \]
\[ \text{LRESIMS} \ text{m/z 438 (100%) [M+H]^+}. \]
\[ \text{HRESIMS found 438.2300, calcd for C}_{26}\text{H}_{32}\text{NO}_5, 438.2280 \text{ [M+H]^+}. \]
\[ ^1\text{H NMR (500 MHz, CDCl}_3) \delta 7.36 - 7.21 \text{ (10H, m, ArH)}, 5.03 \text{ (1H, d, } J = 11.6 \text{ Hz, OCH}_2\text{Ph}), 4.89 \text{ (1H, t, } J = 5.5 \text{ Hz, H8b)}, 4.59 \text{ (1H, d, } J = 11.6 \text{ Hz, OCH}_2\text{Ph}), 4.54 \text{ (1H, dd, } J = 5.8, 4.1 \text{ Hz, H3a)}, 4.46 \text{ (2H, s, OCH}_2\text{Ph)}, 4.16 \text{ (1H, dd, } J = 8.5, 1.5 \text{ Hz, H7)}, 3.96 \text{ (1H, ddd, } J = 8.6, 3.5, 2.1 \text{ Hz, H6)}, 3.69 \text{ (1H, dd, } J = 5.2, 1.5 \text{ Hz, H8a)}, 3.65 \text{ (1H, dd, } J = 10.4, 3.5 \text{ Hz, H1'}), 3.43 \text{ (1H, dd, } J = 10.5, 2.1 \text{ Hz, H1'')}, 3.30 \text{ (1H, qd, } J = 7.2, 4.0 \text{ Hz, H4)}, 1.47 - 1.41 \text{ (6H, m, C2–CH}_3\text{, H1'}), 1.26 \text{ (3H, s, C2–CH}_3\text{).} \]
\[ ^1\text{C NMR (125 MHz, CDCl}_3) \delta 211.8 \text{ (C8)}, 138.0 \text{ (ArC)}, 137.9 \text{ (ArC)}, 128.4 \text{ (ArCH)}, 128.2 \text{ (ArCH)}, 128.12 \text{ (ArCH)}, 128.11, 127.8 \text{ (ArCH)}, 127.7 \text{ (ArCH)}, 112.5 \text{ (C2)}, 84.4 \text{ (C3a)}, 83.5 \text{ (C8b)}, 80.9 \text{ (C7)}, 73.4 \text{ (OCH}_2\text{Ph)}, 73.0 \text{ (OCH}_2\text{Ph)}, 72.4 \text{ (C8a)}, 69.0 \text{ (C1')} \text{, 61.01 (C6)}, 60.97 \text{ (C4)}, 26.2 \text{ (C2–CH}_3\text{)}, 22.9 \text{ (C2–CH}_3\text{)}, 12.0 \text{ (C1').} \]
(3\(R\),4\(R\),6\(R\),7\(R\),8\(a\)R,8\(b\)S)-7-(Benzyloxy)-6-((benzyloxy)methyl)-2,2,4-trimethylhexahydro-8\(H\)-[1,3]dioxolo[4,5-a]pyrrolizin-8-one (233)

Compound 233 was synthesized from the tricyclic alcohol 149 (71 mg, 0.16 mmol) by using the general method for the Swern oxidation. The resultant yellow oil 233 (71 mg) was used for the next step without further purification.

**LRESIMS** \(m/z 438 (100\%) [M+H]^+\).

**General method for the reduction of a ketone to a secondary alcohol with L-selectride®**

(3\(a\)S,4\(S\),6\(R\),7\(R\),8\(S\),8\(a\)S,8\(b\)R)-7-(Benzyloxy)-6-((benzyloxy)methyl)-2,2,4-trimethylhexahydro-4\(H\)-[1,3]dioxolo[4,5-a]pyrrolizin-8-ol (236)

To a cooled (−78 °C) solution of the cyclic ketone 231 (52 mg, 0.12 mmol) in THF (5 mL) was added a solution of L–selectride® (1.0 M solution in THF, 476 µL, 0.476 mmol). The reaction mixture was stirred for 1 h at −78 °C then allowed to warm to room temperature and stirred for 2 h. By this time, TLC analysis confirmed full consumption of the starting material, by which time the reaction mixture was quenched with the addition of an NH\(_4\)Cl solution (1.0 M, 7 mL). The product was extracted with EtOAc (3 x 10 mL), dried (MgSO\(_4\)), filtered and concentrated in vacuo. Purification by FCC (3:97 MeOH/CH\(_2\)Cl\(_2\)) afforded the title product 236 as an orange oil (50 mg, 96%).

\(R_t(3:97\text{ MeOH/CH}_2\text{Cl}_2) = 0.45\)

\[\alpha\]\(_D^{25}\) +25.3 (c 1.00, CHCl\(_3\))
Compound 237 was synthesized from the tricyclic ketone 232 (28 mg, 0.064 mmol) by using the general method for the reduction of a ketone to a secondary alcohol with L–selectride®. The product was purified by FCC (5:95 MeOH/CH₂Cl₂) to give the target compound 237 as an orange oil (19 mg, 66%).

\[ \text{Rf} (5:95 \text{ MeOH/CH₂Cl₂}) = 0.25 \]
\[ [\alpha]^{25}_D +52.1 \ (c 1.00, \text{CHCl}_3) \]

IR \( \nu_{\max} (\text{cm}^{-1}) \): 3290, 2989, 2926, 2873, 1585, 1484, 1095, 694 cm\(^{-1}\)

LRESIMS \( m/z \) 440 (100%) [M+H]+.

