# Studies towards the synthesis of the aminopolyol antibiotic zwittermicin A

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**Doctor of Philosophy**
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## Declaration:

This thesis is submitted in part of fulfillment of the requirements for the degree of Doctor of Philosophy at The University of Edinburgh. Unless otherwise stated the work described in this thesis is original and has not been submitted previously in whole or in part for any degree or other qualification at this, or any other university. In accordance with the regulations this thesis does not exceed 70,000 words in length.

Nina A. Dobrovinskaya

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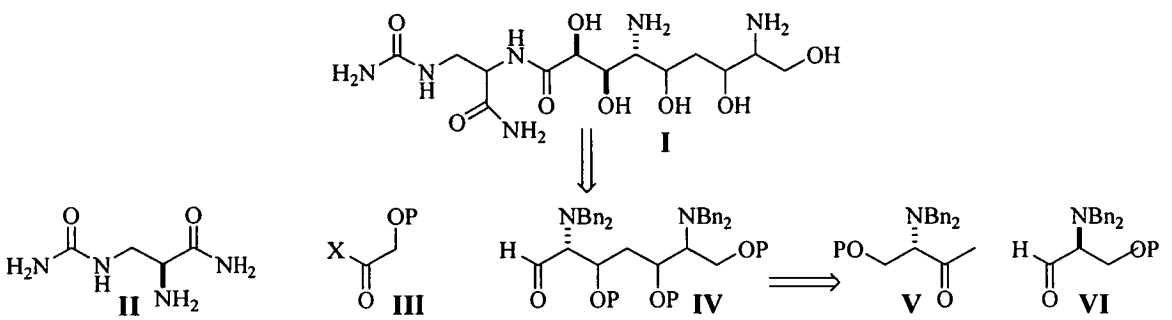
Finally, I would like to thank my parents and my brother for their continuous support, love and encouragement.

## Abstract:

The problems caused by *Phytophthora medecaginis*; together with the isolation, biological activity and biosynthesis of zwittermicin A (which shows potential as an inhibitor of this disease) are reviewed. Studies towards the synthesis of the aminopolyol antibiotic zwittermicin A **I** will be described.



Chapter 2 outlines the approaches investigated towards the synthesis of the nitrogen-rich fragment **II** which include: enzymatic separation of racemic 2,3-diaminopropionic acid using a *D*-amino acid oxidase; and synthetic routes based on a Hofmann rearrangement of *N*-protected asparagine.

Chapter 3 describes studies towards the synthesis of aminopolyol species **IV** from threonine-derived ketone **V** and the serine-derived aldehyde **VI**. Methodology for the "matched" lithium-mediated aldol reaction between these two components was developed, together with directed reductions of the resultant aldol adducts to give either *syn* or *anti* diol relationships. The future application of this methodology to the total synthesis of zwittermicin A is discussed in Chapter 4.

Chapter 5 presents a preliminary study of high pressure conditions for enantioselective organocatalytic aldol reactions.

## Contents:

# Chapter 1: Introduction.

## 1.1. Isolation and structural determination of zwittermicin A.

The problem of the struggle against *Phytophthora sp.* has existed for almost two hundred years. The first species of *Phytophthora* to be discovered was *Phytophthora infestans.* This is the most feared disease of potatoes and tomatoes. The homeland of this disease is Mexico; however, in 1844 potato disease appeared in USA and Europe. More than two million people died of starvation in Ireland during the next 7 years.

Unfortunately, the *Phytophthora* species is famous not only because of *Phytophthora infestans.* There are many other kinds of *Phytophthora* diseases which affect different plants all over the world.[1]

There is one more very widespread *Phytophthora* species – *Phytophthora medicaginis.* This is the most serious causal agent of root rot in both alfalfa (*Medicago sativa L.*) and chickpeas (*Cicer arietinum L.*).[2] Alfalfa is a well-known plant; the ancient Greeks first used it in 480 B.C. as a good livestock feed. Later it was imported to Spain, Mexico and America. Nowadays, alfalfa is one of the most important feedstocks and it is cultivated worldwide. It is also used as an excellent natural fertilizer,[3] and has applications in medicine since it contains a complex mixture of biologically active compounds, e.g., saponins, coumarins, alkaloids, and almost all known vitamins and minerals. *Phytophthora medicaginis* infects the roots, lower stems and seedlings of alfalfa and chickpea plants causing a necrotic lesion[2] and "damping-off" of the plant. Root rot of alfalfa occurs in nearly every region of the world where alfalfa is cultivated.[4] This disease is especially dangerous because *Phytophthora medicaginis* produces oospores, which can survive for years[2] and they may spread very easily with the movement of infected soil, plant material, by people or animals and also may be transported by water (**Figure 1**).

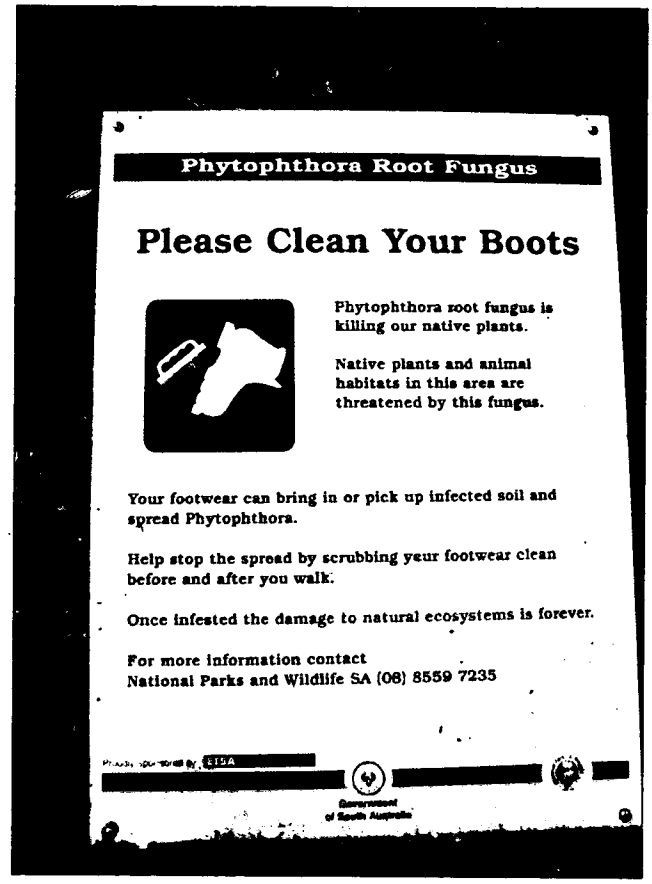


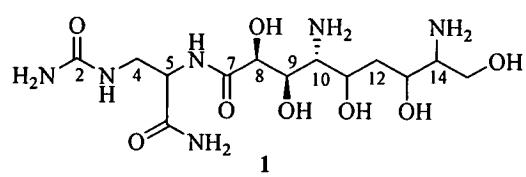

**Figure 1:** Sign illustrating the effort to control the spread of Phytophthora in South Australia.

As mentioned above, the "damping-off" of alfalfa roots and seedlings is a very serious problem. For this reason many investigations have been carried out to control the spread of this disease, particularly at the Department of Plant Pathology, University of Wisconsin. Researchers at this department were trying to find biological agents to control *Phytophthora medicaginis*. Since certain bacteria and fungi[5] have been shown to control other diseases caused by members of the genus *Phytophthora* on other host plants, biocontrol of *Phytophthora medicaginis* appears to be worthy of investigation.

Handelsman *et al.*[5] initiated their search for a biocontrol agent for alfalfa "damping-off" by screening bacteria that were associated with the roots of symptomless

alfalfa plants. A total of 700 bacteria were isolated from the roots of alfalfa grown in a variety of Wisconsin soils. The only culture that reduced mortality to 0% was an isolate designated UW85. Later UW85 was identified as *Bacillus cereus* on the basis of standard bacteriological criteria. It was discovered that the bacteria were producing extracellular biologically-active secondary metabolites during sporulation giving rise to the biocontrol activity. Since many other *Bacillus* strains have been tested and UW85 was found to be the only one that suppressed "damping-off", this activity appeared to be completely unusual and unique to UW85.

The next step was to isolate these biologically-active secondary metabolites. The isolation was carried out by using a column containing carboxymethyl-Sephadex cation-exchange matrix in the ammonium form.[6] Fractions were collected and assayed for inhibition of *Phytophthora medicaginis*. Further purification of the active phase was conducted using high-voltage paper electrophoresis. Silo-Suh *et al.*[6] identified two spots on the paper electrophoretograms associated with inhibitory activity in the agar plate diffusion assay. The first was designated zwittermicin A and the second antibiotic was given the provisional designation antibiotic B. The structures of both antibiotics were determined by spectroscopic methods ($^1$H, $^{13}$C and 2D NMR).[7] Zwittermicin A is an amorphous, colourless powder with molecular formula of $C_{13}H_{28}N_6O_8$. Fast atom bombardment mass spectroscopy (FAB-MS) indicated that zwittermicin A has a molecular mass of 397.20 Da. Both $^1$H and $^{13}$C NMR data (**Table 1**) indicate that C-8, C-9, C-11, C-13 and C-15 are attached to oxygens while C-2, C-4, C-10 and C-14 are attached to nitrogens.[7] The proposed structure of zwittermicin A is shown in **Figure 2**.



**1**

**Figure 2:** The proposed structure of zwittermicin A.[7]

| Atom | $^{13}C$ (100 MHz) in $D_2O$ | $^{1}H$ (400 MHz) in $D_2O$ |
|---|---|---|
| 1 | 164.6 (s) | |
| $NH_2$-1 | | |
| 2 | | |
| 3 | 43.5 (t) | 3.62 (dd, 14.5, 3.5)<br>3.49 (dd, 14.5, 7.0) |
| 4 | 57.3 (d) | 4.45 (dd, 7.0, 3.5) |
| 5 | 177.1 (s) | |
| $NH_2$-5 | | |
| NH-6 | | |
| 7 | 177.9 | |
| 8 | 74.7 (d) | 4.55 (d, 2.0) |
| 9 | 72.1 (d) | 4.35 (dd, 4.5, 2.0) |
| 10 | 60.6 (d) | 3.56 (dd, 6.0, 4.5) |
| NH-10 | | |
| 11 | 68.4 (d) | 4.28 (m) |
| 12 | 37.3 (t) | 1.80 (m)<br>1.76 (m) |
| 13 | 68.8 (d) | 4.19 (br d, 10.0) |
| 14 | 59.7 (d) | 3.43 (ddd, 8.5, 4.0, 4.0) |
| NH-14 | | |
| 15 | 60.1 (t) | 3.94 (dd, 12.5, 4.0)<br>3.78 (dd, 12.5, 8.5) |

**Table 1:** NMR spectra data for zwittermicin A.[7]

It is important to point out that not all of the absolute stereochemistry of this antibiotic has been established yet. Nevertheless, the absolute configurations of three of the chiral centres have been determined as being 8*S*, 9*S*, and 10*R* (**Figure 2**).

## 1.2. Biological activity of zwittermicin A.

Zwittermicin A has a broad therapeutic spectrum and inhibits the growth of a variety of gram-positive bacteria (e.g., *Staphylococcus aureus*, *Bacillus cereus* strains that do not produce zwittermicin A, *Lactobacillus acidophilus*, etc.), and gram-negative bacteria (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, etc.). Moreover, this antibiotic strongly inhibits many plant pathogenic fungi in all groups: Ascomycetes, Basidiomycetes and Deuteromycetes. Most importantly, zwittermicin A has shown a high activity for inhibiting the growth of Oomycetes (for example: *Phytophthora medicaginis)* in very low concentration (MIC[i] – 1 μg/filter disk).[8] The life cycle of *Phytophthora medicaginis* is represented in **Figure 3**. Zwittermicin A does not interfere with germination of the *Phytophthora medicaginis* cysts; however, it inhibits elongation of germ tubes derived from these cysts (**Figure 4** and **Figure 5**).[6]

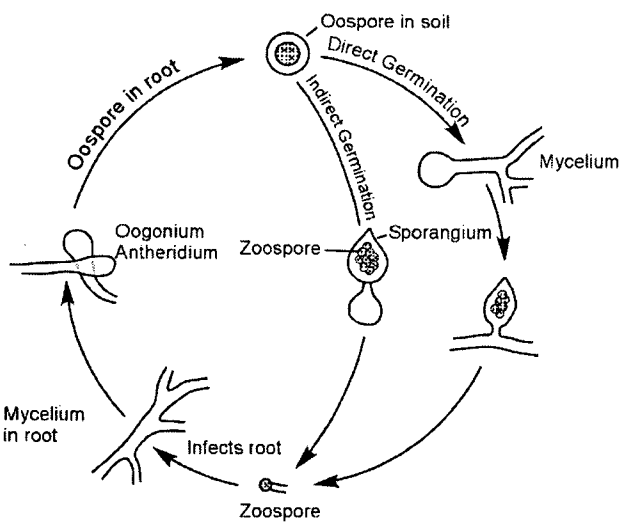



**Figure 3:** Life cycle of *Phytophthora medicaginis.*

[i] MIC indicates the minimum inhibitory concentration of antibiotic required to produce a zone of inhibition on agar plates.[8]

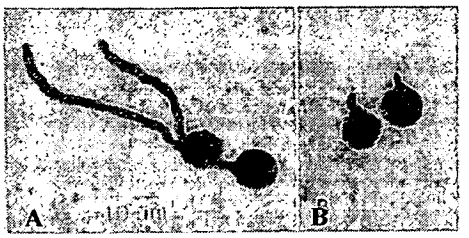



**Figure 4**

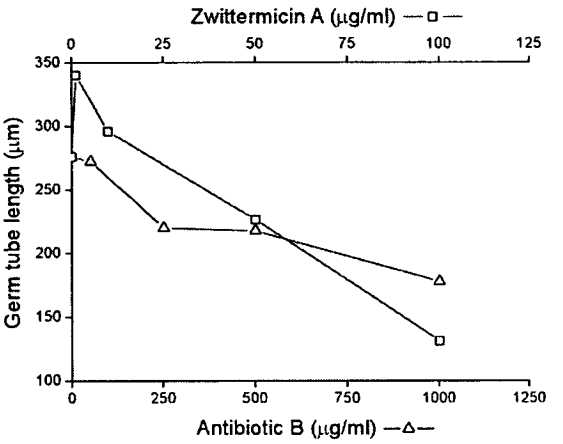



**Figure 5**

**Figure 4:** Effect of zwittermicin A on germination cysts of *P. medicaginis.* **A** – No treatment; **B** – 100 μg of zwittermicin A per ml.[6]

**Figure 5:** Inhibition of germ tube elongation by zwittermicin A and antibiotic B.[6]

As described above, *Bacillus cereus* produces two antibiotics – zwittermicin A and antibiotic B.[6] The structure of the antibiotic B was determined only in 1996[9] as an aminoglycoside antibiotic (3-amino-3-deoxy-D-glucose) called kanosamine (**Figure 6**).

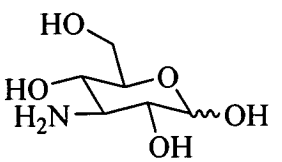



**Figure 6:** Structure of kanosamine.[9]

This antibiotic also has the ability to suppress disease in alfalfa caused by *Phytophthora medicaginis*, by reducing germ tube elongation. However, zwittermicin A provided better disease suppression than kanosamine at lower concentrations (**Figure 7A**).[6] To determine the effect of zwittermicin A in the presence of kanosamine, these two antibiotics were tested together. It was found out that they acted synergistically

against *Escherichia coli* (**Figure 7B**) and additively against *Phytophthora medicaginis* (**Figure 7C**).[8]

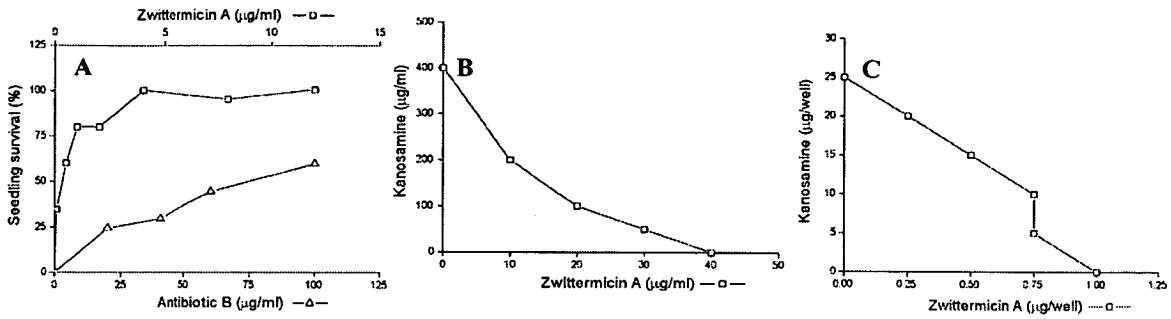



**Figure 7A:** Effect of zwittermicin A and antibiotic B on "damping-off" of alfalfa seedings caused by *Phytophthora. medicaginis*.[6]

**Figure 7B:** Combined activity of zwittermicin A and kanosamine against *Escherichia coli*.[8]

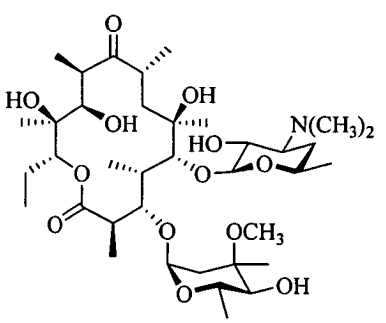
**Figure 7C:** Combined activity of zwittermicin A and kanosamine against *Phytophthora medicaginis*.[8]

Additionally, the activities of zwittermicin A at different pH were tested and it was determined that the antibiotic was more active at higher pH (7-8) than at lower pH (5-6) against bacteria and fungi.[8]

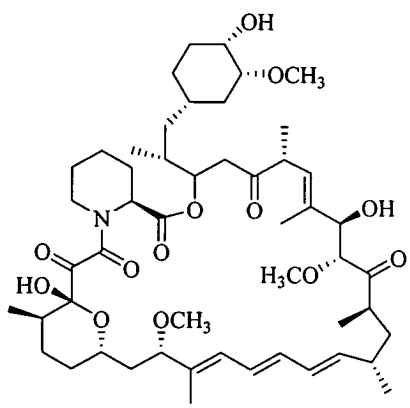
Zwittermicin A is the only known linear aminopolyol and recent studies (discussed in section 1.6) have suggested that it is the product of mixed polyketide (PKS) synthase/nonribosomal peptide (NRPS) synthase pathways. Polyketide, nonribosomal peptide, and mixed polyketide/nonribosomal peptide synthases pathways are reviewed in the next three sections.

## 1.3. Polyketide biosynthesis.

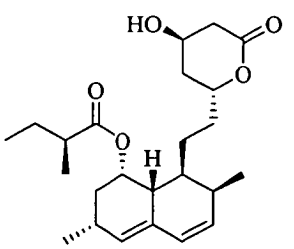
Along with essential primary metabolites (carbohydrates, proteins, nucleic acids), microorganisms make a wealth of unusual metabolites (e.g. terpenes, alkaloids, steroids, prostaglandins, etc.) that have a secondary role in the organism's ontogeny. As the need arises, they could be used in self-defense,[10,11] aggression, or even communication. Polyketides are a remarkable class of natural products, numbering over 10,000 compounds:[12] many of them are known to be extremely pharmacologically active. The most commercially important polyketides include antibiotics (erythromycin A, tetracycline), immunosuppressants (rapamycin, FK506), anticancer agents (doxorubicin, adriamycin), antifungal agents (amphotericin B), antihelmintic (avermectin), cholesterol-lowering agents (lovastation) and other agents (**Figure 8**).[12-14]



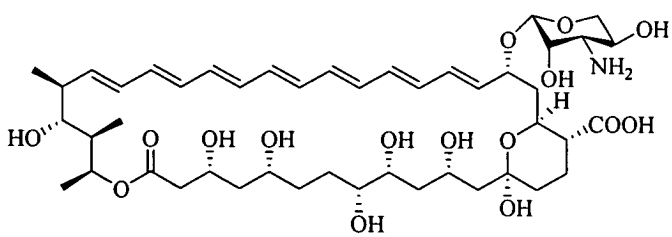
erythromycin A



rapamycin



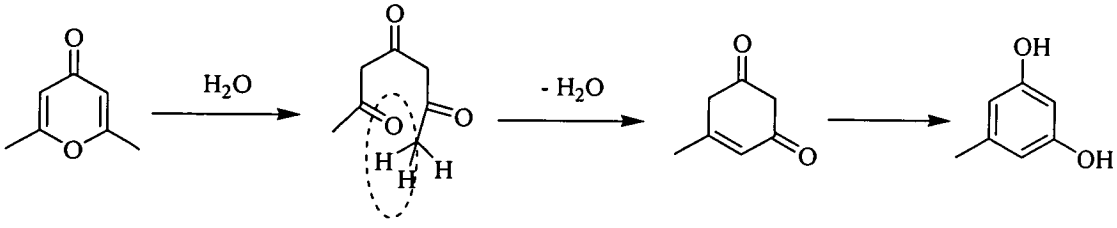
lovastatin



amphotericin B

**Figure 8:** Examples of some pharmaceutically important polyketides.

The first progress in understanding polyketide chemistry was realised by James Collie in 1893. While proving the structure of dehydroacetic acid, he—unexpectedly—obtained orcinol as a product of his manipulations. He explained the mechanism of orcinol formation through a polyketone intermediate (**Figure 9**).[13] This proposition was inspirational.




Dehydroacetic acid Polyketone intermediate Orcinol

**Figure 9:** Collie's explanation of orcinol formation.[13]

However, the most important work in the formulation of a "polyketide hypothesis" was carried out by Arthur Birch in the 1950s. He proposed that the polyketones could be generated from acetate units by repeated condensation reactions, and used 1- and 2-$^{14}$C-labelled acetates to prove his theory.[15] For confirmation he fed the *Penicilium patulum* mould with labelled acetate, and analysed the resulting 6-methylsalicylic acid using degradation experiments (**Figure 10**).

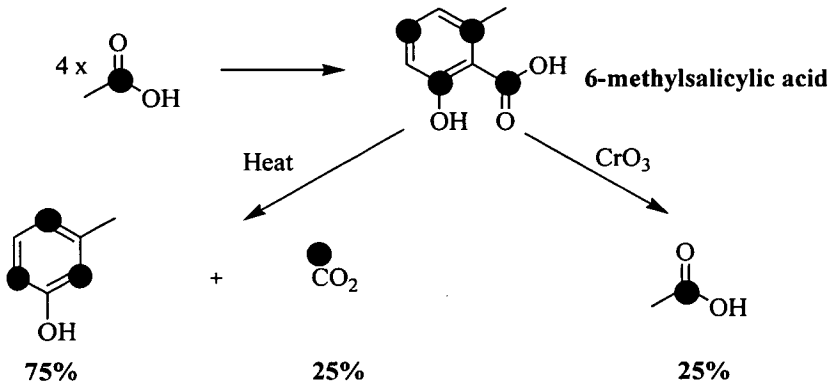



**Figure 10:** Birch's demonstration that 6-methylsalicylic acid is derived from four acetate units.[13]

The development of NMR techniques in the 1960s and 70s played an essential role in polyketide chemistry. Since the majority of polyketide-producing organisms readily take up isotopic-labelled precursors, use of NMR helped to determine: (i) that polyketides are built up from multiple acetate units; (ii) the full chemical structure of huge number of polyketide natural products; and also (iii) the types of biosynthetic pathways employed in polyketide chemistry.

From all the above information it can be said that polyketides are synthesised by sequential reactions from simple building blocks such as acetyl-CoA, propionyl-CoA, methylmalonyl-CoA and butyryl-CoA. The key C–C bond-forming reaction is the Claisen condensation. The biocatalytic assembly of polyketides is carried out by exceptionally large, multifunctional proteins: so-called polyketide synthases (PKSs). PKSs are organised into co-ordinated groups of active enzymes called modules, where each module catalyses one cycle of polyketide chain elongation.[16] Typically, three types of module are distinguished within one PKS: a loading module, an extension module, and an ending module (**Figure 11**).

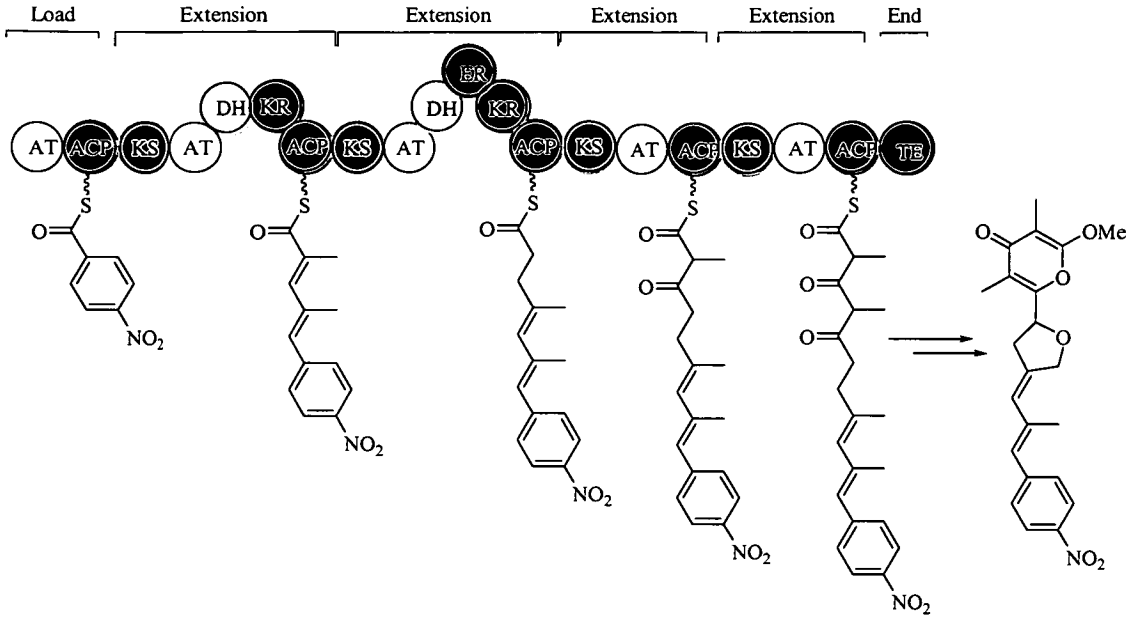



**Figure 11:** A schematic view of a typical PKS. Biosynthesis of aureothin.[17]

The loading module consists of two domains: an acyltransferase (AT) and an acyl carrier protein (ACP). The acyltransferase selects the first acyl-CoA unit and transfers it to the acyl carrier protein. The extension module contains at least three domains: AT, ACP and ketosynthase (KS). Extension of the chain occurs when the activated acyl unit transfers from the ACP to the KS, which catalyses the Claisen condensation with a second pre-loaded acyl-ACP. Additionally, some extension modules can contain ketoreductase (KR), dehydratase (DH), and enoyl reductase (ER). The ending module has one domain—thioesterase (TE), which releases the final product by hydrolysis or lactonisation.[12]

Few attempts to classify the PKSs have been undertaken historically, but the modern classification is based on the fundamental differences in the organisation and operation of the enzymes. Typically, polyketide synthases are divided into two types; however, some researchers suggest that an additional third type also exists.[14] Type I PKSs are multifunctional enzymes, which are organised into modules. Each module performs a unique function and catalyses only one cycle of polyketide chain elongation. The gigantic (~350 kDa) multienzyme polypeptides 6-deoxyerythronolide B synthase

(DEBS) 1, 2 and 3 from the biosynthesis of the reduced polyketides erythromycin A exemplify the type I PKS (**Figure 12**).

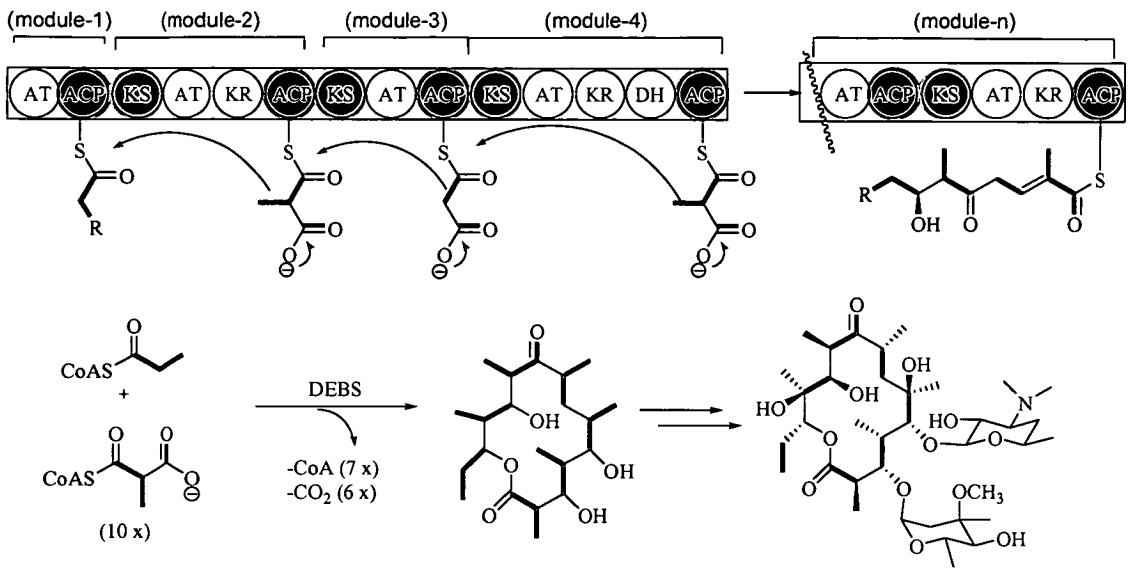
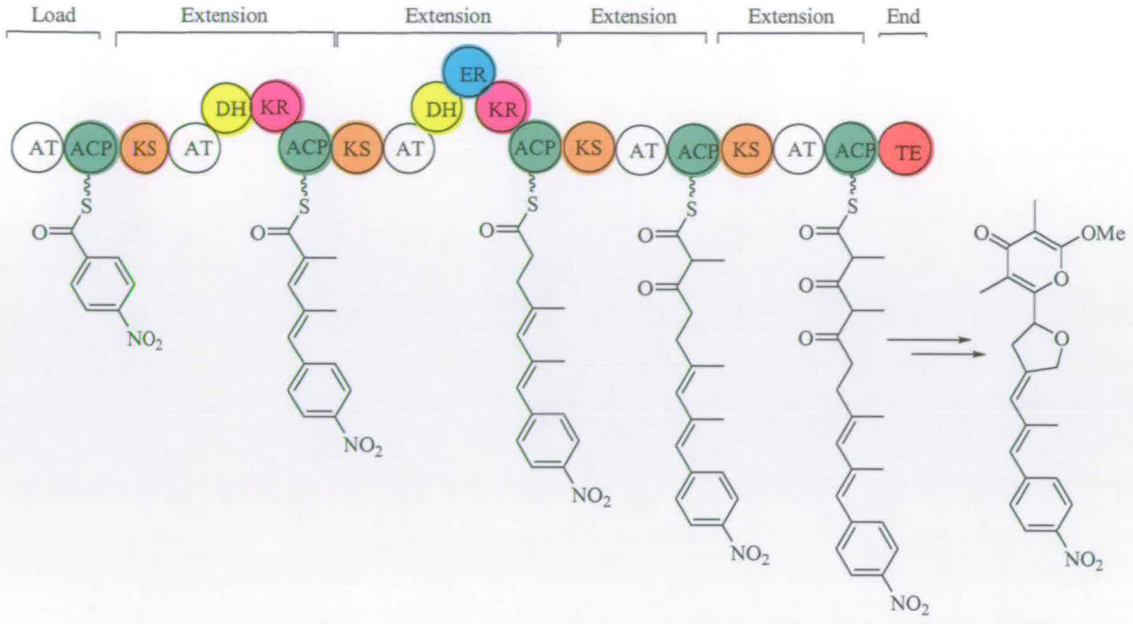



**Figure 12:** Biosynthesis of erythromycin A: an example of Type I PKS (non-iterative).[14]

Type II PKSs (for example, the tetracenomycin and actinorhodin PKSs) are multienzyme complexes that perform an iterative function, and catalyse the formation of aromatic and/or cyclic compounds (**Figure 13**).[14]

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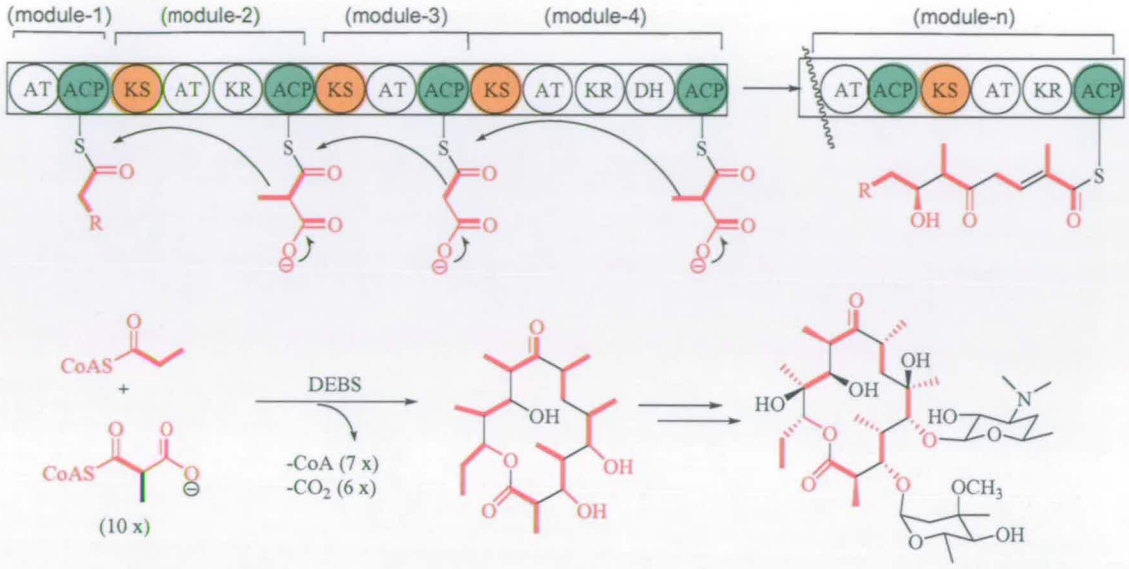



**Figure 12:** Biosynthesis of erythromycin A: an example of Type I PKS (non-iterative).[14]

Type II PKSs (for example, the tetracenomycin and actinorhodin PKSs) are multienzyme complexes that perform an iterative function, and catalyse the formation of aromatic and/or cyclic compounds (**Figure 13**).[14]

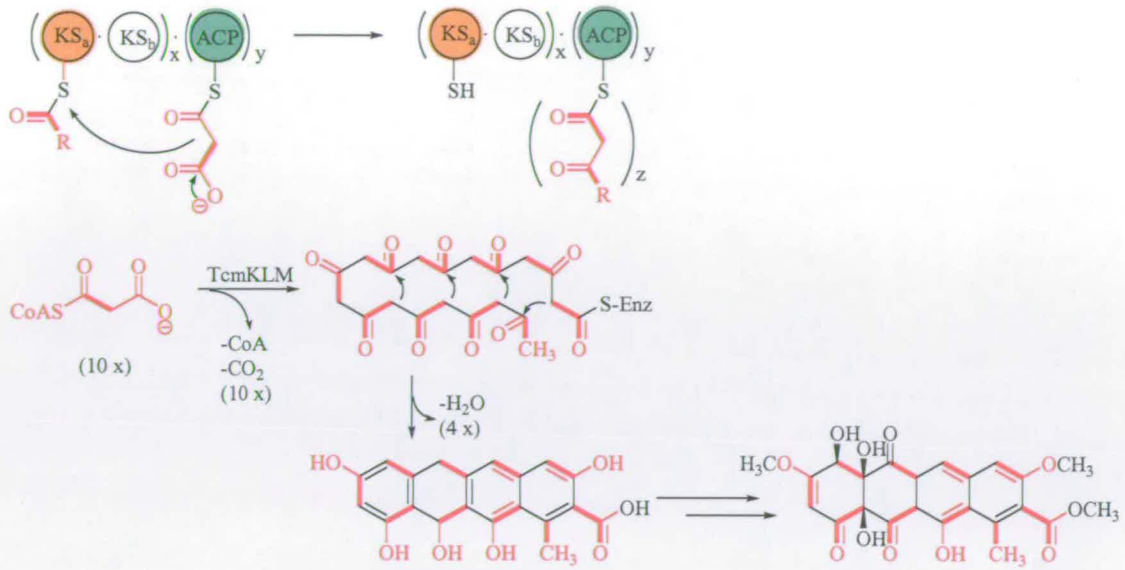



**Figure 13:** Biosynthesis of tetracenomycin C: an example of Type II PKS (iterative).[14]

Type III PKSs are relatively small homodimeric, iteratively acting enzymes, which catalyse the biosynthesis of chalcones, stilbenes and polyhydroxy phenols. This type of PKS is involved in flavonoid biosynthesis (**Figure 14**).[14,18]

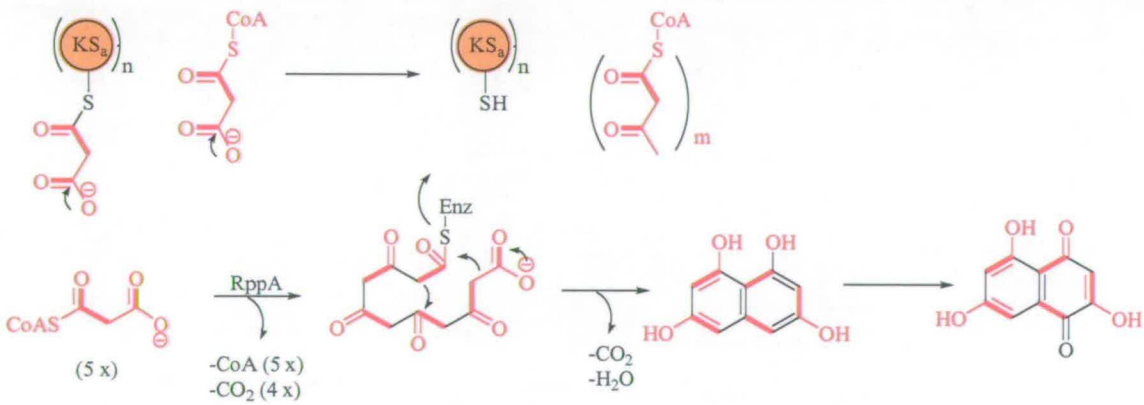



**Figure 14:** Biosynthesis of flavolin: an example of Type III PKS (ACP-independent and iterative).[14]

There is some evidence that bacterial type I PKSs may be iterative, and that not only type II PKSs can produce aromatic rings.[19-22] These examples have shown that there is much more diversity in both mechanism and structure than the type I, II and III model suggests.[14,23] The most notable combination is the working together of polyketide (PKS) and nonribosomal peptide (NRPS) synthases to produce huge numbers of pharmaceutically active compounds.

## 1.4. Nonribosomal peptide biosynthesis.

Along with polyketides, there is another significant class of secondary metabolites that result from iterative chain extension—nonribosomal peptides (NRP). A large number of important pharmaceutical, veterinary, agricultural and other agents arise from this class. The most pharmaceutically important nonribosomal peptides include antibiotics (penicillin, bacitracin, vancomycin, pristinamycin, gramicidin), antifungal drugs (echinocandin), cytostatic agents (bouverdins), and immunosuppressants (cyclosporin) (**Figure 15**).[24-26]

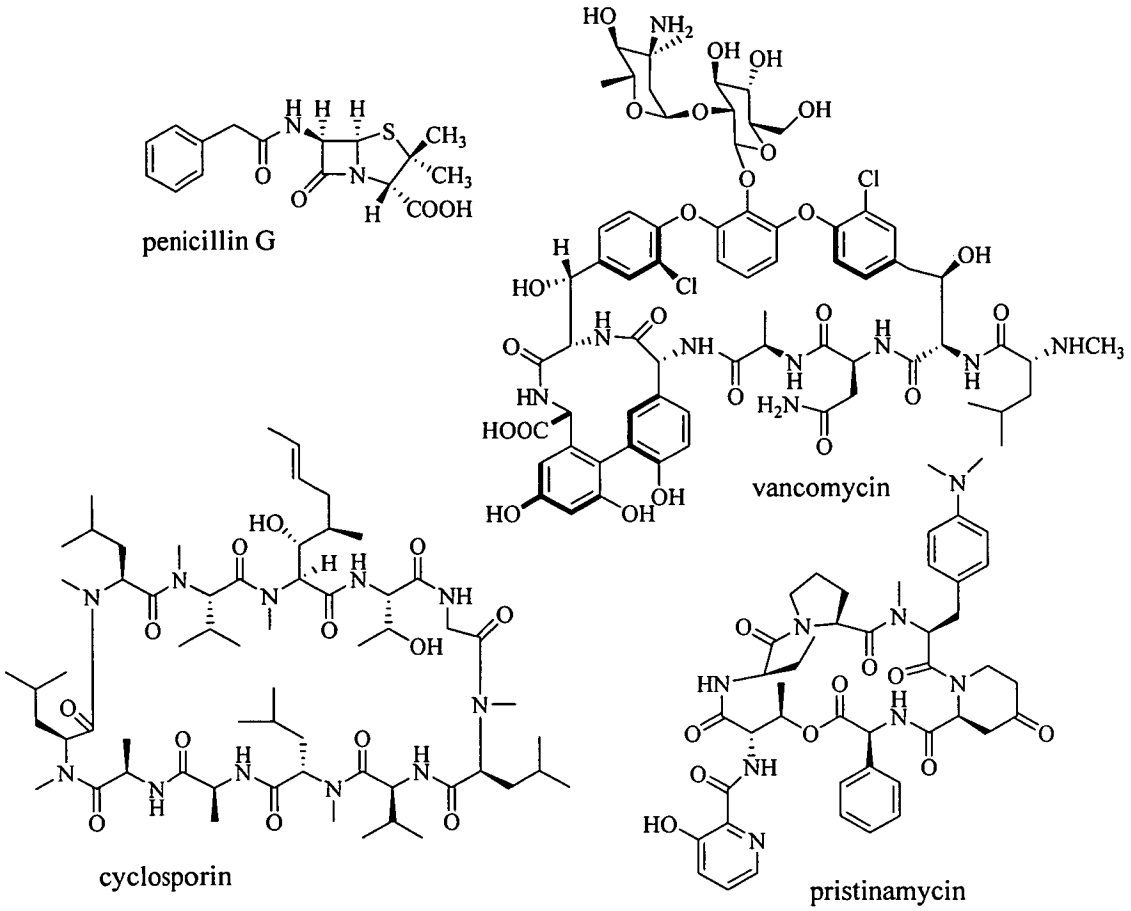



**Figure 15:** Examples of some nonribosomal peptide natural products.

Nonribosomal peptides are synthesised via a template-directed, nucleic acid-independent nonribosomal mechanism. This mechanism is catalysed by the largest enzymes known in nature, called nonribosomal peptide synthases (NRPSs).[27] In a similar fashion to polyketide synthases, nonribosomal peptide synthases are organised in co-ordinated groups of iterative modules. Each module is responsible for catalysing one single cycle of the peptide chain elongation, and modifications of the resulting functional group. A minimum elongation module consists of adenylation (A), peptidyl carrier protein (PCP), condensation (C), and thioesterase (TE) domains. Some modules can additionally have cyclization (Cy), methyltransferase (MT), and epimerisation (Er) domains (**Figure 16**).

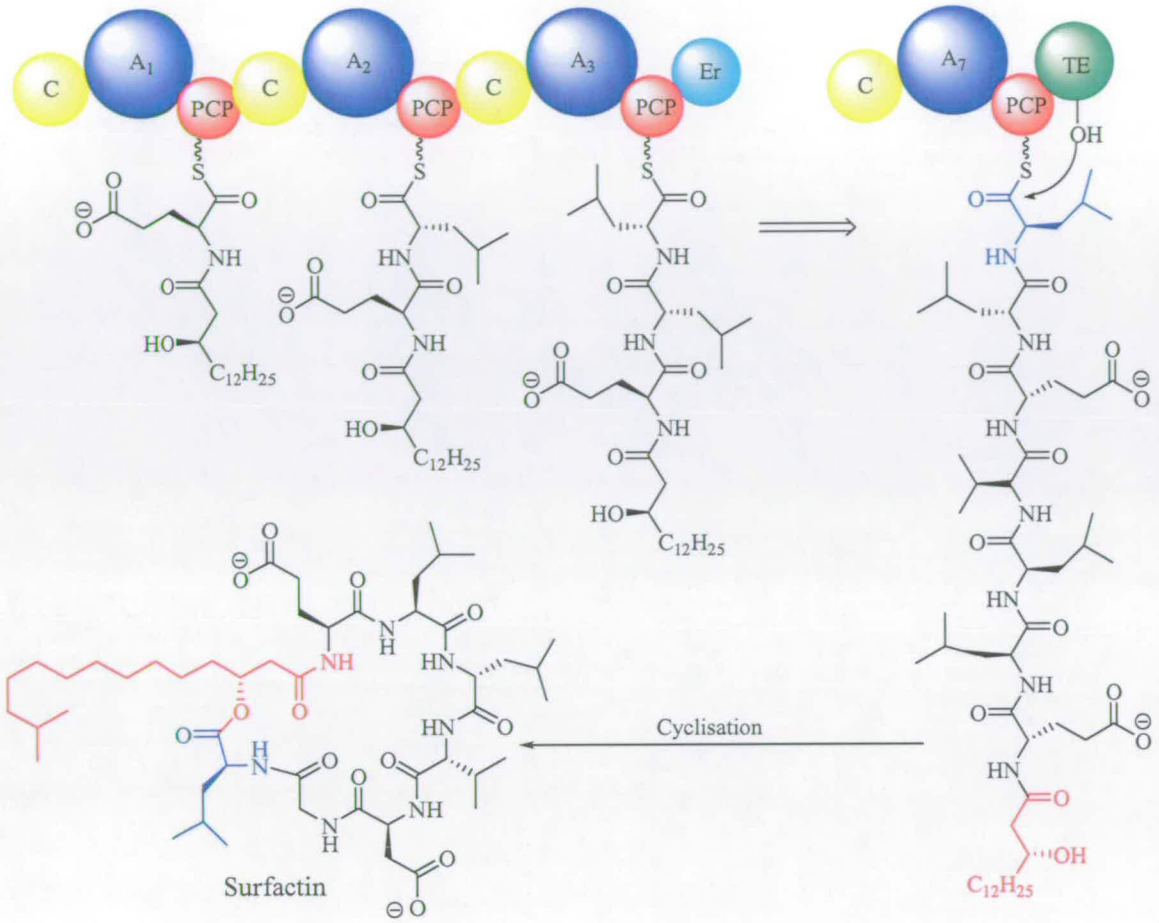



**Figure 16:** Biosynthesis of surfactin: an example of NRPS.[25]

The structure of the adenylate domain was first determined in 1997 by Marahiel.[28] Adenylation activates an amino acid using ATP to generate the adenylated carboxylate,[26] (**Figure 17**)[10] and uses the amino acid's positively charged amino group to co-ordinate the ribose phosphate region of ATP to bring them to the right conformation for the reaction.[24]

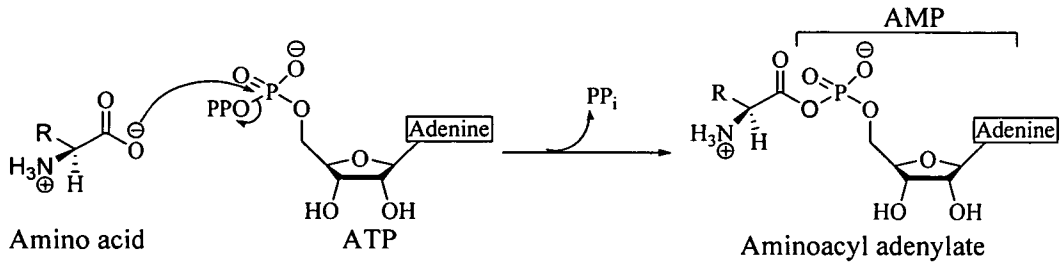



**Figure 17:** Schematic activation mechanism of adenylate.[10]

The peptidyl carrier protein normally exists in an inactive apo form. Before the biosynthesis can start, the PCP domain has to be activated to give its holo form. Activation takes place by the covalent attachment of a 4'-phosphopantetheine (Ppant) cofactor from coenzyme A to a conserved serine residue (**Figure 18**). This modification is catalysed by phosphopantetheinyl transferase (PPTase).[24]

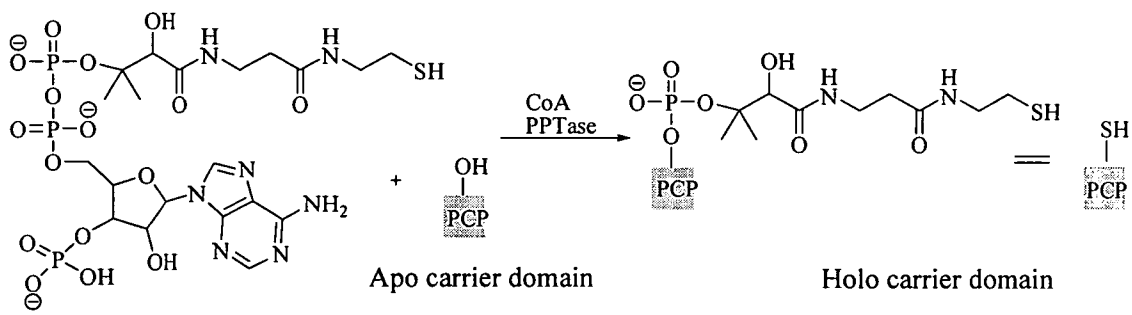



**Figure 18:** CoA activation of carrier domain.[26]

The condensation domain catalyses peptide bond formation between activated aminoacyl- or peptidyl-S-PCP, resulting in peptide elongation by one residue fixed to the PCP domain. The importance of the C domain has been demonstrated by deletion and mutation experiments. However, the catalytic mechanism of peptide bond formation is still unknown. Further modification can take place, such as cyclisation, methylation or

epimerisation, while the amino acid or the growing peptide is still attached to a carrier domain. When the peptide chain reaches the desired length, the final product is released from the last carrier domain via cyclisation, or via hydrolysis by a thioesterase domain (TE).

## 1.5. Hybrid polyketide and nonribosomal peptide biosynthesis.

To summarise sections 1.3 and 1.4: nonribosomal peptides and polyketides are two large families of natural products that include many pharmaceutically active drugs and other important compounds.[29] Both classes use a very similar strategy for the assembly of natural products by sequential condensation of amino acids (in nonribosomal peptide synthesis) or carboxylic acids (in polyketide synthesis). The biosynthesis of these two classes of natural products is catalysed by multifunctional enzymes: nonribosomal peptide synthases (NRPSs) and polyketide synthases (PKSs), organised in modules. The structure of the final natural product depends on the number and order of the modules in the assembly line. Both systems use carrier proteins: peptidyl carrier protein (PCP) for NRPS and acyl carrier protein (ACP) for PKS. Due to their similarity, it is not surprising that these two classes can work together to create new, more complicated natural products.[30,31]

Hybrid peptide–polyketide natural products derive from amino acids and carboxylic acids, e.g., cyclosporin, coronatine, bleomycin,[31] rifamycin,[32] leinamycin,[33] myxothiazol,[34] etc. Known hybrid compounds are divided into two classes: (i) those whose hybrid peptide–polyketide backbone is assembled via other mechanisms that do not require direct functional hybridization between NRPS and PKS proteins; and (ii) those whose hybrid peptide–polyketide backbone is assembled by a hybrid NRPS–PKS system that mediates the direct elongation of a NRPS-bound peptidyl intermediate by a PKS module, or vice versa.[35]

The biosynthetic pathways of coronatine and cyclosporin can be used to illustrate the first class of the hybrid natural products. In the biosynthesis of coronatine, the

peptide and polyketide moieties are synthesised separately and subsequently combined by a ligase (**Figure 19**).[35]

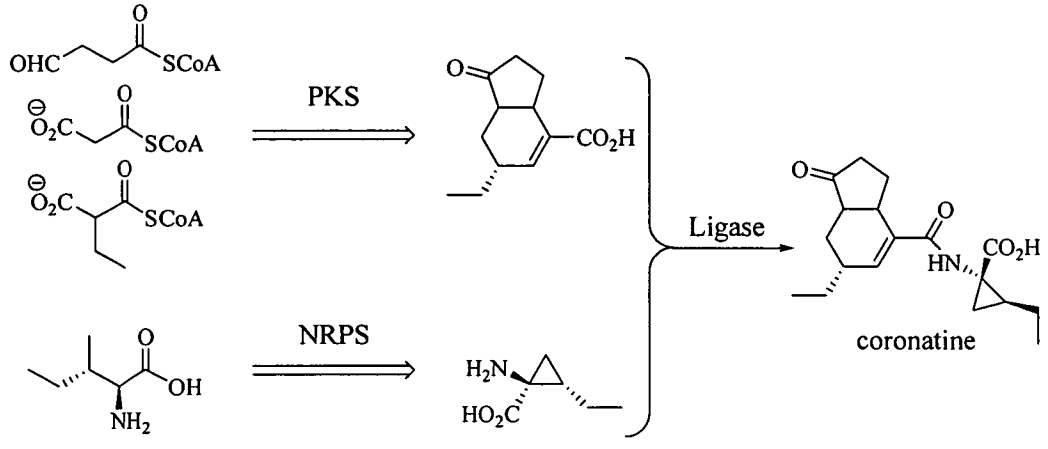



**Figure 19:** Biosynthetic pathway for coronatine.[35]

In the biosynthesis of cyclosporin, on the other hand, the polyketide moiety is firstly converted into an amino acid, and then the resulting intermediate is incorporated into a nonribosomal peptide pathway (**Figure 20**).[35]

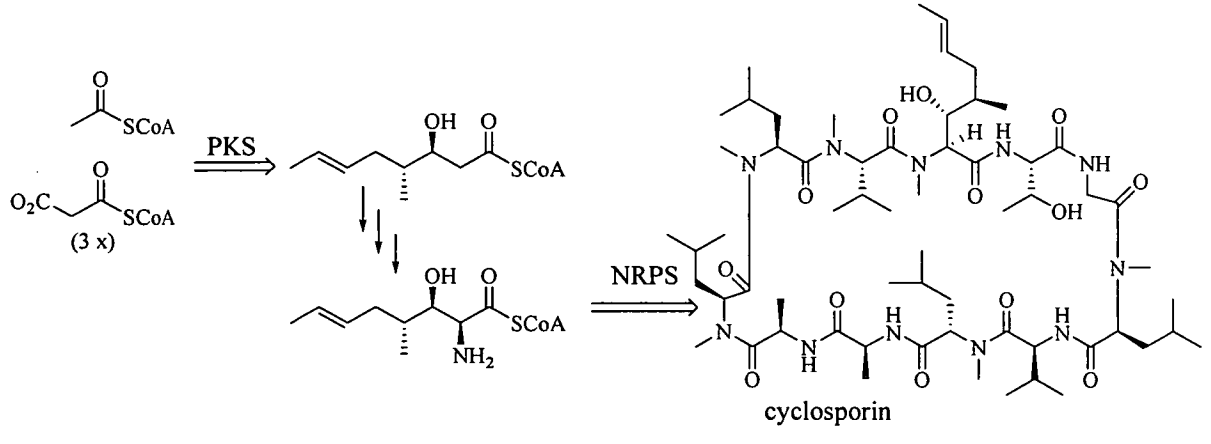



**Figure 20:** Biosynthetic pathway for cyclosporine.[35]

Most of the hybrid peptide–polyketide metabolites, however, involve direct transfer of the activated peptidyl intermediate onto a PKS module, or vice versa. A schematic representation of the module organisation in a hybrid NRPS–PKS is illustrated in **Figure 21**.[35]

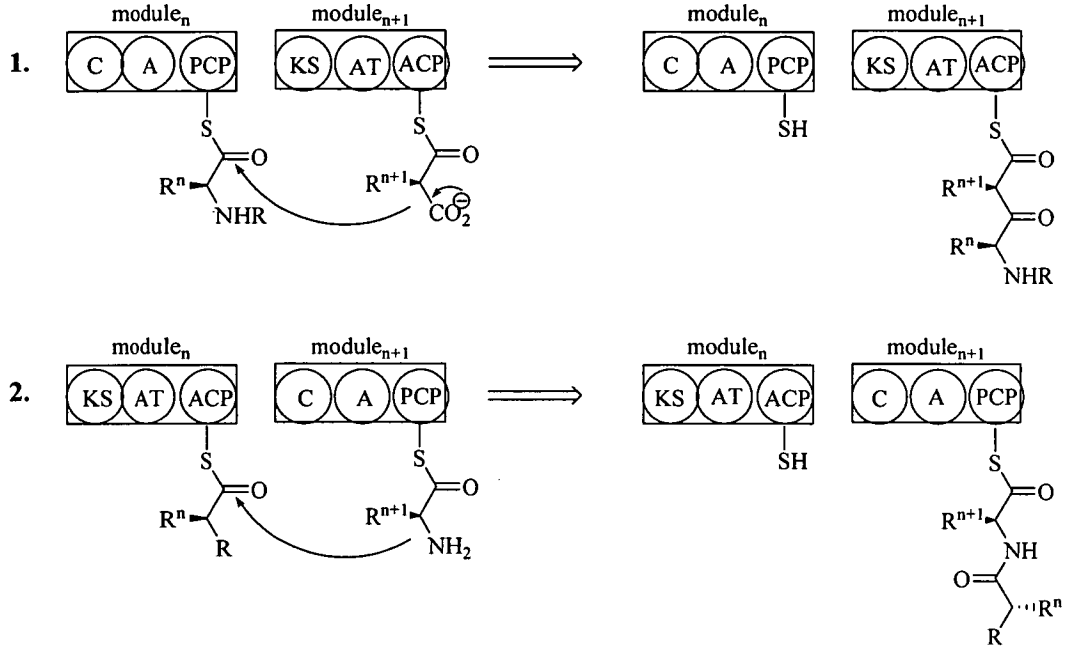



**Figure 21:** (1) C–C bond formation for hybrid peptide-polyketide biosynthesis; (2) C-N bond formation for hybrid polyketide–peptide biosynthesis.[35]

As an example of this class of hybrid NRPS–PKS biosynthesis, the biosynthesis of pristanamide can be considered. This biosynthetic pathway was first established by Kingston *et al.* through feeding experiments.[36] **Figure 22**[35] presents a schematic biosynthetic pathway towards pristanamide. It shows that this natural product can be assembled by a hybrid NRPS–PKS system, which mediates the transfer of the growing peptide or polyketide intermediates between NRPS and PKS modules three times.

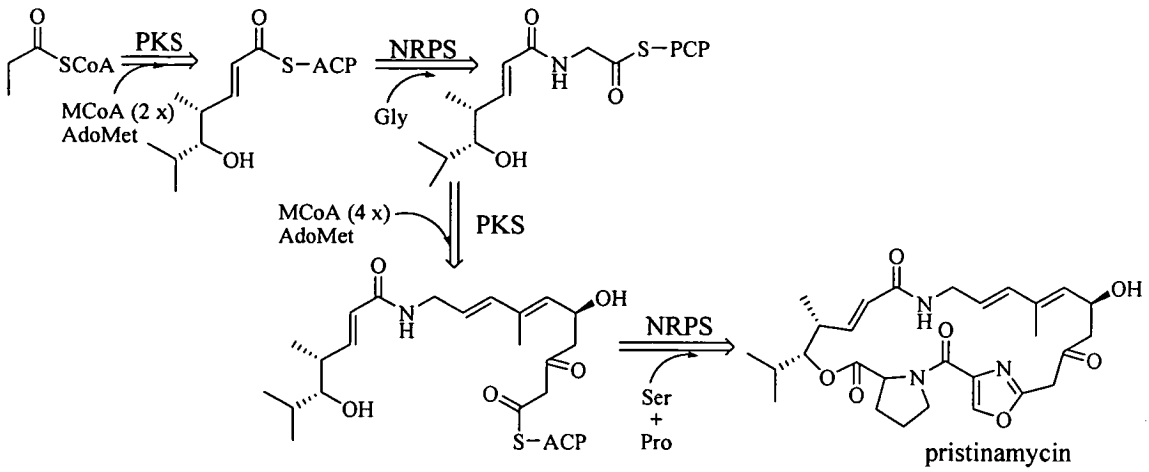



**Figure 22:** Biosynthetic pathway for pristinamycin.[35]

In the previous three sections introductions to polyketide, nonribosomal peptide and hybrid polyketide–peptide biosynthetic pathways were presented. These areas give a better understanding of zwittermicin A biosynthesis by mixed polyketide–nonribosomal peptide (PKS–NRPS) synthases as discussed in the following section.

### 1.6. Genetics and biosynthesis of zwittermicin A.

Zwittermicin A is the only known linear aminopolyol antibiotic, and has structural features in common with peptide and polyketide antibiotics (**Figure 23**). The hydroxyl groups on the carbon backbone are similar to those of a partially reduced polyketide structure; the nitrogen-rich end of zwittermicin A is derived from an amino acid, similar to peptide antibiotics.[37,38]

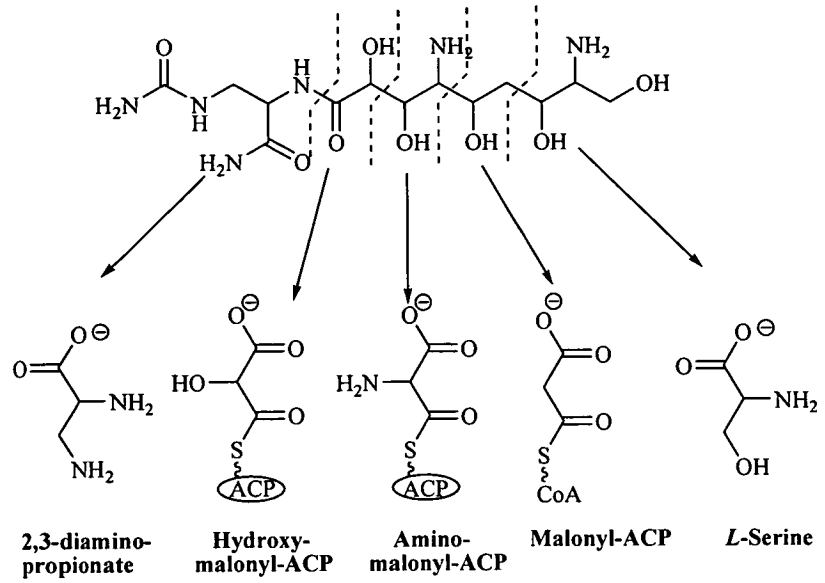



**Figure 23:** Chemical structure of zwittermicin A and the proposed precursors for its biosynthesis.[38]

The intriguing structure of zwittermicin A makes understanding of the mechanism of biosynthesis of this antibiotic very interesting. Unfortunately, the vast majority of genetic studies of polyketide antibiotic biosynthesis have been carried out in *Streptomyces* spp.[13] and almost nothing is known about other polyketide antibiotics (for example: difficidin[39] and aurantinin[40]) in *Bacillus* spp. Such a lack of information has provided the impetus for many investigations into the possible mechanism of biosynthesis of zwittermicin A.

Milner *et al.*[41] have identified, isolated and determined the sequence of a zwittermicin A–resistance gene[ii], *zmaR*, from *Bacillus cereus* UW85, which produces zwittermicin A. Mutants of UW85 that do not produce zwittermicin A contain large genomic deletions that span *zmaR*;[41] however, some strains that do not produce

[ii] Antibiotic-producing organisms require antibiotic self-resistance genes to protect themselves from the action of their toxic metabolites: these genes are often found in a cluster with antibiotic biosynthesis genes.[41]

zwittermicin A also contain the self-resistance gene *zmaR*.[9] It was demonstrated by radiochemical assay that the protein encoded in the *zmaR* gene is an acetyltransferase.[37] Extracted cells of the resistance culture of *Escherichia coli* containing *zmaR* inactivated zwittermicin A by acetylation; however, purified *zmaR* protein inactivated zwittermicin A in the same way only when cofactor acetyl-CoA is present.

In the initial studies of *zmaR*[41] and the adjacent DNA[37] three open reading frames (ORFs) (*orf* 1 to 3) were identified. Proteins encoded by those ORFs showed similarity to proteins involved in polyketide and peptide biosynthesis.[38] Five additional ORFs (*orf4* to *orf8)* and one partial ORF (*orf9*) were disclosed later by sequence analysis.[38] All of these additional five ORFs have the same transcriptional orientation as *orf1* to *orf3* and *zmaR* (**Figure 24**). Emmert *et al.* suggested that these five newly identified ORFs are also responsible for encoding proteins similar to known proteins involved in the biosynthesis of polyketides and nonribosomal peptides.[38]

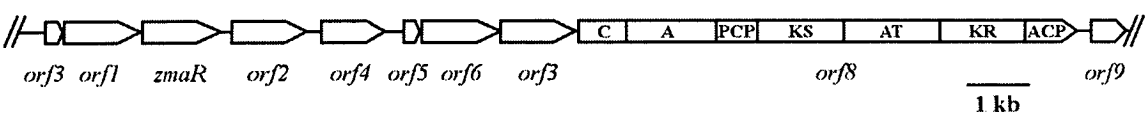



**Figure 24:** Organization of genes identified in the zwittermicin A biosynthetic cluster of *Bacillus cereus* UW85.[38]

Using the BLAST[iii] algorithm[37] it has been determined that the putative proteins encoded by *orf4* to *orf6* are similar to enzymes involved in the synthesis of methoxymalonyl-acyl carrier protein (ACP). *Orf5* encodes proteins that are similar to two ACPs that are also expected to be involved in the formation of methoxymalonyl-ACP. The proteins from *orf5*, *orf6* and *orf3*, *orf1* are predicted to be similar, suggesting that one group of genes may have arisen through duplication of the other.[38] Gene disruption showed that protein encoded by *orf2* is necessary for zwittermicin A

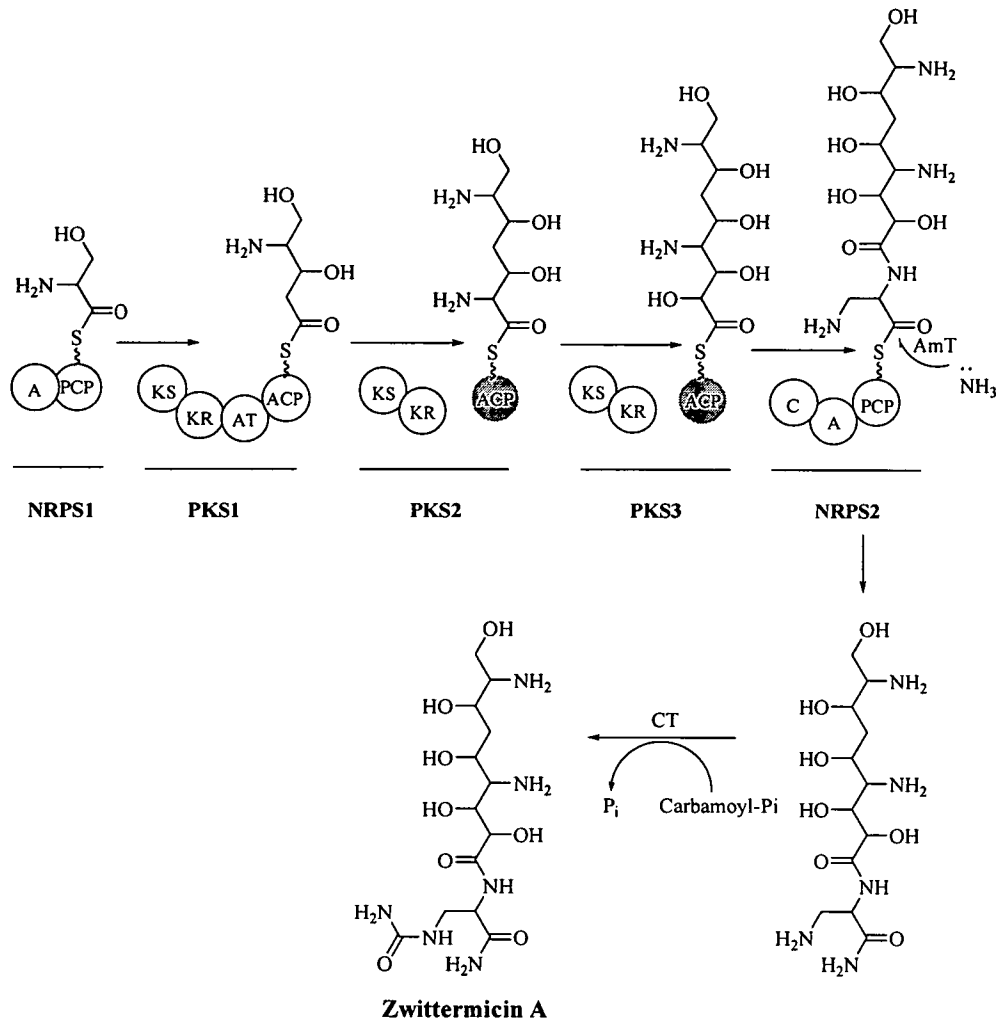
[iii] B LAST database—a protein database search program, which allows comparing amino acid query sequences and nucleotide query sequences against a protein-sequence database.

production, and is a putative malonyl-CoA transacylase.[38]

Whilst the gene sequence of *orf7* is highly similar to genes encoding the nonribosomal peptide synthases (NRPSs). It has been shown that protein encoded by *orf7* is responsible for serine adenylation.[38]

*Orf8* encodes a large putative protein that appears to be a multifunctional hybrid of NRPS and a type I polyketide synthase (PKS). One portion of this protein contains a module typical for nonribosomal peptide synthases (NRPSs), which includes the condensation, adenylation and peptidyl-carrier protein.[38] The other portion of this protein, however, contains regions with a high similarity to domains typical for type I PKSs. This large multifunctional protein is supposed to catalyse the condensation of short-chain carboxylic acids into the growing polyketide structure.[38] Emmert *et al.*[38] showed that the last 1500 amino acids of *orf8* contained regions that were similar to ketoacyl synthase, acyltransferase, ketoreductase, and ACP domains from PKS type I enzymes.

The results of the sequence and bioinformatic analyses presented by Emmert *et al.*[38] suggest the probable mechanism of zwittermicin A biosynthesis (**Scheme 1**).




**Scheme 1:** Proposal for the minimum biosynthetic pathway needed for zwittermicin A assembly.[38]

Abbreviations: A—adenylation; AT—acyltransferase; C—condensation; AmT—amidotransferase; CT—carbamoyltransferase.

According to Emmert *et al.*[38] the following zwittermicin A biosynthesis has been predicted (**Scheme 1**): NRPS1 would activate and tether serine to its peptidyl-carrier protein (PCP) domain; PKS1 would tether a malonyl moiety from malonyl-CoA to its ACP domain, and bring about a condensation with the above serine; the ketoreductase (KR) domain from PKS1 would reduce the carbonyl group from serine; the two subsequent condensation and reduction reactions, with aminomalonyl and hydroxymalonyl moieties, would be catalysed by ketosynthase (KS) and ketoreductase (KR) as shown on PKS2 and PKS3; finally, the NRPS2 would activate, tether and condense 2,3-diaminopropionate with the PKS3-tethered intermediate.

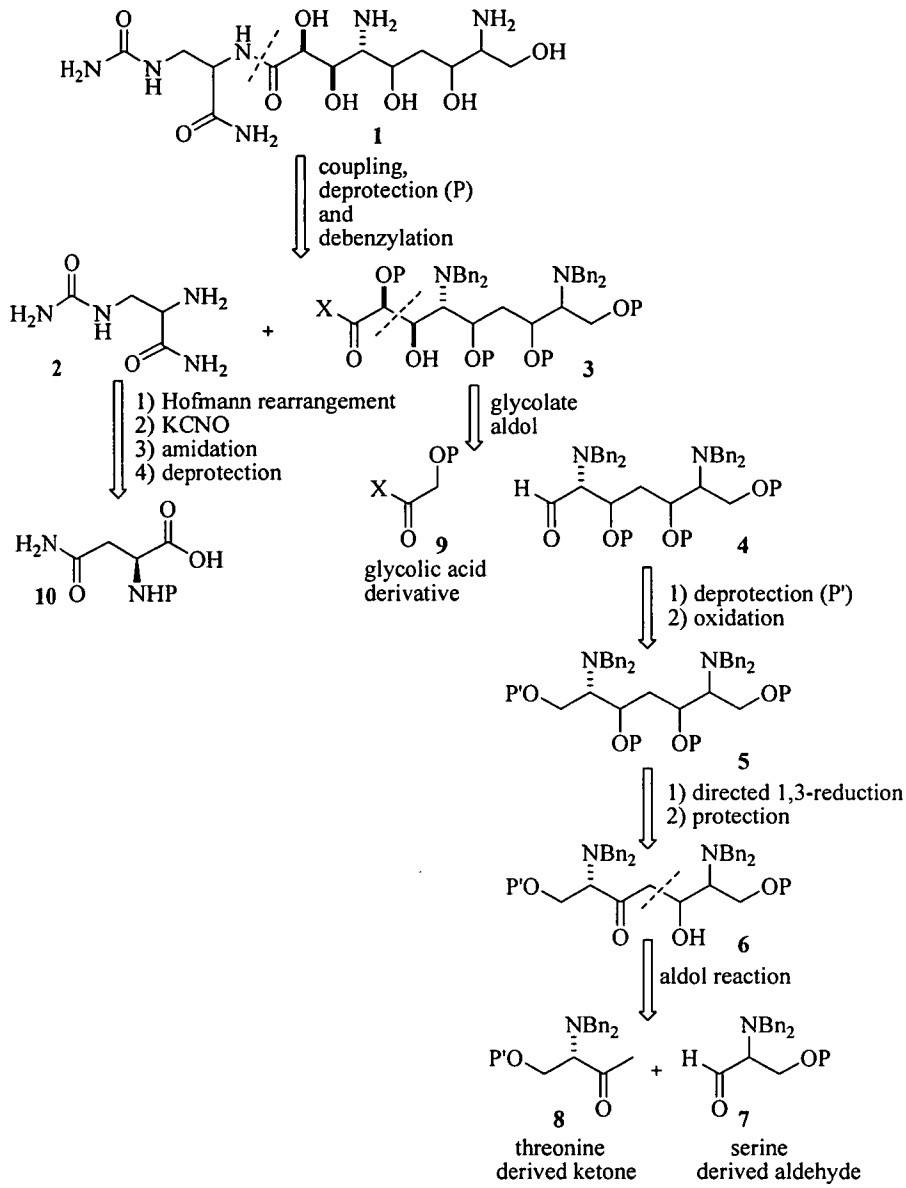
The proposed mechanism of zwittermicin A biosynthesis has not been fully validated: however, this work has considerably increased the understanding of the genetics of zwittermicin A production.

## 1.7. Retrosynthetic analysis of zwittermicin A.

Emmert *et al.*[38] have proposed that the biosynthesis of zwittermicin A starts with a serine unit (**Scheme 1**) and builds onto this using a polyketide synthase type pathway. In a similar fashion, we proposed to start our synthesis with an aldol reaction of a threonine derived ketone and serine derivative aldehyde. Our retrosynthetic analysis of zwittermicin A is shown in **Scheme 2.**

Disconnection of zwittermicin A **1** as shown in **Scheme 2** gave us two species: diaminopolyhydroxyacid derivative **3** and the nitrogen rich end of zwittermicin A **2**. We proposed to start the synthesis of **2** from *N*-monoprotected-asparagine **10** using the known methodology for Hofmann rearrangement[42] followed by urea formation, amidation and final deprotection. Diaminopolyhydroxyketone **3** could be made by glycolate aldol reaction of glycolic acid derivative **9** and aldehyde **4**. This molecule may be synthesised by selective deprotection (P') and oxidation of diaminopolyol **5**. Directed 1,3-reduction and protection of species **6** gives us diaminopolyol **5**. The molecule **6** is to be made by the aldol reaction of serine **7** and threonine **8** derivatives. Since the

diastereoselectivity of this part of zwittermicin A is unknown, it is useful to synthesise different diastereomers to discover which one has biological activity.



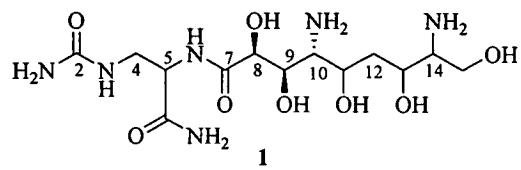

**Scheme 2:** Proposed retrosynthesis of zwittermicin A.

# Chapter 2: Results and Discussion 1.

# Synthesis of the nitrogen rich fragment $N_1$–$N_6$

## 2.1. Absolute stereochemistry and previous approaches to nitrogen-rich fragment 2.

When the structure of zwittermicin A **1** was first established in 1994,[7] the absolute stereochemistry of only three of the chiral centres was determined: as 8*S*, 9*R*, and 10*R* (**Figure 25**).



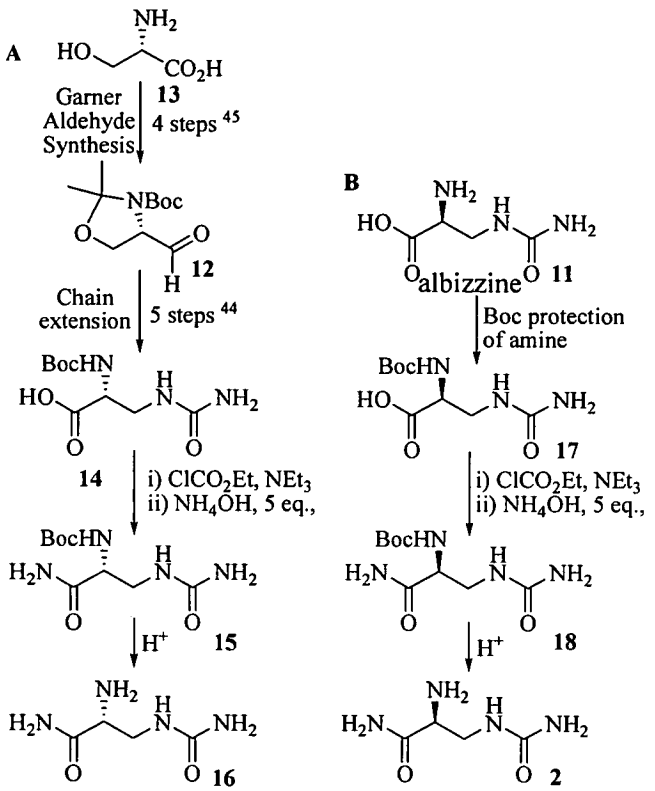

**1**

**Figure 25:** The proposed structure of zwittermicin A.[7]

However, in collaboration with Prof. Michael G. Thomas from the University of Wisconsin–Madison, we obtained additional information about the unknown stereocentres. It is now known (unpublished results) that the likely absolute configuration of C(14) is 14*S*; and that the nitrogen-rich fragment $N_1$–$N_6$ is likely to be derived from *L*-2,3-diaminopropionate. With this extra information in hand, the following synthetic strategies were suggested.

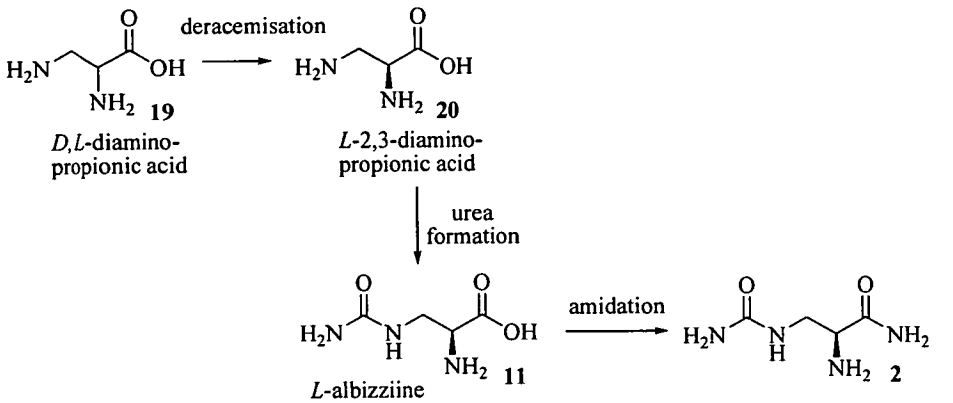
The desired nitrogen-rich fragment can be obtained from the unusual amino acid albizziine **11**. Two previous approaches to the synthesis of *D*-albizziine have been reported.[43,44] In the most recent paper, a five-step synthesis is used to convert the Garner aldehyde **12**,[45] derived from *L*-serine **13**, into *Nα*-Boc-*D*-albizziine **14** (**Scheme 3**).[44] The total sequence of nine steps may be achieved in an overall yield of 26%. In the second approach, enantioselective cleavage of the hydantoin derivative of racemic albizziine

with an *Agrobacterium radiobacter* bacterial culture is employed.[43] Neither of these two synthetic routes is suitable for large scale synthesis, or for the production of significant quantities of isotopically labelled material.




**Scheme 3:** (**A**) Previous synthesis of **2** from *D*-albizziine;[44] (**B**) proposed synthesis of **2** from *L*-albizziine **11**.

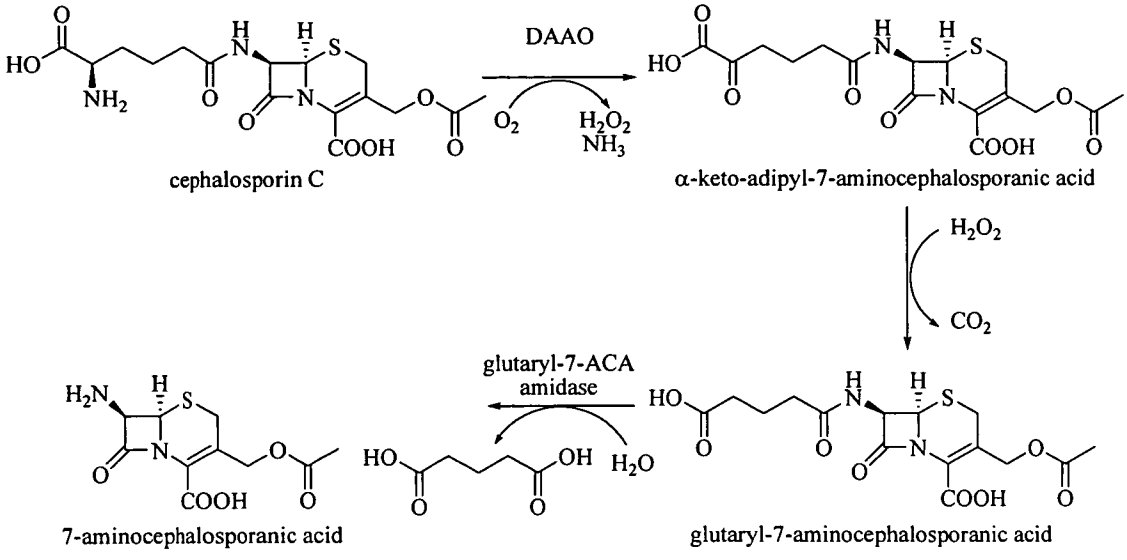
It would be most obvious to synthesise the nitrogen-rich fragment **2** from *L*-albizziine **11** using a simple amidation reaction. Although *L*-albizziine **11** itself was commercially available when we started this project, it was prohibitively expensive. In fact, its sale has since been discontinued. So, this synthetic approach was deemed to be unreliable to work with. Based on a literature precedent[44,46,47] we therefore proposed to start the synthesis of the nitrogen-rich end (**2**) from racemic 2,3-diaminopropionic acid, as illustrated in **Scheme 4.**




**Scheme 4:** The initial proposed synthetic path for nitrogen-rich fragment **2**.

## 2.2. Enzyme catalysed enantioseparation of racemic 2,3-diaminopropionic acid.

Niida *et al.* have reported that both enantiomers of albizziine can be made from the corresponding 2,3-diaminopropionic acid in 24% yield.[47] For the synthesis of zwittermicin A **1**, since the production of *L*-albizziine **11** was essential, we were required to start synthesis from *L*-2,3-diaminopropionic acid **20**. The high cost of this compound, however, made us look for an alternative source of starting material. The use of *D*-amino acid oxidases (DAAO) for the kinetic resolution of racemates is widely known in nature,[46] and broadly utilized in research and industry. The most important industrial application of DAAO is the first step of the production of 7-aminocephalosporanic acid (7-ACA) from cephalosporin C (**Scheme 5**).[48] 7-ACA is widely used as a synthetic material in cephalosporin analogue production. Another very important application of DAAO is in production of enantiomerically pure *L*-amino acids (**Scheme 6**).[46,49]

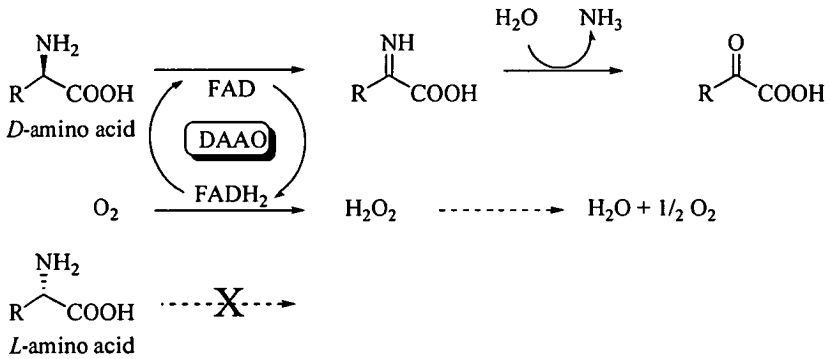



**Scheme 5:** Use of DAAO for the production of 7-ACA from cephalosporin C.[48]

Two sources of DAAO are known: yeasts and non-microbial sources, such as porcine kidney. The use of yeast as a source of DAAO has a few advantages. First of all, large amounts of enzyme are readily available by fermentation; secondly, the isolated DAAOs are tightly bound with cofactor, so that it is not necessary to add the coenzyme flavin adenine dinucleotide (FAD) during the biotransformation process.[46] Usually, whole or cross-linked cells, or immobilized DAAO are used, because these forms of the biocatalyst are reusable.

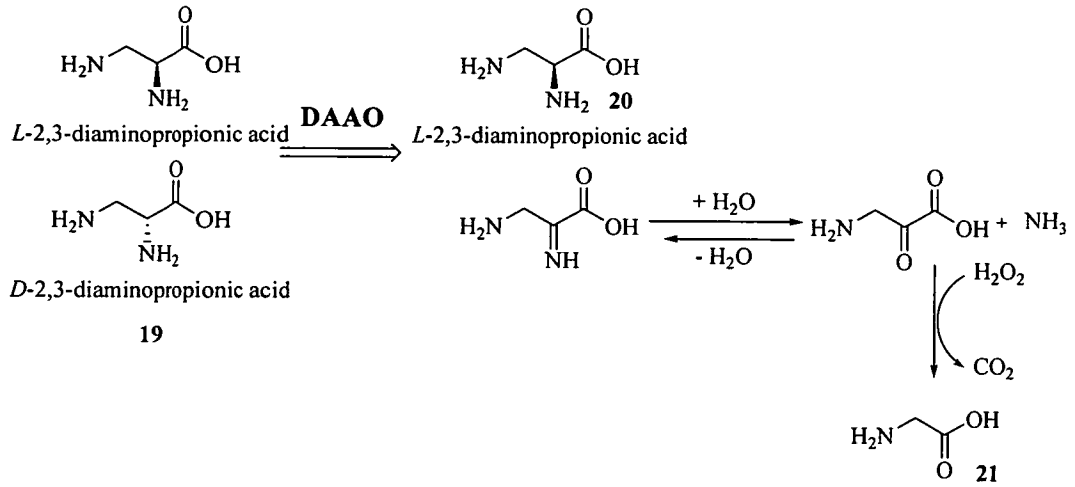
*D*-amino acid oxidase catalyses the oxidative deamination of a wide range of *D*-amino acids to their corresponding imino acids. These acids undergo non-enzymatic hydrolysis to the respective α-keto acid and ammonia (**Scheme 6**).[46,49,50] Oxygen, produced in the reaction, re-oxidaze the coenzyme flavin adenine dinucleotide ($FADH_2$), resulting in formation of hydrogen peroxide as a by-product of the reaction. In *vivo*, DAAO is situated in peroxysomes or microsomes of cells and tissues, and co-exists with catalases, which are responsible for the conversion of hydrogen peroxide to water and molecular oxygen. However, DAAO used in *vitro* is usually found with only a trace of catalase and is therefore not able to decompose $H_2O_2$. As a result, additional

decarboxylation of α-keto acids takes place, unless the hydrogen peroxide is immediately removed from the reaction mixture:[46] for example, by the addition of free catalases.



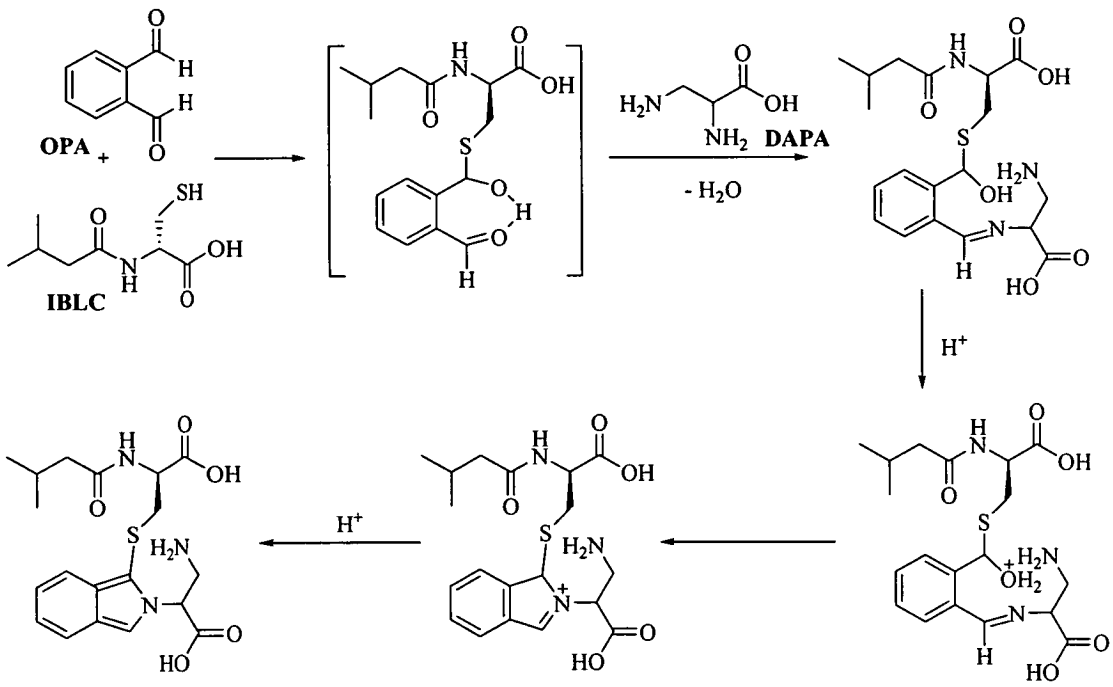

**Scheme 6:** Reaction scheme for the production of pure *L*-amino acids and α-keto acids using *D*-amino acid oxidase.[46,49]

D-amino acid oxidases are used for the purification of a broad range of α-monoamino acids and for the preparation of α-keto acids. Nevertheless, only a few examples of using D-amino acid oxidases to act on diamino acids are known. The action of D- and L-amino acid oxidases on racemic α,γ-diaminobutyric acid (2,4-diaminobutyric acid) and α,ε-diaminopimelic acid (2,6-diaminopimelic acid) has been reported previously.[51,52] However, there is no precedent in the literature for the use of D-amino acid oxidase for kinetic resolution of 2,3-diaminopropionic acid **19**. Chen *et al.* showed that in the case of 2,4-diaminobutyric acid, oxidation of only the α-amino group was observed.[51] As a result of their finding, we expected to obtain similar results in the case of racemic 2,3-diaminopropionic acid (**Scheme 7**).




**Scheme 7:** Production of pure *L*-2,3-diaminopropionic (DAPA) acid using a *D*-amino acid oxidase.

To proceed with this investigation (**Scheme 7**), suitable conditions for HPLC analysis had to be found, in order to follow the reaction. The reaction was monitored by HPLC using a Waters 2695 module and a 5-micron reverse-phase Gemini column, fitted with a Gemini guard column. A UV detector tuned to 338 nm was used to detect the fluorescent derivatives, which were obtained using standard derivatization conditions. The derivatization involved reaction of the amino acid with *o*-phthalaldehyde (OPA) combined with the chiral thiol *N*-isobutyryl-*L*-cysteine (IBLC).[53-55] It has been found that premixing the *o*-phthalaldehyde and thiol before adding the amino acid is necessary to increase the yield of UV-active isoindoles.[55] The proposed mechanism and one of the possible products are shown in **Scheme 8**.[54,55] Since the derivatization reaction is equally possible for two amino functionalities, three products are possible (**Figure 26**). However, because an excess of the reagents was used, we suggest that the derivatized product **24** is preferable.




**Scheme 8:** Proposed derivatization reaction of 2,3-diaminopropionic acid (DAPA).[54,55]

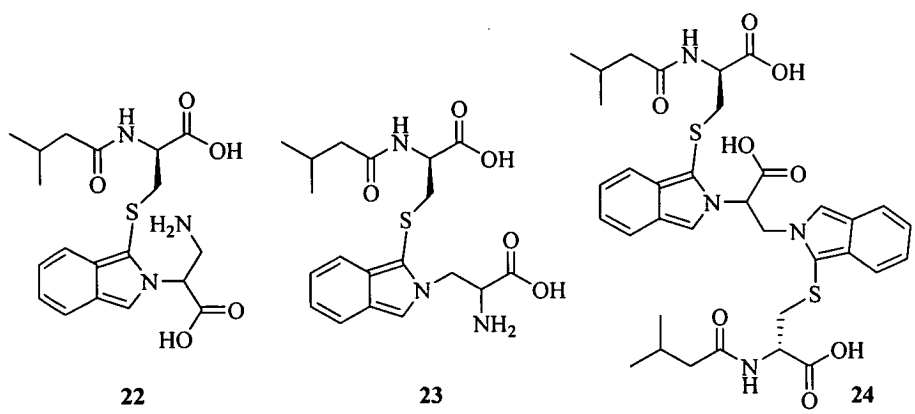
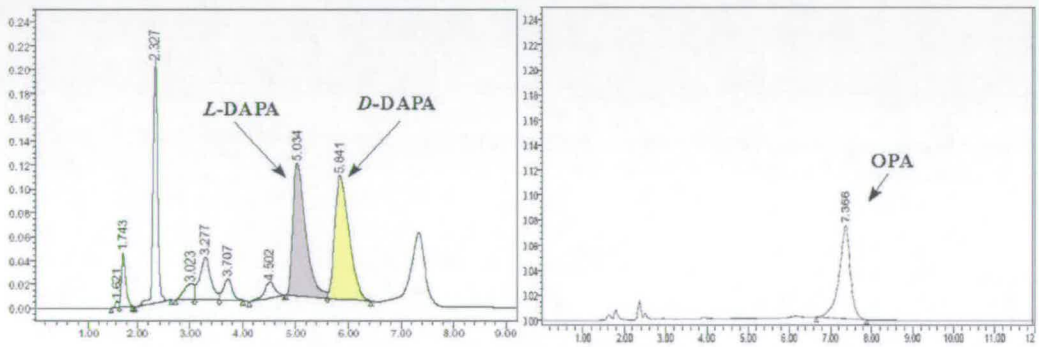



**Figure 26:** Three possible products of the derivatization reaction of 2,3-diaminopropionic acid.

In order to find good, working HPLC conditions, different solvents and derivatization reagent ratios, along with different derivatization times were applied. Application of 73% sodium phosphate buffer (pH 7) and 27% acetonitrile gave the best separation of the *D*- and *L*-2,3-diaminopropionic acid peaks. Using this solvent system, experimentally, it was found that the best HPLC traces were obtained if the 1:2:2 mixture of (sample of 2,3-diaminopropionic acid, or water):(premixed solution of OPA and IBLC):(potassium borate 0.4 M buffer pH~10) was retained in the injection loop for 4 min to allow derivatization to occur before being injected onto the column for analysis.

HPLC of *D,L*-2,3-diaminopropionic acid derivatized using these conditions was run first (**Figure 27A**). To identify which peak corresponds to which 2,3-diaminopropionic acid enantiomer, the same experiment using *D*-2,3-diaminopropionic acid (a cheap, commercially available compound) was run (**Figure 27C**). Since extra peaks of unknown nature were observed, a blank run using water instead of 2,3-diaminopropionic acid was carried out to identify the OPA (**Figure 27B**).

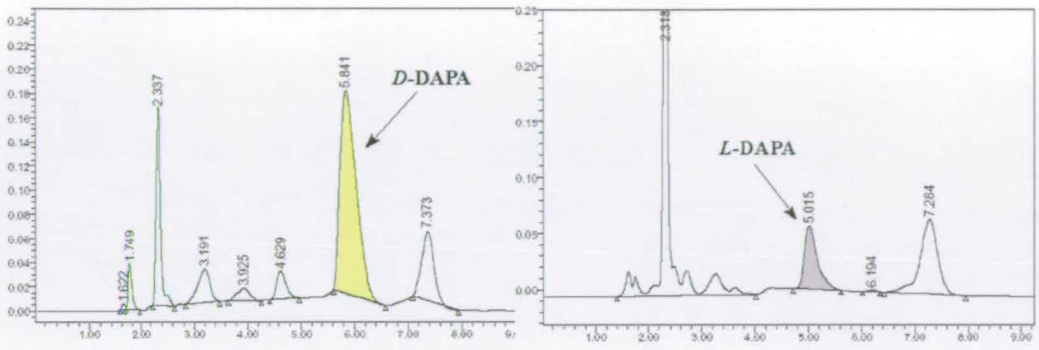



**A** **B**

**Figure 27: A**—HPLC traces of *D,L*- 2,3-diaminopropionic acid; **B**—HLPC trace of blank experiment to identify OPA and derivatization products peaks.

Actual enzymatic separation of the racemate of 2,3-diaminopropionic acid using *D*-amino acid oxidase was subsequently carried out. The enzymatic separation was performed in collaboration with Ingenza Ltd. using optimised conditions (10% w/v of enzyme, 24 h, 37 °C) for the reaction with resin-bound DAAO (Dr. Archer, I., Ingenza

Ltd, unpublished results). The HPLC trace of the result of this experiment is shown in **Figure 27D**.

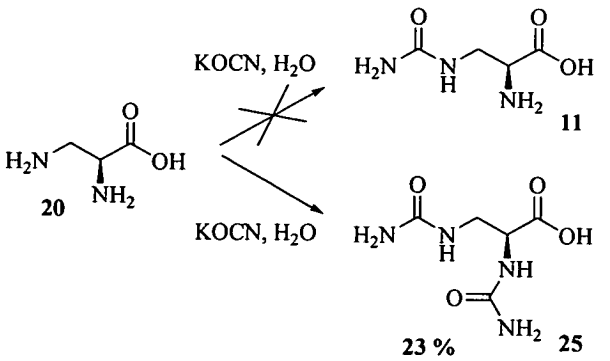



**C** **D**

**Figure 27:** (**C**)—HPLC traces of *D*- 2,3-diaminopropionic acid; (**D**)—HLPC trace of enzymatic resolution using DAAO.

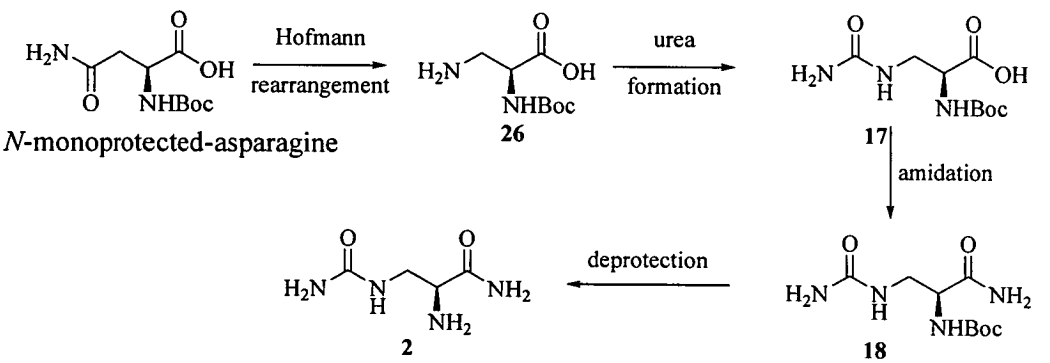
**Figures 27A–D** clearly show that the enzymatic separation reaction has proceeded to completion, and no trace of *D*-2,3-diaminopropionic acid was observed. The reaction mixture was filtered and concentrated, and NMR analysis was carried out (**Appendix 1**). Both the desired *L*-2,3-diaminopropionic acid **20** and the glycine **21** by-product can be observed in the NMR spectrum. Separation of these two products was never carried out, since the attempted regioselective reaction of racemic 2,3-diaminopropionic acid **19** with potassium cyanate failed. In contrast with published results,[47] where only the primary β-amino group reacted to give *L*-albizziine **11**, $^{13}C$ NMR showed two newly formed urea peaks at 162.1 and 161.3 ppm, suggesting that the diurea product **25** was formed in our case[iv] (**Scheme 9**).[56]

[iv] The carbonyl peak for urea comes at 163.0 ppm,[56] which excludes the possibility that urea had been produced as a by-product of this reaction.




**Scheme 9:** Reaction of 2,3-diaminopropionic acid with potassium cyanate.

Since our first proposed route failed, a new synthetic idea was required. The use of a Hofmann rearrangement was considered as a first step in the direction of the nitrogen-rich fragment synthesis. This second proposed synthesis is shown in **Scheme 10**.

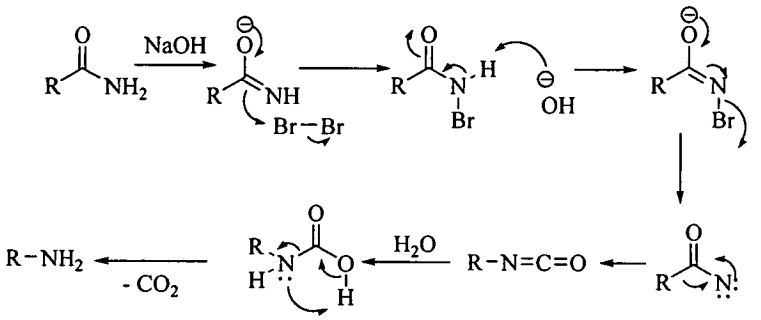



**Scheme 10:** Second proposed synthesis of the nitrogen-rich fragment **2**.

## 2.3. Hofmann rearrangement–based route to the nitrogen-rich fragment 2.

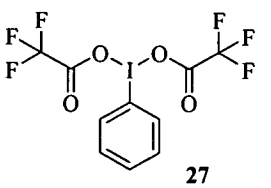
### 2.3.1. First synthetic route toward the nitrogen-rich fragment 2.