HRESIMS found 440.2430, calcd for C\(_{26}\)H\(_{34}\)NO\(_5\), 440.2437 [M+H]+.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.37 – 7.20 (10H, m, ArH), 4.75 – 4.61 (3H, m, OCH\(_2\)Ph), 3.49 – 3.41 (2H, m, H\(_4\)), 1.52 (3H, s, C\(_2\)–CH\(_3\)), 1.32 (3H, s, C\(_4\)–CH\(_3\)), 1.13 (3H, d, \( J = 6.7 \text{ Hz, H1''} \)).
dd, $J = 8.1, 4.7$ Hz, H7), 3.76 – 3.69 (2H, m, OH, H6), 3.49 (1H, dd, $J = 7.3, 4.6$ Hz, H8a), 3.42 (1H, dd, $J = 9.8, 3.4$ Hz, H1'), 3.30 (1H, dd, $J = 9.8, 6.3$ Hz, H1'), 3.12 (1H, qd, $J = 7.0, 4.2$ Hz, H4), 1.43 (3H, s, C2–CH3), 1.33 (3H, d, $J = 7.1$ Hz, H1''), 1.26 (3H, s, C2–CH3).

$^{13}$C NMR (125 MHz, CDCl3) δ 138.7 (ArC), 138.2 (ArC), 128.3 (ArCH), 128.1 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 127.3 (ArCH), 111.8 (C2), 85.4 (C3a), 82.6 (C7), 82.4 (C8), 73.3 (OCH2Ph), 72.8 (OCH2Ph), 72.4 (C1'), 71.3 (C8a), 70.4 (C8b), 63.2 (C6), 58.9 (C4), 25.7 (C2–CH3), 23.3 (C2–CH3), 11.9 (C1'').

(3aR,4R,6R,7R,8S,8aS,8bS)-7-(Benzyloxy)-6-((benzyloxy)methyl)-2,2,4-trimethylhexahydro-4H-[1,3]dioxolo[4,5-a]pyrrolizin-8-ol (238)

Compound 238 was synthesized from the crude tricyclic ketone 233 (71 mg, 0.16 mmol) by using the general method for the reduction of a ketone to a secondary alcohol with L–selectride®. The product was purified by FCC (5:95 MeOH/CH2Cl2) to give the target compound 238 as an orange oil (46 mg, 65%).

$\text{Rf}$(5:95 MeOH/CH2Cl2) = 0.45

$[\alpha]^{25}_D$ –72.0 (c 1.00, CHCl3)

IR $\nu_{\text{max}}$ (cm$^{-1}$): 3340, 2967, 2872, 1516, 1496, 1140, 1066, 866, 635 cm$^{-1}$.

LRESIMS m/z 440 (100%) [M+H]+.

HRESIMS found 440.2446, calcd for C26H34NO5, 440.2437 [M+H]+.

$^1$H NMR (500 MHz, CDCl3) δ 7.39 – 7.19 (10H, m, ArH), 4.86 (1H, dd, $J = 6.3, 3.2$ Hz, H8b), 4.58 (1H, d, $J = 11.7$ Hz, OCH2Ph), 4.55 – 4.47 (3H, m, OCH2Ph), 4.23 (1H, t, $J = 5.7$ Hz, H3a), 4.19 (1H, d, $J = 4.7$ Hz, H8), 3.87 (1H, t, $J = 4.3$ Hz, H7), 3.61 (1H, t, $J = 4.1$ Hz, H8a), 3.47 – 3.36 (2H, m, H1', H4), 3.36 – 3.26 (2H, m, H1', H6), 1.50 (3H, s, C2–CH3), 1.32 (3H, s, C2–CH3), 1.21 (3H, d, $J = 7.0$ Hz, H'').

$^{13}$C NMR (125 MHz, CDCl3) δ 138.4 (ArC), 137.5 (ArC), 128.6 (ArCH), 128.4 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 127.59 (ArCH), 127.56 (ArCH), 112.7 (C2), 87.3 (C3a),
Compound 239 was synthesized from 236 (50 mg, 0.11 mmol) using the general method for hydrolysis of an acetonide. The product was purified by FCC (5:95 MeOH/CH$_2$Cl$_2$) to give the desired product 239 as a pale–yellow oil (36 mg, 79%).

$\text{R}_f$(5:95 MeOH/CH$_2$Cl$_2$) = 0.15
$[\alpha]^{25}_D$ = –67.4 (c 1.00, CHCl$_3$)

IR $\nu_{\text{max}}$(cm$^{-1}$): 3334, 3063, 2925, 2868, 1679, 1512, 1082, 656 cm$^{-1}$

LRESIMS $m/z$ 400 (100%) [M+H]$^+$. 

HRESIMS found 400.2118, calcd for C$_{23}$H$_{30}$NO$_5$, 400.2124 [M+H]$^+$. 

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.38 – 7.20 (10H, m, ArH), 4.59 – 4.46 (4H, m, OCH$_2$Ph), 4.34 (1H, t, $J = 4.3$ Hz, H7), 4.31 (1H, t, $J = 5.5$ Hz, H1), 4.03 (1H, dd, $J = 7.4$, 4.0 Hz, H6), 3.70 – 3.58 (2H, m, H2, H7a), 3.49 (2H, d, $J = 4.5$ Hz, H1$'$), 3.13 (1H, dt, $J = 8.0$, 4.5 Hz, H3), 2.86 (1H, quint, $J = 6.3$ Hz, H5), 1.19 (3H, d, $J = 6.2$ Hz, H1$''$).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 138.2 (ArC), 136.9 (ArC), 128.7 (ArCH), 128.4 (ArCH), 128.4 (ArCH), 128.1 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 82.6 (C7), 79.4 (C1), 73.4 (C6), 73.1 (C7a), 72.7 (OCH$_2$Ph), 72.2 (OCH$_2$Ph), 71.0 (C1$'$), 67.4 (C2), 66.7 (C5), 65.7 (C3), 19.4 (C1$''$).
Compound 240 was synthesized from 237 (19 mg, 0.042 mmol) using the general method for hydrolysis of an acetonide. The product was purified by FCC (8:92 MeOH/CH₂Cl₂) to give 240 as a yellow oil (15 mg, 87%).