The use of Hofmann rearrangement conditions is a well-known means to convert aliphatic carboxylic acid amides into the corresponding amines.[42,57-61]




**Scheme 11:** The original Hofmann rearrangement reaction.[57]

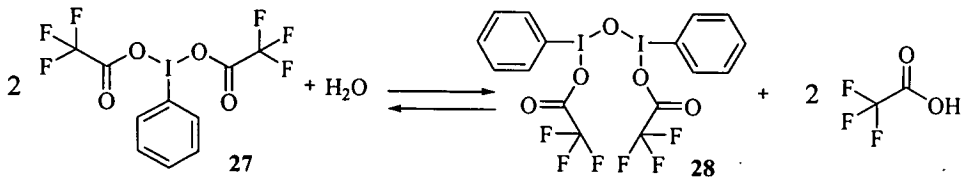
This rearrangement reaction usually starts with in *situ* formation of sodium hypobromide from sodium hydroxide and bromine, which transforms the primary amide into an isocyanate, followed by hydrolysis to form a primary amine and carbon dioxide (**Scheme 11**).[57] Later, milder alternatives to bromine, such as hypervalent iodine reagents, were discovered. Historically, the first such reagent to be used in this reaction was [*I,I*-Bis(trifluoroacetoxy)-iodo]benzene (PIFA) **27**.[58]



**27**

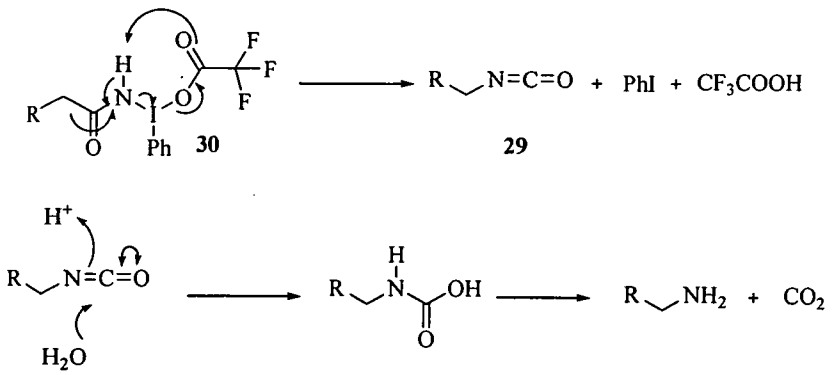
It has been shown that PIFA dissolves in aqueous acetonitrile to give an acidic solution. The acidity results from formation of free trifluoroacetic acid. It has been

proved that increasing PIFA concentration increases the acidity of the solution. Using this and previously known[62] information, Loudon and Boutin[59] suggested that PIFA can exist in aqueous solution in two forms depending on pH: as PIFA **27** itself, or a PIFA dimer **28** (**Scheme 12**). Before the addition of amide the PIFA **27** and PIFA dimer **28** exist in equilibrium.



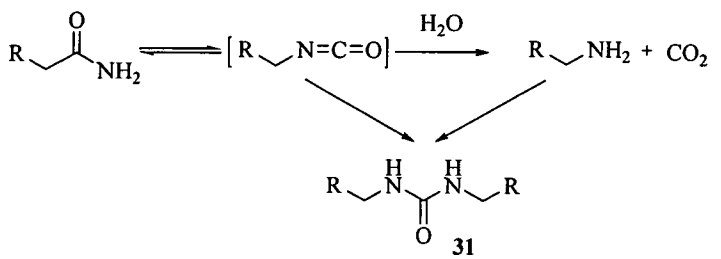

**Scheme 12:** Proposed behaviour of PIFA in aqueous solutions.[59]

Studies of pH showed that the rearrangement of the amides to the corresponding isocyanates **29** (**Scheme 13**) resulted in the release of 1 equivalent of trifluoroacetic acid per mole of amide. The hydrolysis of the isocyanate in turn, produced a basic amine which, under the reaction conditions, was protonated.[59] Boutin and Loudon suggested that the first step in the reaction mechanism using PIFA as a reagent, is the formation of the complex **30** between the PIFA **27** or PIFA dimer **28** and amide.[59] However, the mechanism of this formation was not explained. The proposed mechanism of the Hofmann rearrangement using PIFA is shown in **Scheme 13**.[59]




**Scheme 13:** Proposed mechanism for the Hofmann rearrangement.[59]

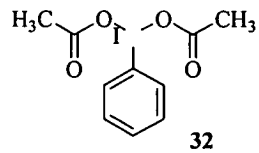
Trifluoroacetic acid, released during the reaction, can both catalyse the attack of water on the isocyanate and protonation of the product amine, removing it from its participation in the side-formation of urea derivatives such as **31** (**Scheme 14**).[58,60] However, the generated acid can also cause removal of some protecting groups (for example the Boc protecting group).[60]




**Scheme 14:** Possible urea formation between isocyanate and amine.[58]

The use of iodosobenzene diacetate (PIDA) **32** has been considered as an analogue of PIFA **27**[42] According to Zhang *et al.*, even better results can be achieved using this alternative. Zhang *et al.* claimed that under the less acidic, milder PIDA conditions, the reaction rate was faster, that there was no evidence of side-formation of ureas, and that the isolated product was much cleaner. Additionally, no sign of epimerisation has been

observed under such mild and slightly acidic conditions.[42]

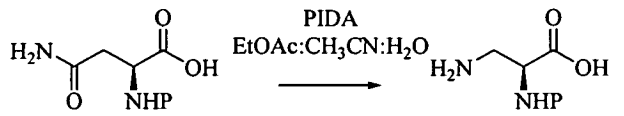


**32**

However, it has been noticed that the right organic solvent:water ratio has to be maintained. The amount of water was found to be crucial to the product purity. Too much water resulted in a voluminous and gelatinous mass, which was hard to filter; too little water led to the formation of a cyclic urea, which was difficult to separate from the product.[42]

Additional studies of the rearrangement mechanism were carried out, using $^{1}H$ NMR techniques. Zhang's group successfully proved that the mechanism proposed by Loundon and Boutin (**Scheme 13**) was correct.

Using the conditions reported by Zhang *et al.*, Cbz- and Boc-*Nα*-protected *L*-2,3-diaminopropionic acids **33** and **26** were synthesised in high yields (93% and 80% respectively) (**Scheme 15**). This compares well with Zhang's results, which were 87% in case of Cbz-*Nα*-protected *L*-2,3-diaminopropionic acids and 62% in case of Boc-*Nα*-protected *L*-2,3-diaminopropionic acids.[42] The purity of these compounds was proved by $^{1}H$ and $^{13}C$ NMR and m.p. in comparison with the literature.[42,63] The $[\alpha]_D$ of the synthesised Boc-*Nα*-protected *L*-2,3-diaminopropionic acid [–24.1 (c=0.96, MeOH)] compared favourably to the literature value [–16.5 (c=3, $H_2O$)].[63] Since the $[\alpha]_D$'s have been measured in different solvents, the extent of any racemisation could not be assessed at this stage.




*N*-monoprotected-asparagine
P = Cbz or Boc

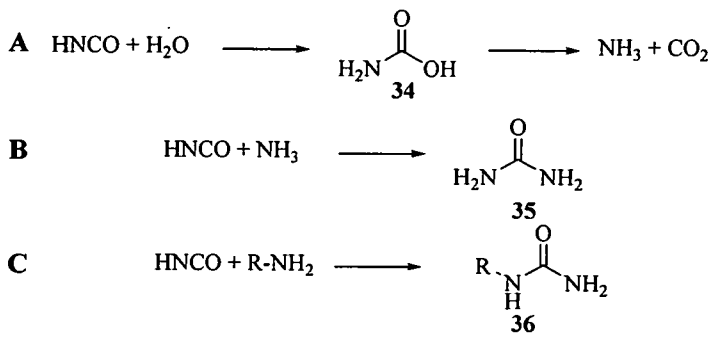
**33** P = Cbz (93%)
**26** P = Boc (80%)

**Scheme 15:** Synthesis of Cbz- or Boc-monoprotected *L*-2,3-diaminopropionic acid.

After successful formation of monoprotected *L*-2,3-diaminopropionic acid, a return to the initial idea of urea formation using potassium cyanate seemed to be reasonable (**Scheme 9**). Since only the $N_\beta$-amino group was unprotected now, the reaction was expected to go with the desired regioselectivity.

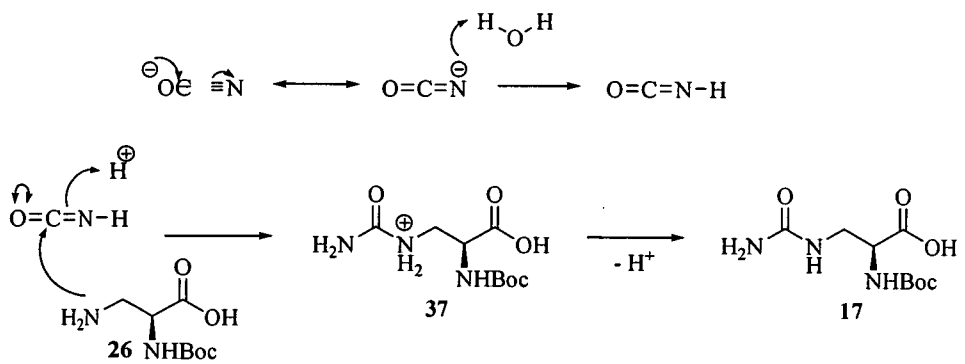
*N*-carbamoylation reactions are considered to be one of the most important reactions in peptide synthesis and biochemistry.[64-66] These reactions also play an important role in the investigation of metabolic pathways. Amongst the few, known synthetic routes towards *N*-carbamoyl amino acids: urea degradation,[67,68] hydantoin hydrolysis[69] or enzymatic reaction,[70] the cheapest and easiest way to prepare them is by reaction of the free amino acid with an aqueous solution of mineral cyanate.[68,71,72]

A few investigations have been carried out to study the mechanism of the cyanate reaction.[68,72,73] Firstly, in water, potassium (or sodium) cyanate exists in equilibrium with isocyanic acid. The isocyanic acid can be exposed to nucleophilic attack by water, ammonia or amines, forming carbamates **34**, ureas **35** or *N*-carbamoyls **36** (**Scheme 16**). This nucleophilic attack is rate limiting and, in the case of attack by water, it is followed by fast decomposition of the carbamate intermediate **34** into ammonia and carbon dioxide. The ureas, however, are much more stable and are not likely to undergo decomposition.




**Scheme 16:** Reaction of isocyanic acid with water (**A**), ammonia (**B**) and amine (**C**).

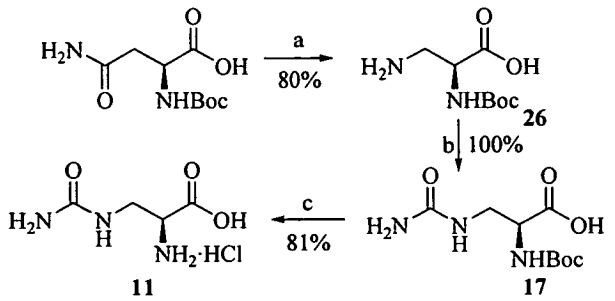
Computer–simulated studies of the potassium cyanate reaction kinetics[68] showed that domination of *N*-carbamoyl compound formation over the side reactions requires maintenance of very low concentrations of ammonia and carbonates. It was found to be possible to achieve these conditions by performing reactions in the pH range 7–8. Additional studies performed by Taillades *et al.*[68] showed that independently from pH, selectivity between *N*-carbamoyl amino acids and urea also depends on temperature. It was found that selectivity decreases as the temperature increases. Having all of this information in hand, it was decided that the best temperature range for the reaction was 40–50 °C. The mechanism of the desired *N*-carbamoylation reaction is shown in **Scheme 17**.




**Scheme 17:** Mechanism of *N*-carbamoylation of *L*-2,3-diaminopropionic acid.[68]

Using conditions optimised by Taillades *et al.*[68] (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17** was synthesised in quantitative yield. Since the only difference between the starting material [(2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26**] and the product **17** by NMR was the appearance of a new quaternary carbon peak at 159.6 ppm in the $^{13}C$ NMR spectra (**Appendix 2** and **Appendix 3**), the reaction progress was monitored by electrospray mass spectrometry. The disappearance of the starting material **26** peak at *m/z* = 205 in the positive-ion mass spectrum suggested that reaction had gone to completion. Alternatively, reaction progress could be followed by the appearance of the product **17** peak at *m/z* = 246 using negative-ion electrospray mass spectrometry.

Our attempts to recrystallize (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17** from aqueous HCl resulted in loss of the Boc-protecting group and *L*-albizziine hydrochloride salt [(*S*)-2-amino-3-ureidopropanoic acid hydrochloride **38**] was obtained (**Scheme 18**) (**Appendix 4**). Hence, successful synthesis of *L*-albizziine was achieved from $N_\alpha$-*tert*-butoxycarbonyl-*L*-asparagine in two steps in 65% overall yield. The purity of the obtained product was proved by $[\alpha]_D$ and m.p. in comparison with literature. The $[\alpha]_D$ of the synthesised *L*-albizziine was measured as –16.9 (c=0.71, MeOH) (lit. $[\alpha]_D$ – 63.4 (c=1, $H_2O$),[47] whereas the m.p. was measured to be 210–212 °C (lit. m.p. 218–220 °C).[47] In a similar fashion, we envisage that synthesis of *D*-albizziine could be accomplished starting from $N_\alpha$-*tert*-butoxycarbonyl-*D*-asparagine, if required.

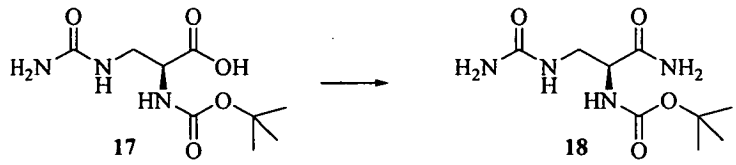



(a) PIDA, EtOAc:$CH_3CN$:$H_2O$ in the ratio 2:2:1; (b) KOCN, $H_2O/H^+$, pH~7.5;

(c) recrystallisation from aq. HCl.

**Scheme 18:** Synthesis of *L*-albizziine **11**.

According to the proposed synthetic strategy (**Scheme 10**), successful synthesis of (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17** was to be followed by an amidation reaction to give the amido derivative **18**. The Boc-protected starting material **26** was chosen because of the relative ease of the subsequent deprotection procedure. Initially, formation of a mixed anhydride and reaction with aqueous ammonia was attempted as reported by Vallee *et al.* (**Table 2, entry 1**).[44,74] However, after several attempts, this route appeared to give irreproducible results. Only quantitative recovery of the starting material **17** was observed. A recently reported procedure for the preparation of primary amides from carboxylic acids and urea, which uses microwave irradiation, was applied (**Table 2, entry 2**).[75] Khalafi-Nezhad *et al.* reported that this simple and solvent-free procedure gave various aliphatic and aromatic primary amides in high yield.[75] Imidazole, in this case, was used to promote reaction, since it formed the polar carboxylic acid salt, which is more efficient for microwave energy absorption. However, this strategy failed as well. After a few attempts only the unreacted starting material **17**, imidazole and urea were recovered. Standard peptide-coupling reagents such as 1-(3,3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDCI) were used,[76] but were also unsuccessful (**Table 2, entry 3, 4, 5**).



**Scheme 25:** Amidation reaction of (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17**.

| Entry | Ammonia source | Conditions | Solvent | Product |
|---|---|---|---|---|
| 1 | $NH_4OH$ | $ClCO_2Et$, $NEt_3$ | THF | Quantitative recovery of starting material **17** |
| 2 | $NH_2CONH_2$ | imidazole, μw | – | Quantitative recovery of starting materials **17**, imidazole and urea |
| 3 | $NH_4OH$ | EDCI, HOBt | THF | Unreacted EDCI and HOBt were recovered |
| 4 | $NH_4OH$ | EDCI, HOBt | DMF | Unreacted EDCI and HOBt were recovered |
| 5 | $NH_4OH$ | EDCI, HOBt | $CH_3CN$ | Unreacted EDCI and HOBt were recovered |
| 6 | $NH_4HCO_3$ | $(Boc)_2O$, pyridine | THF : MeOH | No material was recovered |
| 7 | $NH_4HCO_3$ | $(Boc)_2O$, pyridine | DMF | No material was recovered |
| 8 | $NH_4HCO_3$ | $(Boc)_2O$, pyridine | 1,4-dioxane | No material was recovered |
| 9 | $BnNH_2$ | $(Boc)_2O$, pyridine | 1,4-dioxane | Starting material **17** and $BnNH_2$ were recovered |
| 10 | $NH_4OH$ | DMT–MM | MeOH | Unreacted starting material **17** and 4,6-dimethoxy-1,3,5-triazin-2-one **39** were recovered |
| 11 | 7M $NH_3$ in MeOH | DMT–MM | MeOH | Unreacted starting material **17** and 4,6-dimethoxy-1,3,5-triazin-2-one **39** were recovered |
| 12 | $NH_3$ gas | DMT–MM | MeOH | Methyl-(2*S*)-2-(*tert*-butoxycarbonylamino)-3-ureido-propanoate **40** was isolated in 59% yield |
| 13 | $NH_2Bn$ | DMT–MM | MeOH | Undefined mixture of products |

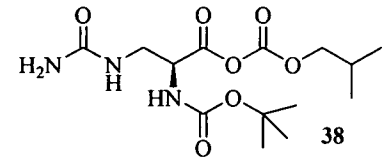
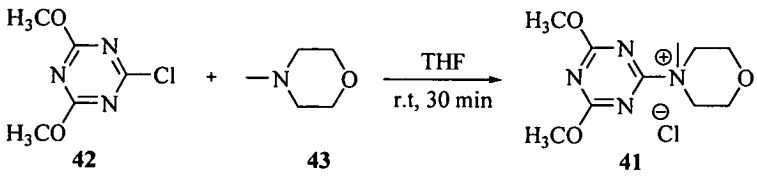
| | | | | |
|---|---|---|---|---|
| 14 | $NH_4HCO_3$ | NMM, IBC | THF : MeOH |  **38** was recovered in 15% yield |

**Table 2:** Conditions and solvents used for the amidation of (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17**.

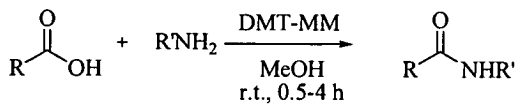
Other mixed-anhydride methods were applied including: a combination of *N*-methylmorpholine (NMM) and isobutyl chloroformate (IBC);[77] and, a combination of di-*t*-butyldicarbonate [$(Boc)_2O$] and pyridine[78-80] in a range of solvents. However, none of the desired product was obtained. In the case of NMM and IBC, an unusually stable mixed anhydride **38** (**Table 2, entry 14**) was recovered in 15% yield. It is believed that these attempts failed due to the low solubility of the starting material **17**. Generally, the reagents described above are used in less-polar solvents such as DCM, THF, MeCN or aprotic polar solvents such as DMF and DMSO. However, it was found that $N_\alpha$-Boc-protected *L*-albizziine **17** is not very soluble in any of those solvents. The use of a polar solvent such as MeOH was required.

Kunishima *et al.* reported that the recently developed 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT–MM) reagent **41** was highly suitable for the formation of carboxamides in water, MeOH and EtOH.[81] The same group also developed the simple and quantitative one-step synthesis of DMT–MM starting from 2-chloro-4,6-dimethoxy-1,3,5-triazine **42** and *N*-methylmorpholine **43** (**Scheme 20**).[82]




**Scheme 20:** Synthesis of DMT–MM **41**.[82]

It has been shown that addition of this reagent to the mixture of carboxylic acids and amines resulted in formation of the corresponding amides in high yields and with high amide/ester selectivity. These conditions were applicable to aliphatic, aromatic, sterically hindered, and α,β-unsaturated acids (**Scheme 21**).[81]

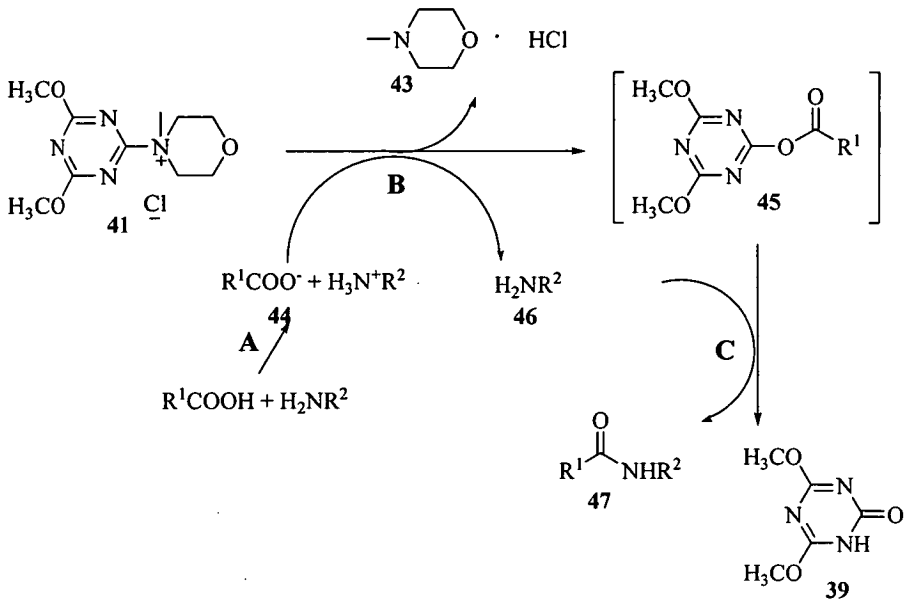



| R | R′ | Yield (%) |
|---|---|---|
| $Ph(CH_2)_2$ | $Ph(CH_2)_2$ | 98 |
| $CH_3(CH_2)_4$ | $Ph(CH_2)_2$ | 96 |
| PhCH=CH | $Ph(CH_2)_2$ | 92 |
| *t*-Bu | $Ph(CH_2)_2$ | 84 |
| Ph | $Ph(CH_2)_2$ | 97 |
| *p*-MeO–$C_6H_4$ | $Ph(CH_2)_2$ | 100 |

**Scheme 21:** Some examples of condensation of carboxylic acids with amines.[81]

Kunishima's group proposed that the first step in the reaction mechanism is reaction of the carboxylic acid with an amine (**Scheme 22A**) to give rise to an activated carboxylate **44** and protonated amine. The carboxylate is now ready for the nucleophilic attack on the DMT–MM, while the amine is prevented from attacking the reagent by itself. The highly electrophilic carbon atom in the triazine next to the positively charged nitrogen is

attacked by the carboxy-anion resulting in an activated ester intermediate **45**. Furthermore, *N*-methylmorpholine is formed to deprotonate the ammonium cation (**Scheme 22B**). In the next step (**Scheme 22C**) the free amine readily reacts with the activated ester **45** to give the desired amide **47** and the additional by-product 4,6-dimethoxy-1,3,5-triazin-2-one **39**.




**Scheme 22:** Mechanism of amide coupling with DMT-MM **41**.[82]

When the DMT–MM strategy was applied to the amidation of (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17**, unexpected results were achieved. In contrast with the literature precedent,[81] when DMT–MM coupling was carried out in neat MeOH the methyl ester of $N_\alpha$-Boc-protected albizziine **40** was isolated as the only product in 59% yield (**Table 2, entry 12**). The $^1$H NMR spectrum (**Appendix 5**) clearly shows the presence of an extra $CH_3$ peak at 3.76 ppm, which is strongly indicative of $OCH_3$. Additionally, $^{13}$C NMR (**Appendix 5**) shows shifting of the carboxylic acid peak of (2*S*)-2-*tert*-butyloxycarbonylamino-3-ureidopropanoic acid **17** from 175.9 ppm to 171.1 ppm, which points to methyl ester formation. The structure of the compound **40**

was proved by 2D NMR (**Figure 28**). In the HMBC spectrum, correlation between the $CH_3$ peak (3.76 ppm) and the quaternary carbon peak (171.08 ppm) is clearly shown. In combination with the results of high resolution mass spectrometry, this proves that the product is indeed the methyl ester.

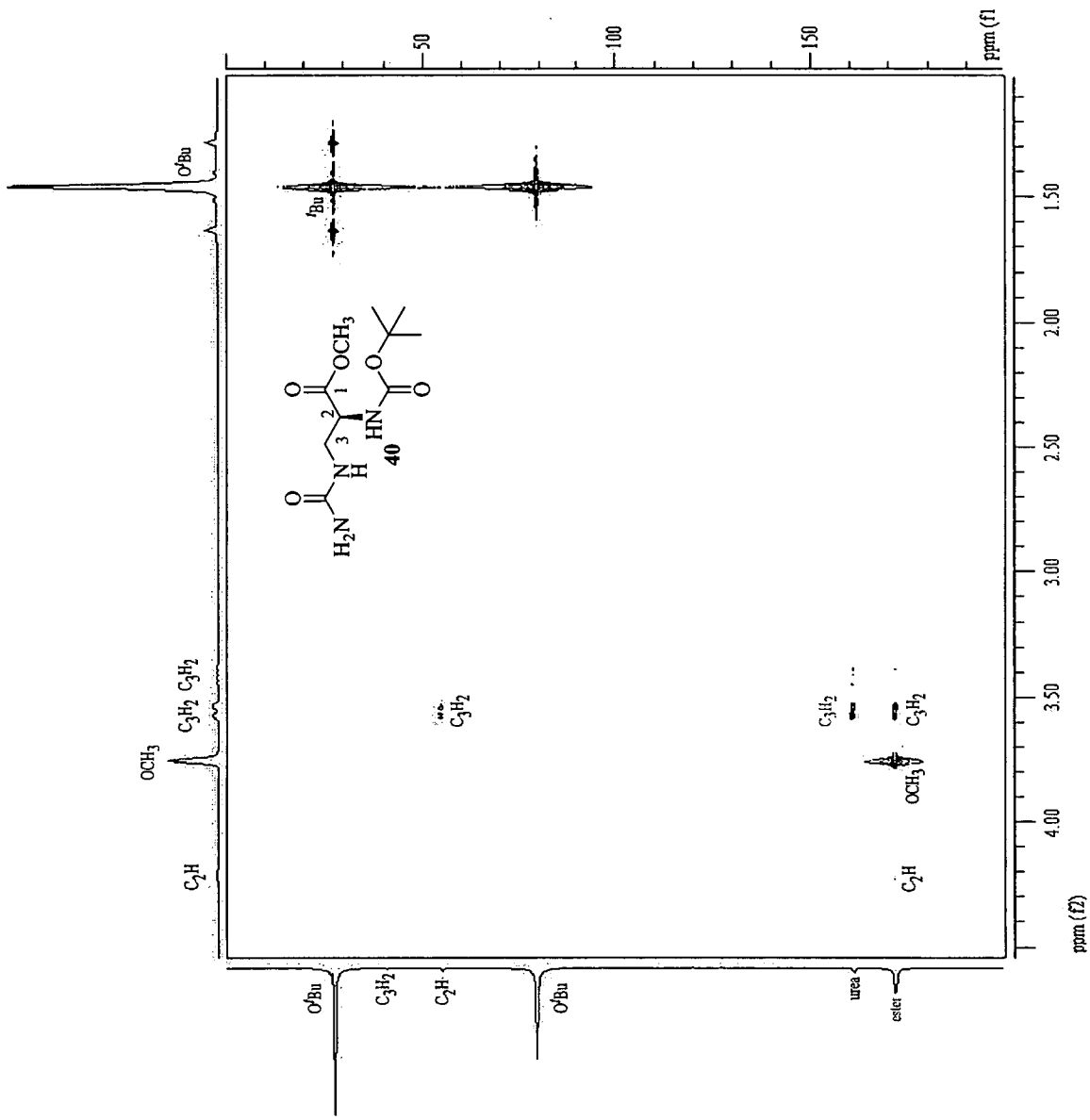
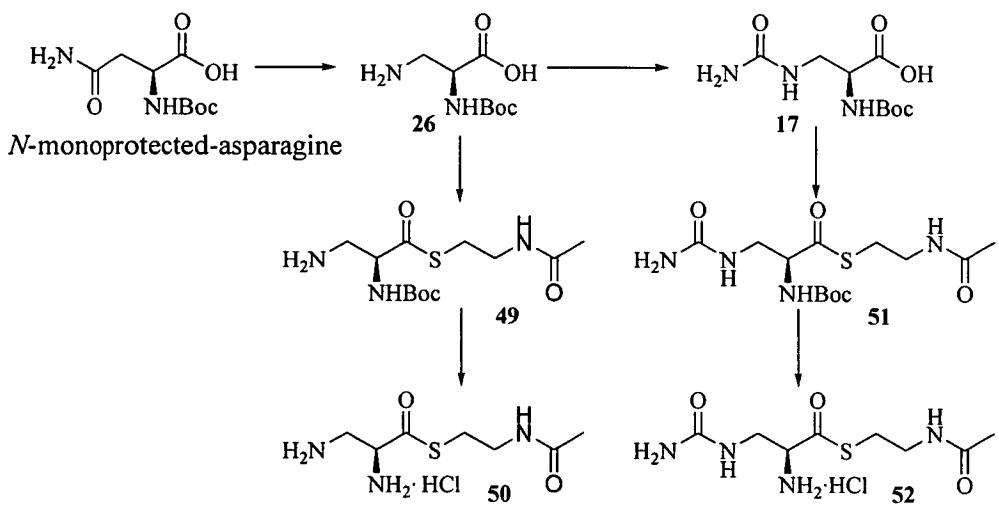



**Figure 28:** HMBC spectrum of $N_\alpha$-Boc-protected albizziine methyl ester **40** (in MeOH).

As part of our research, collaboration with Prof. Michael G. Thomas from the University of Wisconsin–Madison was discussed. Prof. Thomas's laboratory is interested in understanding the sequence of the zwittermicin A **1** producing gene cluster. This joint interest led us to a discussion of the synthesis of unusual precursors for feeding experiments. It is known that *N*-acetyl cysteamine thioester (SNAC) derivatives are accepted as substrates by some polyketide[83,84] and nonribosomal peptide[85] synthases. When SNAC derivatives are used for feeding experiments, these unusual precursors are recognised as Acyl-S-CoA substitutes. So, the trans-thioesterification reaction takes place next, and the desired precursor is loaded into the biosynthetic machinery. Having this knowledge in mind, the synthesis of SNAC analogues of *L*-2,3-diaminopropionic acid **20** and *L*-albizziine **11** was considered.

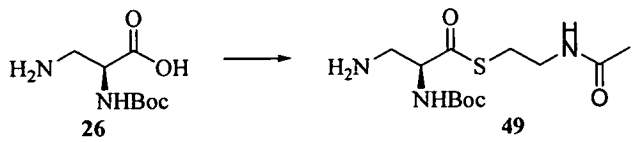
The same synthetic strategy as for synthesis of amido derivative **2** (**Scheme 10**) was applied to the synthesis of the SNAC derivative **50** and **52** (**Scheme 23**).




**Scheme 23:** Proposed synthesis of SNAC derivatives **50** and **52**.

During our investigations, the same problem as with the amidation reaction was faced. It appeared to be impossible to couple *N*-acetylcysteamine **53** to (2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26** or (2*S*)-2-*tert*-butoxycarbonylamino-3-

ureidopropanoic acid **17**. The summary of conditions and solvents used for the reactions between SNAC **53** and (2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26**; and between SNAC and (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17**, are presented in **Tables 3** and **4**, respectively. In all these cases the reactions failed; however, some interesting results were noticed using Boc anhydride (**Table 3, entry 5, 6** and **Table 4, entry 2, 3**) and DMT–MM (**Table 4, entry 4**). In the first case, a mixture of unreacted SNAC **53** and the unexpected coupling product *S*-2-acetamidoethyl-*O*-*tert*-butyl carbonothioate **54** was isolated (**Scheme 26**) together with unreacted starting materials **26** and **17**. In the second case, an unusual coupling between 4,6-dimethoxy-1,3,5-triazine and *N*-acetyl cysteamine thioester was observed, giving rise to *N*-(2-(4,6-dimethoxy-1,3,5-triazin-2-ylthio)ethyl)acetamide **55** (**Scheme 26**).



**26** **49**

**Scheme 24:** Coupling reaction of (2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26** and SNAC **53**.

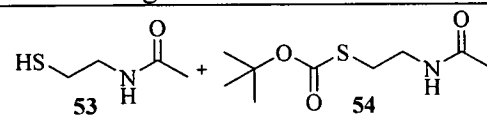
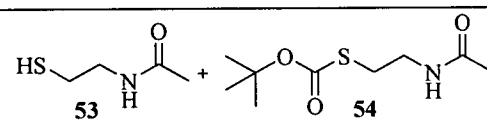
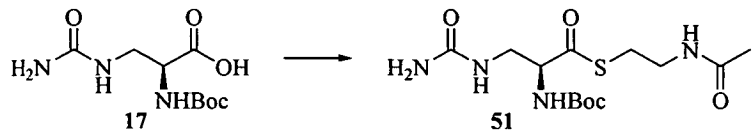
| Entry | Conditions | Solvent | Product |
|---|---|---|---|
| 1 | DCC, HOBt, SNAC | $CH_3CN$ | Unreacted starting material **26** and reagents were recovered |
| 2 | DCC, DMAP, SNAC | DCM | Unreacted starting material **26** and reagents were recovered |
| 3 | DIC, DMAP, SNAC | DCM | Unreacted starting material **26** and reagents were recovered |
| 4 | DIC, DMAP, SNAC | THF | Unreacted starting material **26** and reagents were recovered |
| 5 | $(Boc)_2O$, pyridine, SNAC | THF |  **53** **54** |
| 6 | $(Boc)_2O$, pyridine, SNAC | THF : MeOH (16 : 1) |  **53** **54** + unreacted starting material **26** were recovered |
| 7 | $(Boc)_2O$, pyridine, SNAC | DCM | Unreacted starting material **26** was recovered |

**Table 3:** Conditions and solvents used for the coupling of (2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26** and SNAC **53**.



17 51

**Scheme 25:** Coupling reaction of (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17** and SNAC **53**.

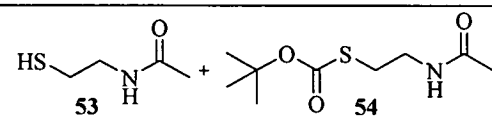
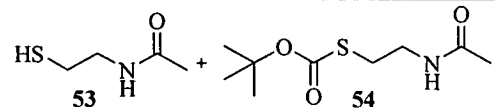
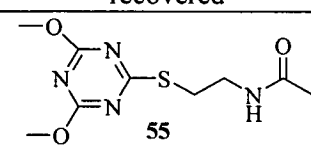
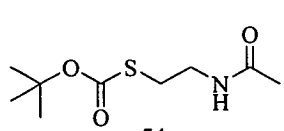
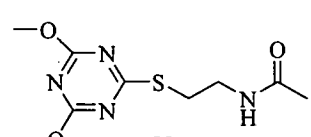
| Entry | Conditions | Solvent | Product |
|---|---|---|---|
| 1 | DCC, HOBt, SNAC | $CH_3CN$ | No products were recovered |
| 2 | $(Boc)_2O$, pyridine, SNAC | THF |  **53** **54** + unreacted starting material **17** were recovered |
| 3 | $(Boc)_2O$, pyridine, SNAC | DCM |  **53** **54** + unreacted starting material **17** were recovered |
| 4 | DMT-MM, SNAC | MeOH |  **55** |

**Table 4:** Conditions and solvents used for the coupling of (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17** and SNAC **53**.



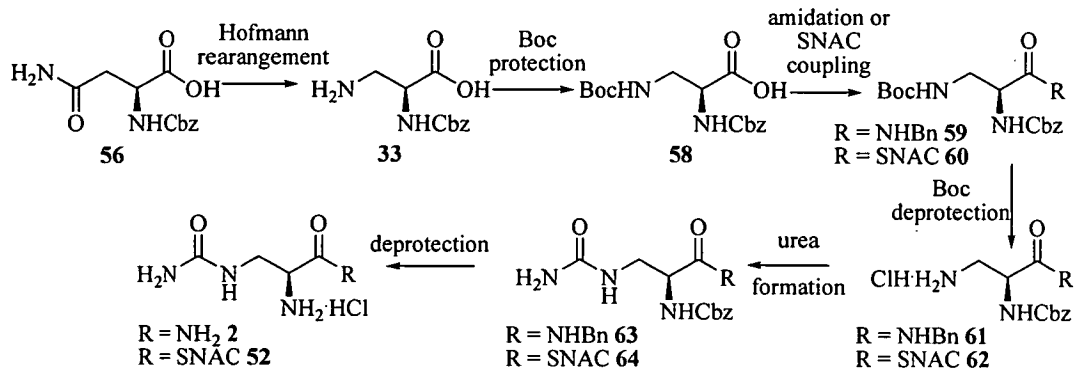
**54**



**55**

**Scheme 26:** Structures of *S*-2-acetamidoethyl-*O*-*tert*-butyl carbonothioate **54** and *N*-(2-(4,6-dimethoxy-1,3,5-triazin-2-ylthio)ethyl)acetamide **55**.

After over 100 unsuccessful attempts, this synthetic strategy was rejected. A new synthetic scheme (**Scheme 27**) was developed, changing the order of reactions with the expectation of escaping solubility issues.

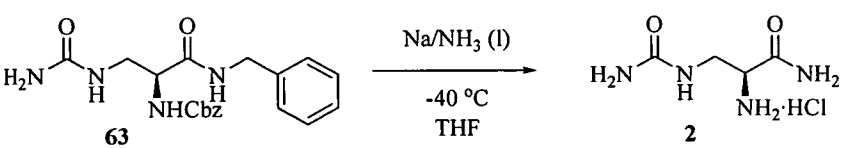



**Scheme 27:** Second proposed synthesis of nitrogen-rich fragment **2**.

### 2.3.2. Second synthetic route toward the nitrogen-rich fragment 2.

This time $N_{\alpha}$-benzyloxycarbonyl-*L*-asparagine **56** was chosen as a starting material: Hofmann rearrangement of which gave (2*S*)-3-amino-2-*tert*-benzyloxycarbonyl-amino-propanoic acid **33** in 93% yield. The decision to change the $N_{\alpha}$-protected group from Boc to Cbz was based on the need to deprotect the $N_{\beta}$-amino group selectively, later in the strategy. It is known that deprotection of Boc is relatively easy in comparison to a Cbz protecting group. So, complication-free monodeprotection was expected. Using Zhang's conditions, $N_{\alpha}$-monoprotected diaminopropionic acid **33** was converted into (2S)-*N*-2-Benzyloxycarbonylamino-*N*-3-butyloxycarbonylamino-propanoic acid **58** in good yield (90%).[86] The most commonly used peptide coupling reagents (DIC and DCC) were considered for use in the next step, since the compound **58** was found to be soluble in DCM. DIC and DCC gave rise to desired products **59** and **60** in good yields: however, it appeared to be difficult to separate the products by column chromatography.

Nevertheless, the EDCI coupling agent gave easy-to-purify products **59** and **60** in high yields: 92% and 93%, respectively. Successful Boc deprotections with 1 M hydrochloric acid in ether (60% **61** and 77% **62**), based on Gibson's report,[87] were followed by reaction with potassium cyanate to give compounds **63** and **64** as described in section 2.3.1. This reaction appeared to proceed in good yield for the amide derivative **63** (77%), whilst formation of the SNAC derivative **64** also appeared to have been successful: although complete characterisation of this compound was not achieved. The reaction progress was determined by monitoring the disappearance of peaks in the positive-ion electrospray mass spectrum assigned to the starting materials **61** and **62**. The only differences observed between starting materials **61** and **62** by NMR was the appearance of a new quaternary (urea) carbon peak at 160.8 ppm (in case of amide **63**) and at 171.0 ppm (in case of SNAC derivative **64**) in the $^{13}C$ NMR spectra. The last step in this synthetic strategy was not so successful, however. Since the benzyl (2*S*)-2-benzyloxycarbonylamino-3-ureido-propanamide **63** has two protecting groups to be removed (Cbz and benzyl), the well-known deprotection method using Na and liquid ammonia seemed suitable[88,89] (**Scheme 28**). Unfortunately, no product was isolated as a result of this reaction.

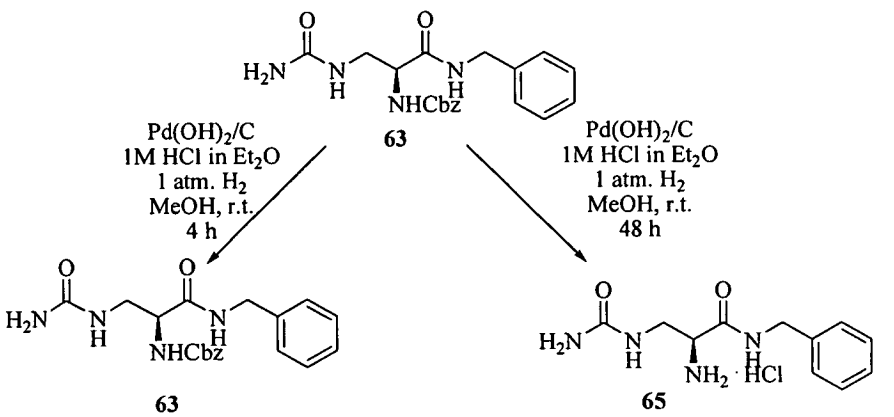



**63** **2**

**Scheme 28:** Deprotection of benzyl (2*S*)-2-benzyloxycarbonylamino-3-ureido-propanamide **63** with Na and liquid ammonia.[88,89]

After this attempt failed, common hydrogenation conditions in the presence of palladium hydroxide were used[90] (**Scheme 29**). However, after running the reaction for 4 hours, no product **2** was observed and only starting material **63** was isolated. The same reaction was repeated again, and was left to run for two days, after which the reaction mixture

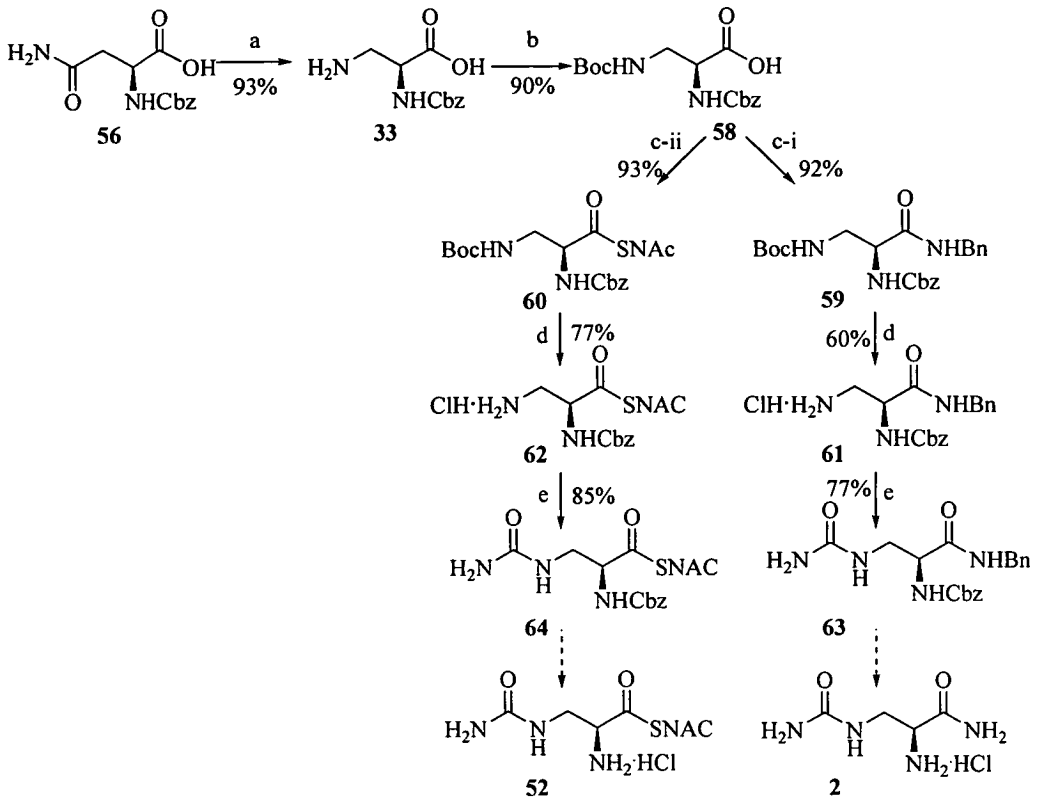
was filtered, concentrated and NMR analysis was carried out. Disappearance of two quaternary peaks at 157.5 ppm and 140.6 ppm and five CH peaks in the aromatic region, in addition with the loss of a $CH_2$ peak at 67.1 ppm, suggests that Cbz-mono-deprotected product **65** was formed (**Scheme 29**).




**Scheme 29**: Deprotection of (2*S*)-2-Benzyloxycarbonylamino-3-ureido-benzylpropanamide **63** with $Pd(OH)_2/C$.[90]

One attempt to deprotect S-2-acetamidoethyl (2*S*)-2-(benzyloxycarbonyl-amino)-3-ureido-propanethioate **64** was undertaken, under using the $Pd(OH)_2$ hydrogenation conditions described above. However, after 5 hours no product was observed. Since the reaction was performed on a very small scale, no starting material **64** was recovered.

To summarise, the attempted synthetic strategy to the nitrogen rich fragment **2** and its SNAC analogue **52** is shown in **Scheme 30**.




(a) PIDA, EtOAc:$CH_3CN$:$H_2O$ in the ratio 2:2:1; (b) $(Boc)_2O$, $Na_2CO_3$, 1,4-dioxane; (c-i) EDCI, DMAP, $NH_2Bn$, DCM; (c-ii) EDCI, DMAP, SNAC, DCM; (d) 1 M HCl in $Et_2O$, DCM; (e) KCON, $H_2O/H^+$, pH~7.5.

**Scheme 30:** Attempted synthesis of (*S*)-1-(2,3-diamino-3-oxopropyl)urea hydrochloride **2,** and S-2-acetamidoethyl (2*S*)-2-amino-3-ureido-propanethioate hydrochloride **52**.

## 2.4. Conclusion and future work.

During our attempts to synthesise the nitrogen-rich fragment **2**, an effective route for the enzymatic separation of racemic 2,3-diaminoproponic acid **19** using *D*-amino acid oxidase was developed. Several different approaches to the synthesis of **2** were explored. During these investigations, an effective synthesis of *L*-albizziine **11** was achieved from $N_\alpha$-*tert*-butoxycarbonyl-*L*-asparagine in two steps, in 65 % overall yield.

In a similar fashion, it is envisaged that the synthesis of *D*-albizziine could be accomplished starting from $N_{\alpha}$-*tert*-butoxycarbonyl-*D*-asparagine.

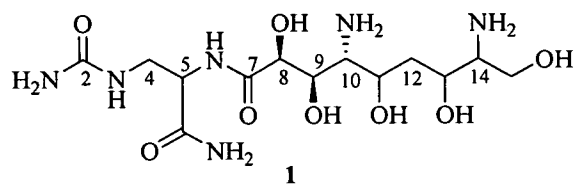
Our final synthetic strategy (**Scheme 30**) gave very encouraging results, leading to the protected benzylamide **63** and the SNAC derivative **64**. Even though normal deprotection attempts failed using a range of standard conditions, the application of high pressure conditions for the hydrogenation reaction might be a solution in this case, giving rise to the desired products **2** and **52**.

# Chapter 3: Results and Discussion 2.

# Synthesis of the $C_{10}$–$C_{16}$ fragment.

## 3.1. Absolute stereochemistry and retrosynthesis of $C_{10}$–$C_{16}$ fragment.

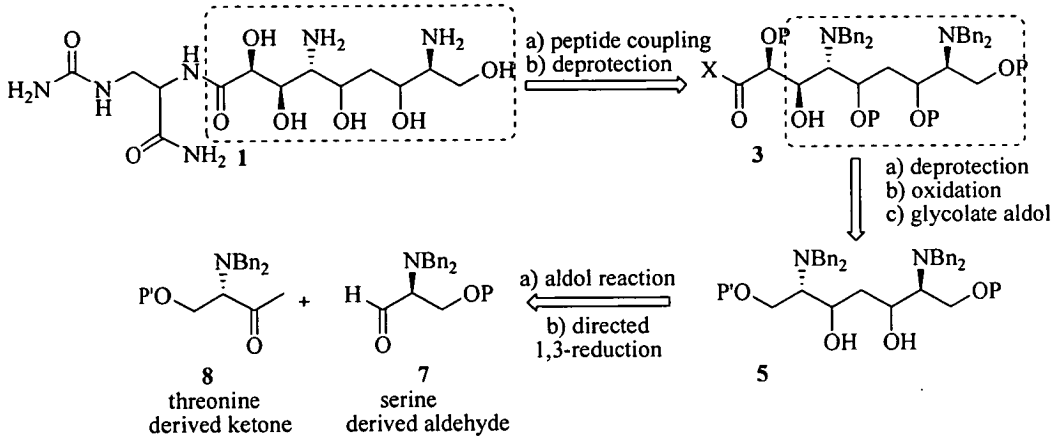
As discussed above (section 2.1), in collaboration with Prof. Michael G. Thomas from the University of Wisconsin–Madison, the essential information regarding the unknown stereochemistry was obtained. At the time this project was started, the absolute stereochemistry of only three chiral centres of zwittermicin A **1** had been published: as 8*S*, 9*R*, and 10*R*[7] (**Figure 29**). However, the information about the absolute configuration of C(14) as 14*S* was kindly provided by Prof. Thomas (unpublished results). With this extra information in hand, the following synthetic strategies were suggested.




**1**

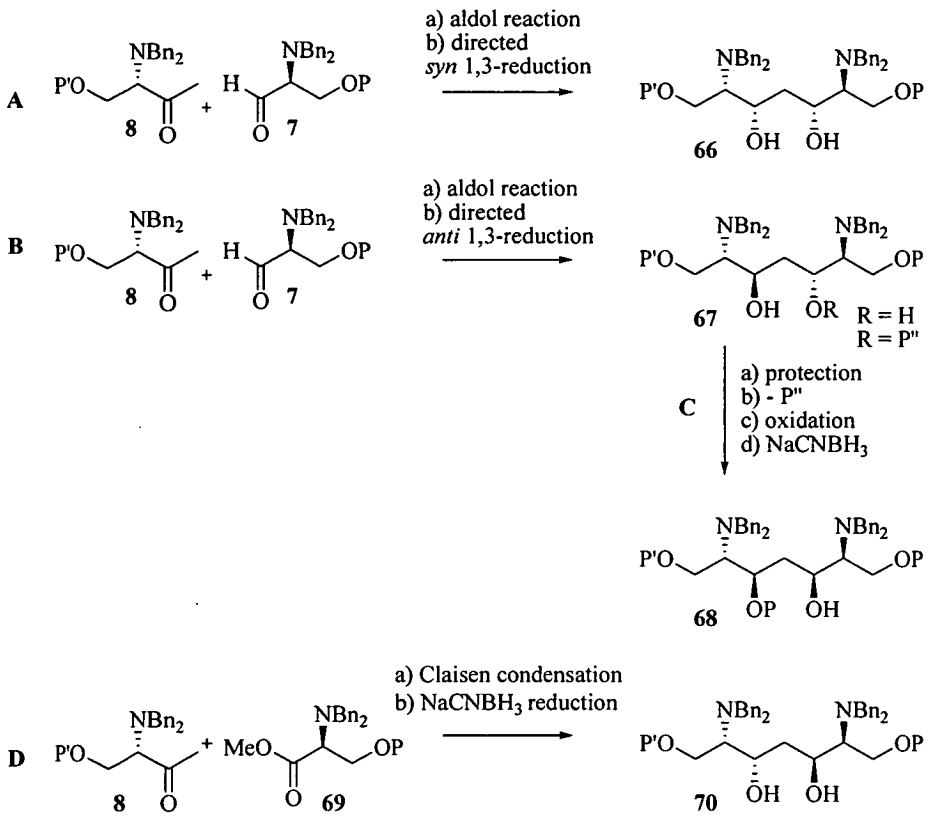
**Figure 29:** The proposed structure of zwittermicin A.[7]

As discussed in the retrosynthetic analysis (section 1.7), synthesis of the desired $C_{10}$–$C_{16}$ fragment can be started from the aldol reaction of a threonine-derived ketone **8** and serine-derived aldehyde **7**, followed by directed 1,3-reduction to give compound **5** (**Scheme 32**).




**Scheme 32:** Concise retrosynthetic analysis of zwittermicin A.

Since the stereochemistry of the C(11) and C(13) stereocentres remains unknown (**Figure 29**), synthesis of four possible diastereoisomers was considered as shown in **Scheme 33**. Comparison of NMR data for the four diastereoisomers obtained by this approach and zwittermicin A **1**[7] should allow the absolute conformation of C(11) and C(13) to be determined.