R<sub>f</sub> (8:92 MeOH/CH₂Cl₂) = 0.25
[α]<sup>25</sup> +12.2 (c 1.00, CHCl₃)
IR δ<sub>max</sub> (cm<sup>-1</sup>): 3340, 3119, 2926, 2893, 1568, 1478, 1058, 634 cm<sup>-1</sup>
LRESIMS <i>m/z</i> 400 (100%) [M+H]<sup>+</sup>.
HRESIMS found 400.2133, calcd for C<sub>23</sub>H<sub>30</sub>NO<sub>5</sub>, 400.2124 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (500 MHz, CDCl₃) δ 7.39 – 7.22 (10H, m, ArH), 4.60 (1H, d, <i>J</i> = 12.2 Hz, OCH₂Ph), 4.53 – 4.45 (3H, m, OCH₂Ph), 4.44 (1H, dd, <i>J</i> = 8.2, 5.0 Hz, H7), 4.18 (1H, t, <i>J</i> = 3.8 Hz, H1), 4.02 (1H, dd, <i>J</i> = 8.0, 3.4 Hz, H2), 3.81 (1H, t, <i>J</i> = 4.5 Hz, H6), 3.72 (1H, dd, <i>J</i> = 8.2, 3.4 Hz, H7a), 3.61 (1H, dt, <i>J</i> = 8.1, 4.0 Hz, H3), 3.47 (2H, dd, <i>J</i> = 3.9, 1.6 Hz, H1’), 3.42 – 3.34 (1H, m, H5), 1.24 (3H, d, <i>J</i> = 7.1 Hz, H1”).

<sup>13</sup>C NMR (125 MHz, CDCl₃) δ 138.3 (ArC), 137.1 (ArC), 128.7 (ArCH), 128.4 (ArCH), 128.3 (ArCH), 128.0 (ArCH), 127.8 (ArCH), 127.6 (ArCH), 82.3 (C2), 74.9 (C6), 74.4 (C7), 73.4 (OCH₂Ph), 73.1 (OCH₂Ph), 71.3 (C1’), 70.8 (C1), 66.8 (C7a), 60.4 (C3), 57.9 (C5), 10.9 (C1’).
Compound 241 was synthesized from alcohol 238 (40 mg, 0.09 mmol) using the general method for hydrolysis of an acetonide. The product was purified by FCC (8:92 MeOH/CH₂Cl₂) to give the desired product 241 as a brown oil (20 mg, 55%).

Rf(5:95 MeOH/CH₂Cl₂) = 0.30
[α]D²⁵ +68.2 (c 1.00, CHCl₃)
IR νmax (cm⁻¹): 3387, 3100, 2878, 1657, 1520, 1467, 1075, 696 cm⁻¹
LRESIMS m/z 400 (100%) [M+H]⁺.
HRESIMS found 400.2127, calcd for C₂₃H₃₀NO₅, 400.2124 [M+H]⁺.

¹H NMR (500 MHz, CDCl₃) δ 7.39 – 7.21 (10H, m, ArH), 4.54 – 4.45 (4H, m, OCH₂Ph), 4.39 (1H, t, J = 5.1 Hz, H7), 4.13 (1H, t, J = 3.9 Hz, H1), 3.88 (1H, dd, J = 7.9, 3.8 Hz, H2), 3.80 (1H, t, J = 4.4 Hz, H6), 3.53 – 3.43 (3H, m, H1’, H7a), 3.40 – 3.30 (2H, m, H5, H3), 1.10 (3H, d, J = 7.0 Hz, H1”).

¹³C NMR (125 MHz, CDCl₃) δ 138.3 (ArC), 137.3 (ArC), 128.6 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 128.0 (ArCH), 127.6 (ArCH), 127.5 (ArCH), 83.5 (C2), 79.0 (C6), 73.3 (OCH₂Ph), 72.7 (OCH₂Ph), 71.9 (C1’), 71.5 (C7a), 69.1 (C7), 67.5 (C1), 60.7 (C5), 58.8 (C3), 14.3 (C1’).

(1S,2R,3R,5S,6S,7R,7aR)-3-(Hydroxymethyl)-5-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol [(–)-5-Epi hyacinthacine C₁] (242)
(−)-5-epi-hyacinthacine C$_1$ 242 was synthesized by the general method for $O$–benzyl deprotection from 239 (34 mg, 0.085 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo returning a clear film. The compound was isolated through basic ion–exchange chromatography followed by concentration in vacuo providing the title compound 242 as a colourless film (15 mg, 80%).

[α]$^D_{25}$ = −14.1 (c 1.00, H$_2$O)

IR $\nu$ max (cm$^{-1}$): 3331, 2956, 1590, 1578, 1439, 1067, 812, 732, 643 cm$^{-1}$

LRESIMS $m/z$ 220 (100%) [M+H]$^+$. 

HRESIMS found 220.1177, calcd for C$_{9}$H$_{18}$NO$_{5}$, 220.1185 [M+H]$^+$. 

$^1$H NMR (500 MHz, D$_2$O) $\delta$ 4.41 (1H, t, $J$ = 4.9 Hz, H1), 4.37 (1H, t, $J$ = 5.0 Hz, H7), 3.97 (1H, dd, $J$ = 7.3, 4.4 Hz, H2), 3.72 – 3.60 (3H, m, H6, H8), 3.58 (1H, t, $J$ = 5.4 Hz, H7a), 3.08 – 3.01 (2H, m, H3, H5), 1.21 (3H, d, $J$ = 6.4 Hz, H9).

$^{13}$C NMR (125 MHz, D$_2$O) $\delta$ 78.4 (C6), 74.5 (C2), 72.6 (C1), 72.0 (C7), 70.6 (C3), 65.1 (C5), 64.3 (C7a), 62.5 (C8), 17.5 (C9).