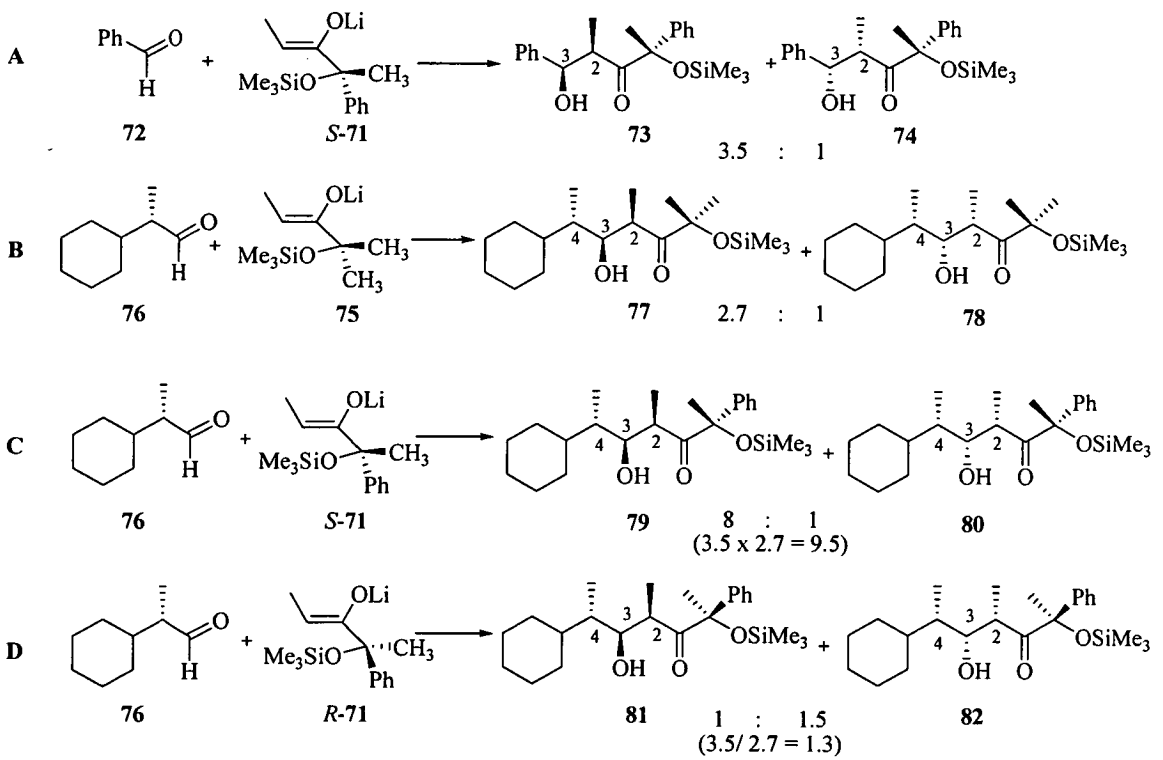

**Scheme 33:** Four possible diastereoisomers produced from the reaction of threonine **8** and serine **7** derivatives.

### 3.2. Double asymmetric induction in aldol reactions.

According to Masamune *et al.*, in single asymmetric reactions where the stereochemistry of the substrate defines the stereochemistry of the new chiral centre formed as a result of reaction, the substrate can be designed in such way that the desired chirality of the product will be achieved. This raises the question of whether the stereochemistry of the product can be controlled in the same way in double asymmetric reactions.[91,92]

Masamune *et al.* considered several examples of aldol, Diels–Alder, catalytic

hydrogenation, and epoxidation reactions, where the reactions between both chiral substrates and achiral reactants were run first, followed by determination of diastereofacial selectivity. The reactions between two chiral substrates were then carried out and the diastereoselectivity was determined. One example of the set of such reactions is illustrated in **Scheme 34.** First, the reaction between chiral compound *S*-**71** and achiral benzaldehyde **72** was performed to provide aldol products **73** and **74** in a 3.5:1 ratio (**Scheme 34A**). The reaction between achiral compound **75** and chiral aldehyde **76** was then carried out to give products **77** and **78** in 2.7:1 ratio (**Scheme 34B**). In both cases, the C(2) and C(3) of the products have *syn* relative stereochemistry, when C(3) and C(4) have *anti* stereochemistry. When the reaction between chiral aldehyde **76** and chiral compound *S*-**71** was carried out, an increase in diastereoselectivity was observed (**Scheme 34C**). However, use of the chiral compound *R*-**71** as a reagent showed a decrease in diastereoselectivity (**Scheme 34D**).[92]




**Scheme 34:** Example of "matched" and "mismatched" pairs.[92]

Masamune *et al.* noticed that, in examples where the diastereofacial selectivities of both chiral reactants favour the same product (i.e., a "matched" pair), increases in diastereoselectivity are apparent. Where the diastereofacial selectivities favour different products (i.e., a "mismatched" pair), decreases in diastereoselectivity occur. Based on these observations, the hypothesis which allows a prediction of diastereoselectivity of the products in double asymmetric induction was proposed as: "The degree of asymmetric induction is approximated to be (a × b) for a 'matched' pair and (a / b) for a 'mismatched' pair, where a and b are the diastereofacial selectivities of a substrate and a reagent, respectively."[92]

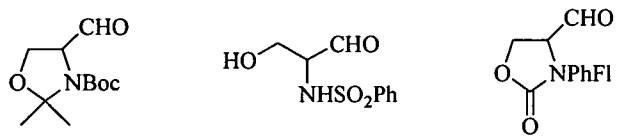
Since our proposed synthetic strategy starts with an aldol reaction between two chiral compounds, threonine-derived ketone **8** and serine-derived aldehyde **7**, it was essential to find if this pair was "matched" or "mismatched". In a similar vein to Masamune's experiments, it was proposed to carry out an aldol reaction of chiral ketone **8** with an achiral aldehyde, followed by an aldol reaction of chiral aldehyde **7** with an achiral ketone. After determination of diastereoselectivities of these pairs, an aldol reaction between the two chiral compounds should be carried out.

## 3.3. α-Amino acid derivatives in synthesis.

### 3.3.1. Use in natural product synthesis.

Usually α-amino acids are transformed into different classes of compounds: e.g., α-amino alcohols, aldehydes and ketones before incorporation into natural product synthesis. The most commonly used class of compounds is *N*-protected-α-amino aldehydes. These compounds have wide applications in synthesis, especially for diastereoselective C–C bond formation through aldol, Grignard, Diels–Alder and other reactions.[93,94] *N*-protected serinal has special importance as the presence of a β-hydroxy group in the side chain of serine affords easy access to a variety of new compounds. Several chiral protected-derivatives of it are known, some examples are shown in **Figure 30**: Garner aldehyde **12**, Rapaport's acyclic *N*-(phenylsulfonyl)-protected aldehyde **83**,

and *N*-(9-phenylfluoren-9-yl) cyclic carbamate **84**.[95]

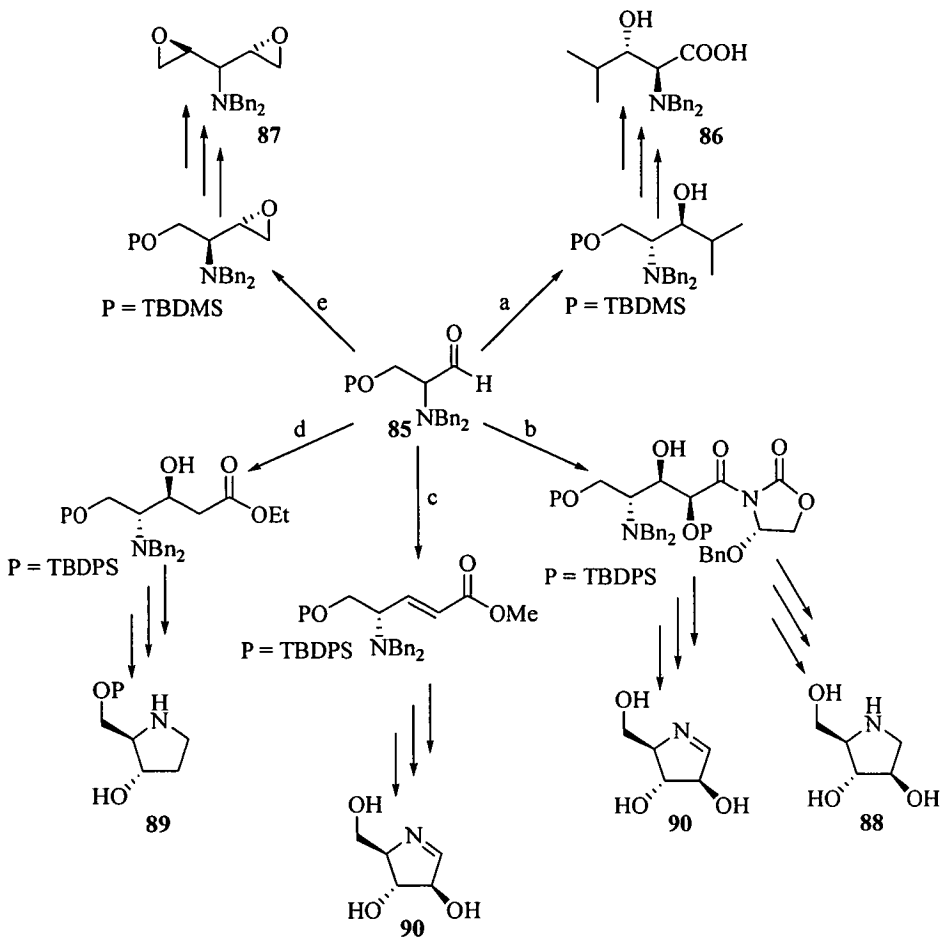



**12** **83** **84**

**Figure 30:** Examples of known *N*-protected serinals.

The Garner aldehyde **12** is one of the most important compounds which have been used as a starting material for a wide range of natural products.[93,96] Although **12** can be easily prepared in four steps from serine in almost enantiomerically pure form (ee = 95%)[96] and has been successfully used in a number of applications, the resultant diastereoselectivity is not always satisfactory.[94,96]

*N,N*-dibenzylamino serinal **85**, on the other hand, is well known in the literature as a chiral building block for a broad range of natural products. Examples of some natural products are shown in **Scheme 35**. In 1998, Zhu *et al.* reported synthesis of (2*S*,3*S*)-β-hydroxyleuceine **86**—a key constituent of a variety of natural peptide antibiotics.[95] Later, Concellón *et al.* published their synthesis of pseudo-$C_2$-symmetric *N,N*-dibenzyl-1,2:4,5-deipoxypentan-3-amine **87**.[97] During recent years, several natural products were synthesised by the Hulme group, starting from *N,N*-dibenzylamino serinal: such as, 1,4-dideoxy-1,4-imino-*D*-arabinitol (DAB-1) **88**,[98] TBDPS-protected hydroxypyrrolidine CYB-3 **89**[99] and nectrisine **90**.[100]



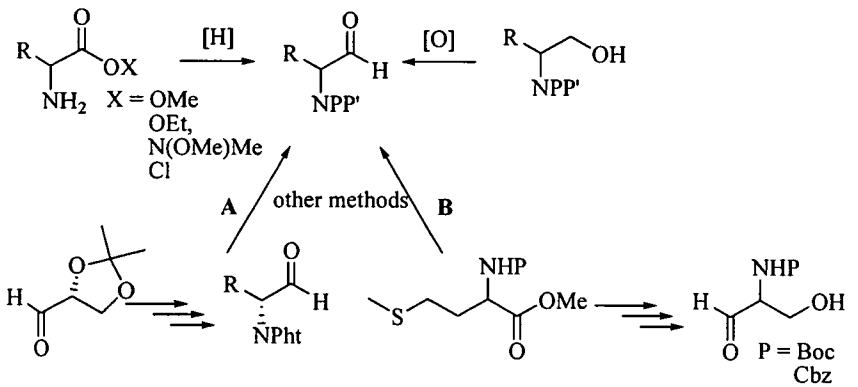

(a) Grignard addition of $^{i}$PrMgCl; (b) boron-mediated aldol reaction; (c) Horner–Wadsworth–Emmons reaction; (d) lithium-mediated aldol reaction; (e) [$LiCH_2I$].

**Scheme 35:** Applications of *N,N*-dibenzylamino serinal **85**.

### 3.3.2. Preparation of α-amino aldehydes and ketones.

Since α-amino aldehydes are not chemically stable, the free amino functionality has to be protected.[94] Usually, *N-tert*-butoxycarbonyl (Boc), *N*-benzyloxycarbonyl (Cbz) or *N-iso*-propoxy carbonyl (Poc) α-amino aldehydes are used. As an alternative to the described *N*-protected α-amino aldehydes, use of *N,N*-dibenzylamino aldehydes was considered.[94] It has been shown that the presence of two protective benzyl groups has a crucial influence on the direction and degree of diastereoselectivity.[101]

Protected α-amino-aldehydes can be obtained from: (a) α-amino acids *via* esters or amides, followed by reduction, using DIBAL, $LiAlH_4$ or Pd/C; (b) oxidation of α-amino alcohols [various methods using activated DMSO, pyridinium dichromate (PDC) or pyridinium chlorochromate (PCC)]; (c) other methods.[93]

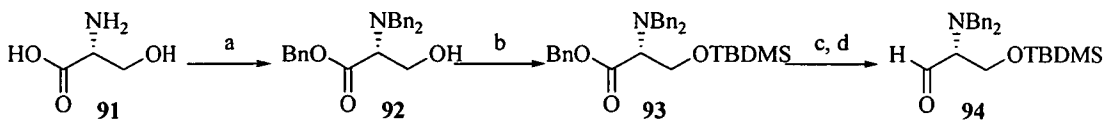



**Scheme 36:** Preparation of *N,N*-protected-α-amino-aldehydes.

However, some of these reagents are not very suitable: for example, application of DIBAL and $LiAlH_4$ can lead to over-reduction to α-amino alcohols; PDC can lead to racemisation to various extents, depending on α-amino aldehyde structure, etc.[93] As another method, synthesis of Pht-*L*-α-amino aldehydes from 2,3-*O*-isopropylidene-*D*-glyceraldehyde (**Scheme 36–A**)[102] and *N*-Boc or Cbz serinals from *L*- or *D*-methionine derivatives (**Scheme 36–B**)[103] can be considered. Although the above-mentioned Boc-, Cbz- and Poc-protected α-amino aldehydes can be handled in cold ether without noticeable racemisation, when they participate in the vast majority of reactions (for example, with Grignard reagents or in the aldol reaction), mixtures of diastereoisomers (1:1 to 1:3) are recovered as products.[93]

Since the key building block in our synthesis is *N*-protected serinal **94**, the synthesis of *N,N*-dibenzylamino serinal according to Reetz's report was considered.[94] Reetz proposed a four-step synthesis of *N,N*-dibenzylamino aldehydes starting from α-amino acids (**Scheme 37**), where the α-amino acid **91** was converted to its benzyl *N,N*-

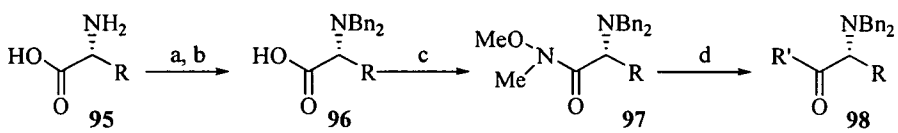
dibenzylamino ester **92**, the free alcohol was protected with TBDMS, the ester **93** was reduced to the *N,N*-dibenzylamino alcohol, followed by Swern oxidation. This synthesis was achieved in 51% overall yield and > 98% ee.[94]




(a) BnBr, $K_2CO_3$, $H_2O$; (b) TBDMSCl, imidazole; (c) $LiBH_4$; (d) Swern oxidation.

**Scheme 37:** Reetz's synthesis of the aldehyde **94**.[94]

On the other hand, the second key building block in our synthesis is a threonine-derived α-amino ketone. Again, application of an *N,N*-dibenzyl protecting group was considered. One of the most common and elegant syntheses of *N,N*-dibenzylamino ketones is shown in **Scheme 38**.[94]




(a) BnBr, $K_2CO_3$, $H_2O$; (b) KOH or $H_2$/Pd; (c) MeN(OMe)H•HCl, DCC, $Et_3N$; (d) R'MgX or R'Li.

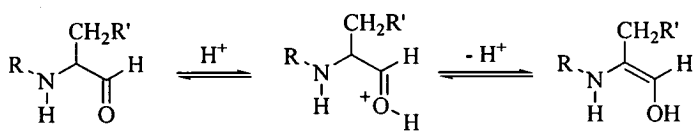
**Scheme 38:** Synthesis of *N,N*-dibenzylamino ketones.[94]

The *N,N*-dibenzyl protecting group is also attractive because deprotection of *N,N*-dibenzylamino aldehydes and ketones usually proceeds in high yields under hydrogenation conditions using Pearlman's catalyst [$Pd(OH)_2$, $H_2$].[94,95,98-100] However, the monodebenzylation of these compounds is usually hard to achieve. Davies *et al.* have recently published a new and highly effective debenzylation method using *N*-iodosuccinimide (NIS).[104] Using the proposed conditions mono- and di-debenzylation can take place in the presence of other protecting groups, such as methyl, benzyl or TBDMS ethers.

### 3.3.3. *N,N*-dibenzylamino aldehyde and ketone stability.

Usually *N,N*-dibenzylamino aldehydes and ketones are colourless oils, which are relatively unstable chemically and configurationally—especially in solution.[93] It is known that *N*-Boc- or *N*-Cbz-amino aldehydes and ketones are very unstable and have to be handled in cold ether[94] to avoid rearrangement or decomposition.[105] However, it has been shown that *N,N*-dibenzylamino aldehydes and ketones are much more stable and do not require manipulation under reduced temperatures.[106]

Chromatographic purification of mono- and di-*N*-protected-α-amino aldehydes and ketones should be avoided due to partial racemisation[107] that can take place.[93] According to Ito *et al.*, *N*-α-amino aldehydes and ketones can be racemised through keto-enol tautomerism as shown in **Scheme 39**.[107] The same mechanism is applicable for the *N,N*-protected-α-amino aldehydes and ketones.




**Scheme 39:** Proposed mechanism of racemisation of *N*-protected α-amino aldehydes and ketones.[107]

It has been shown that the optical lability of crude aldehydes depends on their structure, independent of the *N*-protecting group. For example, *N*-Boc-*L*-phenylalanine would racemise more readily than *N*-Boc-*L*-leucinal.[107,108] Additionally, Evans *et al.* found that the degree of racemisation of *N*-Boc-α-amino aldehydes and ketones depends on the temperature during storage and handling processes.[108] Less than 5% racemisation occurred after storing compounds at –30 °C for 9 days, whereas relatively rapid racemisation was noticed after storage at room temperature for 9 days.

For the reasons described above, optical rotation (due to racemisation) and

elemental analysis (due to decomposition) of *N,N*-protected-α-amino aldehydes and ketones can—at best—be considered as approximate.[93]

All of the disadvantages of *N*-protected α-amino aldehydes and ketones described above, also to some extent apply to *N,N*-dibenzylamino aldehydes and ketones. Despite all these potential problems, *N,N*-dibenzylamino aldehydes and ketones are very useful building blocks in organic synthesis. However, it has been found that they should be used in their crude form and should be synthesised using clean, enantiomerically pure synthetic routes developed earlier, such as **Scheme 37** and **Scheme 38**.[94]

### 3.3.4. Diastereoselective reactions of *N,N*-dibenzylamino aldehydes.

Reetz showed that *N,N*-dibenzylamino aldehydes and ketones can participate in a variety of diastereoselective C–C bond formation reactions, producing nonchelation-controlled products with high diastereoselectivity.[94] In attempts to understand and explain such remarkable behaviour of *N,N*-dibenzylamino aldehyde and ketones, a large number of experiments have been carried out: for example, organometallic reactions (PhMgBr, MeLi, MeTi(O*i*-Pr)$_3$, MeCeCl e.g.), aldol reactions with a variety of metal enolates, nitro-aldol reactions, Diels–Alder reactions, etc.[94] Unexpectedly, all of them were found to proceed in high yields with high diastereoselectivity towards non-chelation products.[94] Moreover, knowing that reactions with *N*-Boc- or *N*-Cbz-protected amino aldehydes or ketones usually give rise to unselective (1:1 – 3:1), low-yielding products,[93,94] and that non-chelation control in these reactions is difficult to achieve,[109] these results were very surprising.

A number of explanations of these results have been proposed. The experimental results for the reaction of (*S*)-α-amino aldehydes, raised from *L*-α-amino acids, showed that the nucleophile attacks preferably at the *re* face of the aldehyde, which can be explained with the Felkin–Anh model. Originally, this model was proposed to explain the stereochemical outcome of reactions of chiral α-chloro and α-alkoxy carbonyl compounds.[110] The most reactive conformer is the conformer in which C–C or C–O σ-

bond is aligned with the π-system of the carboxyl moiety. As a result they can overlap to form a new lower-energy LUMO. Such conformers have the highest reactivity. Application of traditional Felkin–Anh models to *N,N*-dibenzylamino aldehyde and ketones **99** and **100** are shown in **Figure 31**. Lower steric interactions between the incoming reagent and the R group, in the case of **99**, suggests that non-chelation controlled products will be observed.[94]

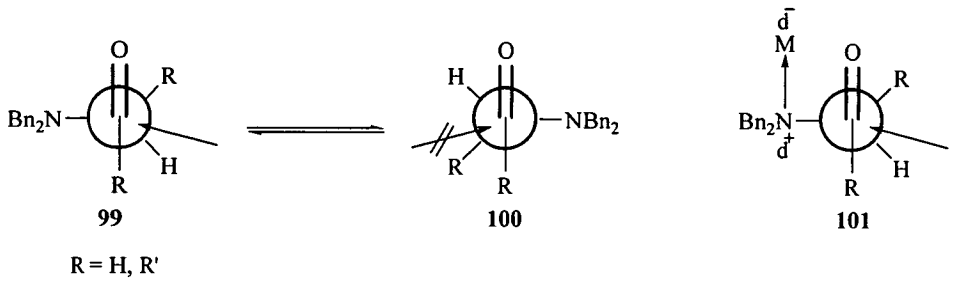



**Figure 31:** Proposed Felkin–Anh models for *N,N*-dibenzylamino aldehydes and ketones.[94]

However, this interpretation does not take into account that the $\sigma^*_{C-N}$ orbital is relatively high-lying compared with $\sigma^*_{C-O}$ or $\sigma^*_{C-Cl}$. To explain this behaviour, it was proposed that the metal coordinates to the amino functionality without undergoing chelation to the carbonyl group. In fact, this would have a dramatic influence on the electronegativity of the amino group, leading to drastic lowering of the $\sigma^*_{C-N}$ orbital, and considerable lowering of the LUMO in comparison with the carbonyl group.[94] Some experimental results,[94] where metal-free reactions showed a relatively low degree of non-chelation control, give weight to the idea of the existence of a modified version of the Felkin–Anh model **101** (**Figure 31**).

In spite of the fact that this theory is supported by experimental results, it is still not clear why chelation to the carbonyl does not take place. It is known that in the case of α-benzyloxy aldehydes and ketones reacting with $TiCl_4$ or $SnCl_4$, five-membered

chelate intermediates have been characterised by NMR and crystallography.[111] However, *N,N*-dibenzylamino aldehydes and ketones generally failed to chelate. Reetz has explained this anomaly by steric factors: depending on the nature and size of the nucleophile, the reaction can go via the hypothetical chelate **102**, acyclic adduct **103** (**Figure 32**) or, in the case of steric inhibition of chelation, by the Felkin–Anh model **99** (**Figure 31**).[94]

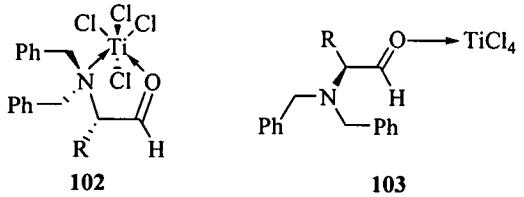



**Figure 32:** Proposed cyclic **102** and acyclic **103** chelates.[94]

Pedrosa *et al.*, however, reported the reaction of *N,N*-dibenzylamino aldehydes with dialkylzinc reagents. These reactions proceeded with excellent selectivity (>99:1) for *syn* addition, presumably due to a chelation-controlled mechanism.[112] A Cram chelate complex **104** was proposed to explain these results (**Figure 33**).[113]

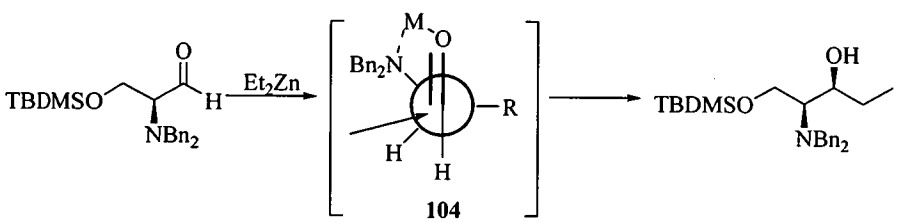



**Figure 33:** Proposed transition state for the *syn* addition of dialkylzinc.[113]

To conclude, in the case of *N,N*-dibenzylamino aldehydes and ketones, non-chelation controlled products are preferable. However, according to Reetz, chelation control can be achieved if required, but is more difficult.[94]

### 3.3.5. Synthesis of the serine-derived aldehydes 7.

Based on the previous work developed within the Hulme group,[98,99] the use of *N,N*-dibenzylamino serinal with three protecting groups **7a**[98-100]/**7b**[95,97,112]/**7c**[114] was considered.

As discussed above (section 3.3.2) Reetz[94] reported the four-step synthesis of *N,N*-*L*-dibenzylamino serinal in 51% overall yield and > 98% ee (**Scheme 37**). However, the one-pot benzylation proposed by Reetz was found to be rather lower yielding,[115] so an alternative synthetic route was considered (**Scheme 40**). *L*-serine methyl ester **105** was used as a starting material for the four-step synthesis of serine-derived aldehyde **7**.[98,99] In our hands, the ester was first *N,N*-dibenzyl protected under non-aqueous conditions to give **106** in 75% yield. The free hydroxyl group was protected with different protecting groups [TBDPS (74%), TBDMS (94%)] to investigate the influence of the steric demands of the protecting group on the diastereoselectivity of the subsequent aldol reaction (**Scheme 40**). The TBDPS protecting group was chosen[98] as one of the most suitable orthogonal protecting groups due to its stability to routine synthetic procedures: it survives aqueous work-up and column chromatography on silica gel. Nevertheless, we decided also to use the TBDMS protecting group as it has the same reactivity and stability, but is less bulky than the TBDPS group, which could affect the stereoselectivity of the aldol reaction. Both of these groups could be introduced and removed in high yield without affecting the rest of the molecule.[116]

Reduction of the protected methyl esters **107** and **108** was achieved with $LiBH_4$ in high yield. After flash chromatography purification, monoprotected alcohols **109** and **110** were obtained as pale yellow oils. Swern oxidation[117] provided the aldehydes **7a** and **7b** in quantitative yield, which were used in subsequent reactions without further purification to avoid possible racemisation.[107] This optimised route provided the enantiomerically pure aldehydes **7a** (>98% e[bb])[98] and **7b**.[95]

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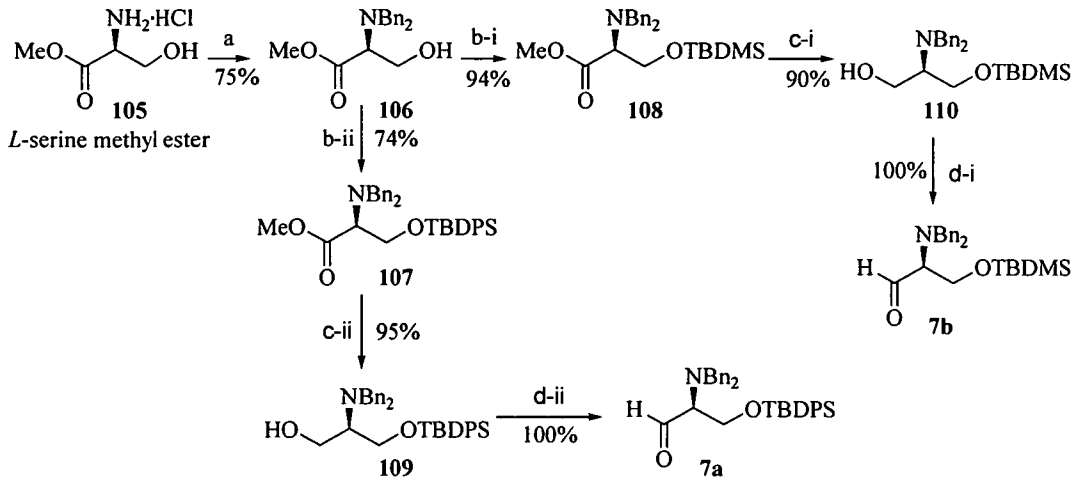
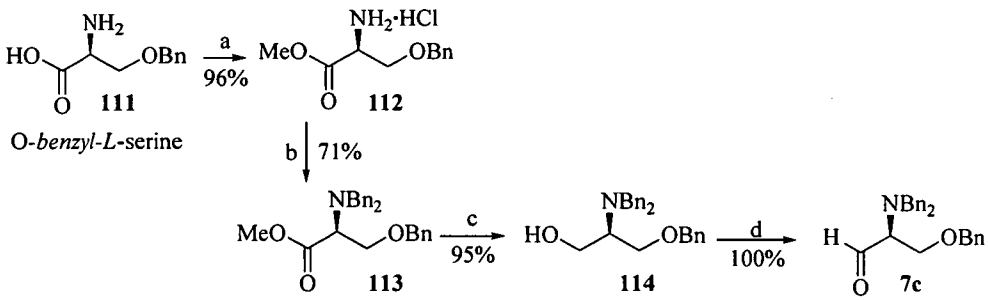
[bb] The enantiomeric excess was determined by C. H. Montgomery.




(a) BnBr, $K_2CO_3$, $CH_3CN$, r.t., 36 h; (b-i) TBDMSCl, imidazole, DMF, r.t., 36 h; (b-ii) TBDPSCl, imidazole, DMF, r.t., 36 h; (c-i) $LiBH_4$, $Et_2O$, MeOH, 45 °C, 4 h; (c-ii) $LiBH_4$, $Et_2O$, MeOH, 45 °C, 4 h; (d-i) $(COCl)_2$, DMSO, $NEt_3$, DCM, –78 °C, 1.5 h; (d-ii) $(COCl)_2$, DMSO, $NEt_3$, DCM, – 78 °C, 1.5 h.

**Scheme 40:** Four-step synthesis of serine-derived aldehydes **7a** and **7b**.

To investigate the influence of the protecting group size and nature on the diastereoselectivity of aldol reactions, Bn-protected *N,N*-dibenzylamino serinal **7c** was also synthesised in our hands in 65% overall yield (**Scheme 41**).

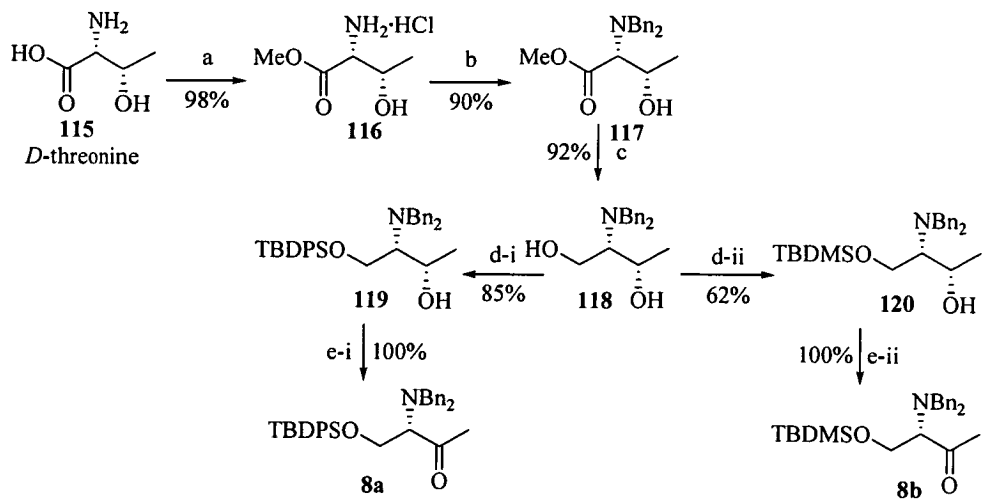



(a) $CH_3COCl$, MeOH, 80 °C; (b) BnBr, $K_2CO_3$, $CH_3CN$, r.t., 36 h; (c) $LiBH_4$, $Et_2O$, MeOH, 45 °C, 4 h; (d) $(COCl)_2$, DMSO, $NEt_3$, DCM, –78 °C, 1.5 h.

**Scheme 41:** Four-step synthesis of serine-derived aldehyde **7c**.

### 3.3.6. Synthesis of threonine-derived ketone 8.

Another key building block in our synthesis was threonine-derived *N,N*-dibenzyl α-amino ketones **8**. A five-step synthesis of the required threonine-derived ketones **8a** and **8b** was carried out using conditions developed by Curley for ketone **8a**.[115] Since the absolute stereochemistry of C(10) is 10(*R*), the use of *D*-threonine as a starting material was necessary. Firstly, *D*-threonine **115** was converted into its methyl ester **116** in 98% yield, followed by dibenzylation of the free amino group to afford **117** in 90% yield (**Scheme 42**). Interestingly, the single enantiomer of **116** was a clear oil whereas (±)-**116** was a colourless solid, as was expected for the hydrochloric salt. The dibenzylamino methyl ester **117** was reduced to dibenzylamino diol **118** in 92% yield. Successful monoprotection of the primary hydroxyl group using two different protecting groups (TBDPS and TBDMS) was carried out (**Scheme 42**). The choice of protecting groups was discussed in section 3.3.5.



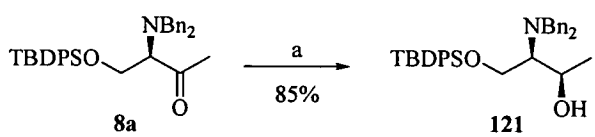

(a) $CH_3COCl$, MeOH, 80 °C, 4 h; (b) BnBr, $K_2CO_3$, $CH_3CN$, r.t., 36 h; (c) $LiBH_4$, $Et_2O$, MeOH, 45 °C, 4 h; (d-i) TBDPSCl, imidazole, DMF, r.t., 36 h; (d-ii) TBDMSCl, imidazole, DMF, r.t., 36 h; (e-i) $(COCl)_2$, DMSO, $NEt_3$, DCM, –78 °C, 1.5 h; (e-ii) $(COCl)_2$, DMSO, $NEt_3$, DCM, –78 °C, 1.5 h.

**Scheme 42:** Five-step synthesis of threonine-derived ketones **8a** and **8b**.

Using the conditions proposed by Curley[115] (1.2 eq. of TBDPSCl for 1.0 eq. of aminodiol **118**) for protection of the primary alcohol, we obtained an inseparable mixture of the monoprotected alcohol and TBDPSOH. Further purification was carried out by HPLC (15% EtOAc in hexane) providing **119** in 40% yield. We decided to decrease the amount of TBDPSCl to 0.9 eq for 1.0 eq of amino diol **118** to get the clean TBDPS-protected alcohol **119** after column chromatography with silica gel. This resulted in an improved yield of 62%. Such a problem did not appear when the TBDMS protecting group was used, since it was possible to remove TBDMSOH by flash chromatography.

Finally a Swern oxidation[117] was carried out to produce the methyl ketones **8a** and **8b** in quantitative yield (**Scheme 42**). The ketones were used in subsequent reactions without further purification since column chromatography can lead to racemisation (see section 3.2.2).[107]

The enantiomeric excess of ketone **8a** was determined by Curley.[115] She chose to reduce the freshly prepared ketone **8a** into alcohol **121** using DIBAL-H (**Scheme 43**) before determination of the %ee to avoid problems associated with the instability of α-amino ketones on silica gel. In parallel, synthesis of the racemic alcohol **121** was carried out as shown in **Scheme 42**. HPLC analysis (5% IPA in Hexane) of both chiral and racemic alcohols was performed. The optical purity of the alcohol in the single enantiomer series was found to be > 99% ee.[115]




(a) DIBAL-H, toluene, –78 °C.

**Scheme 43:** DIBAL-H reduction of threonine-derived ketone **8a**.[115]

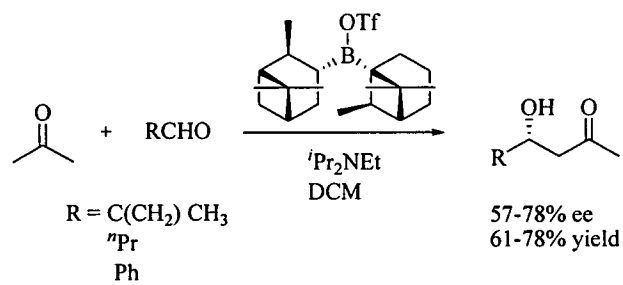
## 3.4. Asymmetric aldol reactions.

The aldol reaction is one of the most important reactions in synthetic organic chemistry due to its ability to form new C–C bonds in a regio-, diastereo- and enantio-selective manner.[118,119] Diastereoselective aldol reactions have appeared as one of the most efficient methods available for the construction of a wide range of optically active compounds. Aldol reactions are also widely adopted in fatty acid and polyketide natural products synthesis.[120-123]

### 3.4.1. Acetate aldol reactions.

Very high diastereoselectivities have been reported for aldol reactions involving chiral enolates derived from ethyl or higher alkyl-substituted ketone derivatives.[124] However, this approach for methyl ketones is much less successful. Comparatively few examples of highly diastereoselective aldol reactions of chiral methyl ketones[125-128] or aldol reactions of methyl ketones with chiral reagents[118,129-132] have been reported.

In 1989, Paterson *et al.* noticed that in the case of (–)-$(Ipc)_2$ boron-mediated aldol reactions of a methyl ketone (acetone) and a variety of aldehydes, each product formed had an (*R*) absolute configuration (**Scheme 44**).[131]




**Scheme 44:** Enantioselective aldol reactions of acetone and variety of aldehydes.[131]

The observed selectivity was explained through the twist-boat transition state **I**, as

shown in **Figure 34**. The transition state **I** is favourable, since steric interactions between the bulky boron ligands L and the R group of the enolate are avoided, whereas in the chair transition state **II** these steric interactions will take place.[131,133]

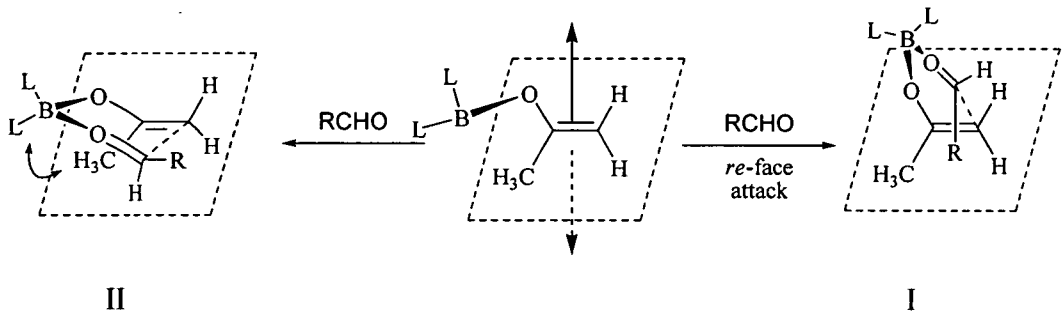
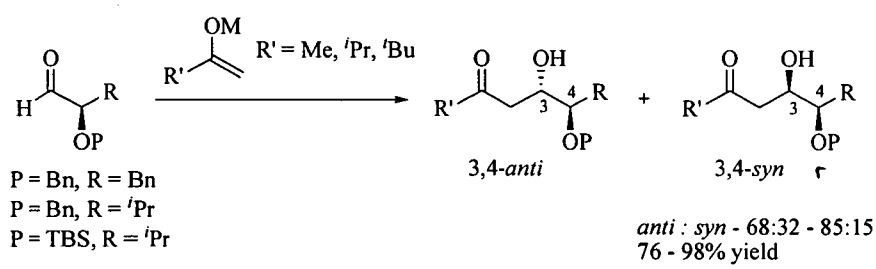



**Figure 34:** Proposed twist-boat transition state **I**.[131]

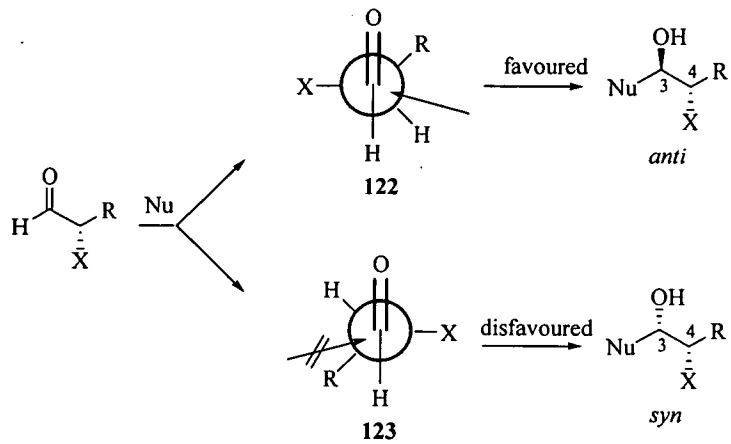
Later, Evans *et al.* studied the boron-mediated aldol addition of methyl ketones to α-alkoxy aldehydes.[132] The formation of the 3,4-*anti* product as the major diastereoisomer was observed (**Scheme 45**).




**Scheme 45:** Boron-mediated aldol reactions of α-alkoxy aldehydes (M = 9-BBN).[132]

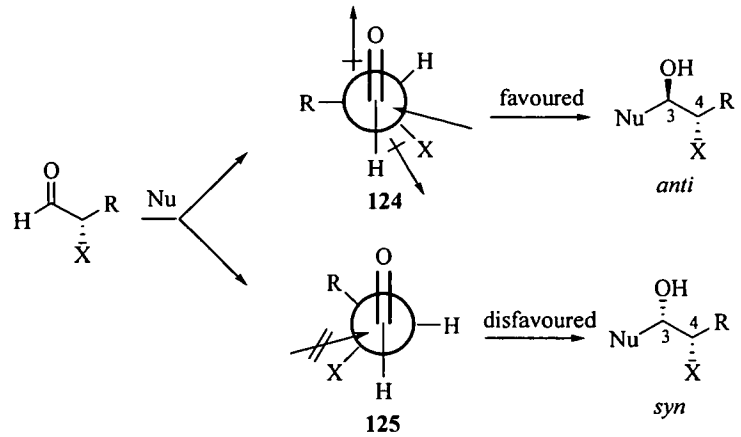
Evans *et al.*[134] reported that nucleophilic addition of carbonyl compounds bearing α-substituents could be explained by both the Cornforth[135] and polar Felkin–Anh[110,136] models. Both models account for the preferential formation of the 3,4-*anti* product diastereomer on the basis of differing transition state control elements deriving from rotation about the aldehyde C(1)–C(2) bond.

In the polar Felkin–Anh model, the hyperconjugative interaction will be maximised when the forming bond and the C–OP bond are antiperiplanar, as shown in **Scheme 46**. The transition state **122** is favourable, since the nucleophilic approach is close to the hydrogen, rather than being destabilized by the alkyl substituent in **123**.



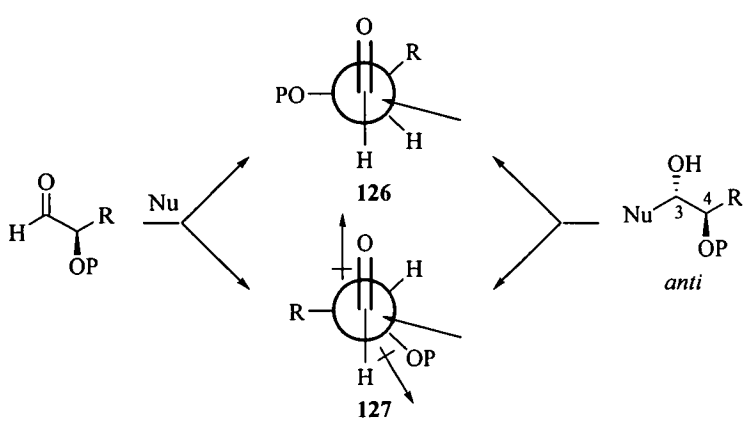

**Scheme 46:** Felkin–Anh transition state for nucleophilic addition to α-alkoxy aldehydes.[134]

On the other hand, the Cornforth model is based on the fact that dipole effects dictate an antiparallel dihedral angle relationship between the carbonyl and α-substituent (**Scheme 47**).[134] Two transition states **124** and **125** have the electronegative substituent and carbonyl in a dipole-minimized orientation. They can be further distinguished by steric interactions between nucleophile and R group. As can be seen in **Scheme 47**, the transition state **124** is favoured since the nucleophile approaches between the α-substituent and hydrogen.



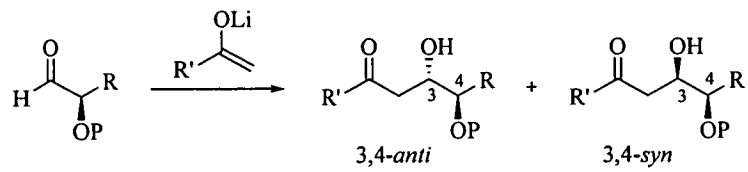

**Scheme 47:** Cornforth transition state for nucleophilic addition to α-alkoxy aldehydes.[134]

According to Evans *et al.*,[132,134] the Cornforth model more accurately describes asymmetric induction in enolborane additions to α-alkoxy aldehydes (**Scheme 48**). While less-electronegative substituents, such as $NMe_2$, favour the polar Felkin–Anh model.




**Scheme 48:** Nucleophilic addition models for α-alkoxy aldehydes; **126**—polar Felkin–Anh model; **127**—Cornforth model.[132]

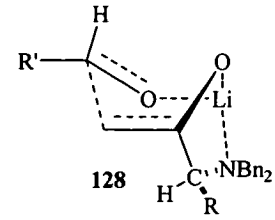
However, when the lithium-mediated aldol addition of methyl ketones to α-alkoxy aldehydes[132] was investigated, even higher selectivity towards the 3,4-*anti* diastereoisomer was discovered (**Scheme 49**) in cases where the α-alkyl substituent is branched.[137]




| Aldehyde (P, R) | Enolate (R') | *anti*:*syn* | Yield (%) |
|---|---|---|---|
| P = Bn, R = $^{i}$Pr | Me | 94:6 | 88 |
| | $^{i}$Pr | 91:9 | 84 |
| | $^{t}$Bu | 89:11 | 76 |
| P = TBS, R = $^{i}$Pr | Me | 85:15 | 84 |
| | $^{i}$Pr | 88:12 | 53 |
| | $^{t}$Bu | 91:9 | 78 |

**Scheme 49:** Lithium-mediated aldol reactions of α-alkoxy aldehydes.[132]

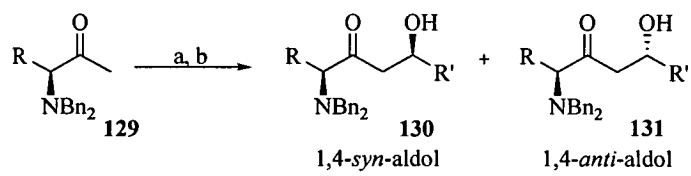
Unexpectedly high diastereoselectivity (80:20 to >98:2) was achieved in the aldol reactions of the lithium enolate of simple α-(*N*,*N*-dibenzylamino) alkyl methyl ketones.[138] Generally, metal enolisation (especially lithium enolisation) of chiral methyl ketones gave poor diastereoselectivity, which was explained by lack of control of the rotamer population around the nonreactive C–C(O) bond.[138] In the case of α-(*N*,*N*-dibenzylamino) alkyl methyl ketones, high diastereoselectivity was observed: presumably due to the unique ability of the dibenzylamino group to participate in lithium chelation at the same time as controlling the facial selectivity of the approaching aldehyde as shown in **Figure 35**.[138]




**128**

**Figure 35:** Chelated-boat transition states **128**.[138]

Previously published results showed that the diastereoselectivity of aldol reactions depends on the bulkiness of the enolate and metal ligands.[139,140] Liotta and co-workers[138] illustrated this statement with the *N,N*-dibenzylamino ketones **129** (**Scheme 50**).




**129** **130** **131**

1,4-*syn*-aldol 1,4-*anti*-aldol

(a) LDA, THF, –78 °C; (b) R'CHO, THF.

| Entry | R | R' | Yield, % | ds | abs. config. of C(4) |
|---|---|---|---|---|---|
| 1 | $CH_3$ | PhCHO | 84 | 80:20 | *R* |
| 2 | $CH_3$ | $(CH_3)_2CHCHO$ | 91 | 88:12 | *R* |
| 3 | $CH_3$ | $(CH_3)_3CCHO$ | 81 | 89:11 | *R* |
| 4 | Bn | $(CH_3)_3CCHO$ | 76 | 92:7 | *R* |
| 5 | *i*-Pr | PhCHO | 90 | >98:2 | *R* |
| 6 | *i*-Pr | $(CH_3)_3CCHO$ | 88 | >98:2 | *R* |

**Scheme 50:** Examples of lithium-mediated diastereoselective aldol reactions.[138]

The chelation model proposed by Reetz,[141] where the *N,N*-dibenzylamino group lies in the plane of the lithium enolate, and diastereofacial selectivity depends on the

differences in size between R' and H, predicts formation of the *S,S*-1,4-*anti* product **131**. However, in Liotta's experiments, the formation of *S,R*-1,4-*syn* products **130** was observed[138] (**Scheme 50**).

Based on previous results where the diastereoselectivity of boron-[140] and titanium-mediated[142,143] aldol reactions of methyl ketones was explained by chair transition states, the same transition structures were proposed for the lithium-mediated reaction (**Figure 36**). However, computational studies of the relative energies of the possible intermediates suggested that the chair transition states (**Figure 36**) are not the favoured conformations in the case of lithium-mediated aldol reactions of *N,N*-dibenzylamino methyl ketones.[138]

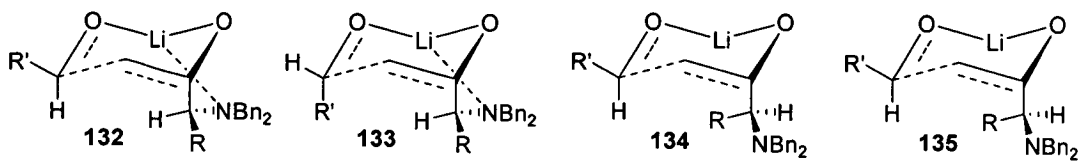



**Figure 36:** Chair transition states.[138]

Based on computational studies, Liotta[138] proposed a chelated-boat transition state model to rationalize the exceptionally high diastereoselectivity observed in lithium-mediated aldol reactions of *N,N*-dibenzylamino methyl ketones (**Figure 37**).

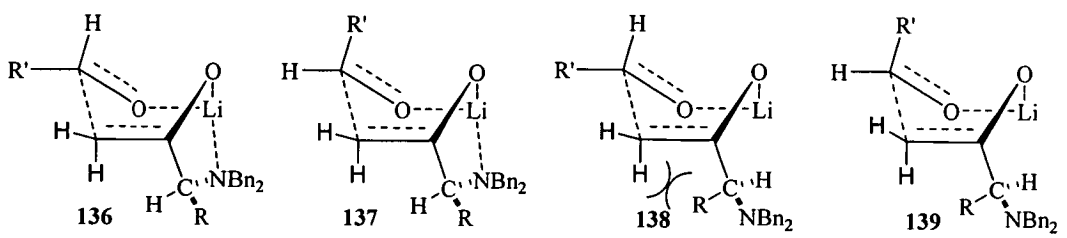
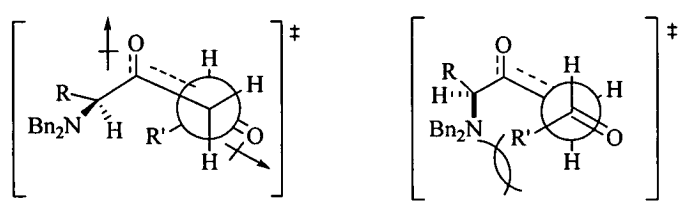



**Figure 37:** Chelated-boat transition states.[138]

Clearly, transition states **137** and **139** are not favourable conformations, due to the destabilisation from placing the R′ group in the sterically hindered "flagpole" position. Between **136** and **138**, **136** is preferable since **138** suffers from inherent $A_{1,3}$-strain.[138]

All the aldol reactions that were carried out in Liotta's work[138] proceeded with high diastereoselectivity (80:20–>98:2) and in excellent yield (64–91%) in favour of 1,4-*syn*-aldol adducts (**Scheme 50**).

In subsequent work, Lagu and Liotta[144] proposed that changing the reaction parameters may give rise to enhanced diastereoselectivity. However, changing the base from LDA to LiHMDS did not give any worthwhile results. Based on the proposed transition state (**Figure 37**), it was anticipated that a counter-ion such as sodium or potassium, which generally forms ionic bonds with oxygen, would disrupt the internal chelation present in the boat transition state and provide lower diastereoselectivity. In fact, surprisingly the potassium enolate gave the aldol product with only slightly lower selectivity than lithium. Even more surprisingly, the sodium enolate gave far higher diastereoselectivity than the lithium enolate.[144] Since these new results could not be explained using the boat transition state (**Figure 37**), another possible model with an open transition state (**Figure 38**) was proposed.[144]




I II

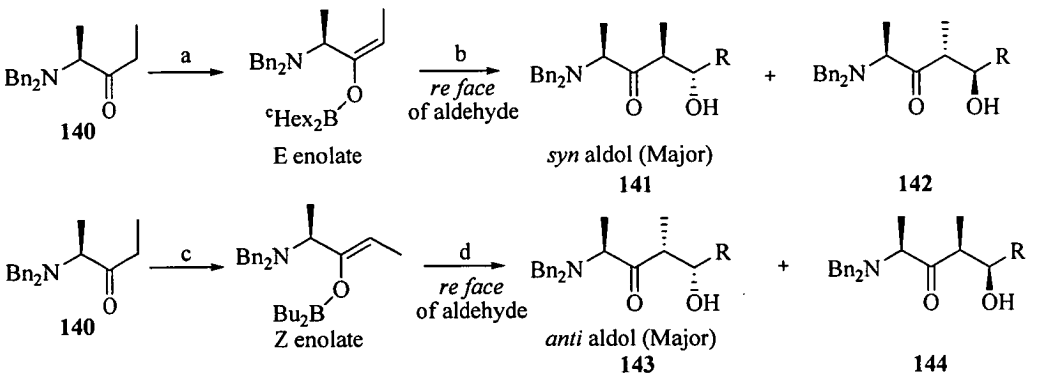
**Figure 38:** Open transition state proposed for the sodium-mediated aldol reaction.[144]

In model **I** the aldehyde approaches in a manner whereby the two oxygen atoms are orientated in opposite directions to minimize the interaction between the dipole moments. The *re* face of the aldehyde will therefore be attacked by the enolate to produce the *syn* aldol adduct. Attack on the *si* face of the aldehyde would be less likely to occur due to unfavourable steric interactions between the *N,N*-dibenzyl group and the

R' group as shown in model **II** (**Figure 38**).[144]

### 3.4.2. Propionate aldol reaction.

The most appropriate conditions to control the stereoselectivity of the α-(*N,N*-dibenzylamino)alkyl ethyl ketones aldol reaction is the boron-mediated aldol reaction. According to Paterson[119] either the *syn* or *anti* aldol adducts can be formed, depending on the choice of boron reagent and base (**Scheme 51**).




(a) $^{c}Hex_2BCl$, $Me_2NEt$; (b) RCHO; (c) $Bu_2BOTf$, $^{i}Pr_2NEt$; (d) RCHO.

**Scheme 51:** Boron-mediated aldol reaction.[119]

Boron enolate aldol reactions for a variety of aldehydes proceeded with high diastereoselectivities (84:16 – 89:11) for *anti* adducts and comparable results (86:14 – 97:3) were obtained for *syn* aldol adducts. The reactions were carried out in reasonable yield (65 - 100%) (**Table 5 and Table 6**).[119]

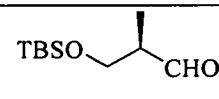
| Entry | R | Yield, % | dr (**141**:**142**) |
|---|---|---|---|
| 1 | Me | 90 | 85:15 |
| 2 | $^{i}$Pr | 95 | 85:15 |
| 3 | $H_2C$=C(Me) | 80 | 89:11 |
| 4 |  | 95 | >98:2 |

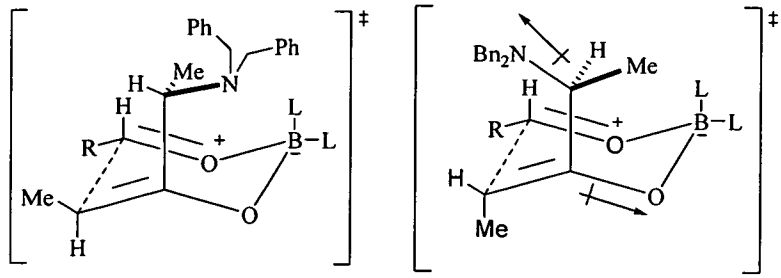


**Table 5:** *Anti*-selective aldol reactions of ketone (*S*)-**140**.[119]

| Entry | R | Yield, % | dr (**143**:**144**) |
|---|---|---|---|
| 1 | Et | 95 | 97:3 |
| 2 | $^{i}$Pr | 96 | 86:14 |
| 3 | $H_2C$=C(Me) | 90 | 87:13 |
| 4 | Ph | 100 | 93:7 |

**Table 6:** *Syn*-selective aldol reactions of ketone (*S*)-**140**.[119]

It is thought that boron-mediated aldol reactions go through a highly ordered six-membered Zimmerman–Traxler transition state. In the case of the *anti* aldol adduct, the transition state **I** is preferred in which 1,3-diaxial interactions are minimised. In this transition state the bulky dibenzylamino group is directed outside, and the methyl group is oriented inwards. The formation of the *syn* aldol product is thought to go through transition state **II**, where the enolate C–O and C–N dipoles are opposed and the methyl group is oriented outwards (**Figure 39**).

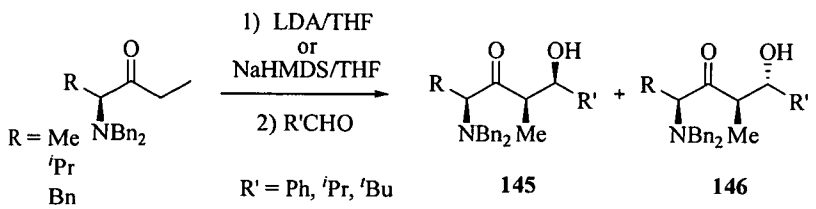


*anti* aldol I

*syn* aldol II

**Figure 39:** Chair transition state proposed for the boron-mediated aldol reaction.[119]

Liotta *et al.* reported their investigations of the lithium- and sodium-mediated aldol reaction of α-(*N,N*-dibenzylamino) ethyl ketones.[145] In all experiments, sodium enolates gave better yields and better diastereoselectivities than lithium ones (**Scheme 52**). The major products (**145**) isolated from reaction with either lithium or sodium enolates, were found to be the same.

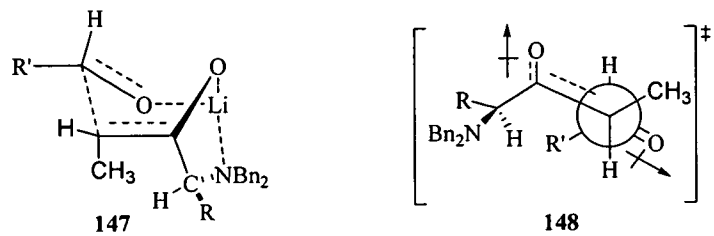



| | LDA | NaHMDS |
|---|---|---|
| d.r. **145:146** | 63:37–>95:5 | 94:6–>95:5 |
| Yield (%) | 31–56 | 55–93 |

**Scheme 52:** Lithium- and sodium-mediated aldol reactions of α-(*N,N*-dibenzylamino) ethyl ketones.[145]

Since the sodium enolate reactions of methyl ketones are known to be unlikely to proceed via a chelated boat transition state **147**,[138] and the same absolute configuration

(1,4-*syn*) was observed in case of the enolates of ethyl ketones, the lithium and sodium enolate aldol reaction of α-(*N,N*-dibenzylamino) ethyl ketones was proposed to go via the open transition state **148** (**Scheme 53**).[145] Additionally, to produce the 1,4-*syn* aldol adducts, the Z-enolate should be formed, but this is impossible in the chelated-boat transition state **147**.

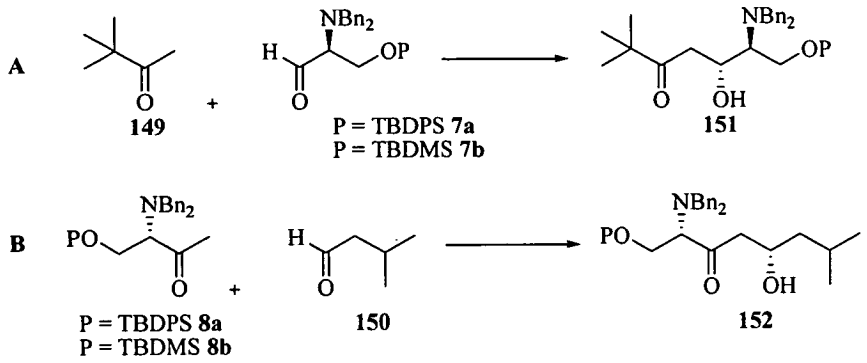



**Scheme 53:** Proposed transition states for aldol reactions of α-(*N,N*-dibenzylamino) ethyl ketones.[145]

### 3.5. Aldol reactions of serine-derived aldehyde 8 and threonine-derived ketone 7.

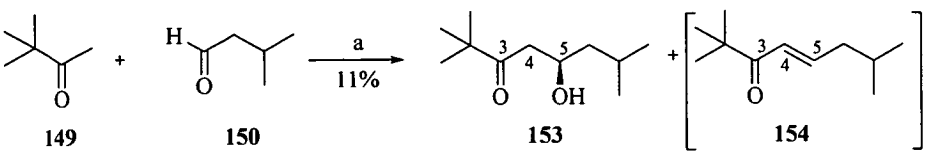
#### 3.5.1. Model studies of the conditions of the aldol reaction.

As described in section 3.2, to predict the stereochemical outcome of the double asymmetric aldol reaction between two chiral compounds, serine-derived aldehyde 7 and threonine-derived ketone **8**, additional reactions using simple achiral ketones and aldehydes needed to be carried out. In our case, reaction of serine-derived aldehyde 7 with pinacolone **149** (**Scheme 54A**), and reaction of threonine-derived ketone **8** with isovaleraldehyde **150**, were carried out (**Scheme 54B**). Pinacolone **149** was chosen due to its bulkiness to mimic the threonine-derived ketone **8**. Isovaleraldehyde **150** was chosen based on the previous investigation of this reaction carried out by Curley.[115]




**Scheme 54:** Reactions between chiral serine **7** and threonine **8** derivatives and achiral ketone **149** and aldehyde **150**.

Based on Liotta's results,[144] use of LiHMDS as a base for the aldol reaction was considered. First of all, however, the conditions optimized by Curley[115] were tested on the model compounds **149** and **150** (**Scheme 55**). Despite the high yield (65–80%) observed in Koga's studies,[130] our model reaction proceeded in only 11% yield. Formation of **153** was proved by disappearance of the aldehyde peak at 9.75 ppm in the $^1$H NMR and appearance of an ABX system at 2.60–2.47 ppm corresponding to C(4)$H_2$, which clearly suggests the formation of a new C–C bond. Additionally, the presence of the new $CH_2$ peak at 43.4 ppm in $^{13}$C NMR supported this suggestion.

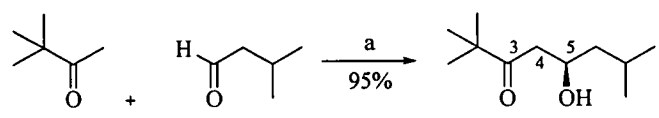



(a) (i) LiHMDS, THF, –78 °C, 1.5 h; (ii) isovaleraldehyde **150**, –78 °C, 0.5 h.

**Scheme 55:** Model studies of the lithium-mediated aldol reaction.

Since the first attempt of the aldol reaction between ketone **149** and aldehyde **150** was so low yielding, the use of the other bases was considered. It has been found by Paterson[131] that the boron-mediated aldol reactions of methyl ketones proceed in good

yields. The enolisation conditions optimised by Hulme $Bu_2BOTf/^iPr_2NEt$[146] gave product **153** in an unexpectedly high yield (95%) (**Scheme 56**). Unfortunately, a clear explanation of the difference between these two metal enolates was not found: however, some speculations can be made. For example, isolation of the dehydration product **154** (10% yield) (**Scheme 55**) as a by-product of the lithium-mediated aldol reaction, suggests that the LiHMDS base is too strong in this case, and further elimination reactions occurred which significantly reduced the yield of the aldol adduct **153**. Additionally, Cainelli *et al.* suggested that the diastereoselective outcome of an aldol reaction could depend on the solvent used.[147]

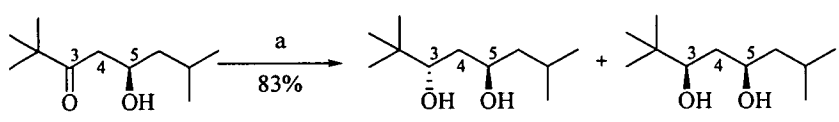



**149** **150** **153**

(a) (i) $^nBu_2BOTf$, $^iPr_2NEt$, DCM, –78 °C, 2 h; (ii) isovaleraldehyde **150**, –78 °C, 2 h, then 0 °C, 1 h.

**Scheme 56:** Model studies of the boron-mediated aldol reaction.

Since aldol adducts obtained from serine **7** and threonine **8** derivatives are expected to be unstable, further directed 1,3-reductions needed to be carried out immediately (section 3.5). *Anti*-reduction using $Me_4NBH(OAc)_3$ was performed on the aldol adduct **153** in order to investigate previously published conditions[148] (**Scheme 57**).




**153** **155** **156**

90 : 10

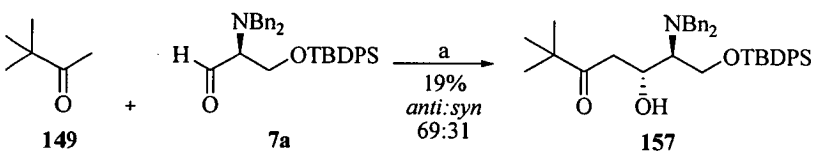
(a) (i) $Me_4NBH(OAc)_3$, $CH_3CN$, $CH_3COOH$, r.t., 0.5 h; (ii) **153**, –40 °C, 18 h.

**Scheme 57:** $Me_4NBH(OAc)_3$ *anti* reduction of **153**.

Product **155:156** was isolated in 83% yield and 90:10 diastereoselectivity. The diastereometic ratio was determined by $^1$H NMR integral measurements of the C(5)*H* peak of the major diastereoisomer (*anti*) located at 4.11 ppm, and the C(5)*H* peak of the minor diastereoisomer (*syn*) located at 3.60 ppm. These results suggested that Paterson's reduction conditions are suitable for our compounds.

### 3.5.2. Aldol reactions of pinacolone 149 and serine-derived aldehydes 7.

As was described above (section 3.5.1), in the case of pinacolone **149** and isovaleraldehyde **150** the boron-mediated aldol reaction proceeded better than the lithium-mediated aldol reaction. So, when aldol reactions between pinacolone **149** and the serine-derived aldehyde **7a** were investigated, boron enolisation conditions were considered as favourable. However, the resulting aldol adduct **157** was isolated in only 19% yield with 69:31 diastereoselectivity (**Scheme 58**).

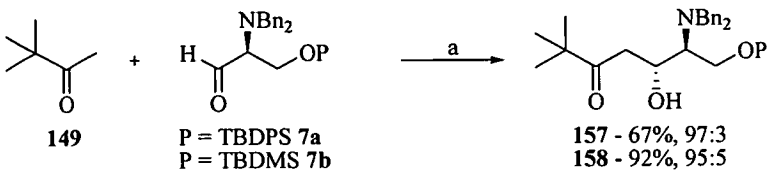



(a) (i) $^n$Bu$_2$BOTf, $^i$Pr$_2$NEt, DCM, –78 °C, 2 h; (ii) **7a**, –78 °C, 2 h, then 0 °C, 1 h.

**Scheme 58:** Boron-mediated aldol reaction of pinacolone **149** and aldehyde **7a**.

Despite previous lack of success with the lithium-mediated aldol reaction, in the case of reaction of pinacolone **149** and serine-derived aldehydes **7a** and **7b**, the reactions proceeded in high yields with excellent diastereoselectivities (**Scheme 59**). Enolisation of pinacolone **149** was carried out at –78 °C in the presence of LiHMDS for one hour followed by addition of aldehydes **7a** and **7b**. After 30 minutes, t.l.c. showed that all of the starting material had been consumed, and therefore the reaction was quenched. Chromatographic purification provided an inseparable mixture of diastereoisomers **157**

and **158** in 66% and 92% yield, respectively.




(a) (i) LiHMDS, THF, –78 °C, 1 h; (ii) **7a** or **7b**, –78 °C, 0.5 h.

**Scheme 59:** Lithium-mediated aldol reaction.

The diastereoselectivities were calculated by measurement of the integrals from the $^1$H NMR corresponding to the protons at $CH_2$ of the *N,N*-dibenzylamino groups. In the major products **157** and **158** two sets of doublets were observed, whereas only one doublet was clearly seen for the minor diastereoisomers.

The lack of reactivity under boron-mediated aldol conditions can be explained in terms of the[131] twist-boat transition state. Increased steric congestion between the *tert*-butyl group of pinacolone **149** and the bulky serine-derived aldehyde **7a** is apparent: thus, making this reaction unfavourable (**Figure 40**).

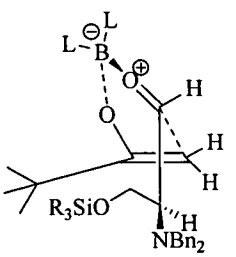



**Figure 40:** Proposed twist-boat transition state.[131]

However, in the case of the lithium enolate, the diastereoselective outcome can be explained by the non-chelation Felkin–Anh model.[94,110,149] Conformer **A** predicts the

*anti*-selectivity, whereby the incoming nucleophile attacks from the less hindered face of the aldehyde (**Figure 41**).

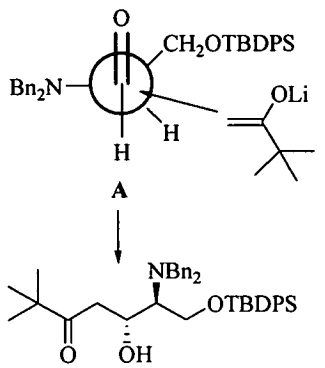
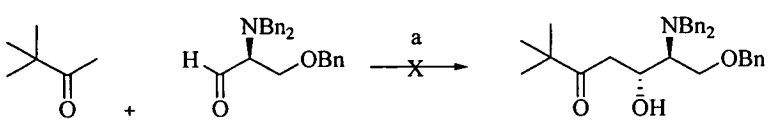



**Figure 41:** Felkin–Anh model for the lithium-mediated aldol reaction.

To recap, the lithium-mediated aldol reactions of pinacolone **149** and serine-derived aldehydes **7a** and **7b** proceeded in high yield and high diastereoselectivity in comparison with the boron-mediated aldol reaction. The resulting *anti*-selectivity was explained by the Felkin–Anh model. Additionally, the diastereoselectivity was proved by a crystal structure of the reduced material as discussed in section 3.6.5 (**Appendix 6**). The two different hydroxyl protecting groups that were used (TBDPS and TBDMS) did not noticeably affect the diastereoselectivity of the aldol reaction products **157** and **158**. In the case of the benzyl protecting group, no aldol adduct **159** was isolated and no starting material **7c** was recovered. This could be due to the decomposition of the aldehyde **7c** (**Scheme 60**).




**149** **7c** **159**

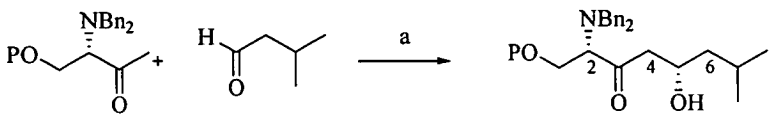
(a) (i) LiHMDS, THF, –78 °C, 1 h; (ii) **7c**, –78 °C, 0.5 h.

**Scheme 60:** Unsuccessful attempt at the aldol reaction of pinacolone **149** and **7c**.

Freshly prepared aldol adducts **157** and **158** were utilised in the directed 1,3-reduction (section 3.6) without further purification.

### 3.5.3. Aldol reactions of threonine-derived ketones 8 and isovaleraldehyde 150.

Based on results reported by Curley,[115] lithium-mediated aldol reactions of threonine-derived ketones **8** and isovaleraldehyde were carried out (**Scheme 61**).




P = TBDPS **8a** | **150** | **160** - 50%, 85:15
P = TBDMS **8b** | | **161** - 96%, 87:13

(a) (i) **8a** or **8b**, LiHMDS, THF, –78 °C, 1 h; (ii) isovaleraldehyde **150**, –78 °C, 0.5 h.

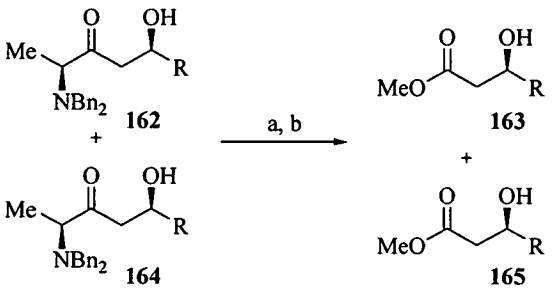
**Scheme 61:** Lithium-mediated aldol reaction.

Enolisation of **8a** and **8b** was carried out as described above (section 3.4.2) followed by addition of aldehyde **150**. However, in this case, the t.l.c. analysis showed full consumption of the starting materials **8a** and **8b** in just 10 minutes. Chromatographic purification resulted in an inseparable mixture of diastereoisomers **160** and **161** in 50% and 96% yield, respectively.

The diastereoselectivity of the reaction of the threonine-derived methyl ketones **8a** and **8b** with isovaleraldehyde **150** was obtained directly from integration of the C(4)$H_2$ $^1$H NMR signals for the two diastereomers. In the case of the major (*syn*) diastereoisomers **160** and **161**, two doublets with large coupling constants were analysed; whereas in the case of the minor (*anti*) diastereoisomers, two doublets of doublets were monitored.

The stereochemistry of the aldol adducts **160** and **161** was assigned based on Liotta's precedent,[138,145] where the conformation of the new stereogenic centre of the

crude aldol adducts **162** and **164** was established by conversion to the corresponding 3-hydroxy methyl esters **163** and **165** (**Scheme 62**). Lanthanide-induced $^1$H NMR shifts of the methoxy group of the two enantiomers were significantly different and confirmed the absolute stereochemistry by comparison with literature values.[145]

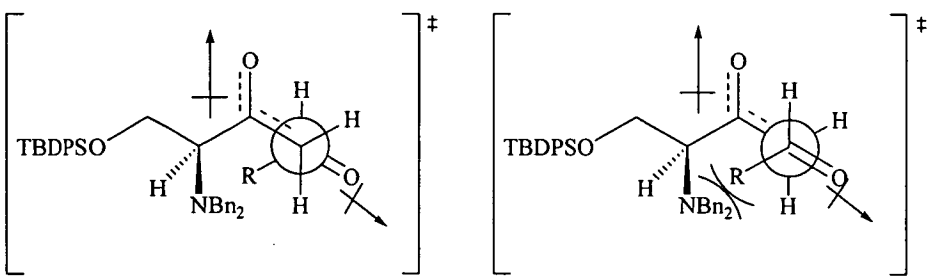



R = Ph, Et, $^i$Pr

(a) $CH_3CO_3H$; (b) $CH_2N_2$.

**Scheme 62:** Liotta's method for establishing the stereochemistry of aldol adducts.[138]

By considering the transition states (as discussed in section 3.4.1) for the threonine-derived ketone **8a** with isovaleraldehyde **150** (**Figure 42**), it can be clearly seen that model **I** is favourable, thus generating *syn* adducts preferably. Hence, on the basis of Liotta's results[138,144,145] assignment of the major diastereoisomer as the *syn* adduct was made.




I II

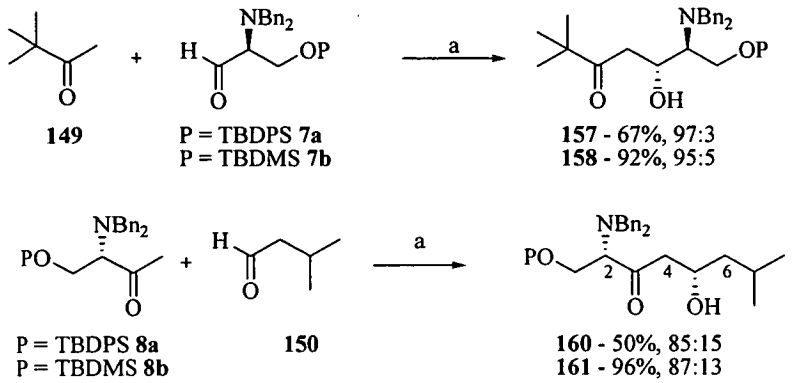
**Figure 42:** Open transition state for aldol reaction of **8a** and isovaleraldehyde **150**.

In summary, the lithium-mediated aldol reactions of threonine-derived ketones **8a** and **8b** and isovaleraldehyde **150** proceeded in good yield and moderate diastereoselectivity. The resulting *syn*-selectivity was explained by the open transition state model proposed by Liotta's group.[138,145] The two different hydroxyl protecting groups that were used (TBDPS and TBDMS) did not noticeably affect the diastereoselectivity of the aldol reaction products **160** and **161**.

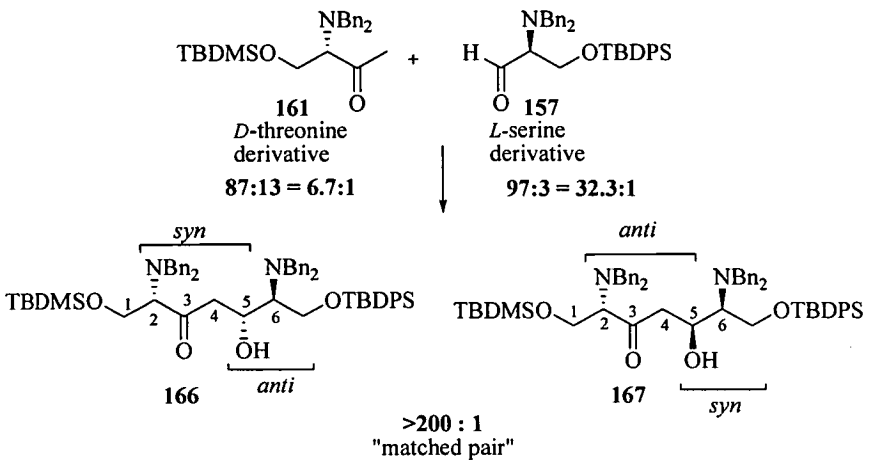
Since Liotta[144] showed that unexpectedly high diastereoselectivity (98:2) was obtained using NaHMDS as a base, some investigations were carried out in this direction. However, this investigation was carried out before it was noticed that storage of the aldol adducts—even under reduced temperatures (fridge)—leads to epimerisation of the *N,N*-dibenzylamino centre adjacent to the ketone group. So, the diastereoselectivity of the resultant aldols observed was 50:50–55:45 with only moderate yields (40–82%). Time constraints did not permit a re-evaluation of this attempted enolate methodology at a later stage.

### 3.5.4. Aldol reactions of threonine-derived ketone 8b and serine-derived aldehyde 7b.

As has been discussed in section 3.2, if both chiral reactants favour the same product, the diastereofacial selectivity increases (i.e., a "matched" pair), whereas if both chiral reactants favour different products, the diastereofacial selectivity decreases (i.e., a "mismatched" pair). Based on the results discussed in sections 3.4.2 and 3.4.3, both the serine-derived aldehydes **7a** and **7b** and the threonine-derived ketones **8a** and **8b** are in favour of the same products (**Scheme 63**). Thus, according to Masamune,[92] this suggests that the aldol reaction between these two chiral compounds would go with an increase of selectivity up to >200 : 1 (**Scheme 64**).

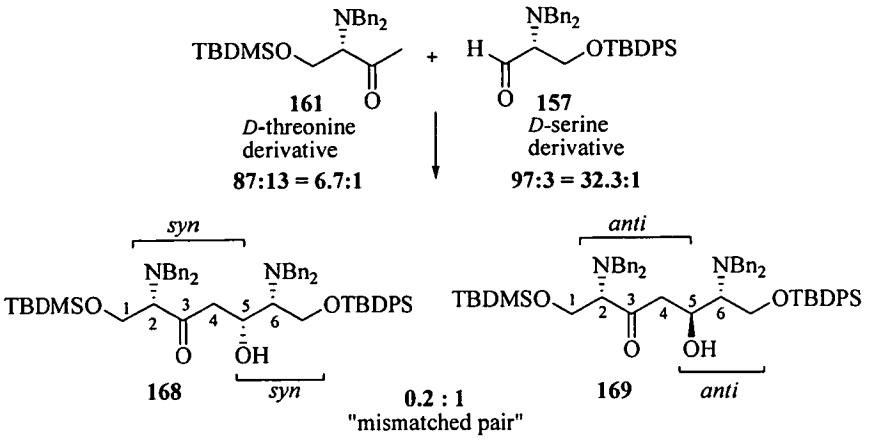



**Scheme 63:** Summary of the aldol reactions of serine and threonine.



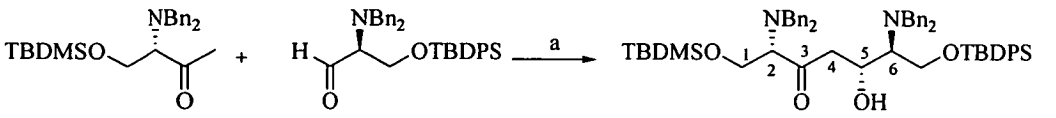

**Scheme 64:** "Matched" pair in a double asymmetric aldol reaction.

However, if *D*-serine is substituted in place of *L*-serine, the diastereoselectivity would decrease to 0.2 : 1 (**Scheme 65**).




**Scheme 65:** "Mismatched" pair in a double asymmetric aldol reaction.

Having these expectations in mind, further investigations were carried out with the aldol reaction of threonine-derived ketone **8b** and serine-derived aldehyde **7a** as shown in **Scheme 66**. Such protecting groups were chosen due to the order of the subsequent deprotection reactions. As can be seen from the retrosynthetic analysis (section 3.1, **Scheme 31**) the protecting group of the threonine-derived ketone has to be removed selectively, while not affecting the protecting group of the serine-derived aldehyde. It is known that deprotection of TBDMS requires milder reaction conditions and a shorter reaction time. Hence the choice of this particular combination of reactants.[116] When the lithium enolate aldol reaction conditions (section 3.4.2) were applied, no product was observed by t.l.c. even after 1.5 hours. The reaction was quenched anyway, and $^1$H NMR showed two unreacted starting materials **8b** and **7a**. To rule out the possibility that enolisation did not occur, a second attempt was carried out. This time again after 1.5 hours, only starting materials **8b** and **7a** were observed by t.l.c. Isovaleraldehyde **150** was added and the t.l.c. spot of the threonine derived ketone **8b** disappeared in 10 minutes, proving that enolisation of the ketone had been successfully accomplished. A new spot corresponding to **161** was observed. Several different reaction conditions were applied (**Table 7**), but unfortunately, none of them led to formation of the desired product **166**.




**8b** **7a** **166**

(a) (i) LiHMDS, THF, –78 °C, 1.5 h; (ii) **7a**, –78 °C, 0.5 h.

**Scheme 66:** Lithium enolate aldol reaction of **8b** and **7a**.

| Entry | Enolisation conditions | Reaction conditions | Result |
|---|---|---|---|
| 1 | **8b**, LiHMDS, THF, –78 °C, 1 h | **7a**, –78 °C, 1.5 h | no product **166** formed |
| 2 | | **7a**, –78 °C, 1.5 h, then isovaleraldehyde **150**, 10 min | aldol adduct **161** and unreacted **7a** |
| 3 | | **7a**, –78 °C, 1.5 h, then –30 °C for 1.5h | no product **166** formed |
| 4 | | **7a**, –78 °C, 1.5 h, then 0 °C for 1.5h | no product **166** formed |

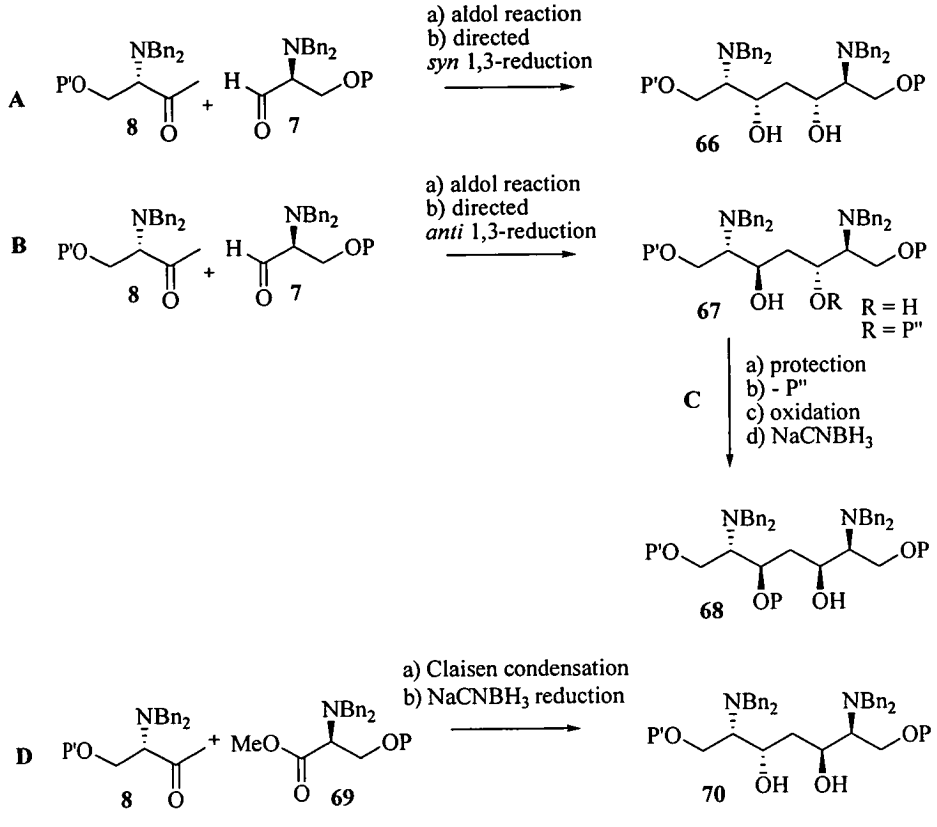
**Table 7:** Reaction conditions applied for aldol reaction of **8b** and **7a**.

Such low reactivity between **8b** and **7a** can be explained by the extreme steric hindrance, since four large protecting groups ($Bn_2$ × 2, TBDPS and TBDMS) are present in the starting materials. Unfortunately, due to a shortage of time, no further reaction conditions were applied to obtain **166**.

### 3.6. Directed 1,3-reductions.

As discussed in section 3.1, since the stereochemistries of C(11) and C(13) are still unknown, the synthesis of all four possible diastereoisomers was considered as shown in **Scheme 67**. During our investigation, the synthesis of two out of four diastereoisomers

was studied. Different conditions for *syn* and *anti* reductions were applied for the serine- and threonine-derived aldol adducts as shown in **Scheme 70** and **78,** and **Scheme 72** and **81.**




**Scheme 67:** Four possible diastereoisomers produced from the reaction of threonine **8** and serine **7** derivatives.

### 3.6.1. Directed *anti* 1,3-reduction.

*Anti* reduction was performed under tetramethylammonium triacetoxyborohydride-mediated and Evans–Tishchenko conditions. $Me_4NBH(OAc)_3$ reduction is a well-known procedure for directed hydride reduction and has a wide application in modern synthetic

chemistry: for example, synthesis of bryostatin 2 **170**,[150] superstolide A **171**,[148] and (+)-discodermolide **172**[151] (**Figure 43**).

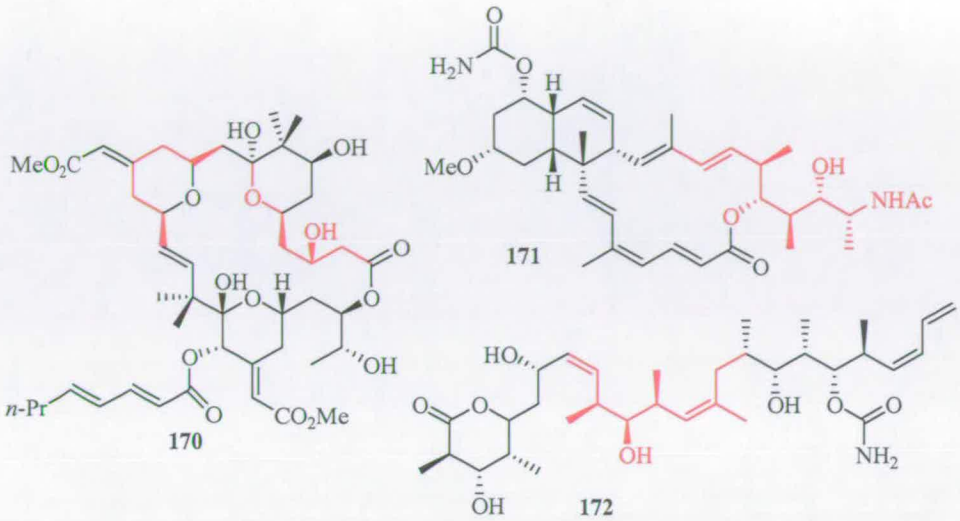
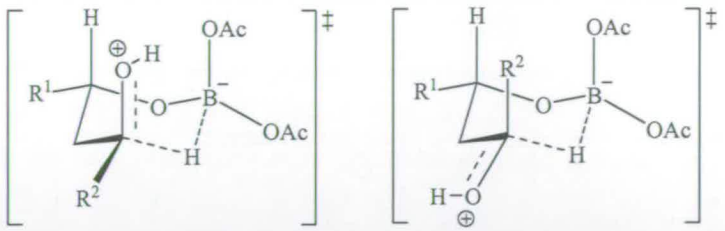



**Figure 43:** Structure of bryostatin 2 **170**,[150] superstolide A **171**,[148] (+)-discodermolide **172**.[151]

The formation of the *anti* diol has been explained through chair-like transition states (**Figure 44**).[152] According to Evans *et al.*, the mechanism of this reaction involves an acid-promoted ligand exchange of acetate for alcohol by the triacetoxyborohydride anion. The resultant hydride intermediate (probably an alkoxydiacetoxyborohydride) reduces the proximal ketone by intramolecular hydride delivery. Transition states **I** and **II** exist in competition. However, **I** is favourable, since 1,3-diaxial interactions between $R^2$ and OAc would destabilize **II** to a greater extent than 1,3-diaxial interactions between $HO^+$ and OAc in **I**.

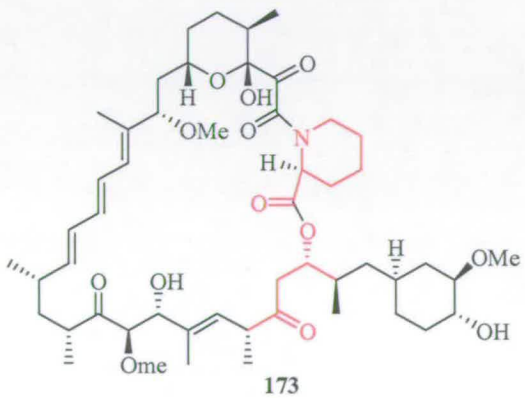


*anti* reduction *syn* reduction

I II

**Figure 44:** Transition states for $Me_4NBH(OAc)_3$ reduction.[152]

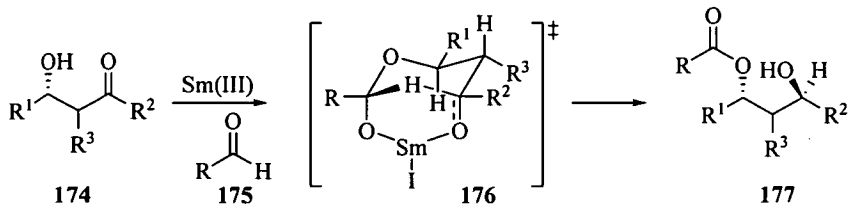
The second approach employed was the Evans–Tishchenko reaction, which is known to be an efficient method for the *anti* reduction of β-hydroxy ketones. This reaction has been widely used in the synthesis of natural products: for example, Schreiber's synthesis of rapamycin **173** (**Figure 45**).[153]



**173**

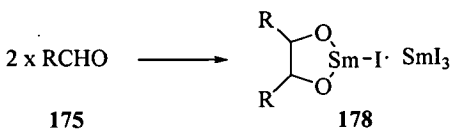
**Figure 45:** Structure of rapamycin **173**.[153]

The samarium-catalysed intramolecular Tishchenko reduction of β-hydroxy ketones was discovered in 1990 by Evans.[154] The reaction involves the coupling of ketone **174** and aldehyde **175** to afford the *anti* diol monoester **177** (**Scheme 68**) in high yields (85-90%) and with excellent level of stereochemical control (>99:1).[154]




**Scheme 68:** Intermolecular Evans–Tishchenko reaction.[154]

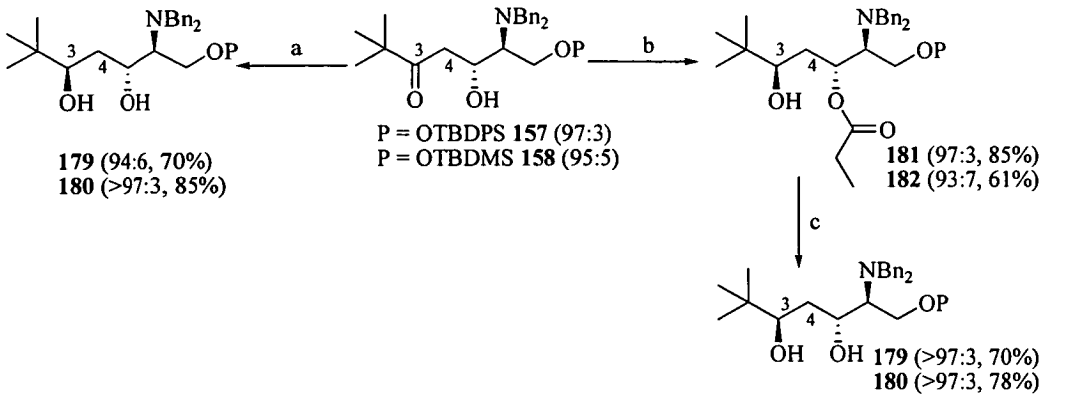
The mechanism proposed by Evans involves intramolecular hydride transfer from an intermediate hemiacetal *via* a transition state **176** as shown in **Scheme 68.**[154] It believed that the active catalyst in the Evans–Tishchenko reaction is a $SmI_3$•$SmI(RCHO)_2$ pinacol adduct **178**, which can be pre-formed or generated *in situ.* The formation of the pinacol adduct may be observed by a colour change. $SmI_2$ is a very dark blue solution; when it is added to aldehyde (usually acetaldehyde, propionaldehyde, or benzaldehyde), a Sm(III) pinacol adduct is formed and this process can be seen by the change of the colour to yellow (**Scheme 69**).[154]




**Scheme 69:** Proposed active Sm(III) pinacol adduct catalyst **178.**[154]

### 3.6.2. Directed *anti* 1,3-reductions of serine-derived aldol adducts 157 and 158.

Experimental conditions optimised by Evans *et al.*[150,152] ($Me_4NBH(OAc)_3$ (4.9 eq), $CH_3CN$:$CH_3COOH$—1:1, –40 °C) gave rise to the *anti* diol **179** in 70% yield with 94:6 selectivity, and to the *anti* diol **180** in 85% yield with selectivity >97:3, respectively (**Scheme 70**).




(a) $Me_4NBH(OAc)_3$, $CH_3CN$, $CH_3COOH$, –40 °C, 18 h; (b) propionaldehyde, $SmI_2$ (0.1 M in THF), THF, –10 °C, 1.5 h; (c) $K_2CO_3$, MeOH, $H_2O$, r.t., 16 h.

**Scheme 70:** 1,3-*anti* reductions of **157** and **158**.

The reactions were monitored by t.l.c. and NMR. The appearance of the C(3)*H* peak in the $^1H$ NMR at 3.44 ppm for **179** and 3.29 ppm for **180**, and the slight upfield shift of C(4)$H_2$ in the 1.83–1.37 ppm region, clearly showed the formation of a 1,3-diol. Additionally, the reaction can be monitored by disappearance of the quaternary ketone carbon in the $^{13}C$ NMR spectra. The diastereoselectivity of **179** was measured using the integral ratio of the diagnostic peaks (4H, m, Ar*H*). In the case of **180** no other diastereoisomer was observed by $^1H$ NMR.

The Evans–Tishchenko reactions for both β-hydroxyketones **157** and **158** proceeded in good yields and with high diastereoselectivity (**Scheme 70**). The reactions were monitored in the same way as described above. Additionally, the appearance of a $CH_3$ peak as a triplet at 0.97 ppm for **181** and 1.10 ppm for **182**, and appearance of a $CH_2$ peak as a multiplet at 2.15 ppm for **181** and 2.27 ppm for **182** in the $^1H$ NMR indicates the formation of desired products.

The hydrolysis of **181** and **182** proceeded in good yield with excellent selectivity to afford 1,3-diols **179** and **180**.

The *anti* relative configuration of 1,3-diols **179** and **180** was proved using Rychnovsky's [$^{13}C$] acetonide method.[155,156] He proposed that 1,3-diols could be converted into their acetonides and their stereochemistry assigned from the $^{13}C$ chemical

shifts of the acetal methyl groups and acetal carbon. This method relies on the conformational properties of the corresponding 1,3-diol acetonides. It is known that *syn*-1,3-diol acetonide exists in a chair conformation **183**, in which the C(4) and C(6) alkyl substituents are in an equatorial position, one of the acetal methyl groups is in an axial position, and the other is in an equatorial position (**Figure 45**). The axial methyl group has a $^{13}C$ chemical shift of approximately 20 ppm, whereas the equatorial methyl group has shift of approximately 30 ppm.[156,157]

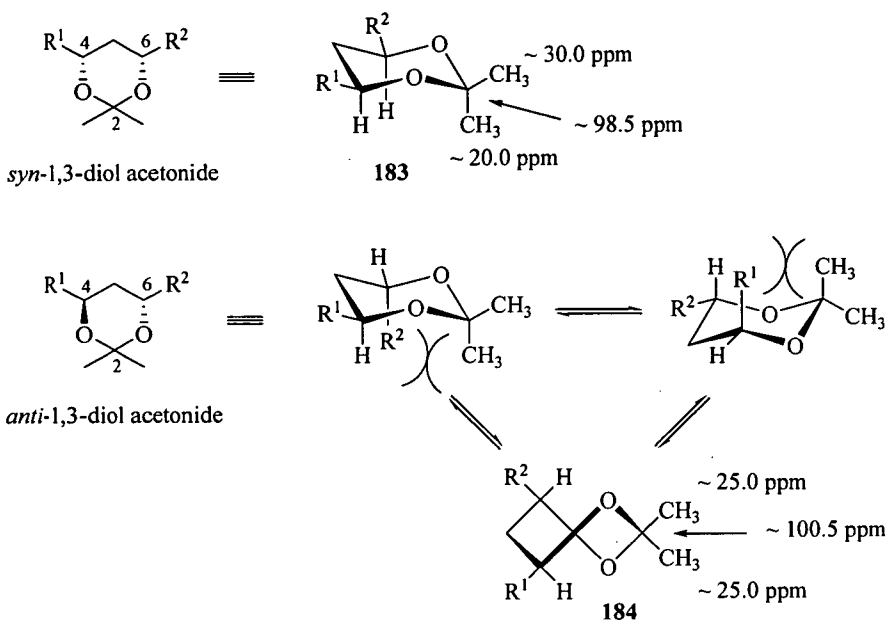
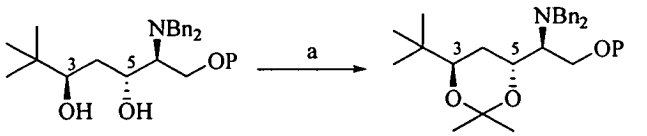



**Figure 45:** Conformations and $^{13}C$ NMR chemical shift correlations for *syn*- and *anti*-1,3-diol acetonides.[156,157]

An *anti*-acetonide, on the other hand, exists in a twist-boat conformation **184** in order to avoid 1,3-diaxial interactions which would be present in a chair conformation (**Figure 45**). In this twist-boat conformation, two acetal methyl groups are in practically identical environments and both have the same $^{13}C$ chemical shift of approximately 25 ppm.[156,157] Additionally, the quaternary acetal carbon of *syn*-1,3-diol acetonide has a $^{13}C$ chemical shift at approximately 98.5 ppm, while the quaternary acetal carbon of *anti*-

1,3-diol acetonide shifts at approximately 100.5 ppm.[157]

The acetonation of **179** and **180** under standard conditions proceeded in moderate to good yields affording the *anti*-3,5-diol acetonides **185** and **186** as shown in **Scheme 71.**




P = OTBDPS **179**
P = OTBDMS **180**

P = OTBDPS **179A** (52%)
P = OTBDMS **180A** (80%)

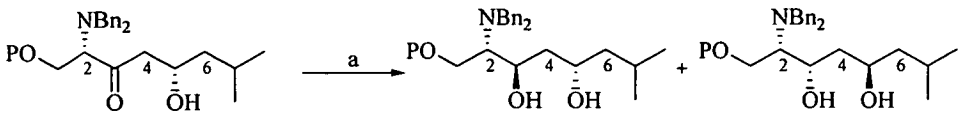
(a) $(MeO)_2CMe_2$, CSA, DCM, r.t., 20 h.

**Scheme 71:** Formation of **179** and **180** *anti*-3,5-diol acetonides.

In the case of **179A**, the $^{13}C$ NMR chemical shift for the quaternary acetal carbon was 99.9 ppm, whereas the acetal methyl groups came at 24.4 ppm and 24.1 ppm. In the case of **180A**, the quaternary acetal carbon had the same shift (99.9 ppm) and the acetal methyl groups came at 24.5 ppm and 24.1 ppm. These results suggest that both tetramethylammonium triacetoxyborohydride-mediated and Evans–Tishchenko conditions gave rise to *anti* 1,3-diols **179** and **180.**

### 3.6.3. Directed *anti* 3,5-reductions of threonine-derived aldol adducts 160 and 161.

The same conditions as described in section 3.6.2 were applied for the tetramethylammonium triacetoxyborohydride-mediated reduction and Evans–Tishchenko reaction of **160** and **161**. $Me_4NBH(OAc)_3$ reductions proceeded affording **185** and **186** in poor yields (19% and 45%, respectively) with moderate diastereoselectivity (**Scheme 72**). Crude aldol adducts **160** and **161** were utilised in the reduction reactions without any purification, which suggests the presence of lithium salt in the reaction mixture. This possibly affected the reaction outcome by reducing the yields.




P = OTBDPS **160a:160b** (85:15)
P = OTBDMS **161a:161b** (87:13)

**185a:185b** (82:18, 19%)
**186a:185b** (85:15, 45%)

(a) $Me_4NBH(OAc)_3$, $CH_3CN$, $CH_3COOH$, -40 °C, 18 h.

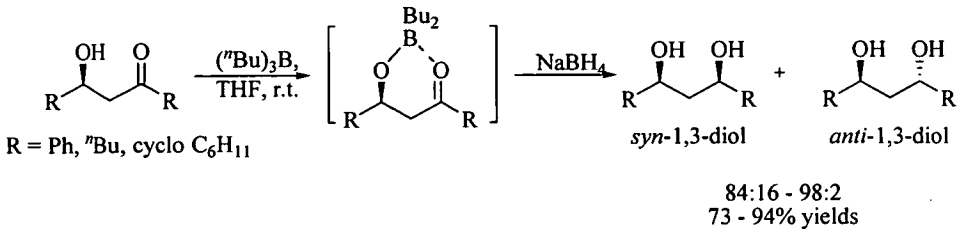
**Scheme 72:** 3,5-*anti* reductions of **160** and **161**.