(1S,2R,3R,5R,6S,7R,7aR)-3-(Hydroxymethyl)-5-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol [Corrected structure for (+)-hyacinthacine C$_1$] (243)
The compound for the corrected structure of (+)-hyacinthacine C1 243 was synthesized by the general method for O–benzyl deprotection from 240 (11 mg, 0.025 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo returning a clear film. The compound was isolated through basic ion–exchange chromatography followed by concentration in vacuo providing the title compound 243 as a colourless film (3.0 mg, 52%).

\[ \alpha^D_{25} +3.0 \text{ (c 1.00, H}_2\text{O)} \]

**IR** \( \nu_{\text{max}} \text{ (cm}^{-1} \text{): 3349, 2925, 1598, 1420, 1071, 967, 651 \text{ cm}^{-1} \)

**LRESIMS** \( m/z \) 220 (100%) \([\text{M+H}]^+\).

**HRESIMS** found 220.1183, calcd for C9H18NO5, 220.1185 \([\text{M+H}]^+\).

**1H NMR** (500 MHz, D2O) \( \delta \) 4.53 (1H, dd, \( J = 8.6, 4.7 \text{ Hz, H7)\), 4.18 (1H, t, \( J = 3.8 \text{ Hz, H1)\), 4.02 (1H, dd, \( J = 9.2, 3.7 \text{ Hz, H2)\), 3.92 (1H, t, \( J = 4.4 \text{ Hz, H6)\), 3.68 – 3.62 (3H, m, H8, H7a), 3.47 (1H, dt, \( J = 9.2, 4.6 \text{ Hz, H3)\), 3.31 (1H, dt, \( J = 7.1, 3.9 \text{ Hz, H5)\), 1.29 (3H, d, \( J = 7.2 \text{ Hz, H9)\).}

**13C NMR** (125 MHz, D2O) \( \delta \) 77.3 (C2), 76.6 (C6), 75.1 (C7), 74.1 (C1), 67.8 (C7a), 65.4 (C8), 64.1 (C3), 59.9 (C5), 12.4 (C9).

\( (1S,2R,3R,5R,6R,7S,7aR)-3-(\text{Hydroxymethyl})-5\text{-methylhexahydro-1H-pyrrolizine-1,2,6,7-tetraol} [(+)-6,7\text{-Di-epi hyacinthacine C1}] (244) \)
(+)-6,7-di-epi-hyacinthacine $C_1$ 244 was synthesized by the general method for $O$-benzyl deprotection from 241 (18 mg, 0.045 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo returning a yellow film. The compound was isolated through basic ion–exchange chromatography followed by concentration in vacuo providing the title compound 244 as a colourless film (6.0 mg, 61%).

$[\alpha]^D_{25} + 13.4 \ (c \ 1.00, \ H_2O)$

IR $\nu_{max}$ (cm$^{-1}$): 3302, 2967, 1620, 1578, 1439, 1190, 1056, 687 cm$^{-1}$

LRESIMS $m/z$ 220 (100%) [M+H]$^+$. 

HRESIMS found 220.1197, calcd for C$_9$H$_{18}$NO$_5$, 220.1185 [M+H]$^+$. 

$^1$H NMR (500 MHz, D$_2$O) $\delta$ 4.33 (1H, t, $J = 4.7$ Hz, H7), 4.17 (1H, t, $J = 4.3$ Hz, H1), 3.99 (1H, dd, $J = 8.1, 4.1$ Hz, H2), 3.86 (1H, t, $J = 5.4$ Hz, H6), 3.66 – 3.57 (2H, m, H8), 3.40 (1H, t, $J = 4.6$ Hz, H7a), 3.21 (1H, quint, $J = 6.7$ Hz, H5), 3.14 (1H, dt, $J = 8.0, 4.9$ Hz, H3), 1.17 (3H, d, $J = 7.0$ Hz, H9).

$^{13}$C NMR (125 MHz, D$_2$O) $\delta$ 77.5 (C6), 74.8 (C2), 70.2 (C6), 69.7 (C1), 69.1 (C7), 62.7 (C8), 60.9 (C3), 59.0 (C5), 13.2 (C9).

(3aS,4S,6R,7R,8S,8aS,8bR)-7,8-Bis(benzyloxy)-6-((benzyloxy)methyl)-2,2,4-trimethylhexahydro-4H-[1,3]dioxolo[4,5-α]pyrrolizine (245)
Compound 245 was synthesized from 236 (46 mg, 0.11 mmol) by the general method for $O$–benzylation. The product was purified by FCC (2:3 EtOAc/$n$-hexane) to give the target compound 245 as a yellow oil (30 mg, 54%).

$R_f$ (2:3 EtOAc/$n$-hexane) = 0.55

$[\alpha]_D^{25} +47.6$ (c 1.00, CHCl$_3$)

IR $\nu_{\max}$ (cm$^{-1}$): 2328, 1645, 1320, 962, 664 cm$^{-1}$

LRESIMS $m/z$ 530 (100%) [M+H]$^+$.  

HRESIMS found 530.2919, calcd for C$_{33}$H$_{40}$NO$_5$, 530.2906 [M+H]$^+$.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.45 – 7.12 (15H, m, ArH), 4.80 – 4.72 (2H, m, OCH$_2$Ph, H8b), 4.71 – 4.64 (1H, m, OCH$_2$Ph), 4.55 – 4.50 (2H, m, OCH$_2$Ph), 4.46 (1H, d, $J$ = 12.2 Hz, OCH$_2$Ph), 4.39 (1H, d, $J$ = 12.0 Hz, OCH$_2$Ph), 4.33 (1H dd, $J$ = 6.4, 4.4 Hz, H3a), 4.13 (1H, t, $J$ = 5.2 Hz, H8), 3.87 (1H, dd, $J$ = 7.4, 5.5 Hz, H7), 3.50 (1H, d, $J$ = 5.2 Hz, H8a), 3.48 – 3.42 (2H, m, H6, H1'), 3.42 – 3.37 (1H, m, H1'), 3.30 (1H, d, $J$ = 6.7, 4.3 Hz, H4), 1.43 (3H, s, C2–CH$_3$), 1.30 (3H, s, C2–CH$_3$), 1.10 (3H, d, $J$ = 6.7 Hz, H1'').

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 139.0 (ArC), 138.50 (ArC), 138.46 (ArC), 128.24 (ArCH), 128.18 (ArCH), 128.1 (ArCH), 127.82 (ArCH), 127.77 (ArCH), 127.69 (ArCH), 127.44 (ArCH), 127.41 (ArCH), 127.3 (ArCH), 113.3 (C2), 88.7 (C3a), 81.6 (C7), 80.7 (C8b), 76.9 (C8), 73.4 (OCH$_2$Ph), 73.2 (OCH$_2$Ph), 72.5 (OCH$_2$Ph), 71.9 (C1'), 67.6 (C6), 66.7 (C8a), 65.8 (C4), 26.7 (C2–CH$_3$), 24.3 (C2–CH$_3$), 19.4 (C1'').

(3aR,4R,6R,7R,8S,8aS,8bS)-7,8-Bis(benzyloxy)-6-((benzyloxy)methyl)-2,2,4-trimethylhexahydro-4H-[1,3]dioxolo[4,5-$\alpha$]pyrrolizine (246)
Compound 246 was synthesized from 238 (62 mg, 0.14 mmol) by the general method for O–benzylation. The product was purified by FCC (1:4 EtOAc/n-hexane) to give the target compound 246 as a yellow oil (39 mg, 52%).

$R_f(2:3 \text{EtOAc/n-hexane}) = 0.30$

$[\alpha]_D^{25} = -12.9$ (c 1.00, CHCl$_3$)

IR $\tilde{\nu}_{\text{max}}$ (cm$^{-1}$): 2978, 1612, 1514, 1254, 1042, 678 cm$^{-1}$

LRESIMS $m/z$ 530 (100%) [M+H]$^+$.  

HRESIMS found 530.2925, calcd for C$_{33}$H$_{40}$NO$_5$, 530.2906 [M+H]$^+$.  

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.39 – 7.20 (15H, m, ArH), 5.10 (1H, dd, $J = 6.6$, 3.0 Hz, H8b), 4.64 – 4.60 (3H, m, OCH$_2$Ph), 4.56 (1H, d, $J = 12.1$ Hz, OCH$_2$Ph), 4.47 (2H, s, OCH$_2$Ph), 4.21 (1H, t, $J = 6.8$ Hz, H3a), 4.13 (1H, dd, $J = 6.3$, 4.3 Hz, H8), 3.84 (1H, dd, $J = 4.4$, 2.2 Hz, H7), 3.71 (1H, dd, $J = 6.5$, 3.0 Hz, H8a), 3.37 – 3.23 (3H, m, H6, H4, H1'), 3.12 (1H, dd, $J = 9.5$, 7.6 Hz, H1'), 1.49 (3H, s, C2–CH$_3$), 1.31 (3H, s, C2–CH$_3$), 1.23 (3H, d, $J = 7.0$ Hz, H1').  

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 138.4 (ArC), 138.3 (ArC), 138.2 (ArC), 128.4 (ArCH), 128.36 (ArCH), 128.35 (ArCH), 127.71 (ArCH), 127.65 (ArCH), 127.6 (ArCH), 113.5 (C2), 86.1 (C3a), 81.0 (C8b), 80.5 (C7), 77.4 (C8), 73.2 (OCH$_2$Ph), 73.1 (C1'), 72.4 (OCH$_2$Ph), 71.8 (OCH$_2$Ph), 71.1 (C8a), 63.2 (C6), 61.2 (C4), 27.8 (C2–CH$_3$), 25.7 (C2–CH$_3$), 14.2 (C1').  

(1R,2S,3S,5R,6R,7S,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2-diol (247)
Compound 247 was synthesized from 245 (64 mg, 0.12 mmol) using the general method for hydrolysis of an acetonide. The product was purified by FCC (5:95 MeOH/CH₂Cl₂) to give the title compound 247 as a yellow oil (50 mg, 85%).

Rf(5:95 MeOH/CH₂Cl₂) = 0.25  
[α]D²⁵ +10.5 (c 2.15, CHCl₃).

IR υmax (cm⁻¹): 3347, 3065, 2943, 2889, 1631, 1035, 673 cm⁻¹

LRESIMS m/z 490 (100%) [M+H]^+.

HRESIMS found 490.2581, calcd for C₃₀H₃₆NO₅, 490.2593 [M+H]^+.

1H NMR (500 MHz, CDCl₃) δ 7.39 – 7.22 (15H, m, ArH), 4.89 – 4.78 (1H, m, OCH₂Ph), 4.76 (1H, d, J = 11.4 Hz, OCH₂Ph), 4.61 (2H, s, OCH₂Ph), 4.56 – 4.47 (2H, m, OCH₂Ph), 4.33 (1H, dd, J = 6.9, 4.3 Hz, H1), 4.08 (1H, apparent q, J = 4.1 Hz, H7), 4.00 (1H, t, J = 4.5 Hz, H2), 3.73 (1H, t, J = 5.7 Hz, H7a), 3.60 (1H, dd, J = 8.7, 4.1 Hz, H6), 3.43 (1H, dd, J = 9.8, 4.6 Hz, H1’), 3.34 (1H, dd, J = 9.8, 5.8 Hz, H1’), 3.23 (1H, q, J = 5.1 Hz, H3), 2.90 (1H, quint, J = 6.5 Hz, H5), 1.20 (3H, d, J = 6.3 Hz, H1’’).

13C NMR (125 MHz, CDCl₃) δ 138.1 (ArC), 137.3 (ArC), 137.2 (ArC), 128.6 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.10 (ArCH), 128.12 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 80.6 (C2), 80.2 (C6), 79.5 (C1), 73.5 (OCH₂Ph), 73.4 (OCH₂Ph), 72.8 (OCH₂Ph), 72.0 (C7), 71.4 (C1’), 68.6 (C3), 66.9 (C5), 65.6 (C7a), 19.1 (C1’’).