The reactions were monitored by t.l.c. and NMR. The appearance of the C(3)*H* peak as triple of doublets in the $^1H$ NMR at 4.30 ppm for **185** and 4.23 ppm for **186**, and the slight upfield shift of C(4)$H_2$ in the 1.83–1.58 ppm region, clearly showed the formation of a 3,5-diol. Additionally, the reaction can be monitored by disappearance of the quaternary ketone carbon in the $^{13}C$ NMR spectra. The diastereoselectivity of **185** and **186** was measured using the integral ratio of the diagnostic peaks (2H, d, $NCH_XH_YPh$).

The 3,5-*anti* relative selectivity was assigned based on literature precedents[152,154] and on the results discussed in section 3.6.2 for the serine aldehyde series.

### 3.6.4. Directed *syn* 1,3-reduction.

The *syn* 1,3-reduction was carried out under sodium borohydride and lithium aluminium hydride conditions. Narasaka *et al.* reported the *syn* stereoselective reduction of β-hydroxyketones *via* boron chelates.[158] They suggested that the formation of a chelate complex between a of β-hydroxyketones and boron compounds, such as tributylborane or triisobutylborane, along with a catalytic amount of activator such as air, would control the approach of the reducing agent (sodium borohydride). In fact, the experiments carried-out proceeded in high yields with excellent *syn* diastereoselectivities (**Scheme 73**).




**Scheme 73:** Narasaka *et al.* results for *syn* 1,3-reductions.[158]

The *syn* selectivity was explained *via* a chair-like transition state **187** as shown in **Figure 46**.[158] It has been suggested that the dibutylboronic ester of the β-hydroxyketone exists in a transition state **187** and that the axial H of the α-C prevents the approach of a reducing agent from the bottom side. Hence, sodium borohydride preferably attacks from top side leading to the formation of *syn*-isomer.[158]

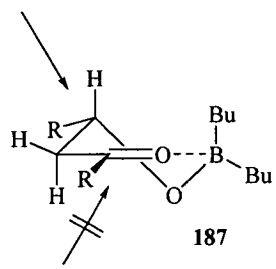
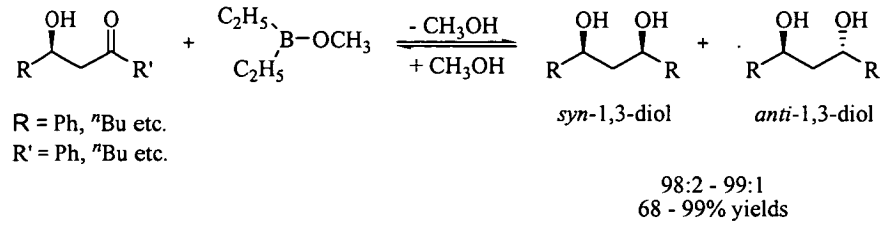



**Figure 46:** Proposed chair-like transition state.[158,159]

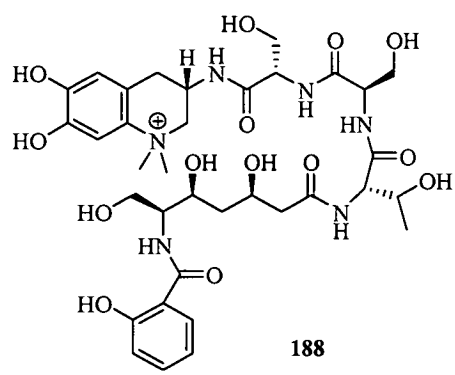
Later Prasad *et al.* reported their studies on *syn* 1,3-reduction of β-hydroxyketones using sodium borohydride.[160] This group suggested that alkoxydialkylboranes could interact directly with hydroxyketones without any additional air or acid activation, forming the boron chelate intermediate, which was then reduced to afford the desired *syn* diols (**Scheme 74**).




**Scheme 74:** Results of Prasad *et al.* for *syn* 1,3-reductions.[160]

The conditions proposed by Prasad *et al.* produced *syn* 1,3-diols with a slightly higher selectivity in comparison with Narasaka's results. The same chair-like transition state **187** was proposed in this case (**Figure 46**).[160]

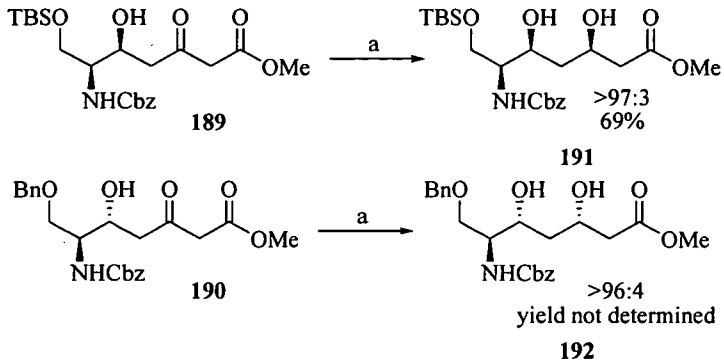
In 2004, Gademann and Bethuel applied modified sodium borohydride reduction conditions in the total synthesis of anachelin H **188** (**Figure 47**).[161]




**188**

**Figure 47:** Structure of anachelin H.[161]

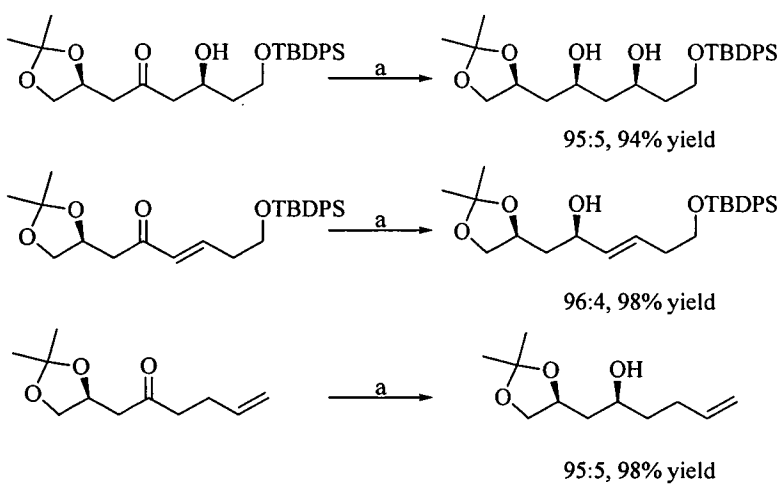
They suggested precomplexation of the substrate with $Et_2BOMe$, which is preformed *in situ* from triethylborane, pivalic acid and methanol. Then the boron chelate intermediate generated was exposed to sodium borohydride. It was found that the addition of pivalic acid was crucial for a successful and selective reaction. The conditions and the results of the performed reaction are shown in **Scheme 75**.[161]




(a) (i) $Et_3B$, pivalic acid, THF, MeOH, r.t., 1 h; (ii) **189** or **190**, THF, $NaBH_4$, - 70 °C, 4 h.

**Scheme 75:** *syn* 1,3-reductions of **189** and **190**.[161]

Lithium aluminium hydride asymmetric 1,3-reduction was carried out, based on the Suzuki *et al.* report.[162,163] This group showed that lithium aluminium hydride in the presence of lithium iodide in ether at –100 °C resulted in the formation of *syn*-diols with excellent selectivity (**Scheme 76**).

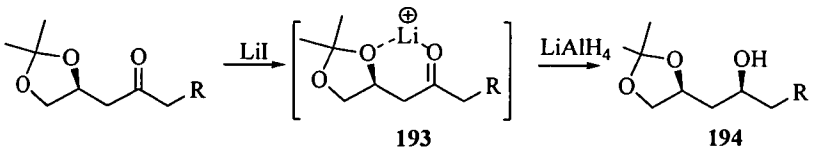



(a) (i) alkoxy ketone, LiI, $Et_2O$, r.t., than –40 °C, 5 min; (ii) $LiAlH_4$, –100 °C, 30 min.

**Scheme 76:** Lithium aluminium hydride *syn* 1,3-reduction.[162]

According to Suzuki *et al.*, the high *syn*-selectivity for 1,3-diols arose from β-chelation of both the ketone and ether oxygen with the lithium cation to form an

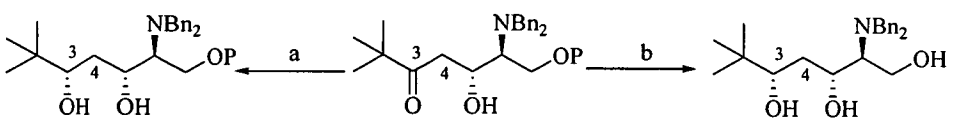
intermediate complex **193** (**Scheme 77**). This locks the conformation of the β-alkoxy ketone chain, and the hydride then attacks from less hindered side, affording the *syn* product **194**.[162]




**Scheme 77:** Chelation control of the 1,3-selectivity.[162]

### 3.6.5. Directed *syn* 1,3-reductions of serine-derived aldol adducts 157 and 158.

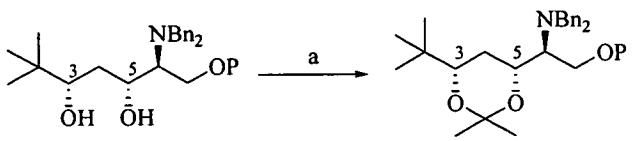
The optimised sodium borohydride reduction conditions[161] were applied to **157** and **158** to afford the desired *syn* 1,3-diols **195** and **196** in good yields and high diastereoselectivity (**Scheme 78**). The reactions were monitored by t.l.c. and $^1$H NMR. The appearance of a C(3)*H* peak as a doublet at 3.50 ppm for **195** and as doublet of doublets at 3.43 ppm for **196**, and the shift of C(4)$H_2$ protons upfield and change of its shape to a doublet with a large coupling constant (*J* 14.5), suggests the formation of the desired products. The diastereoselectivity of **195** and **196** was measured by integration of the C(4)$H_2$ peaks.




(a) (i) $Et_3B$, pivalic acid, THF, MeOH, r.t., 1 h; (ii) **157** or **158**, $NaBH_4$, THF, –70 °C, 1 h; (b) (i) **157**, LiI, THF, –78 °C, 20 min; (ii) $LiAlH_4$, –78 °C, 1.5 h.

**Scheme 78:** 1,3-*syn* reductions of **157** and **158**.

The relative stereochemistry of the 3,5-diol was proved using Rychnovsky's [$^{13}$C] acetonide method, as described in section 3.6.2. The acetonation of **195** and **196** under the standard conditions proceeded in good yields affording the *syn*-3,5-diol acetonides **198** and **199** as shown in **Scheme 79**.




P = OTBDPS **195**
P = OTBDMS**196**

P = OTBDPS **198** (80%)
P = OTBDMS **199** (80%)

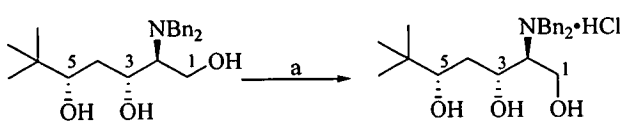
(a) $(MeO)_2CMe_2$, CSA, DCM, r.t., 20 h.

**Scheme 79:** Formation of **198** and **199** *anti*-3,5-diol acetonides.

In the case of **198**, the $^{13}$C NMR chemical shift for the quaternary acetal carbon is 98.1 ppm, whereas the acetal methyl groups come at 29.9 ppm and 19.0 ppm. In the case of **199**, the quaternary acetal carbon has the same shift of 98.5 ppm and the acetal methyl groups come at 30.1 ppm and 20.1 ppm. These results suggest that sodium borohydride reduction conditions gave rise to *syn* 3,5-diols **198** and **199**.

As a result of lithium aluminium hydride reduction of **157,** loss of the TBDPS protecting group was observed and the 1,3,5-triol **197** was isolated in 60% yield with high diastereoselectivity (95:5) (**Scheme 78**).

Compound **197** was converted into its hydrochloride salt in 95% yield (**Scheme 80**) and the crystal structure of **200** was obtained (**Figure 48, Appendix 6**).




**197**

**200** (95%)

(a) **197**, HCl (1 M in $Et_2O$), DCM, r.t., 2 h.

**Scheme 80:** Formation of the 3,5-diol **200**.

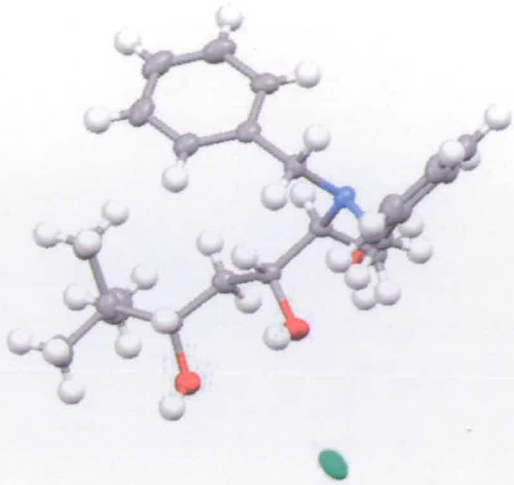
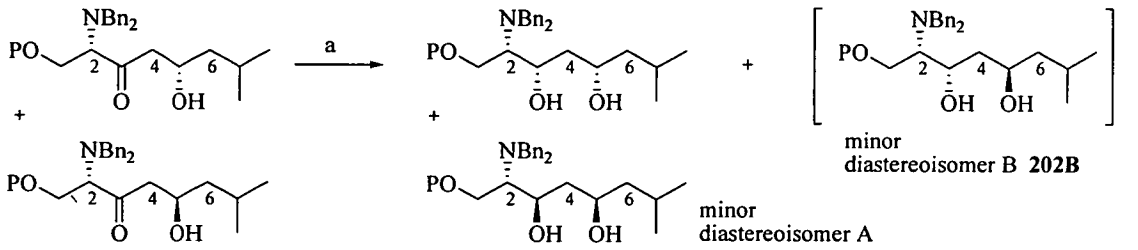


**Figure 48:** Crystal structure of the 3,5-diol **200**.

The relative configuration between C(2)–C(3) as *anti* and between C(3)–C(5) as *syn* is proved by the crystal structure. This information shows that the lithium-mediated aldol reaction between pinacolone **149** and serine-derived aldehydes **7a** and **7b** (section 3.5.2) proceeded *via* the proposed Felkin–Anh transition model (**Figure 41**) to afford the *anti* aldol adducts **157** and **158**. Additionally, it proves that the lithium aluminium hydride reduction gave rise to the 3,5-*syn* diol **197**.

### 3.6.6. Directed *syn* 1,3-reductions of threonine-derived aldol adducts 160 and 161.

The same sodium borohydride conditions as described in section 3.6.5 were applied for **160a/b** and **161a/b**. *Syn* reductions proceeded in reasonable yields (40% and 29%, respectively) with good diastereoselectivity affording **201a/b** and **202a/b** (**Scheme 81**).




P = OTBDPS **160a:160b** (85:15) **201a:201b** (58:42, 40%)
P = OTBDMS **161a:161b** (87:13) **202a:202b:202B** (50:25:25, 29%)

(a) (i) $Et_3B$, pivalic acid, THF, MeOH, r.t., 1 h; (ii) **160** or **161**, $NaBH_4$, THF, -70 °C, 1 h.

**Scheme 81:** 1,3-*syn* reductions of **160** and **161**.

The reactions were monitored by t.l.c and NMR. The slight upfield shift of C(4)$H_2$ in the 1.83-1.58 ppm region, clearly showed the formation of a 3,5-diol. Additionally, the reaction can be monitored by disappearance of the quaternary ketone carbon in the $^{13}C$ NMR spectra.

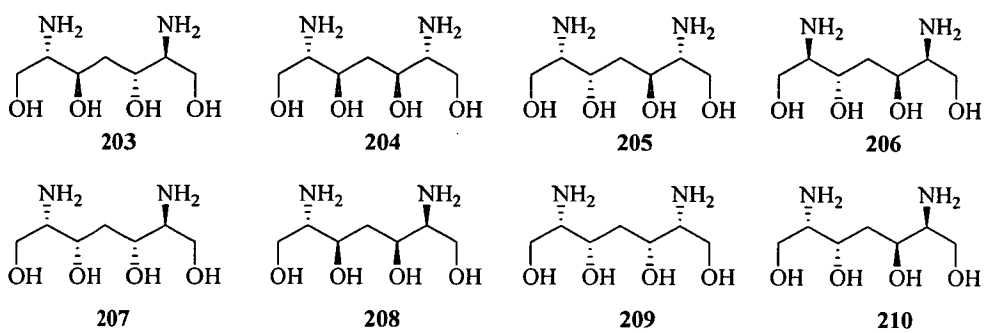
In the case of reduction of the TBDMS-protected adduct **161**, some unreacted starting material (33%) was recovered together with two additional fractions: major product **202a** and a mixture of two minor diastereoisomers, A and B, assigned as **202b** and **202B**. In the case of reduction of the TBDPS-protected adduct **160**, an inseparable mixture of major product **201a** and minor diastereoisomer A (**201b**) was recovered. These results suggest that kinetic resolution has been observed; the minor aldol diastereoisomer appears to have reacted more rapidly hence the ratio between diastereoisomers is approximately 50:50 (or 50:25:25 in case of **202**). The presence of an additional reduction product from the minor aldol diastereoisomer in **161** indicates that as well as reduction of the chelated intermediate there is also significant reduction of the free β-hydroxy ketone, which would give minor diastereoisomer B as a result of Felkin-Anh control. The absence of minor diastereoisomer B in reduction of TBDPS-protected aldol adduct **160** supports the hypothesis that this is an anomalous result. The identity of minor diastereoisomer B as **202B** was confirmed by the correlation within 0.1 ppm of each peak in its $^{13}C$ NMR spectrum with that observed for the 3,5-*anti* reduction

of the minor aldol diastereomer **161b**. It was suggested that if this reaction was allowed to proceed to completion, the starting ratio of approximately 85:15 would be expected.

The 3,5-*syn* relative selectivity was assigned based on literature precedent[152,154] and the $^{13}C$ NMR analysis discussed in section 3.6.5.

## 3.7. Proposed assignment of the relative stereochemistry of zwittermicin A.

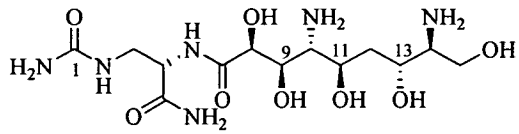
Recently Rogers and Molinski[164] have carried out investigations in order to establish the relative configuration of the C(11) and C(13) stereocentres of zwittermicin A **1**. During their investigations, six out of eight possible diastereoisomers of the C(9)–C(15) fragment of zwittermicin A **1** were synthesised (**Scheme 82**). Two of these model compounds are *meso* compounds **204** and **209**, two are $C_2$ isomers **203** and **210** and two have lack of symmetry **205** and **207**.




**Scheme 82:** Eight possible diastereoisomers of C(9)–C(15) fragment.[164]

Assignment of the relative configuration for the diaminotetraol segment in zwittermicin A **1** was made by pairwise comparisons of the differences in the $^{13}C$ chemical shifts for C(10)–C(15) of **1** and model compounds. Since only six diastereoisomers were synthesised, $^{13}C$ shifts for **206** and **208** were obtained by reverse of the shifts for **205** and **207**, respectively. It was found that the model **203** was the only

compound with a close match to **1** for every carbon, except C(9), which is due to the difference between the model and natural product structures.[164] The structure of zwittermicin A proposed by Rogers is shown in **Figure 49**.



**1**

**Figure 49:** Proposed structure of zwittermicin A **1**.[164]

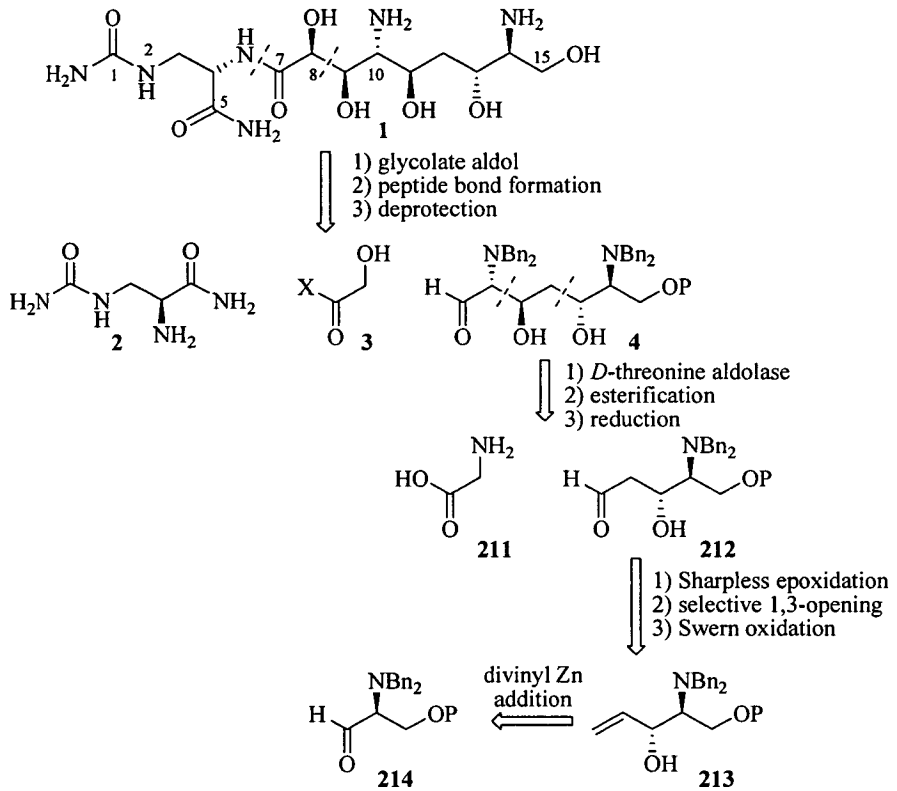
This analysis suggests that, based on the studies accomplished (sections 3.1-3.6), suitable conditions for the synthesis of the C(9)–C(15) moiety with the right diastereochemistry have been found.

### 3.8. Conclusion.

The aldol reactions of serine-derived aldehydes **7a** and **7b** and threonine-derived ketones **8a** and **8b** with achiral pinacolone **149** and isovaleraldehyde **150** were studied. The resultant diastereoselectivity of both sets of aldols was explained. The relative configurations of reduced 3,5-diol **200** were determined from its crystal structure. Several attempts to perform the double asymmetric aldol reaction between serine-derived aldehydes **7a** and **7b** and threonine-derived ketones **8a** and **8b** were made. Application of known *syn* 1,3-reduction and *anti* 1,3-reduction conditions gave the desired products in good yield with moderate to high diastereoselectivity. All of the above results suggest that the synthesis of the C(9)–C(15) fragment of zwittermicin A **1** can be achieved with the correct relative stereochemistry using this strategy.

## Chapter 4: Future work.

Since several attempts to perform the double asymmetric aldol reactions between serine-derived aldehydes **7a** and **7b** and threonine-derived ketones **8a** and **8b** failed so far, an alternative synthetic route was considered, as shown in **Scheme 83**.

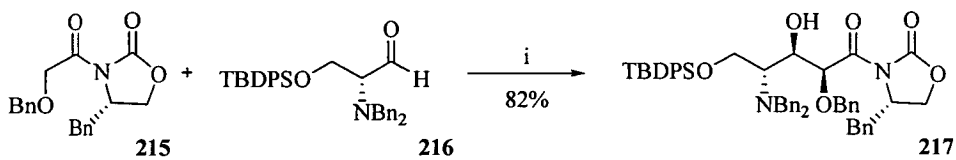



**Scheme 83:** Second proposed retrosynthetic analysis.

We proposed to use *N,N*-dibenzylamino *L*-serinal **214** as the starting material. Diastereoselective addition of the divinyl zinc to **214** would afford the compound **213**. According to literature precedents, the reaction would go through the Felkin–Ahn open transition state in favour of the 1,2-*anti* adduct.[112,165] Then it is proposed to employ this

aminodiol **213** in a Sharpless asymmetric epoxidation,[166,167] followed by selective 1,3-opening and oxidation of the primary alcohol, for example utilising Swern oxidation conditions. The enzyme-catalysed reaction between glycine **211** and the amino-dihydroxy aldehyde **212** obtained from this synthetic sequence could be performed next using a *D*-threonine aldolase to achieve the *anti* selectivity,[168,169] followed by esterification and reduction to afford the aldehyde (**4**).

To accomplish the synthesis of the C(9)–C(15) fragment of zwittermicin A **1**, the glycolate aldol reaction between compounds **3** and **4** is proposed. The application of the Evans oxazolidinone **215** for the boron-mediated glycolate aldol reaction has been extensively utilised in the Hulme group, allowing the formation of *syn* adducts in good yields and excellent selectivity (**Scheme 84**).[98,100,170]

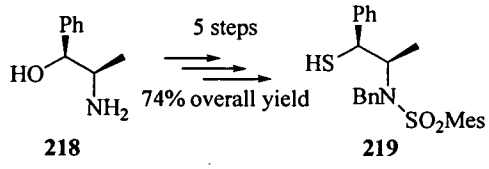



(i) $Et_3N$, $^nBu_2BOTf$, DCM, –78 °C → 0 °C, 3 h; **216**, DCM, –78 °C → 0 °C, 2.5 h.

**Scheme 84:** Previous application of the glycolate aldol in the Hulme group.[98]

Peptide bond formation between the nitrogen-rich fragment **2** and glycolate adduct could be synthesised using standard coupling agents, such as EDCI, DCC or DIC.

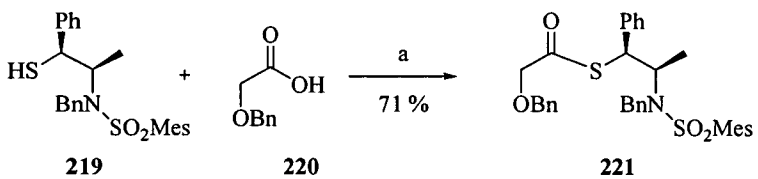
Alternatively, we proposed to use the new thiol auxiliary developed recently in the Hulme group (**Scheme 85**).[171] The auxiliary **219** can be synthesised in five steps from (1*S*,2*R*)-(+)-norephedrine **218** in 74% overall yield.




218 219

**Scheme 85:** Five-step synthesis towards the new thiol auxiliary **219**.[171]

Some investigations toward the utilization of the auxiliary **219** for the glycolate aldol reaction have been carried out by Fanjul.[172] She showed that coupling of benzylprotected glycolic acid to the auxiliary **219** can be performed using standard EDCI coupling conditions in 71% yield (**Scheme 86**).

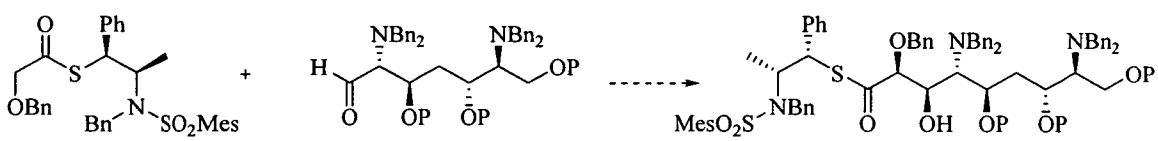



219 220 221

(a) EDCI, DMAP, DCM, r.t., 1.5 h.

**Scheme 86:** Formation of the glycolate aldol substrate.[172]

Boron-mediated glycolate aldol reaction of **221** with a range of aldehydes proceeded in good yields (55–86%) and with the formation of the *syn* diastereoisomer.[172]
Based on these investigations we propose to carry out the glycolate aldol reaction between glycolate substrate **221** and aldehyde **4** (**Scheme 87**).




221 4 222

**Scheme 87:** Proposed route to the glycolate adduct **222**.

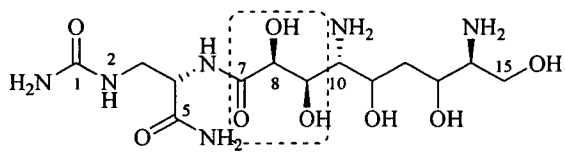
This reaction could be followed by hydrolysis to form the free carboxylic acid followed by peptide bond formation with the nitrogen rich fragment **2** as discussed above.

# Chapter 5: Results and Discussion 3.

# The use of high pressure conditions for enantioselective organocatalytic aldol reactions.

## 5.1. Introduction.

As has been discussed before, we believe that the highlighted diol moiety of zwittermicin A **1** could be synthesised *via* a stereoselective glycolate aldol reaction.



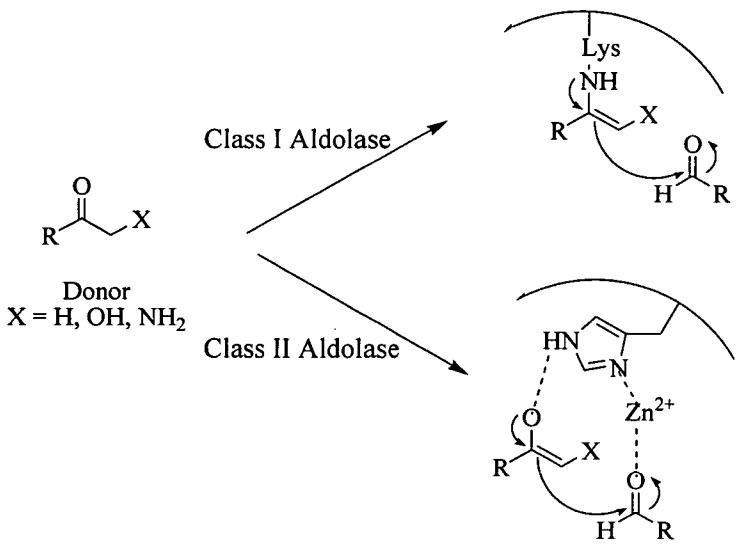
**1**

### 5.1.1. Catalytic aldol reactions.

The catalytic asymmetric aldol reaction as a fundamental C–C bond forming reaction remains—today—one of the fundamental reactions in chemistry[173-175] and biology.[176] Chemically, these reactions usually employ a pre-formed enolate and a chiral transition metal–based catalyst.[177,178] However, these reactions often suffer from problems in chemo- and regio- selectivity. In nature, however, enzymes catalyse the direct aldolisation of two unmodified carbonyl compounds. Two classes of enzymes are known to be involved in the aldol reaction: Class I and Class II. Class I aldolases utilise an enamine based mechanism, while Class II aldolases mediate the reaction by using a zinc cofactor (**Scheme 88**).[178,179] Many of these enzymes can perform aldol reactions with absolute stereocontrol.

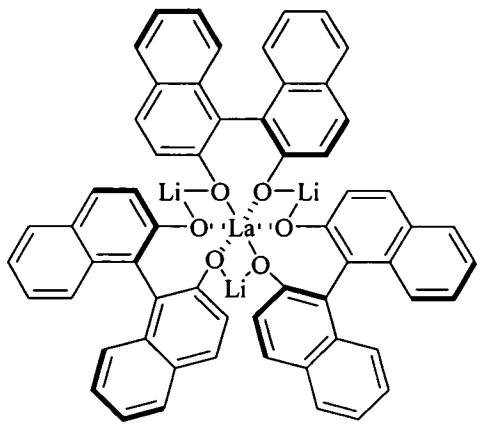
For many years, controlling the stereoselectivity of aldol reaction has been an

ongoing challenge for the synthetic chemists. A variety of different strategies has been applied, including the use of chiral starting materials and chiral auxiliaries.[180,181] However, the ideal solution for the controlled induction of stereochemistry into a molecule would be to find a compound that would catalyse the direct aldol reaction asymmetrically and would not require the pre-generation of enolates or enolate-like species.

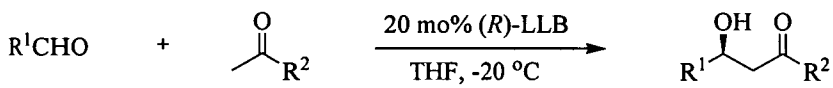



**Scheme 88:** Two Classes of Aldolases.[179]

The first molecules developed to catalyse the aldol reaction were mimics of the metal-containing Class II aldolases, utilising the metal in an organisational role to transfer chiral information and to activate the reagents.[182] The first example of such catalyst was reported in 1997 by Shibasaki *et al.*,[183] who developed a direct, catalytic asymmetric aldol reaction between a range of aldehydes and unmodified ketones using 20 mol% of the catalyst (*R*)-LLB (**Scheme 89**). The reaction affords aldol adducts in good to excellent yields (53–90%), and with moderate to high selectivity (44–94% *ee*).



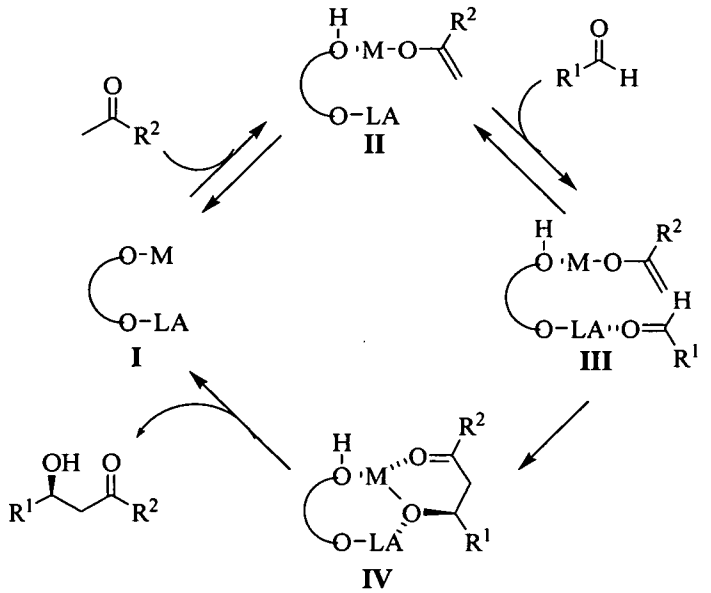

**(*R*)-LLB**




$R^1$ = $^t$Bu, $^i$Pr, cyclohexyl, $Ph(CH_2)_2$
$R^2$ = Ph, 1-naphthyl, Me, Et

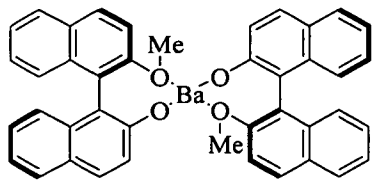
**Scheme 89:** Direct asymmetric aldol reactions catalysed by (*R*)-LLB.

The catalyst contains a central lanthanum atom, which acts as a Lewis acid, and a lithium binaphthoxide moiety, which acts as a Brønsted base. This joint action of both Lewis acid and Brønsted base makes the asymmetric aldol reaction possible without the need for any other activation of the starting material. The proposed mechanism (**Scheme 90**) starts with deprotonation of an α-proton of the ketone by the Brønsted base unit (OM) of catalyst **I** to generate the metal enolate **II**, while at the same time a Lewis acid unit (LA) could activate an aldehyde to give **III**. These two activated species react in the chelation-controlled, asymmetric environment to afford a metal β-oxoalkoxide **IV**. An optically active aldol adduct could then be generated by proton exchange, with full regeneration of the catalyst **I**.[180]

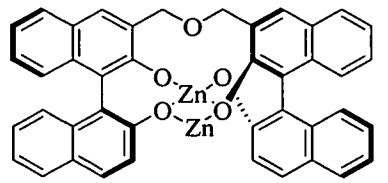


**Scheme 90:** Proposed mechanism for direct aldol reaction with (*R*)-LLB.

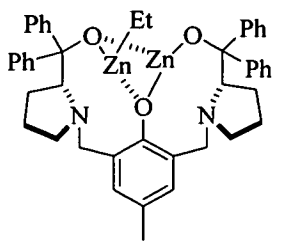
Different modifications of the catalyst (*R*)-LLB have been developed: such as (*R*)-BaB-M **223**, which produces *anti* aldol adducts in excellent yields (77–99%), but unfortunately generates only rather modest enantioselectivities (50–70% *ee*);[184] (*S,S*)-Zn-Zn-linked BINOL **224**, which works in a *syn*-selective manner (*syn:anti* up to 97:3) and in excellent yields (up to 95%) and enantiomeric excess up to 99% *ee*;[185,186] and a recently developed catalyst **225**, which gives aldol adducts in poor to modest yield (24–79%) and in good to excellent selectivities (up to 99% *ee*).[181]



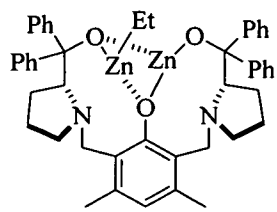

223




224




225

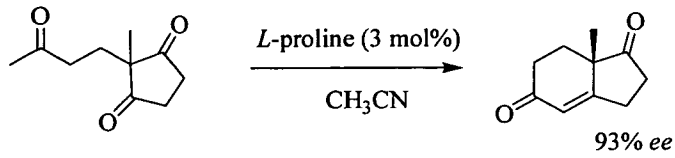



226

Recently, the catalyst **225** has been used by Trost *et al.* in the direct asymmetric aldol reactions with α-hydroxy ketones.[187] These reactions proceeded in good yields (62–98%) and excellent enantiomeric excess (81–96% *ee*). However, improved results were obtained when using a second generation of dinuclear zinc catalyst **226**.[188] The catalyst **226** gave the best results for the direct aldol reactions between acetone and α-unbranched aldehydes (54–89% yield and 76–94% *ee*).

### 5.1.2. Proline-catalysed aldol reactions.

Despite the multiple advantages of aldolase Class II–like catalysts, it was an attractive target to find small organic molecules that would mimic the amine-based asymmetric Class I aldolase. In fact, a small organic molecule, *L*-proline **227** was already known to catalyse the intramolecular aldol reaction in the Hajos–Parrish–Eder–Sauer–Wiechert reaction (**Scheme 91**), first developed in the 1970s.[189] When a catalyst, such as *L*-proline, performs well in one reaction, it can be expected to mediate all similar reactions under optimized reaction conditions.[175] In fact, List *et al.* were the first to report a successful intermolecular variant of this reaction in 2000.[190]

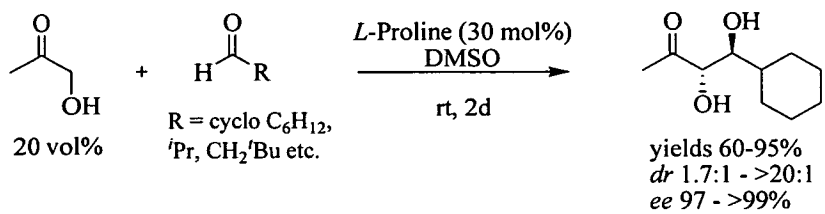



**Scheme 91:** The Hajos–Parrish–Eder–Sauer–Wiechert reaction.[189]

List discovered that *L*-proline **227** can act like an enzyme in promoting direct asymmetric aldol reactions between unmodified acetone and a variety of aldehydes. It was shown that aromatic aldehydes gave aldol products with *ee* values ranging from 60 to 96%. A large range of solvents ($CH_3CN$, THF, DMF, acetone, DMSO) and different commercially available amino acids derivatives were screened. Interestingly, the best results, in terms of reaction time and enantioselectivity, were obtained when anhydrous DMSO was used at room temperature. Even more interesting was the discovery that primary amino acids and acyclic secondary amino acids failed to give any significant amount of the desired product. It became clear that both the pyrrolidine ring and the carboxylate functionality were essential for the catalyst to work.

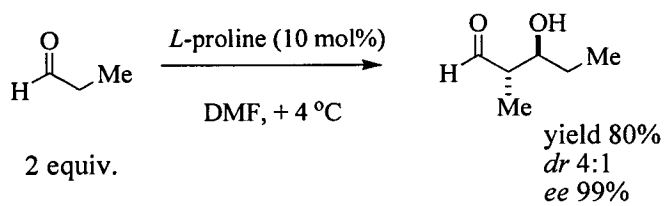
Since the first results of using proline to catalyse the intermolecular aldol reaction were so successful, it became an attractive area for the further research. Additionally, it is important to point out some distinctive features of proline as a catalyst. First of all, it is non-toxic, inexpensive and readily available in both enantiomeric forms. Secondly, it is readily soluble in water and can be removed by simple aqueous extraction. Thirdly, proline is a heavy-metal-free and environmentally-friendly catalyst. Aldol reactions, catalysed by proline do not require prior modification of the carbonyl substrates or inert reaction conditions.[190]

The subsequent work reported by List was devoted to the determination of whether proline is able to catalyse the direct aldol reaction between unprotected hydroxyacetone and a variety of aldehydes.[191] It was found out that these reactions proceeded to give *anti*-diols in good yields, with diastereoselectivities > 20:1 and enantioselectivities > 99% (**Scheme 92**).




**Scheme 92:** First example of aldol reactions with unprotected hydroxyacetone.[191]

The remarkable achievements in the proline-catalysed direct asymmetric aldol reaction between unmodified ketones and aldehydes by List's group, set a new goal for organic chemists: the development of catalytic methods for the direct enantioselective coupling of unmodified aldehyde substrates. The first example of such an aldehyde–aldehyde dimerisation was published by MacMillan's group in 2002.[192] They exposed propionaldehyde to catalytic quantities of *L*-proline in DMF and were pleased to obtain the *anti*-aldol adduct in good yield and with excellent selectivity (**Scheme 93**).

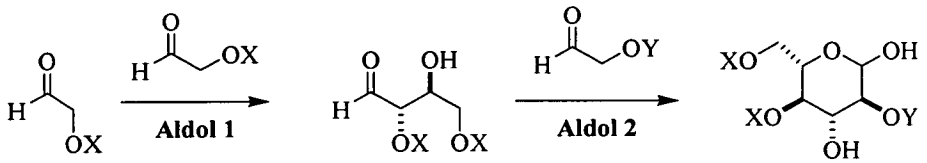



**Scheme 93:** Proline-catalysed aldehyde aldol dimerisation.[192]

The next thing that was examined by MacMillan's group was the capacity of proline to catalyse the aldol reaction between non-equivalent aldehydes. It was found that if the aldehyde donor was added by syringe pump to a series of aldehyde acceptors in the presence of the catalyst, the desired cross-aldol product was observed in excellent yield and selectivity (up to 88% yield, > 99% *ee*).[192]

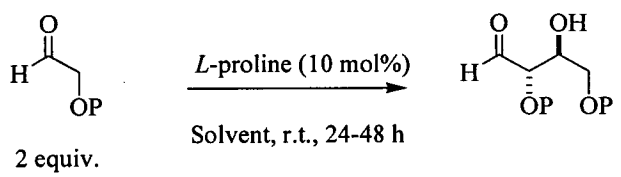
Two years later, MacMillan's group published their work dedicated to the two-step synthesis of carbohydrates (**Scheme 94**).[193,194] They proposed that the first step (Aldol 1)

would be an enantioselective aldol reaction between two α-oxyaldehydes. The second step (Aldol 2), however, will involve a diastereoselective aldol coupling between tri-oxy substituted butanals and the enolate of an α-oxyaldehyde.




**Scheme 94:** Two-step carbohydrate synthesis.[193]

In their paper on Aldol step 1,[193] which is of particular significance to our project, the MacMillan group successfully performed a number of glycolate aldol dimerisation reactions between various protected α-oxyaldehydes. Selected results are presented in **Table 8**.



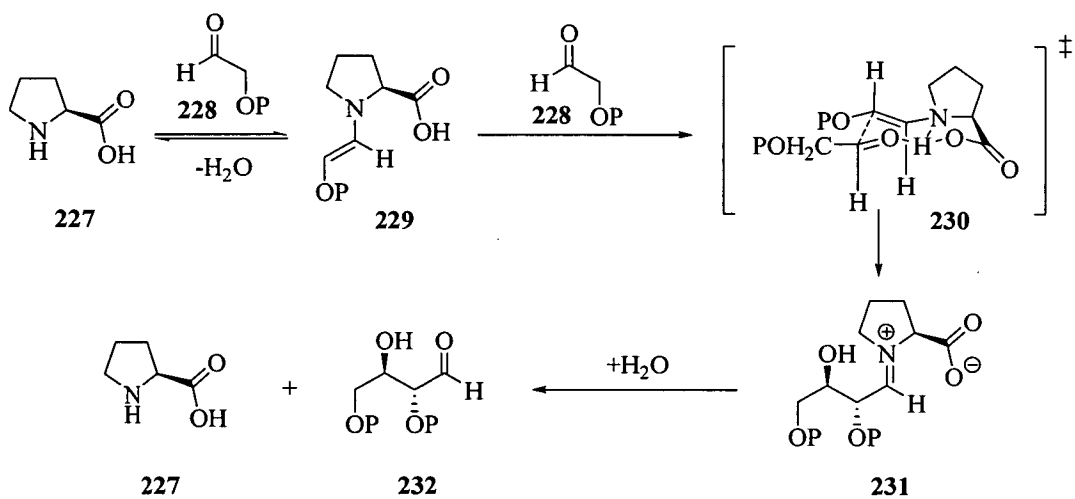

| Protecting group | Solvent | Yield (%) | *ee* (%) | *anti* : *syn* |
|---|---|---|---|---|
| PMB (*para*-methoxybenzyl) | DMF | 64 | 97 | 4 : 1 |
| TIPS (triisopropylsilyl) | DMSO | 92 | 95 | 4 : 1 |

**Table 8:** Proline-catalysed glycolate aldol reactions.[193]

The *para*-methoxybenzyl- and triisopropylsilyl-protected α-oxyaldehydes are highlighted here because these substrates were used in this project for further investigations. As can be seen from **Table 8**, both reactions proceeded in moderate to high yield with excellent enantioselectivities and each with 4:1 *anti:syn* ratio.

### 5.1.3. Mechanism of proline-catalysed aldol reactions.

List's group[177] was the first to propose that the mechanism of the proline-catalysed directed intermolecular aldol reaction closely resembles the aldolase Class I reaction mechanism. According to their proposal, the secondary amine functionality of proline plays the role of a nucleophilic enamine catalyst, while the carboxylic acid is a general Brønsted co-catalyst. The origins of the stereochemistry observed in the previously described reactions have been reported in several papers, and the proposed mechanism is shown in **Scheme 95**.[195-197]




**Scheme 95:** Proposed mechanism of glycolate aldol reaction.

The first stage is the reversible reaction of the *L*-proline-catalyst **227** with one equivalent of α-oxyaldehyde **228** to form the enamine intermediate **229**. Previously, it

has been suggested that the C–C bond formation in the reaction of enamine **229** with the second equivalent of α-oxyaldehyde **228** to form the iminium-ion species **231** is the rate-determining step.[198] However, the calculations performed by Boyd *et al.* showed that the initial complexation between proline and the first equivalent of α-oxyaldehyde **228** is, in fact, a rate-determining step in the proposed mechanism.[195] Finally, hydrolysis of **231** yields the aldol product **232** and free *L*-proline **227**, ready for another catalytic turn-over. Despite the initial proposal that two proline molecules were involved in the intramolecular aldol reaction,[177] List and Houk experimentally proved that only one molecule of proline is required.[199]

The proposed mechanism has been supported by numerous density functional studies[195,200,201] and by additional indirect information:[177] including the fact that *N*-methyl proline is not catalytically active at all, which verifies the presence of iminium and enamine intermediates.

It has also been shown that solvent plays a key role in stabilizing the intermediate zwitterionic structures and providing an alternative, lower-energy pathway by which the reaction may proceed. Usually, polar solvents, such as dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), water or an ionic liquid are employed for these types of reactions. In the presence of such solvents, the proton of the carboxylic acid moiety of proline, rather than the proton from the nitrogen of proline, is transferred to the carbonyl oxygen of the first equivalent of α-oxyaldehyde.

The transition state **230** of the reaction, an expansion of which is shown in **Figure 50**, is key to controlling the stereochemistry. It is proposed to be a metal-free Zimmerman–Traxler six-membered ring chair-like transition state wherein the aldehyde (red) approaches the enamine (blue) in a specific and controlled manner. The stereocontrol is due to three factors:

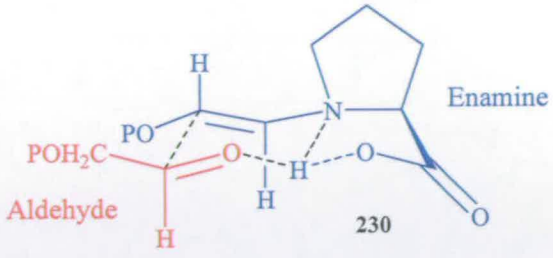
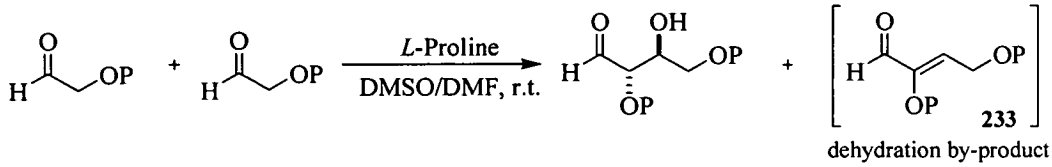



**Figure 50:** Expanded transition state.

1) The enamine bond is *trans* as this is the most thermodynamically stable conformation. In addition, this allows the bulky pyrrolidine and OP groups to be in pseudo-equatorial positions in the six-membered ring transition state.
2) The carboxylic acid group forms a hydrogen bonding interaction with the oxygen of the incoming aldehyde. This means the aldehyde is directed to the front face of the enamine double bond as the carboxylic acid is pointing out of the page. If *D*-proline were to be used, the aldehyde would be directed to the opposite face.
3) The bulky $CH_2OP$ group preferentially takes up the pseudo-equatorial position over the aldehyde hydrogen as this leads to significantly less steric crowding.

The combination of these three factors gives rise to the high enantio- and diastereo-selectivities observed in proline-catalysed direct aldol reactions.

All of the above-mentioned advantages of proline have made it a very widely used catalyst in organic chemistry. However, the particular reactions described above, which we were interested in, have several disadvantages. These include the long reaction time (up to 48 hours); limited solvent compatibility, which requires the use of high-boiling polar solvents (such as DMSO and DMF) due to the insoluble nature of proline **227**; high catalyst loading (up to 20 mol%); and, the formation of significant levels of a dehydration by-product **233** (**Scheme 96**).

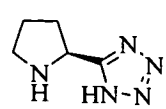



**Scheme 96:** Proline-catalysed glycolate aldol reactions.

In an attempt to overcome these deficiencies we investigated two alternative pathways: use of an alternative catalyst (the tetrazole derivative of proline) and use of high-pressure conditions.

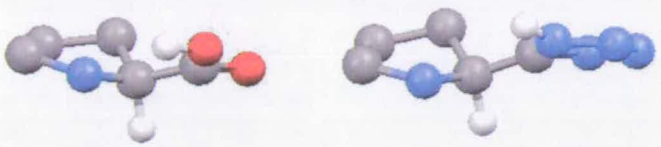
### 5.1.4. Tetrazole-derived catalyst.

As has been pointed out above, the carboxylic acid functionality in proline plays a very important role. First of all, it orients the incoming aldehyde through a hydrogen bond, which results in direction of the reaction onto only one face of the enamine ring. Secondly, it lowers the activation barrier of the reaction by charge stabilisation. Based on the mechanism suggested above (**Scheme 95**), Arvidsson *et al.* reasoned that a stronger hydrogen bond donor should lower the energy of the transition state further, which will lead to increased reactivity.[202] It is well-known that the replacement of a carboxylic acid functionality with a tetrazole derivative has been widely used in medicinal chemistry (due to the similar p$K_a$ of the two groups) in order to increase lipophilicity, and thus the solubility.[76,203,204] So, it was proposed that a suitable catalyst could be obtained by substitution of the carboxylic functionality of proline with tetrazolic acid **234**.



**234**

From the structure of the new catalyst, it is easy to see why this substitution has been contemplated: the N=C–NH portion of the tetrazolic acid appears similar in shape and electronic character to that of a carboxylic acid. The three-dimensional structure of the two molecules shows their similar orientation in space (**Figure 51**).[202] In particular, it is worth noting the similar spacial orientation of the NH and OH groups in these molecules, as the hydrogen bonding through these groups is the key to success of these reactions.