(1S,2R,3R,5R,6R,7S,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2-diol (248)
Compound 248 was synthesized from 246 (38 mg, 0.072 mmol) using the general method for hydrolysis of an acetonide. The product was purified by FCC (5:95 MeOH/CH₂Cl₂) to give the desired product 248 as a brown oil (30 mg, 85%).

$R_f(5:95 \text{ MeOH}/\text{CH}_2\text{Cl}_2) = 0.15$

$[\alpha]_D^{25} +18.4 \ (c \ 1.00, \ \text{CHCl}_3)$

IR $\lambda_{\text{max}} \ (\text{cm}^{-1})$: 3327, 3099, 2912, 2843, 1589, 1075, 678 cm$^{-1}$

LRESIMS $m/z$ 490 (100%) [M+H]+.

HRESIMS found 490.2612, calcd for C$_{30}$H$_{36}$NO$_5$, 490.2593 [M+H]+.

$^1$H NMR (500 MHz, CDCl₃) $\delta$ 7.41 – 7.20 (15H, m, ArH), 4.74 (1H, d, $J = 11.6$ Hz, OCH$_2$Ph), 4.63 (1H, d, $J = 11.7$ Hz, OCH$_2$Ph), 4.59 – 4.39 (5H, m, OCH$_2$Ph, H7), 4.10 (1H, t, $J = 4.2$ Hz, H1), 3.94 – 3.86 (1H, m, H2), 3.83 (1H, t, $J = 4.8$ Hz, H6), 3.68 (1H, t, $J = 5.1$ Hz, H7a), 3.60 – 3.40 (4H, m, H5, H3, H1’), 1.96 (1H, s, OH), 1.16 (3H, d, $J = 6.9$ Hz, C1’).

$^{13}$C NMR (125 MHz, CDCl₃) $\delta$ 138.1 (ArC), 138.0 (ArC), 137.6 (ArC), 128.45 (ArCH), 128.35 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 127.60 (ArCH), 127.58 (ArCH), 82.0 (C2), 78.1 (C6), 75.0 (C1), 73.4 (OCH$_2$Ph), 73.3 (OCH$_2$Ph), 72.6 (OCH$_2$Ph), 71.3 (C7a), 71.1 (C1’), 69.7 (C7), 61.8 (C5), 59.6 (C3), 14.1 (C1”).

(3aS,4S,6R,7R,8S,8aS,8bR)-7,8-Bis(benzyloxy)-6-((benzyloxy)methyl)-4-methylhexahydro-4H-[1,3,2]dioxathiolo[4,5-a]pyrrolizine 2,2-dioxide (249)
Compound 249 was synthesized from the diol 247 (43 mg, 0.088 mmol) by using the general method for the synthesis of a cyclic sulfate. The resultant crude brown oil 249 (48 mg) was used for the next step without further purification.

**LRESIMS m/z 586 (100%) [M+Cl]⁻.**

\((3aR,4R,6R,7R,8S,8aS,8bS)-7,8\text{-Bis(benzyloxy)-6-((benzyloxy)methyl)-4-methylhexahydro-4H-[1,3,2]dioxathiolo[4,5-a]pyrrolizine 2,2-dioxide (250)}\)

Compound 250 was synthesized from the diol 248 (25 mg, 0.051 mmol) by using the general method for the synthesis of a cyclic sulfate. The resultant crude brown oil 250 (28 mg) was used for the next step without further purification.

**LRESIMS m/z 586 (100%) [M+Cl]⁻.**

\((1S,2S,3R,5R,6R,7S,7aR)-6,7\text{-Bis(benzyloxy)-5-((benzyloxy)methyl)-1-hydroxy-3-methylhexahydro-1H-pyrrolizin-2-yl benzoate (251)}\)
Compound 251 was synthesized from the cyclic sulfate 250 (28 mg, 0.051 mmol) using the general method for ring opening of cyclic sulfates with benzoate. Purification by FCC (3:2 EtOAc/n-hexane) returned the title compound 251 (13 mg, 42%) as a brown oil.

Rf (3:2 EtOAc/n-hexane) = 0.25

[α]25D +8.5 (c 1.00, CHCl3)

IR vmax (cm⁻¹): 3380, 3093, 1710, 1576, 1104, 1011, 608 cm⁻¹

LRESIMS m/z 594 (100%) [M+H]+.

HRESIMS found 594.2843, calcd for C37H40NO6, 594.2856 [M+H]+.

1H NMR (500 MHz, CDCl3) δ 8.00 (2H, d, J = 7.7 Hz, o–Bz), 7.54 (1H, dd, J = 7.8 Hz, p–Bz), 7.39 (2H, t, J = 7.8 Hz, m–Bz), 7.36 – 7.17 (15H, m, ArH), 5.30 (1H, t, J = 5.8 Hz, H6), 4.77 (1H, dd, J = 6.9, 5.5 Hz, H7), 4.71 (1H, d, J = 12.0 Hz, OCH2Ph), 4.67 (1H, d, J = 12.0 Hz, OCH2Ph), 4.57 – 4.47 (3H, m, OCH2Ph), 4.42 (1H, d, J = 12.0 Hz, OCH2Ph), 4.13 (1H, t, J = 4.5 Hz, H1), 3.93 – 3.86 (2H, m, H5, H2), 3.69 (1H, q, J = 5.7 Hz, H3), 3.59 (1H, t, J = 5.9 Hz, H7a), 3.44 (2H, d, J = 5.3 Hz, H1‘), 1.25 (3H, d, J = 8.8 Hz, H1”).

13C NMR (125 MHz, CDCl3) δ 167.2 (CO), 138.4 (ArC), 138.3 (ArC), 137.9 (ArC), 133.2 (ArCH), 129.8 (ArCH), 128.40 (ArCH), 128.37 (ArCH), 128.33 (ArCH), 128.26 (ArCH), 127.7 (ArCH), 127.63 (ArCH), 127.58 (ArCH), 127.5 (ArCH), 84.6 (C6), 81.8 (C2), 75.4 (C1), 74.3 (C7), 73.4 (OCH2Ph), 72.9 (OCH2Ph), 72.3 (OCH2Ph), 72.2 (C1’), 70.8 (C7a), 60.3 (C3), 57.2 (C5), 11.8 (C1”).

(1S,2S,3R,5R,6R,7S,7aR)-6,7-Bis(benzyloxy)-5-((benzyloxy)methyl)-3-methylhexahydro-1H-pyrrolizine-1,2-diol (252)
Compound 252 was synthesized from benzoate 251 (11 mg, 0.017 mmol) using the general method for $O$–benzoyl deprotection, except that the reaction was stirred at rt for 1 h. The crude brown oil was purified by FCC (5:95 MeOH/CH$_2$Cl$_2$) to give the product 252 as a clear brown film (7.0 mg, 87%).

$R_f$(5:95 MeOH/CH$_2$Cl$_2$) = 0.20

$[\alpha]_D^{25} +29.8$ (c 1.00, CHCl$_3$)

**IR** $\nu_{max}$ (cm$^{-1}$): 3362, 3101, 2967, 1542, 1489, 1027, 872, 696 cm$^{-1}$

**LRESIMS** $m/z$ 490 (100%) [M+H]$^+$.  
**HRESIMS** found 490.2597, calcd for C$_{30}$H$_{36}$NO$_5$, 490.2593 [M+H]$^+$.

**$^1$H NMR (500 MHz, CDCl$_3$)** $\delta$ 7.39 – 7.22 (15H, ArH), 4.83 (1H, d, $J = 11.2$ Hz, OCH$_2$Ph), 4.68 – 4.51 (5H, m, OCH$_2$Ph), 4.40 (1H, t, $J = 2.2$ Hz, H7), 4.22 (1H, t, $J = 4.5$ Hz, H1), 4.12 (1H, dd, $J = 8.2$, 3.7 Hz, H2), 4.09 – 4.01 (1H, m, H7a), 4.01 – 3.92 (2H, m, H5, H6), 3.76 (1H, dt, $J = 8.5$, 4.5 Hz, H3), 3.67 (1H, dd, $J = 10.4$, 5.0 Hz, H1'), 3.61 (1H, dd, $J = 10.4$, 3.9 Hz, H1''), 3.31 (3H, d, $J = 6.7$ Hz, H1$''$).

**$^{13}$C NMR (125 MHz, CDCl$_3$)** $\delta$ 137.8 (ArC), 136.9 (ArC), 136.6 (ArC), 128.8 (ArCH), 128.6 (ArCH), 128.5 (ArCH), 128.4 (ArCH), 128.34 (ArCH), 128.30 (ArCH), 128.1 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 127.7 (ArCH), 81.1 (C6), 80.9 (C2), 75.8 (C1), 75.4 (C7), 74.3 (OCH$_2$Ph), 73.7 (OCH$_2$Ph), 73.5 (OCH$_2$Ph), 73.3 (C7a), 68.7 (C1'), 61.0 (C5), 60.6 (C3), 10.2 (C1$''$).

(1$S$,2$R$,3$R$,5$R$,6$S$,7$S$,7$aR$)-3-(Hydroxymethyl)-5-methylhexahydro-1$H$-pyrrolizine-1,2,6,7-tetraol [(+)-7- Epi hyacinthacine C1] (253)
(+)-7-epi-hyacinthacine C$_1$ 253 was synthesized by the general method of O–benzyl deprotection from 252 (6.0 mg, 0.012 mmol). The crude product was filtered through a pad of celite and washed with additional MeOH (6 mL). The combined filtrates were concentrated in vacuo returning a yellow film. The title compound 253 was isolated through basic ion–exchange chromatography followed by concentration in vacuo, as a colourless film (2.0 mg, 75%).

$[\alpha]_{D}^{25} +6.5$ (c 1.00, H$_2$O)

**IR $\nu_{\text{max}}$ (cm$^{-1}$):** 3303, 2981, 1702, 1570, 1412, 1087, 1078, 656 cm$^{-1}$

**LRESIMS $m/z$ 220 (100%) [M+H].**

**HRESIMS** found 220.1181, calcd for C$_9$H$_{18}$NO$_5$, 220.1185 [M+H]$^+$.  

**$^1$H NMR (500 MHz, D$_2$O) $\delta$:** 4.29 (1H, d, $J = 5.8$ Hz, H7), 4.15 (1H, t, $J = 3.9$ Hz, H1), 4.11 (1H, dd, $J = 7.4$, 6.0 Hz, H6), 3.97 (1H, dd, $J = 9.1$, 3.9 Hz, H2), 3.74 (1H, dd, $J = 11.6$, 4.3 Hz, H8), 3.61 (1H, dd, $J = 11.7$, 6.4 Hz, H8), 3.46 (1H, quint, $J = 6.8$ Hz, H5), 3.33 – 3.26 (2H, m, H7a, H3), 1.12 (3H, d, $J = 7.0$ Hz, H9).

**$^{13}$C NMR (125 MHz, D$_2$O) $\delta$:** 78.4 (C6), 74.9 (C2), 71.4 (C7), 69.3 (C1), 68.8 (C7a), 63.0 (C8), 60.6 (C3), 57.0 (C5), 10.3 (C9).
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Figure A.1: $^1$H NMR spectrum (500 MHz, D$_2$O) of 155

Figure A.2: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 155
Figure A.3: gCOSY spectrum (500 MHz, D$_2$O) of 155

Figure A.4: gHSQC spectrum (500 MHz, D$_2$O) of 155
Figure A.5: NOESY spectrum (500 MHz, D$_2$O) of 155
Figure A.6: $^1$H NMR spectrum (500 MHz, D$_2$O) of 156

Figure A.7: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 156
Figure A.8: gCOSY spectrum (500 MHz, D$_2$O) of 156

Figure A.9: gHSQC spectrum (500 MHz, D$_2$O) of 156
Figure A.10: ROESY spectrum (500 MHz, D$_2$O) of 156
Figure A.11: $^1$H NMR spectrum (500 MHz, D$_2$O) of 169