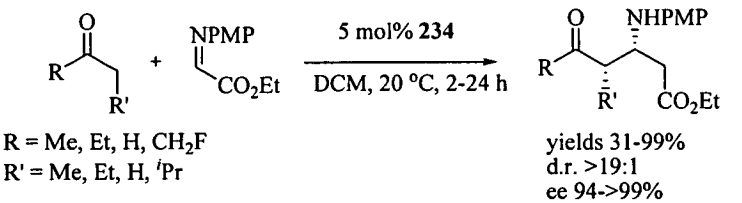
*L*-proline **227** tetrazole derivative **234**

**Figure 51:** 3D models of *L*-proline **227** and its tetrazole derivative **234**.

It is expected that the aldol reaction will proceed through a Zimmerman–Traxler transition state, since the tetrazole functionality is known to exist predominantly in its *1H*-tautomeric form in polar solvents such as DMSO. Catalyst **234** is also expected to stabilize the developing negative charge in this transition state by delocalisation of charge into the tetrazole ring.[202] Examination of the transition state **230** (**Figure 50**) shows that successful reaction requires movement of electron density around the six-membered ring onto the oxygen of the carboxylic acid group. As a result, the product iminium ion **230** (**Scheme 95**), has a full negative charge centred on this oxygen. When this oxygen is replaced by nitrogen, as part of a tetrazole, the negative charge could be delocalised into the ring, thereby lowering the energy of the transition state. This would lead to an improved rate of reaction, and the increased reactivity might also allow lower

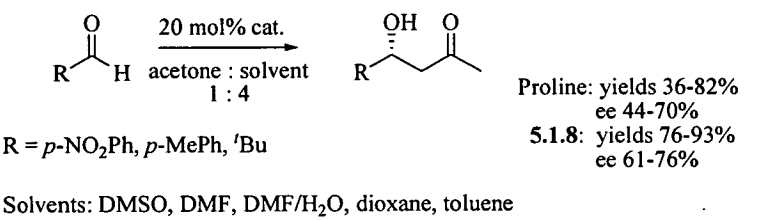
catalyst loadings.

Ley *et al.* reported the first successful utilisation of the catalyst **234** for a range of Mannich-type reactions[204] (**Scheme 97**). It was found that under these conditions using *L*-proline as a catalyst no reaction took place, but when catalyst **234** was used the reaction proceeded in good yields with a >19:1 *syn* diastereoselectivity.




**Scheme 97:** Mannich-type reaction catalysed by **234**.[204]

Later Arvidsson *et al.* applied catalyst **234** to the aldol reaction between acetone and a selection of aldehydes (**Scheme 98**).[202]



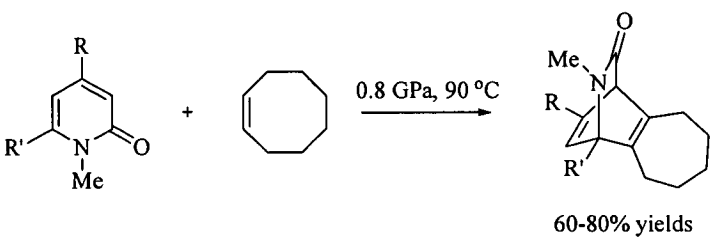

**Scheme 98:** Aldol reactions catalysed by **234**.[202]

They found that catalyst **234** has a higher reactivity than *L*-proline **227** in a variety of solvents. However, no significant difference in enantioselectivities was observed.

Based on these results, we expect that the tetrazole derivative **234** would catalyse the glycolate aldol reaction in the similar fashion to proline **227**.

### 5.1.5. Application of high pressure conditions.

It is well known fact that most organic reactions require some sort of activation, such as heating, light, sonoactivation, microwave activation, etc.[205] The use of high pressure is another possible method of activation. Despite its popularity in many scientific fields, such as physics and geosciences, this method is used relatively rarely in organic synthesis. However, utilisation of high-pressure conditions for the Diels–Alder reaction is well known, for example the reaction of *N*-methyl-2(1*H*)-pyridones with cyclooctyne (**Scheme 99**).[206] This reaction did not result in cycloaddition product formation at atmospheric pressure ($1.01 \times 10^5$ Pa). However, application of 0.3 GPa gave the desired product in 60–80% yield.




**Scheme 99:** Example of Diels–Alder reaction under high-pressure conditions.

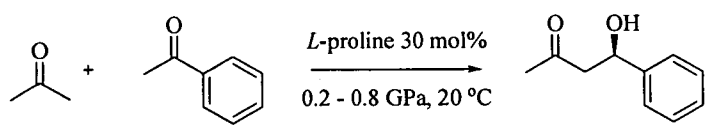
The application of high pressure can very often increase the rate of the Diels–Alder reaction due to the negative activation volumes of these reactions. The activation volume, $\Delta V^{\neq}$, can be defined as the volume variation due to change in the nuclear positions of the reactants during the formation of the transition state.[207] In other words, the difference in volume between the transition state (in the rate-determining step) and the starting material before any interaction has taken place. According to the Evans–Polanyi equation (**Equation 1**) (where k is the rate constant), when the reaction is characterised by a negative activation volume, an increase in pressure will lead to an increase in the reaction rate.[207]

$$d\,(ln\,k) / dP = -\Delta V^{\neq} / RT$$

**Equation 1:**

Since the Diels–Alder reaction involves the intimate approach of the two substrates *via* a cyclic transition state, which will almost certainly occupy a smaller volume than the free reactants, the activation volume will be large and negative. The proline-catalysed aldol reaction, on the other hand, also goes *via* a highly ordered transition state (section 5.1.2), so a negative activation volume is predicted. The activation volumes for aldol reactions have been investigated and were found to be large and negative.[208,209]

In 2003, Sekiguchi *et al.* reported their results for the proline-catalysed aldol reaction of a series of aldehydes with acetone at high pressure.[210] Acetone was used as a reactant and as a solvent, and the reaction conditions was optimised based on its reaction with benzaldehyde (**Scheme 100**).




**Scheme 100:** High pressure proline-catalysed aldol reaction.[210]

The application of 0.2 GPa gave the best results, producing the desired aldol adduct in 90% yield with 72% enantioselectivity: slightly higher in comparison with the same experiment under atmospheric pressure (62% yield, 60% ee).[190] These comparable values imply that the equilibrium contribution of transition states **I** and **II** is almost the same as at normal pressure (**Figure 52**) and that *re*-facial attack of benzaldehyde is a major process.[210] Pressures exceeding 0.2 GPa led to the discrimination between the two transition states in favour of **II**.

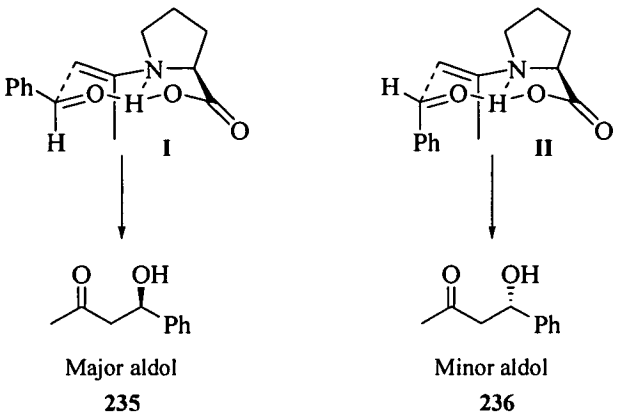



**Figure 52:** Proposed transition states for the proline-catalysed aldol reaction.[210]

The reaction was performed on a variety of substrates. In general, higher yields, shorter reaction times and no significant change of enantioselectivity were observed. Particularly noticeable was the decreased amount of elimination by-product in comparison with the reaction at atmospheric pressure. This could suggest that under high pressure, the transition state leading to the aldol adduct is more preferable than the one leading to the elimination product.[210]

In 2004, Hayashi *et al.* performed similar experiments in DMSO at 0.2 GPa, which was induced by water freezing.[211] The reactions were carried out at –20 °C, which was supposed to give better enantioselectivities and to help to suppress the elimination. In fact, these conditions led to a small increase in enantioselectivity (up to 10%), but the yields were generally lower than those found by Sekiguchi *et al.*[210] This behavior can be explained by a reduction of the reaction rate at lower temperatures.

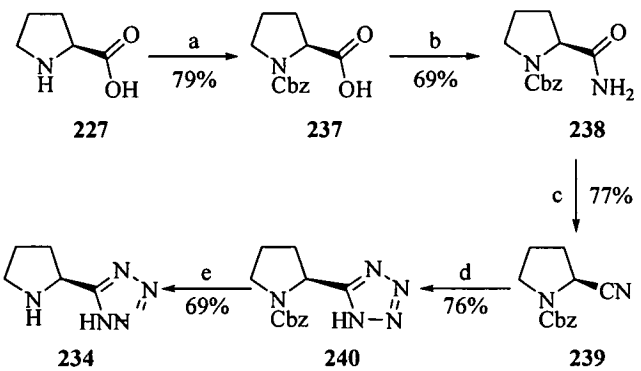
To conclude, intermolecular aldol reactions can be catalysed by amino acids, such as *L*-proline **227**, or its analogues, such as the tetrazole derivative **234**. The application of both catalysts has been tested on a variety of substrates, including aldehydes, ketones and α-oxyaldehydes. Reactions catalysed by L-proline **227** usually require long reaction times and high-boiling solvents, whereas use of tetrazole **234** helps to overcome these disadvantages. Additionally, performance of these reactions under high pressure has

shown encouraging results.

## 5.2. Synthesis of glycolate aldol components.

### 5.2.1. Synthesis of the tetrazole catalyst.

The catalyst **234** was synthesised from *L*-proline **227** in five steps following the published method[76,203] in 22% overall yield, as shown in **Scheme 101**.



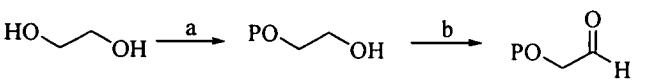

(a) CbzCl, $Na_2CO_3$, THF, 5 °C, 18 h; (b) (i) EDCI, HOBt, THF, r.t., 1.5 h; (ii) $NH_3$ (aq.), r.t., 36 h; (c) *p*-TsCl, pyridine, DCM, r.t., 72 h; (d) $NaN_3$, $NH_4Cl$, DMF, 90 °C, 18 h; (e) 10% Pd/C, $H_2$, AcOH:$H_2O$ (9:1), r.t., 3 h.

**Scheme 101:** Synthesis of the tetrazole derivative of *L*-proline **234**.

In our hands, the synthesis proceeded in comparable yields, except for the amidation reaction, where the observed yield was slightly lower in comparison with the literature value of 100%.[76] The NMR data obtained for the tetrazole catalyst (**5.1.8**) was in a good agreement with the literature.

### 5.2.2. Synthesis of the aldehydes.

The two required aldehydes, either TIPS- or PMB-protected were each synthesised in the same manner (**Scheme 102**). Ethylene glycol **241** was deprotonated with potassium hydroxide and then heated to 145 °C for several hours to remove excess water.[212] The mixture was then treated with the appropriate chloride to give the desired monoprotected alcohols **242** and **243**. Several oxidation conditions were attempted (DMP, IBX), but Swern oxidation conditions gave the best results.[117]




**241** P = TIPS **242** (24%), P = PMB **243** (72%) P = TIPS **244** (67%), P = PMB **245** (82%)

(a) KOH, 145 °C, 18 h, TIPSCl or PMBCl. 35 °C, 18 h; (b) (i) $(COCl)_2$, DMSO, DCM, –78 °C, 10 min, (ii) **242** and **243**, - 78 °C, 1 h, (iii) $NEt_3$, –78 °C to r.t. over 1 h.

**Scheme 102:** Synthesis of aldehydes **244** and **245**.

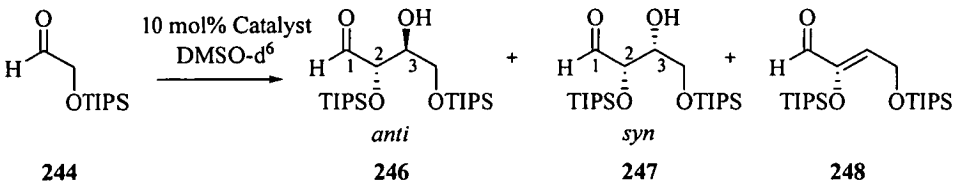
With these materials in hand it was possible to carry out further glycolate aldol experiments.

## 5.3. Atmospheric pressure experiments.

The first step was to compare the outcomes of the aldol reactions of aldehydes **244** and **245** using both catalysts (*L*-proline **227** and tetrazole catalyst **234**) under atmospheric pressure conditions. These experiments were performed on a small scale in deuterated solvents and were monitored by $^1$H NMR. For ease of comparison, the same solvents and catalytic loadings were used as reported by MacMillan *et al.* (**Table 8, page 124**).[193] The progress of the reactions and the appearance of an elimination by-product were monitored over 48 hours and a series of $^1$H NMR spectra were taken.

### 5.3.1. Aldol reaction of TIPS-protected aldehyde.

Two aldol reactions of TIPS-protected α-oxyaldehyde **244** using catalysts **227** and **234** (**Scheme 103**) were examined using the diagnostic peaks for C(2)*H* to obtain integral ratios as shown in **Figure 53**.




**Scheme 103:** Aldol reaction of TIPS-protected aldehyde.

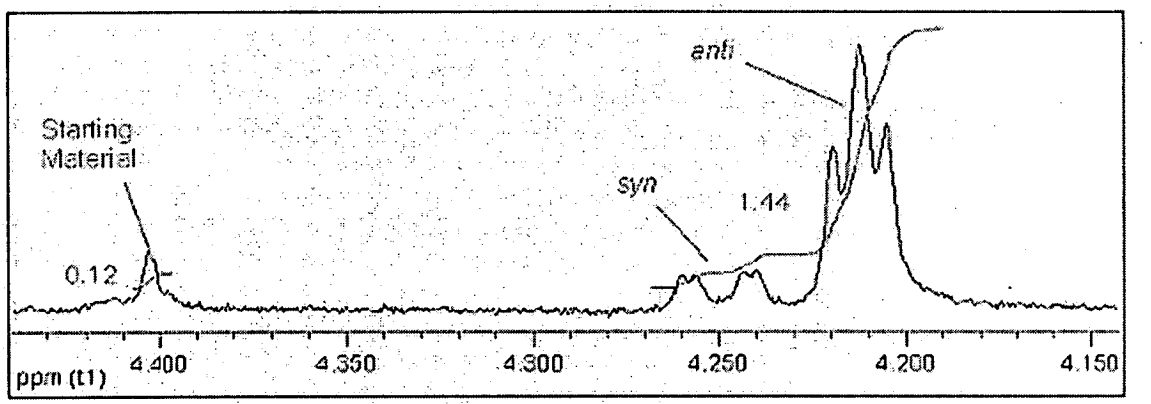



**Figure 53:** Section of the $^{1}$H NMR spectra (after 2 hours).

The *syn* and *anti* diastereoisomers were assigned based on analysis of the coupling constants. The coupling constants for the *syn* doublet of doublets were 1.0 Hz and 4.2 Hz and for the *anti* triplet, the coupling constant was 1.8 Hz.

Examination of the results, shown in **Table 9**, suggested that the reaction catalysed by *L*-proline **227** proceeded faster (almost complete in 5 hours in contrast with MacMillan's results[193]) and with better diastereoselectivity (approximately 6:1).

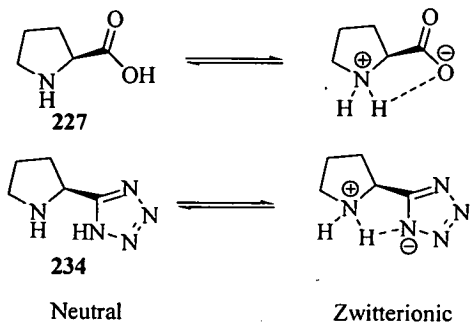
However, the formation of the elimination by-product **248** was evident, and increased with time.

| Catalyst | Time (h) | Product **246** (δ = 4.25) | *anti*:*syn* **246**:**247** (δ = 4.25) | start. mat. **244** (δ = 4.40) | Elimination **248** (δ = 9.28) |
|---|---|---|---|---|---|
| *L*-proline (**227**) | 1 | 1.44 | 6.6:1 | 0.12 | 0.02 |
| | 5 | 1.37 | 6.9:1 | 0.03 | 0.01 |
| | 21 | 1.20 | 5.5:1 | trace | 0.04 |
| Tetrazole (**234**) | 1 | 0.80 | 3.0:1 | 6.64 | none |
| | 5 | 0.95 | 3.1:1 | 4.31 | none |
| | 21 | 2.02 | 3.3:1 | 0.01 | none |

**Table 9:** Analysis of $^{1}$H NMR data for the TIPS-protected aldehyde **244**.

In the case of tetrazole catalyst **244**, the reaction took much longer (up to 21 hours) and the observed diastereoselectivity was lower (approximately 3:1) than in the reaction catalysed by *L*-proline **227**. However, no elimination product was observed.

The different degree of the elimination by the two catalysts could be explained by comparison of their pKa values. Since the polarities of DMSO and DMF are similar, the pKa values are likely to be the similar. The pKa of acetic acid in DMSO is 12.3, whereas the pKa of tetrazole is 8.2.[213] Hence, in both solvents, proline **227** would prefer to exist as a free amine, whereas the tetrazole catalyst **234** would prefer to exist in its zwitterionic form (**Scheme 104**). This would explain why more elimination was seen in the proline-catalysed reaction.

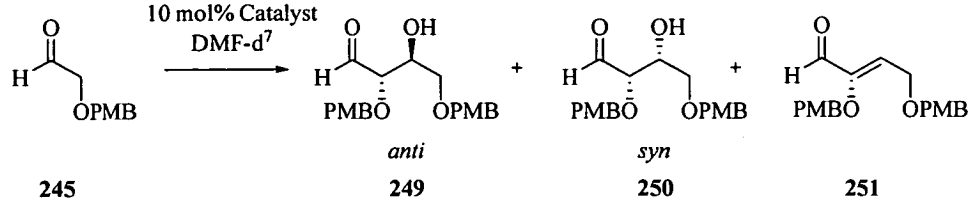



**Scheme 104:** Two possible forms of the two catalysts.

The preference of the tetrazole catalyst to exist in the zwitterionic form may also explain the lower rate of the reaction, since the free amine is required for the formation of the enamine intermediate.

In conclusion, while the proline-catalysed reaction was faster and more selective, the tetrazole-catalysed reaction was cleaner. However, from **Table 9,** it can be seen that before the proline-catalysed reaction reached completion, no significant amount of the elimination product **248** was observed. It is also possible that if the tetrazole-catalysed reaction was left for longer, some elimination would also have taken place.

### 5.3.2. Aldol reaction of PMB-protected aldehyde.




245 249 250 251

**Scheme 105:** Aldol reaction of PMB-protected aldehyde.

| catalyst | *L*-proline **227** | Tetrazole derivative **234** |
|---|---|---|
| Reaction time (h) | ~ 21 | > 21 |
| Elimination product **251** | Significant | None |
| *anti* : *syn* | 2.2 : 1 | 2.5 :1 |
| DMF/DMSO | No difference | No difference |

**Table 10:** Results for the PMB-protected aldehyde.

A similar procedure to monitor the aldol reaction of PMB-protected aldehydes was carried out, using diagnostic $^1$H NMR peaks for starting material **245**, product **249** and **250** and the elimination by-product **251**. Based on the remaining starting material, it could be concluded that the proline-catalysed reaction was almost complete after 21 hours, while the tetrazole-catalysed reaction was still far from completion. Additionally, the proline-catalysed reaction was lower yielding than the tetrazole reaction. This could be explained by the appearance of a significant amount of the elimination product **251**. In the case of the tetrazole-catalysed reaction, no elimination was observed after 21 hours. The proline-catalysed aldol reaction proceeded with 2.2:1 ratio, whereas the tetrazole-catalysed one proceeded with 2.5:1 ratio. In our case, the diastereoselectivities were notably lower than those reported by MacMillan *et al.* (4:1)[193] (**Table 8, page 124**).

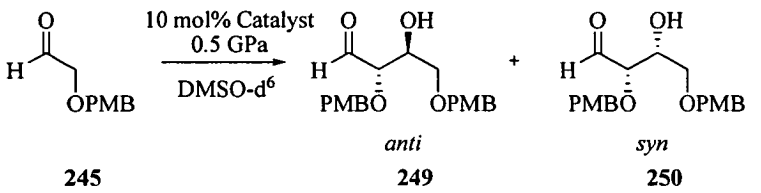
So far, it can be seen that the reaction with the TIPS-protected aldehyde **244** gave

better results. This could be due to the different steric and electronic characters of the PMB and TIPS protecting groups. However, an overall comparison cannot be made directly, since these two reactions were carried out in different solvents. To investigate if solvents can affect the reaction outcome, the aldol reaction of PMB-protected aldehyde **245** was also performed in deuterated DMSO. The results obtained from this experiment were similar to the previous experiment (**Table 10**). Even after 29 hours, the proline-catalysed reaction was not complete, and a significant amount of the elimination product was observed. Again, the tetrazole-catalysed reaction was much slower, but no elimination was observed.

To conclude, the aldol reaction of the TIPS-protected aldehyde **244** gave higher yields and diastereoselectivities with both catalysts **227** and **234**. The reaction rate of the PMB-protected aldehyde **245** was significantly slower and changing the solvent did not affect the outcome. The tetrazole catalyst **234** was found to suppress elimination: however, the reaction suffered from significantly slower turn-over. With this information in hand, an investigation of the influence of high pressure on the reaction rate was possible.

## 5.4. High pressure experiments.

As previously described in section 5.1.5, it was believed that application of high pressure to the glycolate aldol reaction would lead to an increase in the reaction rate.[205] Our investigation into this effect was performed on the PMB-protected aldehyde **245** in DMSO-d$^6$ (**Scheme 106**).




**Scheme 106:** Aldol reaction of PMB-protected aldehyde under high pressure.

A reaction was performed using both catalysts **227** and **234**, for 30 minutes under a pressure of 0.5 GPa. The apparatus used to generate and apply the pressure was an LC10 Liquid Pressure Cell ID 7 mm, as shown in **Figure 53**. Since the diameter of the high pressure chamber is only 7 mm, special sealed reaction vessels had to be designed (**Figure 54**). The vessels invented could hold volumes up to 0.25 ml.


**Figure 53:** High pressure apparatus.

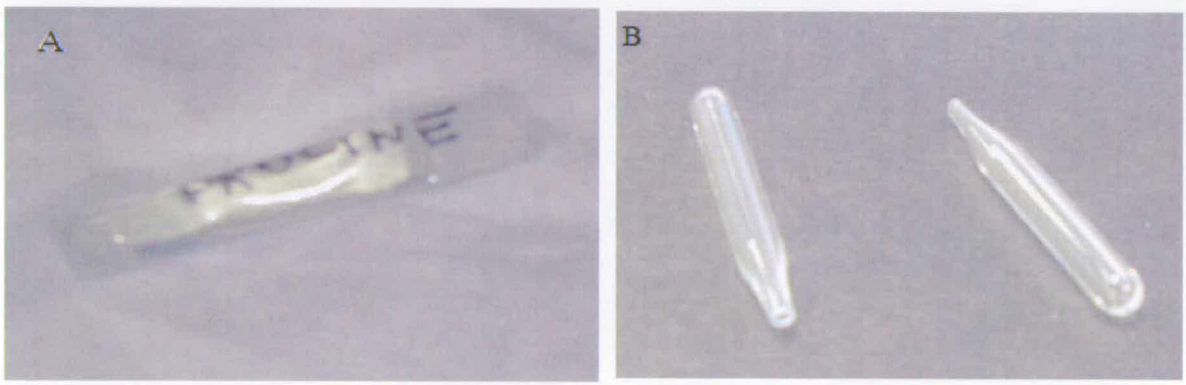



**Figure 54:** Reaction vessels designed: **A**—from plastic; **B**—from glass.

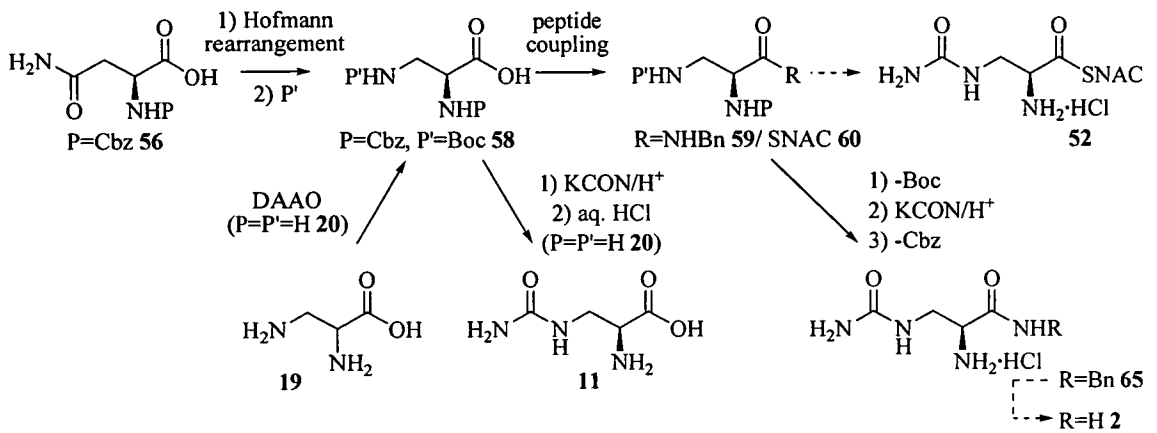
The $^{1}H$ NMR spectra of the crude reaction material after 30 minutes showed significant rate acceleration. The proline-catalysed reaction was found to be at the equivalent 7-hours timepoint when compared with the atmospheric pressure experiment.

The amount of the elimination product was much lower than was observed at 7 hours at atmospheric pressure. Surprisingly, the rate of the tetrazole-catalysed reaction was accelerated even more dramatically. After just 30 minutes under 0.5 GPa, the $^{1}$H NMR spectra resembled that obtained after 29 hours under atmospheric pressure. However, a small amount of elimination by-product **251** had begun to appear.

To conclude, although only two experiments were performed under high pressure conditions, both of them showed significant rate acceleration without further increase of elimination. The use of high pressure conditions for the glycolate aldol reaction requires further investigation, including isolation and characterisation of the products. These could not have been performed at this stage due to the unavailability of a large-scale high-pressure apparatus and limitations on the design of the reaction vessels (which were too small due to the size of the reaction chamber.

## Conclusions.

During our attempts to synthesise the nitrogen-rich fragment **2**, an effective route for the enzymatic separation of racemic 2,3-diaminoproponic acid **19** using *D*-amino acid oxidase was developed. Several different approaches to the synthesis of **2** were explored. During these investigations, an effective synthesis of *L*-albizziine **11** was achieved from $N_\alpha$-*tert*-butoxycarbonyl-*L*-asparagine in two steps, in 65 % overall yield. In a similar fashion, it is envisaged that the synthesis of *D*-albizziine could be accomplished starting from $N_\alpha$-*tert*-butoxycarbonyl-*D*-asparagine (**Scheme 107**).

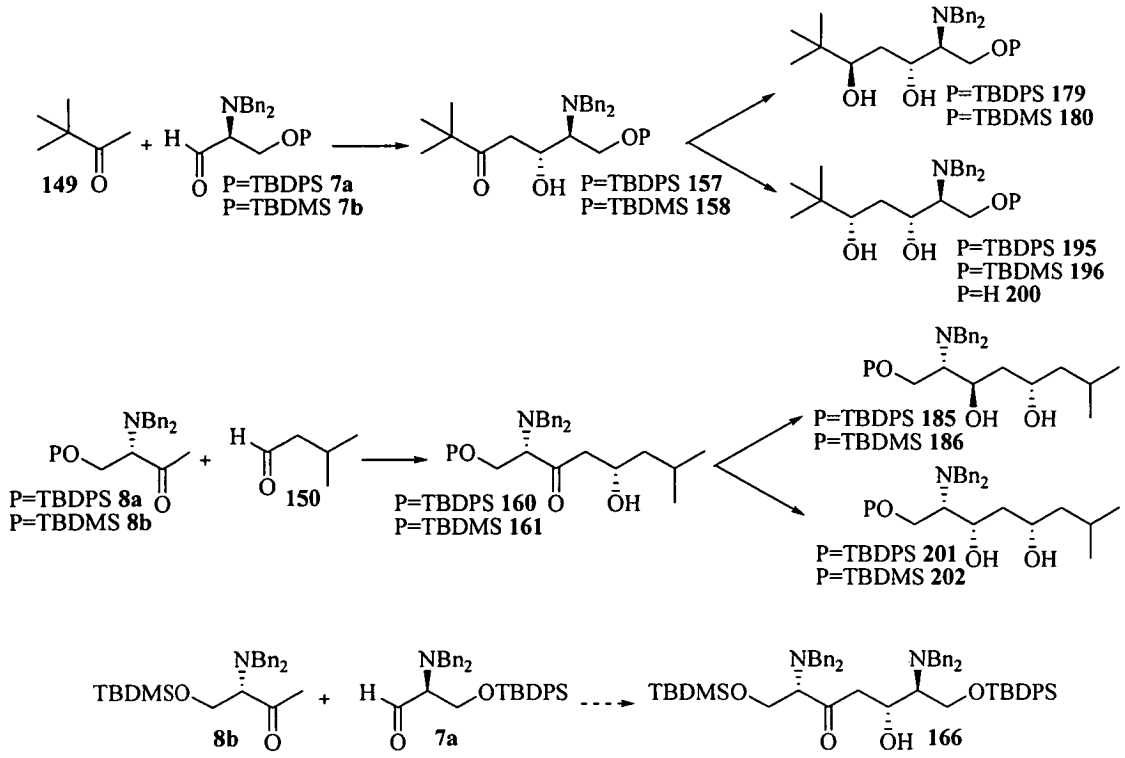



**Scheme 107:** Synthetic achievements towards nitrogen rich fragment **2**.

Our final synthetic strategy led to the monoprotected benzylamide **65** and the SNAC derivative **60**. Even though attempted deprotection failed using a range of standard conditions, we anticipate that the application of high pressure conditions for the hydrogenation reaction might be a solution in this case, giving rise to the desired products **2** and the SNAC derivative **52**.

The aldol reactions of serine-derived aldehydes **7a** and **7b** and threonine-derived ketones **8a** and **8b** with achiral pinacolone **149** and isovaleraldehyde **150** were studied.

The resultant diastereoselectivity of both sets of aldols was explained (**Scheme 108**). Confirmation of the Felkin-Anh selectivity for the addition of the pinacolone enolate to aldehyde **7a** was gained from a crystal structure of the deprotected 3,5-*syn* reduction product **200**. Several attempts to perform the double asymmetric aldol reaction between serine-derived aldehydes **7a** and **7b** and threonine-derived ketones **8a** and **8b** were made; these were unsuccessful, but a lack of material prevented extensive optimisation of the aldol conditions. Nonetheless, application of known *syn* 1,3-reduction and *anti* 1,3-reduction conditions to aldol adducts **157**, **158**, **160** and **161** gave the corresponding reduced products in good yield with moderate to high diastereoselectivity (**Scheme 108**). All of the above results suggest that with further optimisation the synthesis of the C(9)–C(15) fragment of zwittermicin A **1** can be achieved with the correct relative stereochemistry using this strategy. In addition, an alternative synthetic strategy for the aminopolyol backbone C(9)-C(15) is discussed in chapter 4.




**Scheme 108:** Synthetic achievements towards the C(9)-C(15) aminopolyol backbone.

Two final bond connections are required for completion of the synthesis of zwittermicin A **1**; a glycolate aldol coupling to complete the aminopolyol chain **3** and peptide bond formation between this fragment and the nitrogen-rich fragment **2**. Chapter 5 describes preliminary investigations towards an enantioselective organocatalytic glycolate aldol reaction under high pressure conditions.

# Chapter 6: Experimental.

## 6.1. General experimental.

$^{1}H$ nuclear magnetic resonance (NMR) spectra were recorded using an internal deuterium lock for the indicated reference at ambient probe temperatures on Varian Gemini 200 (200 MHz), Bruker AC250 (250 MHz) and Bruker Am360 (360 MHz) Fourier transform instruments. The data is presented as follows: chemical shift (in ppm on the δ scale relative to $\delta_{TMS}=0$), integration, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, qn=quintet, m=multiplet, br=broad), coupling constant and the interpretation. $^{13}C$ NMR spectra were recorded using an internal deuterium lock for the indicated reference at ambient probe temperatures on Varian Gemini 200 (50.3 MHz), Bruker AC250 (62.9 MHz) and Bruker Am360 (90.6 MHz) Fourier transform instruments and are reported in ppm on the δ scale.

Infra-red spectra were recorded on the Perkin Elmer Paragon 1000 FT-IR instrument using 5 mm sodium chloride plates or 0.1 mm sodium chloride solution cells. The wavelengths of maximum absorbance ($\upsilon_{max}$) are quoted in $cm^{-1}$.

Fast atom bombardment (FAB) mass spectra were performed on a Kratos MS50TC mass spectrometer.

Optical rotations were measured on an AA-1000 polarimeter with a path length of 1.0 dm at the sodium D line (589 nm) and are reported as follows: $[\alpha]_D$, concentration (c in g/100 $cm^3$) and solvent. All optical rotations were measured at a temperature of 23 °C.

Elemental analysis was carried out on a Perkin Elmer 2400 CHN Elemental analyzer.

T.l.c. was performed on Merck $60F_{254}$ (0.25 mm) glass backed silica plates and visualised by ultraviolet (UV) light and/or ammonium molybdate or potassium permanganate stain.† Flash column chromatography was carried out on Merck Kieselgel 60 (Merck 9385) under positive pressure by means of a hand pump or air flow. Eluent compositions are quoted as v/v ratios. High performance liquid chromatography (HPLC) was carried out on a Gilson instrument using a Spherisorb column (internal diameter 20 mm) and equipped with a Gilson refractive index detector. A standard flow of 7 $cm^3$/min was used. All HPLC samples were filtered through 45 μm nylon syringe filters prior to analysis. All solvents used for HPLC analysis were vacuum filtered and degassed prior to use.

The high pressure experiments were performed on a LC10 Liquid Pressure Cell ID 7 mm using specially desined sealed reaction vessels.

Reagents were purified by standard means. Dichloromethane (DCM), dimethylformamide (DMF) and triethylamine were distilled from calcium hydride and stored over calcium hydride under an argon atmosphere. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl and stored under a nitrogen atmosphere. All other reagents were used as supplied. Isovaleraldehyde and benzaldehade were distilled over calcium hydride and storaged under nitrogen.

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† Ammonium molybdate dip prepared as follows: to water (950 $cm^3$) was added concentrated sulphuric acid (50 $cm^3$) followed by ammonium molybdate (50 g) and ceric sulfate (3 g). The mixture was stirred until all the solid material had disappeared and a bright yellow solution remained.

Potassium permanganate dip prepared as follows: to water (1000 $cm^3$) was added potassium permanganate (10 g), potassium carbonate (50 g) and sodium hydroxide pellets (40 g). The mixture was stirred until all the solid material disappeared and a purple solution remained.

All experiments were performed under an atmosphere of nitrogen under anhydrous conditions using oven dried apparatus cooled in a desiccator prior to use. Standard techniques for the handling of air-sensitive materials were employed.

## Enzymatic separation of racemic 2,3-diaminopropionic acid

### Preparation of o-phthalaldehyde (OPA)/*N*-isobutyryl-*L*-cysteine (IBLC) reagent.

OPA (50 mg) and IBLC (110 mg) was placed in a volumetrical flask (5 $cm^3$) and dissolved in of MeOH (0.5 $cm^3$). The sample was diluted to 5 $cm^3$ with potassium borate buffer (0.4 M; pH~10.4).

### Preparation of sample solution.

*D,L*-2,3-diaminopropionic acid (1.4 mg) was dissolved in potassium borate buffer (2 $cm^3$, 0.4 M, pH~10.4) and filtered.

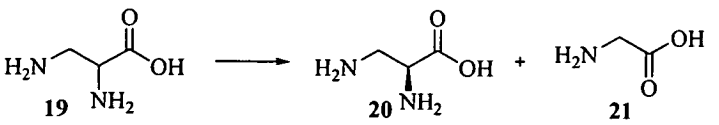
### Preparation of standard solution.

*D*-2,3-diaminopropionic acid (1.4 mg) was dissolved in potassium borate buffer (2 $cm^3$, 0.4 M, pH~10.4) and filtered

### Apparatus.

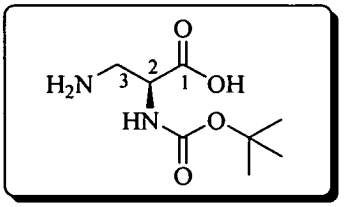
A Waters 2695 Separations Module with a 5 micron reverse phase Gemini column, fitted with a Gemini guard column was used. A Waters 486 tunable absorbance UV detector tuned to the wavelength of 338 nm was used.

**Enzymatic separation experiment.**




*D,L*-2,3-diaminopropionic acid (0.35 g) was dissolved in water (50 $cm^3$) and pH~7 was maintained by addition of NaOH (1 M, aq.). 20 $cm^3$ of prepared solution was placed in reaction flask and DAAO resin (2.17 g) was added. The resulting solution was shacked for 27 h at 37 °C. Mixture then was filtered through Iso-Disc filters and used for the HPLC analysis.

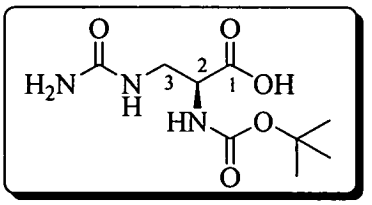
## (2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid 26



A slurry of $N_{\alpha}$-*tert*-butoxycarbonyl-*L*-asparagine (10.0 g, 43.1 mmol), ethyl acetate (48 $cm^3$), acetonitrile (48 $cm^3$), water (24 $cm^3$) and iodosobenzene diacetate (16.6 g, 51.7 mmol) was cooled and stirred at 16 °C in a cooled water bath for 30 min. The temperature was allowed to warm to 20 °C, and the reaction mixture was stirred for 4 h. The mixture then was placed in the fridge and kept there overnight. The resulting precipitate was filtered. The filtrate was washed with ethyl acetate (100 $cm^3$) and dried *in vacuo* to give the title compound **26** (7.01 g, 80.0 %) as a colourless solid. $\mathbf{R_f}$ [BuOH : AcOH : pyridine : $H_2O$ (4 : 1 : 1 : 2)] 0.54; $[\alpha]_D$ – 24.08 (c 0.96, MeOH) (lit.[63] $[\alpha]_D$ -16.5 (c 3, $H_2O$); **mp** 208-210 °C (dec.) (lit.[42] mp 216 °C); $\nu_{max}$ (Nujol)/$cm^{-1}$ 3342, 1685, 1655; $\delta_H$ (250 MHz, DMSO/TFA) 8.08 (3H, br s, $C_3NH_2$, O*H*), 7.39 (1H, d, *J* 8.9, $C_2NH$), 4.37 (1H, td, *J* 8.9, 4.8, $C_2H$), 3.40-3.31 (1H, m, $C_3H_XH_Y$), 3.19-3.08 (1H, m, $C_3H_XH_Y$), 1.54 (9H, s, *'Bu*); $\delta_C$ (62.8 MHz, DMSO/TFA) 171.4 (C), 155.9 (C), 79.1 (C), 51.7 (CH), 39.6 ($CH_2$), 28.3 ($3CH_3$); ***m/z*** (FAB, THIOG) 227 ($[M+Na]^+$, 21%), 205 ($[M+H]^+$, 85), 161 (17), 149 (97); **HRMS** (FAB, THIOG) $C_8H_{17}N_2O_4$ $[M+H]^+$ requires 205.1188, found 205.1189.

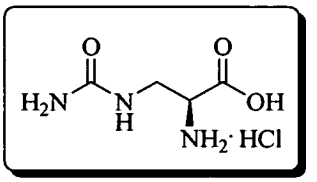
Spectroscopic data in good agreement with the literature.[42]

## (2*S*)-2-*tert*-Butoxycarbonylamino-3-ureidopropanoic acid 17



To a warm (50 °C) stirred solution of (2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26** (1.00 g, 4.89 mmol) in 50 $cm^3$ of water was added potassium cyanide (0.600 g, 7.34 mmol). The pH was then regulated at 7.5 using a pH meter by dropwise addition of 2M HCl. The reaction was followed to completion by mass spectrometry. After completion (~ 5 h), the reaction mixture was cooled to room temperature and concentrated using a freeze drier to give the title compound **17** (1.21 g, 100%) as a colourless solid. $\mathbf{R_f}$ [BuOH : AcOH : pyridine : $H_2O$ (4 : 1 : 1 : 2)] 0.64; $[\alpha]_D$ + 15.2 (c 2.30, MeOH); decomposed at room temperature; $\nu_{max}$ (Nujol)/$cm^{-1}$ 3339, 1686, 1595; $\delta_H$ (250 MHz, MeOH) 4.02 (1H, br t, *J* 6.3, $C_2H$), 3.50 (1H, dd, *J* 13.8, 4.9, $C_3H_XH_Y$), 3.41 (1H, dd, *J* 13.8, 6.3, $C_3H_XH_Y$), 1.48 (9H, s, *'Bu*); $\delta_C$ (62.8 MHz, MeOH) 175.9 (C), 160.7 (C), 156.2 (C), 78.6 (C), 56.2 (CH), 42.1 ($CH_2$), 27.2 ($3CH_3$); ***m/z*** (ESI, –) 246.0 ($[M-H]^-$, 100%), 202.9 (10), 171.8 (65), 131.4 (10), 129.0 (15); **HRMS** (FAB, THIOG) $C_9H_{17}N_3O_5$ $[M-H]^-$ requires 246.9240, found 246.9243.

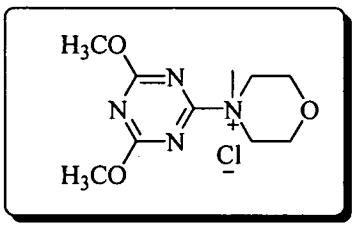
## (2*S*)-2-amino-3-ureidopropanoic acid hydrochloride salt 11



To a warm (50 °C) stirred solution of (2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26** (0.550 g, 2.69 mmol) in water (25 $cm^3$) was added potassium cyanate (0.330 g, 4.07 mmol). The pH was regulated at 7.5 by dropwise addition of 2M HCl .using the pH meter. The reaction was followed to completion by mass spectrometry. After completion (~ 5 h), the reaction mixture was cooled to room temperature and acidifed to pH 1 by dropwise addition of HCl (6M aq.). The resulting mixture was concentrated using a freeze drier and recrystallised from water to give the title compound **11** (0.32 g, 81%) as a colourless solid. $[\alpha]_D$ - 16.9 (c 0.71, MeOH) (lit.[47] $[\alpha]_D$ - 63.4 (c 1, $H_2O$); **mp** 210-212 °C (lit.[47] mp 218-220 °C); $\nu_{max}$ (Nujol)/$cm^{-1}$ 1685, 1660, 1613, 1578; $\delta_H$ (250 MHz, $D_2O$) 4.35 (1H, dd, *J* 5.8, 4.0, $C_2H$), 3.92 (1H, dd, *J* 15.3, 4.0, $C_3H_xH_y$), 3.80 (1H, dd, *J* 15.3, 5.8, $C_3H_xH_y$); $\delta_C$ (62.8 MHz, $D_2O$) 170.8 (C), 161.9 (C), 54.3 (CH), 40.0 ($CH_2$); ***m/z*** (FAB, THIOG) 148 ($[M+H]^+$, 44%), 133 (22), 123 (19), 105 (24), 99 (17); **HRMS** (FAB, THIOG) $C_4H_9N_3O_3$ $[M]^+$ requires 147.0644, found 147.0648.

## 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride

## 41

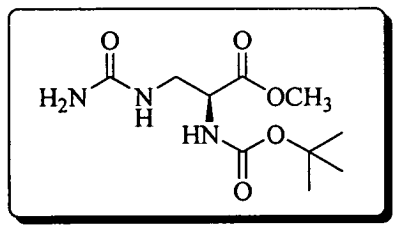



To a solution of dimethoxy-chlorotriazine **42** (1.40 g, 7.97 mmol) in dry THF (20 $cm^3$) was added *N*-methyl-morpholine **43** (0.80 $cm^3$, 7.28 mmol). The resulting mixture was stirred at r.t. for 30 min, and then filtered and washed with dry THF (50 $cm^3$). The resultant crystals were dried under high vacuum to give the desired product **41** as a colourless solid (2.01 g, 99 %). **mp** 116-117 °C (lit.[82] mp 116-117 °C); $\delta_H$ (250 MHz, $D_2O$) 4.71 (3H, s, NC*H*$_3$), 4.60-4.52 (2H, m, C*H*$_2$), 4.02 (6H, s, O*Me*), 4.07-4.00 (2H, m, C*H*$_2$), 3.84-3.74 (4H, m, 2xC*H*$_2$); $\delta_C$ (62.8 MHz, $D_2O$) 174.2 (2C), 170.4 (C), 62.4 ($2CH_2$), 60.4 ($2CH_2$), 57.4 ($2CH_3$), 56.2 ($CH_3$).

Spectroscopic data in good agreement with the literature.[82]

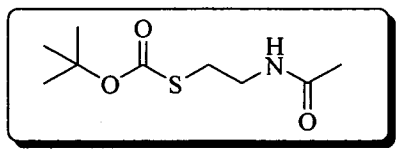
**Attempted synthesis of (2*S*)-2-(*tert*-butoxycarboxylamino)-3-ureido-propanamide 18**

## (*S*)-Methyl-2-(*tert*-butoxycarbonylamino)-3-ureidopropanoate 40



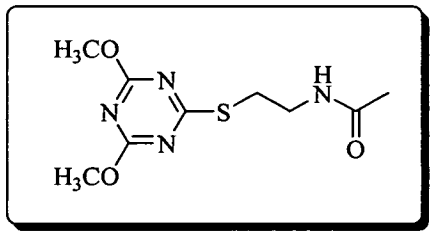
Ammonium gas was cautiously bubbled through dry MeOH (10 $cm^3$) for 40 min (exothermic reaction). The gas cylinder was then taken away (the solution became slightly warm). (2*S*)-2-*tert*-butyloxycarbonylamino-3-ureidopropanoic acid **17** (1.10 g, 4.45 mmol) was then added to the resulting solution and the reaction mixture was stirred at r.t. for 1 h. The reaction mixture was evaporated to remove excess ammonia. The residue was redissolved in dry MeOH (10 $cm^3$) and DMT-MM (2.46 g, 8.89 mmol) then was added and the reaction mixture was stirred at r.t. for 2 h. The resulting solution was evaporated under reduced pressure to give a colourless precipitate, which was identified as (*S*)-methyl-2-(*tert*-butoxycarbonylamino)-3-ureidopropanoate **40** (0.64 g, 59%) as a colourless solid. $\mathbf{R_f}$ [DCM : MeOH (15 : 1)] 0.14; $[\alpha]_D$ – 24.0 (c 0.58, MeOH); **mp** 138-139 °C; $\nu_{max}$ (Nujol)/$cm^{-1}$ 3472, 3407, 3365, 2917, 1731, 1686, 1651; $\delta_H$ (360 MHz, MeOH) 4.22 (1H, t, *J* 4.5, $C_2H$), 3.76 (3H, s, O*Me*), 3.56 (1H, dd, *J* 9.8, 3.4, $C_3H_XH_Y$), 3.42 (1H, dd, *J* 9.8, 4.5, $C_3H_XH_Y$), 1.47 (9H, s, $^tBu$); $\delta_C$ (90.6 MHz, MeOH) 171.1 (C), 160.1 (C), 155.8 (C), 78.7 (C), 54.0 (CH), 50.8 ($CH_3$), 39.9 ($CH_2$), 26.6 ($3CH_3$); ***m/z*** (FAB, THIOG) 262 ($[M+H]^+$, 29%), 206 (51), 162 (70), 145 (37), 102 (22), 90 (11); **HRMS** (FAB, NOBA) $C_{10}H_{20}N_3O_5$ $[M+H]^+$ requires 262.1403, found 262.1408; **CHN** requires (%) C 45.97, H 7.33, N 16.08, found (%) C 45.99, H 7.87, N 16.00.

## *S*-2-acetamidoethyl-*O*-*tert*-butyl carbothioate 54



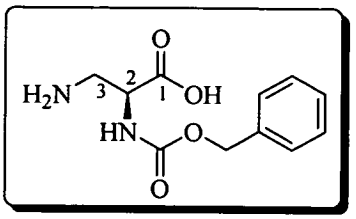
To a solution of (2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26** (0.300 g, 1.47 mmol) in THF (25 $cm^3$) was added di-*t*-butyldicarbonate (0.700 g, 3.23 mmol) followed by pyridine (0.260 $cm^3$, 3.23 mmol). The resulting solution was stirred at r.t. for 3 h. *N*-Acetylcysteamine (0.17 $cm^3$, 1.62 mmol) then was added and mixture was stirred overnight. Solvent was evaporated and the residue was portioned between water (20 $cm^3$) and EtOAc (20 $cm^3$). Aqueous phase was extracted with EtOAc (2 x 20 $cm^3$), washed with water (20 $cm^3$), 1N HCl (20 $cm^3$), brine (20 $cm^3$), and dried ($MgSO_4$) and concentrated to give a mixture of recovered *N*-acetyl cysteamine and the title compounds **54** (0.167 g, 1:1 ration, 20% **54**). $\mathbf{R_f}$ [EtOAc : MeOH : AcOH (9.4 : 0.5 : 0.1)] 0.51; $\nu_{max}$ ($CHCl_3$)/$cm^{-1}$ 3299, 2980, 2934, 1701, 1642; $\delta_H$ (250 MHz, MeOH) 3.37 (2H, t, *J* 6.6, C*H*$_2$), 2.91(2H, t, *J* 6.6, C*H*$_2$), 1.94 (3H, s, C*H*$_3$), 1.49 (9H, s, $^t$*Bu*); $\delta_C$ (62.9 MHz, MeOH) 173.8 (C), 170.7 (C), 86.3 (C), 40.9 ($CH_2$), 31.6 ($CH_2$), 28.9 ($3CH_3$), 23.0 ($2CH_3$); ***m/z*** (FAB, THIOG) 259 ($[M+K]^+$, 24%), 242 ($[M+Na]^+$, 52), 220 ($[M+H]^+$, 46), 186 (37), 164 (77), 142 (41); **HRMS** (FAB, NOBA) $C_9H_{18}NO_3S$ $[M+H]^+$ requires 220.1007, found 220.1007.

## *N*-(2-(4,6-dimethoxy-1,3,5-triazin-2-ylthio)ethyl)acetamide 55



To a solution of (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17** (0.20 g, 0.81 mmol) in dry MeOH (10 $cm^3$) was added *N*-acetylcysteamine (0.11 $cm^3$, 1.1 mmol) and resulting mixture was stirred at r.t. for 30 min. DMT-MM (0.45 g, 1.6 mmol) was then added and the reaction mixture was stirred at r.t. overnight (18 h). The resulting solution was evaporated under reduced pressure, diluted with water and extracted with EtOAc (3 x 30 $cm^3$). Combined organic phases were washed with brine (20 $cm^3$), 1N HCl (20 $cm^3$), brine (20 $cm^3$), dried ($MgSO_4$) and concentrated to give the title compound **55** (0.26 g, 93%) as a colourless solid. $\mathbf{R_f}$ [EtOAc : MeOH : AcOH (8 : 1 : 1)] 0.68; **mp** 120-122°C; $\nu_{max}$ ($CHCl_3$)/$cm^{-1}$ 3670, 2240, 1793; $\delta_H$ (250 MHz, $CDCl_3$) 7.13 (1H, br s, N*H*), 3.83 (6H, s, 2xO*Me*), 3.40 (2H, q, *J* 6.2, C$H_2$NHAc), 3.10 (2H, t, *J* 6.2, C$H_2$S), 1.79 (3H, s, *Me*); $\delta_C$ (62.9 MHz, $CDCl_3$) 184.2 (C), 170.7 (2C), 170.2 (C), 55.1 ($2CH_3$), 38.9 ($CH_2$), 29.7 ($CH_2$), 22.8 ($CH_3$); ***m/z*** (FAB, THIOG) 259 ($[M+H]^+$, 53%), 174 (43), 147 (33), 91 (27); **HRMS** (FAB, NOBA) $C_9H_{15}N_4O_3S$ $[M+H]^+$ requires 259.0865, found 259.0863.