Figure A.12: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 169
Figure A.13: gCOSY spectrum (500 MHz, D₂O) of 169

Figure A.14: gHSQC spectrum (500 MHz, D₂O) of 169
Figure A.15: ROESY spectrum (500 MHz, D₂O) of 169
Figure A.16: $^1$H NMR spectrum (500 MHz, D$_2$O) of the purported hyacinthacine C$_5$

Figure A.17: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of the purported hyacinthacine C$_5$
Figure A.18: gCOSY spectrum (500 MHz, D₂O) of the purported hyacinthacine C₅

Figure A.19: gHSQC spectrum (500 MHz, D₂O) of the purported hyacinthacine C₅
Figure A.20: ROESY spectrum (500 MHz, D$_2$O) of the purported hyacinthacine C$_5$
Figure A.21: $^1$H NMR spectrum (500 MHz, D$_2$O) of 215

Figure A.22: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 215
Figure A.23: gCOSY spectrum (500 MHz, D$_2$O) of 215

Figure A.24: gHSQC spectrum (500 MHz, D$_2$O) of 215
Figure A.25: ROESY spectrum (500 MHz, D$_2$O) of 215
Figure A.26: $^1$H NMR spectrum (500 MHz, D$_2$O) of 216

Figure A.27: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 216
Figure A.28: gCOSY spectrum (500 MHz, D$_2$O) of 216

Figure A.29: gHSQC spectrum (500 MHz, D$_2$O) of 216
Figure A.30: ROESY spectrum (500 MHz, D$_2$O) of 216
Figure A.31: $^1$H NMR spectrum (500 MHz, D$_2$O) of 217

Figure A.32: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 217
Figure A.33: gCOSY spectrum (500 MHz, D$_2$O) of 217

Figure A.34: gHSQC spectrum (500 MHz, D$_2$O) of 217
Figure A.35: 1D-NOE spectrum (500 MHz, D$_2$O) of 217
Figure A.36: $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 218

Figure A.37: $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 218
Figure A.38: gCOSY spectrum (500 MHz, CDCl$_3$) of 218

Figure A.39: gHSQC spectrum (500 MHz, CDCl$_3$) of 218
Figure A.40: 1D-NOE spectrum (500 MHz, D$_2$O) of 218
Figure A.41: $^1$H NMR spectrum (500 MHz, D$_2$O) of 242

Figure A.42: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 242
Figure A.43: gCOSY spectrum (500 MHz, D$_2$O) of 242

Figure A.44: gHSQC spectrum (500 MHz, D$_2$O) of 242
Figure A.45: ROESY spectrum (400 MHz, D$_2$O) of 242
Figure A.46: $^1$H NMR spectrum (500 MHz, D$_2$O) of 243

Figure A.47: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 243
Figure A.48: gCOSY spectrum (500 MHz, D₂O) of 243

Figure A.49: gHSQC spectrum (500 MHz, D₂O) of 243
Figure A.50: ROESY spectrum (400 MHz, D$_2$O) of 243
Figure A.51: $^1$H NMR spectrum (500 MHz, D$_2$O) of 244

Figure A.52: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 244
Figure A.53: gCOSY spectrum (500 MHz, D$_2$O) of 244

Figure A.54: gHSQC spectrum (500 MHz, D$_2$O) of 244
Figure A.55: 1D-NOE spectrum (500 MHz, D$_2$O) of 244
Figure A.56: $^1$H NMR spectrum (500 MHz, D$_2$O) of 253

Figure A.57: $^{13}$C NMR spectrum (125 MHz, D$_2$O) of 253
Figure A.58: gCOSY spectrum (500 MHz, D$_2$O) of 253

Figure A.59: gHSQC spectrum (500 MHz, D$_2$O) of 253
(+)-7-α-D-hyacinthaine C$_1$

Diagram of proton NMR spectra with labels for H1, H2, H3, H5, H6, H7, H7a, H8, and H9.
Figure A.60: 1D-NOE spectrum (500 MHz, D₂O) of 253
Miscellaneous

Here I have included an unconventional section, highlighting a few moments throughout the course of this work that deserve to be included in this thesis.

The first of these is the winning of the C&En May “Chemistry in Photos” contest. Below is the photo I took, along with the small card I received from the C&En office in Washington, DC, USA. The photo shows ammonia sulfate (white solid) and a copper-ammonia complex (deep blue). It was the by-product of the acetonide protection of L-xylose. This work up procedure usually involves physically separating the hydrated copper sulfate (light blue) via vacuum filtration, followed by a separate removal of the precipitated ammonia sulfate using gravity filtration. Instead of gravity filtering the white precipitate, I re-used the vacuum filter (containing copper sulfate) and below is what resulted. I was lucky that the copper sulfate did not re-dissolve into my collecting flask as the copper-ammonia complex, as this would have defeated the purpose of the initial removal of the copper. It made for a nice photo though and was printed in C&En captioned “Ice Faux Floes”. Many thanks to the C&En team, and particularly Dr Reeser for ensuring I received the $50 cash prize.
The second mention was prior to the publishing our second body of work in the ACS Journal of Natural Products. My wife Shenae is extremely talented in arts, graphic and digital design. She has supported this project in numerous ways including her art. Below is an art piece that she drew depicting the corrected structure of hyacinthacine C1. We released ownership of this masterpiece to accompany our manuscript following its publication in the ACS Journal of Natural Products.
Towards the end of 2018, I presented at the RACI NSW Natural Products Chemistry Symposium held at UNSW. I was very fortunate to win the prize for the “Best Student Oral Presentation”. There were some fantastic speakers on the day and so it was amazing to win this award.
The final highlight included in this section was my receiving of the prestigious Larry and Marilyn Hick award for 2018. This award is presented for the “Best Published Article by a Postgraduate Student” for that year. The paper that won this award is discussed in Chapters 2, 3 and 4B and was published in the ACS Journal of Organic Chemistry.