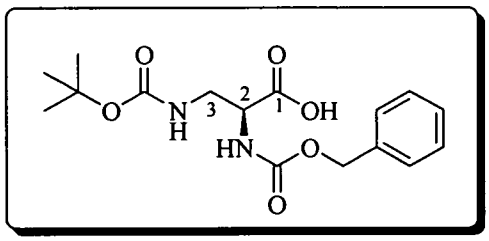
## (2*S*)-3-amino-2- benzyloxycarbonylamino-propanoic acid 33



A slurry of $N_{\alpha}$-benzyloxycarbonyl-*L*-asparagine **56** (1.00 g, 3.76 mmol), ethyl acetate (4.8 $cm^3$), acetonitrile (4.8 $cm^3$), water (2.4 $cm^3$) and iodosobenzene diacetate (1.45 g, 4.51 mmol) was cooled and stirred at 16 °C in a cooled water bath for 30 min. The temperature was allowed to warm to 20 °C, and the reaction mixture was stirred for 4 h. The mixture then was placed in the fridge and kept there overnight. The resulting precipitate was filtered. The filtrate was washed with ethyl acetate (100 $cm^3$) and dried *in vacuo* to give the title compound **57** (0.83 g, 93.0 %) as a colourless solid. **mp** 216-218 °C (dec.) (lit.[42] mp 210 °C); $\nu_{max}$ (Nujol)/$cm^{-1}$ 3301, 2912, 1694, 1595, 1542; $\delta_H$ (360 MHz, DMSO/TFA) 9.53 (1H, br s, *OH*), 8.03 (2H, br s, $C_3NH_2$), 7.75 (1H, d, *J* 8.6, $C_2NH$), 7.39-7.28 (5H, m, Ar*H*), 5.07 (2H, s, $CH_2Ph$), 4.34-4.28 (1H, m, $C_2H$), 3.28-3.22 (1H, m, $C_3H_XH_Y$), 3.07-2.99 (1H, m, $C_3H_XH_Y$); $\delta_C$ (62.8 MHz, DMSO/TFA) 171.2 (C), 156.6 (C), 136.9 (C), 128.7 (2CH), 128.2 (2CH), 128.1 (CH), 66.2 ($CH_2$), 52.1 (CH), 39.7 ($CH_2$); ***m/z*** (FAB, THIOG) 239 ($[M+H]^+$, 49%), 217 (45), 215 (32), 214 (23), 91 (100); **HRMS** (FAB, NOBA) $C_{11}H_{14}N_2O_4$ $[M+H]^+$ requires 239.1032, found 239.1035; **CHN** requires (%) C 55.46, H 5.92, N 11.76, found (%) C 55.35, H 5.82, N 11.59.

Spectroscopic data in good agreement with the literature.[42]

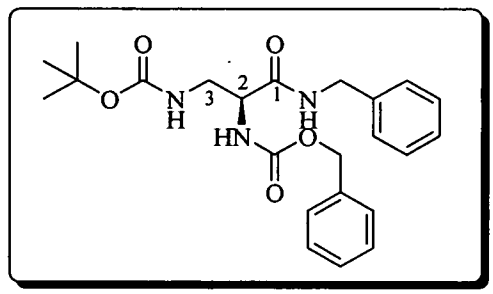
## (2*S*)- 2-Benzyloxycarbonylamino- 3-*tert*-butyloxycarbonylamino-propanoic acid 58



To a solution of (2*S*)-3-amino-2-benzyloxycarbonylamino-propanoic acid **57** (0.50 g, 2.10 mmol) in 10 % $Na_2CO_3$ (5 $cm^3$) were added 1,4-dioxane (3 $cm^3$) and $(Boc)_2O$ (0.55 g, 2.52 mmol) at 0 °C. The reaction mixture was stirred overnight (for 18 h) at r.t. The resulting mixture was then poured into water (100 $cm^3$) after which the mixture was extracted with diethyl ether (3 x 50 $cm^3$). The aqueous layer was acidified with HCl (~20 $cm^3$, 2M aq.) to pH 1in water and the colourless suspension was extracted with EtOAc (3 x 50 $cm^3$). The combined EtOAc layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was crystallized from $Et_2O$ to afford the desired product **58** (0.64 g, 90 %) as a colourless solid. $[\alpha]_D$ – 5.1 (c 0.98, MeOH); **mp** 143-144 °C (lit.[86] mp 144 °C); $\nu_{max}$ (Nujol)/$cm^{-1}$ 3423, 3369, 3300, 2924, 1726, 1702, 1673, 1532; $\delta_H$ (250 MHz, DMSO) 7.40-7.27 (5H, m, Ar*H*), 6.81 (1H, t, *J* 5.8, N*H*), 5.00 (2H, s, C$H_2$Ph), 4.09-3.97 (1H, m, $C_2H$), 3.33-3.10 (2H, m, $C_3H_2$), 1.32 (9H, s, *tBu*); $\delta_C$ (62.8 MHz, DMSO) 173.2 (C), 157.1 (C), 156.8 (C), 137.9 (C), 129.5 (2CH), 128.9 (CH), 128.8 (2CH), 79.2 (C), 66.7 ($CH_2$), 55.3 (CH), 42.2 ($CH_2$), 29.2 ($3CH_3$); ***m/z*** (FAB, THIOG) 338 ($[M]^+$, 5%), 239 (45), 205 (24), 149 (41), 105 (24), 93 (40); **HRMS** (FAB, NOBA) $C_{16}H_{23}N_2O_6$ $[M+H]^+$ requires 339.1556, found 339.1553; **CHN** requires (%) C 56.80, H 6.55, N 8.28, found (%) C 56.70, H 6.99, N 8.31.

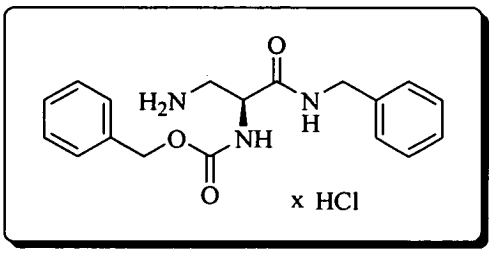
Spectroscopic data in good agreement with the literature.[86]

## Benzyl-(2*S*)-2-benzyloxycarbonylamino-3-*tert*-butyloxycarbonylamino-propanamide 59



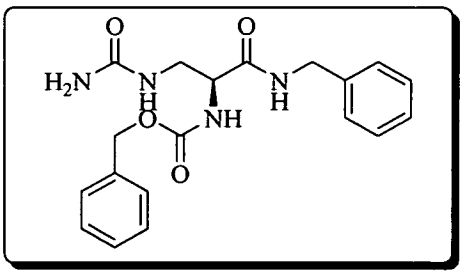
To a solution of (2*S*)-2-Benzyloxycarbonylamino-3-*tert*-butyloxycarbonylamino-propanoic acid **58** (0.440 g, 1.32 mmol) in DCM (10 $cm^3$) was added EDCI (0.300 g, 1.59 mmol), DMAP (few crystals), followed by benzylamine (0.170 $cm^3$, 1.59 mmol). The resulting mixture was stirred at r.t. overnight (18 h). Solution was concentrated and redissolved in EtOAc (10 $cm^3$) and water (10 $cm^3$) and the aqueous layer was extracted with EtOAc (3 x 50 $cm^3$), washed with $NH_4Cl$ (10 $cm^3$, sat), brine (10 $cm^3$) and dried ($MgSO_4$) and concentrated. The remaining residue was chromatographed on silica gel [DCM : $Et_2O$ (4 : 1)] to give the title compound **59** (0.52 g, 92%) as a colourless solid. $\mathbf{R_f}$ [DCM : $Et_2O$ (4 : 1)] 0.35; $[\alpha]_D$ – 14.3 (c 0.21, $CHCl_3$); **mp** 157-159 °C; $\nu_{max}$ (Nujol)/$cm^{-1}$ 3323, 2931, 1685, 1658, 1539; $\delta_H$ (250 MHz, $CDCl_3$) 7.25-7.13 (10H, m, Ar*H*), 6.91 (1H, br s, N*H*), 6.28 (1H, br d, *J* 6.1, N*H*), 5.13 (1H, br s, N*H*), 5.02 (2H, s, C$H_2$Ph), 4.33 (1H, dd, *J* 14.9, 5.5, $C_3H_AH_B$), 4.31 (1H, m, $C_3H_AH_B$), 4.28-4.20 (1H, m, $C_2H$), 3.51-3.35 (2H, m, NHC$H_2$Ph), 1.33 (9H, s, *tBu*); $\delta_C$ (62.9 MHz, $CDCl_3$) 169.9 (C), 156.7 (C), 156.6 (C), 137.6 (C), 135.9 (C), 128.5 (2CH), 128.4 (2CH), 128.1 (CH), 127.9 (2CH), 127.3 (3CH), 80.1 (C), 67.1 (CH), 67.0 ($CH_2$), 43.3 ($CH_2$), 42.5 ($CH_2$), 28.1 ($3CH_3$); ***m/z*** (FAB, THIOG) 427 ([M], 25%), 371 (33), 327 (34), 194 (25), 120 (21), 106 (40), 91(76); **HRMS** (FAB, NOBA) $C_{23}H_{30}N_3O_5$ $[M+H]^+$ requires 428.2186, found 428.2189; **CHN** requires (%) C 64.62, H 6.84, N 9.83, found (%) C 64.75, H 6.80, N 9.75.

## Benzyl-(2*S*)-2-benzyloxycarbonylamino-3-amino-propanamide 61



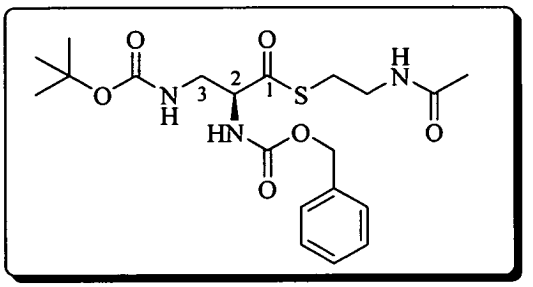

To a solution of benzyl-(2*S*)-2-benzyloxycarbonylamino-3-butyloxycarbonylamino-propanamide **59** (0.500 g, 1.17 mmol) in DCM (20 $cm^3$) was added 1M HCl in $Et_2O$ (10 $cm^3$, 9.9 mmol) and the resulting solution was stirred at r.t. for 20 h. The precipitate which formed was removed from the reaction mixture by filtration and dried (in *vacuo*) to give the title compound as a colouress solid **61** (0.25 g, 60 %) as a white solid. $[\alpha]_D$ -13.3 (c 0.3, MeOH); **mp** 176-178 °C; $\nu_{max}$ (Nujol)/$cm^{-1}$ 3315, 1704, 1687, 1659, 1535, 1462; $\delta_H$ (360 MHz, $D_2O$) 7.40-7.23 (10H, m, Ar*H*), 5.13 (2H, br s, C$H_2$Ph), 4.50-4.47 (1H, m, $C_2H$), 4.40 (1H, d, *J* 15.5, NHC$H_X$H$_Y$Ph), 4.32 (1H, d, *J* 15.5, NHCH$_X$$H_Y$Ph), 3.49 (1H, dd, *J* 13.3, 4.8, $C_3H_AH_B$), 3.26 (1H, dd, *J* 13.3, 9.0, $C_3H_AH_B$); $\delta_C$ (90.6 MHz, $D_2O$) 171.5 (C), 158.5 (C), 138.6 (C), 137.0 (C), 129.9 (3CH), 129.6 (CH), 128.9 (2CH), 128.6 (2CH), 128.2 (2CH), 68.7 ($CH_2$), 53.3 (CH), 44.1 ($CH_2$), 40.9 ($CH_2$); ***m/z*** (FAB, THIOG) 655 ($[2M+H]^+$, 46%), 328 ($[M+H]^+$, 100), 284 (29), 238 (57), 215 (69), 199 (57), 181 (65); **HRMS** (FAB, NOBA) $C_{18}H_{22}N_3O_3$ $[M+H]^+$ requires 328.1661, found 328.1661.

## Benzyl-(2*S*)-2-benzyloxycarbonylamino-3-ureido-propanamide 63



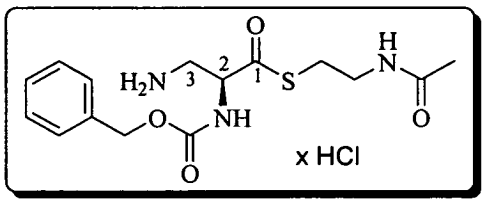
To a warm (50 °C) stirred solution of benzyl-(2*S*)-2-benzyloxycarbonylamino-3-amino-propanamide **61** (0.10 g, 0.27 mmol) in 20 $cm^3$ of water was added potassium cyanate (0.055 g, 0.67 mmol). The resulting mixture was stirred for 1 h and the precipitate formed was removed by filtration. The colourless solid was washed with water (20 $cm^3$) and dried using a freeze drier overnight to give the title compound **63** (0.078 g, 77%) as a colourless solid. $[\alpha]_D$ – 35.3 (c 0.085, MeOH); **mp** 182-184 °C; $\nu_{max}$ (Nujol)/$cm^{-1}$ 3286, 1673, 1645, 1540; $\delta_H$ (360 MHz, DMSO) 8.54 (1H, t, *J* 5.9, N*H*), 7.48 (1H, d, *J* 7.4, N*H*), 7.38-7.24 (10H, m, Ar*H*), 6.17 (1H, t, *J* 5.9 N*H*), 5.69 (2H, br s, N$H_2$), 5.05 (2H, s, OC$H_2$Ph), 4.30 (2H, m, NC$H_2$Ph), 4.07 (1H, td, *J* 8.0, 4.6, $C_2H$), 3.39 (1H, ddd, *J* 13.9, 6.1, 4.6, C3$H_x$H$_y$), 3.18 (1H, ddd, *J* 13.9, 8.0, 6.1, $C_3H_xH_y$); $\delta_C$ (90.6 MHz, DMSO) 171.9 (C), 160.8 (C), 157.5 (C), 140.6 (C), 138.3 (C), 129.9 (2CH), 129.7 (2CH), 129.3 (CH), 129.2 ($2CH_2$), 128.4 (2CH), 128.2 (CH), 67.1 ($CH_2$), 57.8 (CH), 43.5 ($CH_2$), 42.6 ($CH_2$); ***m/z*** (FAB, NOBA) 371 ($[M+H]^+$, 95%), 307 (46), 154 (95), 137 (79), 91 (100); **HRMS** (FAB, NOBA) $C_{19}H_{23}N_4O_4$ $[M+H]^+$ requires 371.1719, found 371.1719.

## *S*-(2'-Acetamidoethyl)-(2*S*)-2-(benzyloxycarbonylamino)-3-(*tert*-butoxycarbonylamino)-propanethioate 60



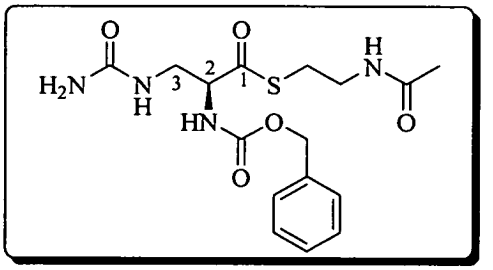
To a solution of (2*S*)-3-*tert*-butoxycarbonyl-2-benzyloxycarbonyl-2,3-diamino-propionic acid **58** (0.20 g, 0.59 mmol) in DCM (10 $cm^3$) was added EDCI (0.14 g, 0.71 mmol), and DMAP (few crystals), followed by *N*-acetylcysteamine (0.070 $cm^3$, 0.71 mmol). The resulting mixture was stirred at r.t. overnight (18 h). Solution was concentrated and redissolved in EtOAc (10 $cm^3$) and water (10 $cm^3$) and the aqueous layer was extracted with EtOAc (3 x 50 $cm^3$), washed with $NH_4Cl$ (20 $cm^3$, sat), brine (20 $cm^3$) and dried ($MgSO_4$) and concentrated. The remaining residue was chromatographed on silica gel [EtOAc : MeOH (9 : 1)] to give the title compound **60** (0.24 g, 93%) as a colourless oil. $\mathbf{R_f}$ [EtOAc : MeOH (9 : 1)] 0.57; $[\alpha]_D$ – 21.8 (c 0.55, $CHCl_3$); $\nu_{max}$ (neat)/$cm^{-1}$ 3295, 1698, 1652, 1547; $\delta_H$ (250 MHz, $CDCl_3$) 7.61-7.48 (5H, m, Ar*H*), 6.62 (1H, br s, N*H*), 5.54 (1H, br d, *J* 4.0, N*H*), 5.29 (2H, s, C$H_2$Ph), 4.61-4.59 (1H, m, $C_2H$), 3.83-3.44 (4H, m, 2x$CH_2$ (SNAC)), 3.29-3.09 (2H, m, $C_3H_2$), 2.13 (3H, s, *Me*), 1.58 (9H, s, *tBu*); $\delta_C$ (62.9 MHz, $CDCl_3$) 199.6 (C), 170.7 (C), 156.8 (C), 156.0 (C), 135.9 (C), 128.4 (2CH), 128.1 (CH), 127.9 (2CH), 80.1 (C), 67.0 ($CH_2$), 62.1 (CH), 42.3 ($CH_2$), 38.2 ($CH_2$), 28.8 ($CH_2$), 28.1 ($3CH_3$), 22.9 ($CH_3$); ***m/z*** (FAB, THIOG) 439 ($[M]^+$, 25%), 383 (32), 339 (61), 301 (20), 225 (26), 193 (36); **HRMS** (FAB, NOBA) $C_{20}H_{30}N_3O_6S$ $[M+H]^+$ requires 440.1856, found 440.1878.

## *S*-(2'-Acetamidoethyl)-3-amino-(2*S*)-2-(benzyloxycarbonylamino)-propanethioate hydrochloride salt 62




To a solution of *S*-(2'-Acetamidoethyl)-(2*S*)-2-(benzyloxycarbonylamino)-3-(*tert*-butoxycarbonylamino)-propanethioate **60** (0.20 g, 0.46 mmol) in DCM (10 $cm^3$) was added HCl (7.0 $cm^3$, 7.0 mmol, 1M in $Et_2O$) and the resulting solution was stirred at r.t. for 40 min. The precipitate which formed was removed from the reaction mixture by filtration and dried (in *vacuo*) to give the desired compound **62** (0.13 g, 77%) as a colourless solid. $\nu_{max}$ (Nujol)/$cm^{-1}$ 2922, 2360, 1732; $\delta_H$ (250 MHz, MeOH) 7.64-7.47 (5H, m, Ar*H*), 5.31 (2H, s, C$H_2$Ph), 4.82-4.80 (1H, m, $C_2H$), 3.78-3.36 (4H, m, 2x$CH_2$), 3.22 (2H, br s, $C_3H_2$), 2.06 (3H, s, $CH_3$); $\delta_C$ (62.9 MHz, MeOH) 200.6 (C), 175.1 (C), 158.7 (C), 138.2 (C), 130.0 (2CH), 129.7 (CH), 129.5 (2CH), 68.8 ($CH_2$), 60.2 (CH), 41.5 ($CH_2$), 40.6 ($CH_2$), 29.6 ($CH_2$), 22.6 ($CH_3$); ***m/z*** (FAB, NOBA) 340 ($[M+H]^+$, 28%), 392 (13), 167 (13), 154 (59), 149 (100), 136 (52); **HRMS** (FAB, NOBA) $C_{15}H_{22}N_3O_4S$ $[M+H]^+$ requires 340.1331, found 340.1337.

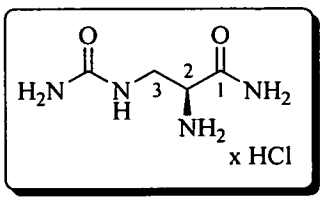
## Attempted synthesis of *S*-2-acetamidoethyl-(*S*)-2-(benzyloxycarbonylamino)-3-ureido-propanethioate 64



To a warm (50 °C) stirred solution of *S*-2-acetamidoethyl (*S*)-3-amino-2-(benzyloxycarbonyl)-propanethioate hydrochloride salt **62** (0.090 g, 0.24 mmol) in water (20 $cm^3$) was added potassium cyanate (0.050 g, 0.60 mmol). The resulting mixture was stirred for 4 h and the precipitate which formed was removed by filtration. This colourless solid was washed with water and dried using a freeze drier overnight to give the title compound **64** as a colourless solid.

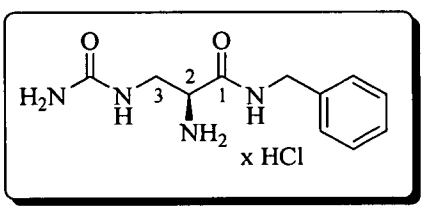
The presence of the new quaternary peak in the $^{13}C$ NMR at 160.7 ppm suggested successful formation of the urea, together with a molecular ion observed in the FAB mass spectrum. ***m/z*** (FAB, NOBA) 383 ($[M+H]^+$, 0.5%), 275 (25), 237 (15), 192 (75), 154 (41), 136.1 (39). However, a clean analytical sample for full NMR analysis was not produced, since purification of the title compound **64** failed.

## Attempted synthesis of (2*S*)-2-amino-3-ureido-propanamide hydrochloride salt 2




After the condensation of approximately 20 $cm^3$ of anhydrous ammonia at – 40 °C , Na (0.011 g, 0.46 mmol) was added in several small pieces. The resulting blue solution was stirred for 10 min and benzyl-(2*S*)-2-benzyloxycarbonylamino-3-ureido-propanamide **63** (0.078 g, 0.21 mmol) as a solution in dry THF (10 $cm^3$) was added over a ten minutes period. The mixture was stirred for 40 min, followed by addition of $NH_4Cl$ (5.0 g). The ammonia was then allowed to distill off and THF (20 $cm^3$) was added to the white slurry. After filtration and washing of the solids with an additional 50 $cm^3$ THF, the combined organics were concentrated. However, no product was isolated as a result of this reaction.

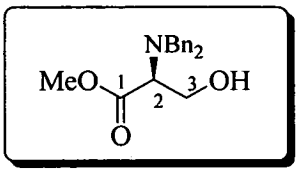
## (2*S*)-2-amino-3-ureido-benzylpropanamide hydrochloride 65




Benzyl-(2*S*)-2-benzyloxycarbonylamino-3-ureido-propanamide **63** (0.064 g, 0.17 mmol) was dissolved in dry MeOH (5 $cm^3$) and $Pd(OH)_2/C$ (0.064 g, 100 wt%) was added. The mixture was stirred for 10 min, followed by addition of 1M HCl in $Et_2O$ (0.35 $cm^3$, 0.17 mmol). The resulting solution was exposed to the $H_2$ atmosphere and stirred vigorously for 48 hour. The reaction mixture was filtered through celite, washed with MeOH and combined solution was concentrated under reduced pressure to give the benzyl-(2*S*)-2-amino-3-ureido-propanamide hydrochloride **65** (0.035 g, 74%) as a white

solid. $\delta_H$ (360MHz, MeOH) 7.49-7.29 (5H, m, Ar*H*), 4.52 (1H, d, *J* 14.9, $C_3H_XH_Y$), 4.41 (1H, d, *J* 14.9, $C_3H_XH_Y$), 4.10-4.08 (1H, m, $C_2H$),3.33 (2H, s, $CH_2$Ph); $\delta_C$ (90.6 MHz, MeOH) 167.9 (C), 160.2 (C), 138.8 (C), 129.8 (CH), 129.2 (2CH), 128.4 ($2CH_2$), 52.1 (CH), 46.8 ($CH_2$), 44.1 ($CH_2$).

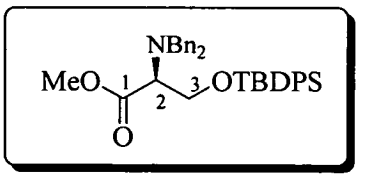
## Methyl (2*S*)-2-*N*,*N*-dibenzylamino-3-hydroxypropanoate 106




To a solution of *L*-serine methyl ester (5.22 g, 43.9 mmol) in anhydrous acetonitrile (300 $cm^3$) was added anhydrous potassium carbonate (30.3 g, 219 mmol) followed by benzylbromide (20.90 $cm^3$, 175.5 mmol). The resulting mixture was stirred for 48 h at room temperature. Water (150 $cm^3$) was added and the aqueous layer was extracted with EtOAc (3 x 100 $cm^3$). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [Hexane : EtOAc (4 : 1)] to give the title compound **106** (11.3 g, 86%) as a colourless oil. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.34; $[\alpha]_D$ -161.8 (c 1.07, $CHCl_3$); $\nu_{max}$ (neat)/$cm^{-1}$ 3446, 1736, 1601, 1542; $\delta_H$ (360 MHz, $CDCl_3$) 7.42-7.29 (10H, m, Ar*H*), 3.97 (2H, d, *J* 13.4, NC$H_x$$H_y$Ph), 3.86 (3H, s, O*Me*), 3.83-3.79 (2H, m, $C_3H_2$), 3.74 (2H, d, *J* 13.4, NC$H_x$$H_y$Ph), 3.63 (1H, t, *J* 7.6, $C_2H$), 2.62 (1H, br s, O*H*); $\delta_C$ (90.5 MHz, $CDCl_3$) 171.6 (C), 138.4 (2C), 128.8 (4CH), 128.4 (4CH), 127.4 (2CH), 61.5 ($CH_3$), 59.1 ($CH_2$), 54.6 ($2CH_2$), 51.4 (CH); ***m/z*** (ESI, +) 299.9 ($[M+H]^+$, 100%), 287.9 (5), 207.8 (5), 180.7 (5); **HRMS** (FAB, NOBA) $C_{18}H_{22}NO_3$ $[M+H]^+$ requires 300.1600, found 300.1600.

Spectroscopic data in good agreement with the literature.[98]

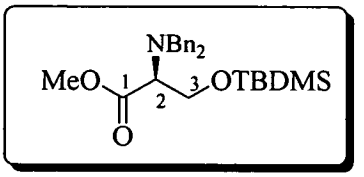
## Methyl (2*S*)-3-*tert*-butyldiphenylsilyloxy-2-*N*,*N*-dibenzylaminopropanoate 107




To a solution of serine methyl ester **106** (1.30 g, 4.34 mmol) in anhydrous DMF (30 $cm^3$) was added *tert*-butyldiphenylsilylchloride (1.00 $cm^3$, 3.90 mmol) followed by imidazole (1.03 g, 15.2 mmol). The mixture was stirred for 20 h at room temperature. Brine (50 $cm^3$) was added and the aqueous layer was extracted with EtOAc (3 × 250 $cm^3$). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [Hexane : EtOAc (4 : 1)] to give the title compound **107** (1.99 g, 85%) as a colourless oil. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.76; $[\alpha]_D$ – 28.4 (c 2.39, $CHCl_3$); $\nu_{max}$ (neat)/$cm^{-1}$ 1730, 1601, 1588, 1520; $\delta_H$ (250 MHz, $CDCl_3$) 7.78-7.65 (4H, m, Ar*H*), 7.65-7.62 (6H, m, Ar*H*), 7.44-7.30 (10H, m, Ar*H*), 4.08 (1H, dd, *J* 10.1, 6.1, $C_3H_xH_y$), 4.03 (2H, d, *J* 14.1, $NCH_xH_yPh$), 3.99 (1H, dd, *J* 10.1, 6.1, $C_3H_xH_y$), 3.78 (3H, s, O*Me*), 3.77 (2H, d, *J* 14.1, $NCH_xH_yPh$), 3.70 (1H, t, *J* 6.1, $C_2H$), 1.05 (9H, s, $^t$Bu); $\delta_C$ (62.8 MHz, $CDCl_3$) 171.8 (C), 139.6 (2C), 135.4 (3CH), 134.8 (CH), 133.0 (2C), 129.5 (2CH), 128.5 (4CH), 128.1 (4CH), 127.5 (4CH), 126.8 (2CH), 63.2 ($CH_2$), 62.8 ($CH_3$), 55.3 ($2CH_2$), 51.0 (CH), 26.6 ($3CH_3$), 19.0 (C); ***m/z*** (FAB, NOBA) 538 ($[M+H]^+$, 54%), 478 (48), 392 (25), 268 (45), 239 (31); **HRMS** (FAB, NOBA) $C_{34}H_{40}NO_3Si$ $[M+H]^+$ requires 538.2778, found 538.2778.

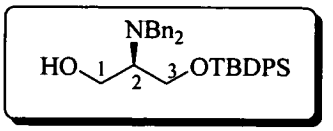
Spectroscopic data in good agreement with the literature.[98]

## Methyl (2*S*)-3-*tert*-butyldimethylsilyloxy-2-*N*,*N*-dibenzylaminopropanoate 108




To a solution of serine methyl ester **106** (1.00 g, 3.35 mmol) in anhydrous DMF (30 $cm^3$) was added *tert*-butyldimethylsilylchloride (0.480 g 3.18 mmol) followed by imidazole (0.800 g, 11.7 mmol). The mixture was stirred for 18 h at room temperature. Brine (50 $cm^3$) was added and the aqueous layer was extracted with EtOAc (3 × 250 $cm^3$). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [Hexane : EtOAc (4 : 1)] to give a title compound **108** (1.05 g, 76%) as a colourless oil. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.80; $[\alpha]_D$ - 140.2 (c 1.17, $CHCl_3$); $\nu_{max}$ (neat)/$cm^{-1}$ 1729, 1601, 1582; $\delta_H$ (250 MHz, $CDCl_3$) 7.42-7.23 (10H, m, Ar*H*), 4.00 (1H, dd, *J* 10.1, 6.1, $C_3H_xH_y$), 3.96 (2H, d, *J* 14.1, $NCH_xH_yPh$), 3.89 (1H, dd, *J* 10.1, 6.1, $C_3H_xH_y$), 3.76 (3H, s, O*Me*), 3.69 (2H, d, *J* 14.1, $NCH_xH_yPh$), 3.56 (1H, t, *J* 6.1, $C_2H$), 0.84 (9H, s, $^t$Bu), 0.00 (6H, s, *Me*Si × 2); $\delta_C$ (62.8 MHz, $CDCl_3$) 171.9 (C), 139.7 (2C), 128.8 (4CH), 128.1 (4CH), 126.8 (2CH), 62.9 ($CH_2$), 62.6 ($CH_3$), 55.3 ($2CH_2$), 50.9 (CH), 25.6 ($3CH_3$), 18.0 (C), -5.2 ($2CH_3$) ; ***m/z*** (FAB, THIOG) 413 ($[M]^+$, 61%), 398 (42), 354 (77), 336 (56), 322 (48); **HRMS** (FAB, NOBA) $C_{24}H_{36}NO_3Si$ [*M* + H]$^+$ requires 414.2465, found 414.2467.

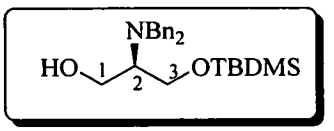
## (2*R*)-3-(*tert*-Butyldiphenylsilyloxy)-2-(dibenzylamino)propan-1-ol 109




To a solution of methyl (2*S*)-3-(*tert*-butyldiphenylsilyloxy)-2-*N,N*-dibenzylamino-propanoate **107** (1.50 g, 2.79 mmol) in $Et_2O$ (30 $cm^3$) at 0 °C was added lithium borohydride (0.36 g, 16.7 mmol) followed by anhydrous MeOH (0.5 $cm^3$). The mixture was stirred at 0 °C until effervescence ceased and then heated to reflux and held at reflux for 4 h. $NH_4Cl$ (50 $cm^3$, sat.) was added cautiously and the aqueous phase was extracted with DCM (3 x 50 $cm^3$). The combined organic phases were washed with brine (30 $cm^3$, sat.), dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **109** (1.16 g, 82%) as a colourless oil which was used without further purification. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.58; $[\alpha]_D$ + 52.3 (c 1.95, $CHCl_3$); $\nu_{max}$ (neat)/$cm^{-1}$ 3458, 1602; $\delta_H$ (250 MHz, $CDCl_3$) 7.95-7.91 (4H, m, Ar*H*), 7.70-7.66 (6H, m, Ar*H*), 7.54-7.47 (10H, m, Ar*H*), 4.14 (1H, dd, *J* 10.7, 6.0, $C_3H_AH_B$), 4.13 (2H, d, *J* 13.3, $NCH_xH_yPh$), 3.99 (1H, dd, *J* 10.7, 6.0, $C_3H_AH_B$), 3.85 (2H, d, *J* 13.3, $NCH_xH_yPh$), 3.82 (2H, d, *J* 7.5, $C_1H_2$), 3.34 (1H, dt, *J* 7.5, 6.0, $C_2H$), 3.16 (1H, br s, O*H*), 1.34 (9H, s, *tBu*); $\delta_C$ (62.8 MHz, $CDCl_3$) 139.9 (2C), 136.0 (2CH), 135.2 (CH), 133.5 (C), 133.4 (C), 130.3 (2CH), 129.9 (4CH), 129.3 (4CH), 128.8 (4CH), 128.2 (CH), 127.6 (2CH), 61.8 ($CH_2$), 60.4 (CH), 59.9 ($CH_2$), 54.4 ($2CH_2$), 27.3 ($3CH_3$), 19.5 (C); ***m/z*** (FAB, THIOG) 510 ($[M+H]^+$, 63%), 508 ($[M-H]^+$, 57), 478 (46), 240 (60), 210 (41), 199 (47); **HRMS** (FAB, THIOG) $C_{33}H_{38}NO_2Si$ $[M-H]^+$ requires 508.2672, found 508.2682.

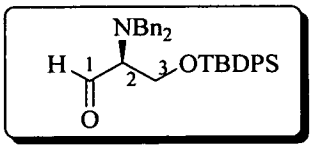
Spectroscopic data in good agreement with literature.[98]

## (2*R*)-3-(*tert*-Butyldimethylsilyloxy)-2-(dibenzylamino)propan-1-ol 110




To a solution of methyl (2*S*)-3-(*tert*-butyldimethylsilyloxy)-2-*N,N*-dibenzylamino-propanoate **108** (0.90 g, 2.2 mmol) in $Et_2O$ (21 $cm^3$) at 0 °C was added lithium borohydride (0.28 g, 13 mmol) followed by anhydrous MeOH (0.35 $cm^3$). The mixture was stirred at 0 °C until effervescence ceased and then heated to reflux and held at reflux for 4 h. $NH_4Cl$ (50 $cm^3$, sat.) was added cautiously and the aqueous phase was extracted with DCM (3 x 50 $cm^3$). The combined organic phases were washed with brine (30 $cm^3$, sat.), dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **110** (0.76 g, 90%) as a colourless oil which was used without further purification. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.55; $[\alpha]_D$ + 59.8 (c 1.26, $CHCl_3$); $\nu_{max}$ (neat)/$cm^{-1}$ 3465, 1602; $\delta_H$ (250 MHz, $CDCl_3$) 7.26-7.13 (10H, m, Ar*H*), 3.82 (2H, d, *J* 13.4, $NCH_xH_yPh$), 3.77 (1H, dd, *J* 10.6, 5.8, $C_3H_AH_B$), 3.65 (1H, dd, *J* 10.6, 5.8, $C_3H_AH_B$), 3.58 (2H, d, *J* 13.4, $NCH_xH_yPh$), 3.50 (1H, dd, *J* 10.6, 8.8, $C_1H_CH_D$), 3.44 (1H, dd, *J* 10.6, 5.8, $C_1H_CH_D$), 2.92 (1H, dq, *J* 8.8, 5.8, $C_2H$), 2.83 (1H, br s, O*H*), 0.84 (9H, s, *tBu*), -0.01 (3H, s, Si*Me*), -0.02 (3H, s, Si*Me*); $\delta_C$ (62.8 MHz, $CDCl_3$) 140.0 (2C), 129.3 (4CH), 128.8 (4CH), 127.5 (2CH), 61.3 ($CH_2$), 60.2 (CH), 59.9 ($CH_2$), 54.5 ($2CH_2$), 26.3 ($3CH_3$), 18.5 (C), -5.7 ($2CH_3$); ***m/z*** (FAB, THIOG) 386 ($[M+H]^+$, 62%), 384 ($[M-H]^+$, 62), 354 (62), 308 (46), 240 (66), 210 (41); **HRMS** (FAB, THIOG) $C_{23}H_{35}NO_2Si$ $[M+H]^+$ requires 386.2515, found 386.2510.

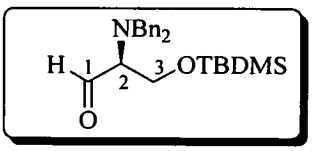
## (2*S*)-3-(*tert*-Butyldiphenylsilyloxy)-2-(dibenzylamino)propanal 7a




To a solution of oxalyl chloride (0.280 $cm^3$, 3.20 mmol) in DCM (10 $cm^3$) at -78 °C was added dropwise DMSO (0.230 $cm^3$, 3.20 mmol) and the mixture stirred for 10 min whereupon it became cloudy. A solution of (2*R*)-3-(*tert*-butyldiphenylsiloxy)-2-(dibenzylamino)propan-1-ol **109** (1.16 g, 2.28 mmol) in DCM (10 $cm^3$) was added *via* cannula and the resulting clear solution was stirred for 1 h. Triethylamine (1.27 $cm^3$, 9.13 mmol) was added, the resulting cloudy solution was allowed to warm to room temperature and stirred for a further hour. The mixture was diluted with water (50 $cm^3$), stirred for 10 min and then extracted with DCM (3 x 50 $cm^3$). The combined organic layers were washed with HCl (50 $cm^3$, 1N aq.), water (50 $cm^3$), $NaHCO_3$ (50 $cm^3$, sat. aq.), brine (50 $cm^3$, sat.), dried ($MgSO_4$) and concentrated *in vacuo* to give crude aldehyde **7a** (1.13 g, 96%) as an oil which was used in the aldol reaction without further purification. $\mathbf{R_f}$ [hexane:EtOAc (9:1)] 0.54; $\mathbf{\upsilon_{max}}$ **(neat)/cm$^{-1}$** 3069, 2711, 1731, 1602, 1589; $\mathbf{\delta_H}$ (250 MHz, $CHCl_3$) 9.79 (1H, s, $C_1H$), 7.79-7.71 (5H, m, Ar*H*), 7.48-7.30 (15H, m, Ar*H*), 4.16 (1H, dd, *J* 11.0, 5.7, $C_3H_AH_B$), 4.10 (1H, dd, *J* 11.0, 5.7, $C_3H_AH_B$), 3.98 (2H, d, *J* 13.8, $NCH_XH_YPh$), 3.91 (2H, d, *J* 13.8, $NCH_XH_YPh$), 3.52 (1H, t, *J* 5.7, $C_2H$), 1.13 (9H, s, *tBu*); $\mathbf{\delta_C}$ (62.9 MHz, $CHCl_3$) 203.3 (C), 139.8 (2C), 136.1 (2CH), 135.9 (2CH), 135.2 (CH), 133.2 (2C), 130.2 (CH), 129.1 (4CH), 128.8 (4CH), 128.2 (4CH), 127.6 (2CH), 68.4 (CH), 61.0 ($CH_2$), 56.1 ($2CH_2$), 27.2 ($3CH_3$), 19.5 (C).

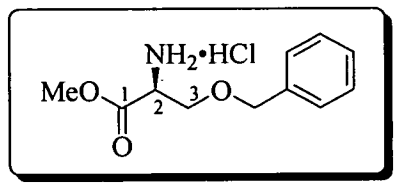
Spectroscopic data in good agreement with literature.[98]

## (2*S*)-3-(*tert*-Butyldimethylsilyloxy)-2-(dibenzylamino)propanal 7b




To a solution of oxalyl chloride (0.84 $cm^3$, 9.7 mmol) in DCM (10 $cm^3$) at -78 °C was added dropwise DMSO (0.68 $cm^3$, 9.7 mmol) and the mixture stirred for 10 min whereupon it became cloudy. A solution of (2*R*)-3-(*tert*-butyldimethylsiloxy)-2-(dibenzylamino)propan-1-ol **110** (2.6 g, 6.9 mmol) in DCM (10 $cm^3$) was added *via* cannula and the resulting clear solution was stirred for 1 h. Triethylamine (3.8 $cm^3$, 27 mmol) was added, the resulting cloudy solution was allowed to warm to room temperature and stirred for a further hour. The mixture was diluted with water (50 $cm^3$), stirred for 10 min and then extracted with DCM (3 x 50 $cm^3$). The combined organic layers were washed with HCl (50 $cm^3$, 1N aq.), water (50 $cm^3$), $NaHCO_3$ (50 $cm^3$, sat. aq.), brine (50 $cm^3$, aq.), dried ($MgSO_4$) and concentrated *in vacuo* to give crude aldehyde **7b** (2.38 g, 94%) as an oil which was used in the aldol reaction without further purification. $\mathbf{R_f}$ [hexane:EtOAc (4:1)] 0.66; $\mathbf{\upsilon_{max}}$ **(neat)/cm$^{-1}$** 3065, 2712, 1722, 1602, 1561; $\mathbf{\delta_H}$ (360 MHz, $CHCl_3$) 9.69 (1H, s, $C_1H$), 7.49-7.09 (10H, m, Ar*H*), 4.03 (1H, dd, *J* 10.8, 5.7, $C_3H_AH_B$), 3.99 (1H, dd, *J* 10.8, 5.7, $C_3H_AH_B$), 3.87 (2H, d, *J* 13.7, $NCH_XH_YPh$), 3.83 (2H, d, *J* 13.7, $NCH_XH_YPh$), 3.35 (1H, t, *J* 5.7, $C_2H$), 0.87 (9H, s, *tBu*), 0.05 (3H, s, Si*Me*), 0.04 (3H, s, Si*Me*); $\mathbf{\delta_C}$ (62.9 MHz, $CHCl_3$) 203.7 (C), 139.9 (2C), 129.2 (4CH), 128.8 (4CH), 127.6 (2CH), 60.4 (CH), 58.3 ($CH_2$), 54.9 ($2CH_2$), 26.3 ($3CH_3$), 18.6 (C), -3.1 ($2CH_3$).

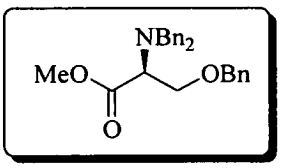
## Methyl (2*S*)-2-amino-3-benzyloxypropanoate hydrochloride 112




Acetyl chloride (3.3 $cm^3$, 46 mmol) was added dropwise to methanol (15 $cm^3$) at 0 °C. The mixture was stirred for *ca.* 15 min and *O*-benzyl-*L*-serine **111** (3.0 g, 15 mmol) was then added portionwise to the solution. The resulting mixture was heated to reflux and held at reflux for 3 h. Concentration under reduced pressure provided hydrochloride salt **112** (3.57 g, 96 %) as a white solid. $[\alpha]_D$ + 3.6 (c 1.38, MeOH); **mp** 140-144 °C ; $\nu_{max}$ (Nujol)/$cm^{-1}$ 1745, 1592; $\delta_H$ (250 MHz, $D_2O$) 7.16-7.08 (5H, m, Ar*H*), 4.36 (1H, d, *J* 12.0, OC$H_x$$H_y$Ph), 4.26 (1H, d, *J* 12.0, OC$H_x$$H_y$Ph), 4.08 (1H, br t, *J* 4.0, $C_2H$), 3.70 (1H, dd, *J* 11.0, 4.2, $C_3H_AH_B$), 3.61 (1H, dd, *J* 11.0, 3.3, $C_3H_AH_B$), 3.52 (3H, s, O*Me*); $\delta_C$ (62.8 MHz, $D_2O$) 169.0 (C), 137.1 (C), 129.1 (2CH), 128.9 (CH), 128.8 (2CH), 73.5 ($CH_2$), 66.7 ($CH_2$), 54.1 ($CH_3$), 53.5 (CH); ***m/z*** (FAB, NOBA) 210 ($[M+H]^+$, 93%), 196 (21), 154 (64), 150 (42), 136 (66), 120 (50), 107 (60); **HRMS** (FAB, NOBA) $C_{11}H_{16}NO_3$ $[M + H]^+$ requires 210.1130, found 210.1127; **CHN** requires (%) C 53.77, H 6.56, N 5.70, found (%) C 53.36, H 6.49, N 5.67.

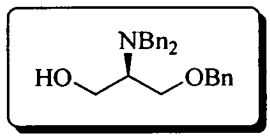
Spectroscopic data in good agreement with the literature.[99]

## Methyl (2*S*)-3-(benzyloxy)-2-(dibenzylamino)propanoate 113



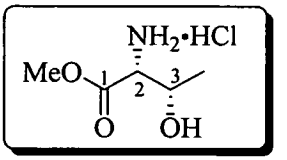

To a solution of the methyl (2*S*)-2-amino-3-benzyloxypropanoate hydrochloride **112** (3.05 g, 1.20 mmole) in acetonitrile (100 $cm^3$) was added anhydrous potassium carbonate (8.60 g, 6.20 mmol) followed by benzyl bromide (3.70 $cm^3$, 3.10 mmol). The resulting mixture was stirred at r.t. for 36 h. Water (100 $cm^3$) was added and the aqueous phase was extracted with EtOAc (3 x 50 $cm^3$). The combined organic phases were washed with brine (50 $cm^3$, sat.), dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **113** (3.43 g, 71%) as a colourless oil after column chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.60; $[\alpha]_D$ – 56.15 (c 1.3, $CHCl_3$); $\nu_{max}$ (Nujol)/$cm^{-1}$ 3062, 3029, 2949, 2855, 1735, 1601, 1585; $\delta_H$ (250 MHz, $CDCl_3$) 7.46-7.28 (15H, m, Ar*H*), 4.52 (2H, s, OC$H_2$Ph), 3.99 (2H, d, J 13.9, NC$H_x$H$_y$Ph), 3.90 (1H, t, *J* 9.2, $C_2H$), 3.92-3.73 (2H, m, $C_3H_2$), 3.84 (3H, s, O*Me*), 3.74 (2H, d, *J* 13.9, NCH$_x$$H_y$Ph); $\delta_C$ (62.8 MHz, $CDCl_3$) 172.4 (C), 140.0 (2C), 138.5 (C), 129.1 (4CH), 128.7 (4CH), 128.6 (4CH), 128.6 (CH), 127.9 (2CH), 127.4 (2CH), 73.5 ($CH_2$), 69.9 ($CH_2$), 61.3 (CH), 55.8 ($2CH_2$), 51.7 ($CH_3$); ***m/z*** (FAB, THIOG) 390 ($[M+H]^+$, 46%), 388 ($[M-H]^+$, 59), 330 (59), 268 (64), 181 (53), 165 (24), 132 (27), 118 (20), 105 (47); **HRMS** (FAB, NOBA) $C_{25}H_{28}NO_3$ $[M+H]^+$ requires 390.2059, found 390.2069.

## (2*R*)-3-(benzyloxy)-2-(dibenzylamino)propan-1-ol 114




To a solution of Methyl (2*S*)-3-(benzyloxy)-2-(dibenzylamino)propanoate **113** (2.00 g, 5.13 mmol) in $Et_2O$ (30 $cm^3$) at 0 °C was added lithium borohydride (0.67 g, 30.8 mmol) followed by anhydrous MeOH (0.5 $cm^3$). The mixture was stirred at 0 °C until effervescence ceased and then heated to reflux and held at reflux for 4 h. $NH_4Cl$ (50 $cm^3$, sat.) was added cautiously and the aqueous phase was extracted with DCM (3 x 50 $cm^3$). The combined organic phases were washed with brine (30 $cm^3$, sat.), dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **114** (1.77 g, 95%) as a colourless oil which was used without further purification. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.3; $[\alpha]_D$ + 84.3 (c 1.37, $CHCl_3$); $\nu_{max}$ (Nujol)/$cm^{-1}$ 3464, 1602, 1581, 1520; $\delta_H$ (250 MHz, $CDCl_3$) 7.84-7.21 (15H, m, Ar*H*), 4.75 (2H, s, $OCH_2Ph$), 4.10 (2H, d, *J* 13.5, $NCH_xH_yPh$), 3.97 (1H, dd, *J* 9.9, 6.3, $C_1H_AH_B$), 3.83 (2H, d, *J* 13.5, $NCH_xH_yPh$), 3.77-3.65 (3H, m, $C_1H_AH_B$+$C_3H_2$), 3.38 (1H, dq, *J* 13.7, 6.3, $C_2H$); $\delta_C$ (62.8 MHz, $CDCl_3$) 139.9 (2C), 138.5 (C), 129.4 (5CH), 128.9 (5CH), 128.2 (CH), 127.9 (2CH), 127.6 (2CH), 73.8 ($CH_2$), 68.4 ($CH_2$), 60.1 ($CH_2$), 58.6 (CH), 54.5 ($2CH_2$); ***m/z*** (FAB, THIOG) 362 ($[M+H]^+$, 67%), 330 (61), 284 (38), 254 (38), 240 (70); **HRMS** (FAB, THIOG) $C_{24}H_{28}NO_2$ $[M+H]^+$ requires 362.2120, found 362.2114.

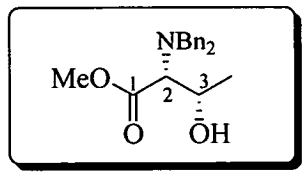
## Methyl (2*R*,3*S*)-2-amino-3-hydroxybutanoate hydrochloride salt 116




Acetyl chloride (30.0 $cm^3$, 3.89 mol) was added dropwise to methanol (140 $cm^3$) at 0 °C. The mixture was stirred for 15 min and *D*-threonine **115** (10.0 g, 0.841 mol) was added portionwise to the solution. The resulting mixture was heated to reflux and held at reflux for 4 h. Concentration under reduced pressure provided the title compound **116** (14.0 g, 98%) as a colourless solid. $[\alpha]_D$ + 7.0 (c 3.425, MeOH); **mp** 101-104 °C; $\upsilon_{max}$ (Nujol)/$cm^{-1}$ 3403, 2853, 1748, 1593, 1456, 1376; $\delta_H$ (250 MHz, $D_2O$) 4.56 (1H, qd, *J* 6.6, 3.8, $C_3HOH$), 4.26 (1H, d, *J* 3.8, $C_2H$), 3.99 (3H, s, O*Me*), 1.47 (3H, d, *J* 6.6, *Me*); $\delta_C$ (62.9 MHz, $D_2O$) 169.6 (C); 65.6 ($CH_3$); 58.7 (CH); 54.1 (CH); 19.2 ($CH_3$); ***m/z*** (ESI,+) 133.8 ($[M+H]^+$, 100%), 115.9 (71), 83.8 (15), 73.8 (54); **HRMS** (FAB, THIOG) $C_5H_{12}NO_3$ $[M+H]^+$ requires 134.0817, found 134.0818.

Spectroscopic data in good agreement with the literature.[115]

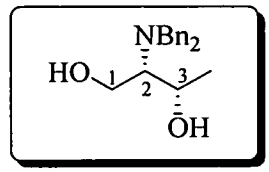
## Methyl (2*R*,3*S*)-2-*N*,*N*-dibenzylamino-3-hydroxybutanoate 117




To a solution of ester **116** (10.0 g, 59.0 mmol) in anhydrous acetonitrile (300 $cm^3$) was added anhydrous potassium carbonate (40.8 g, 295 mmol) followed by benzylbromide (28.1 $cm^3$, 236 mmol). The resulting mixture was stirred for 36 h at room temperature. Water (150 $cm^3$) was added and the aqueous layer was extracted with EtOAc (3 × 100 $cm^3$). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [hexane:EtOAc (4:1)] to give the title compound **117** (13.9 g, 76%) as a yellow oil. $\mathbf{R_f}$ [Hexane:EtOAc (4:1)] 0.45; $[\alpha]_D$ + 223.6 (c 1.825, $CHCl_3$); $\upsilon_{max}$ (neat)/$cm^{-1}$ 3453, 3029, 2950, 1731, 1454; $\delta_H$ (250 MHz, $CDCl_3$) 7.65-7.47 (10H, m, Ar*H*), 4.26 (2H, d, *J* 13.5, NC$H_x$$H_y$Ph × 2), 4.29-4.20 (1H, m, $C_3H$OH), 4.06 (3H, s, O*Me*), 3.75 (2H, d, *J* 13.5, NC$H_x$$H_y$Ph × 2), 3.31 (1H, d, *J* 9.7, $C_2H$), 1.31 (3H, d, *J* 6.0, *Me*); $\delta_C$ (62.9 MHz, $CDCl_3$) 170.5 (C), 137.9 (2C), 129.0 (4CH), 128.4 (4CH), 127.4 (2CH), 67.2 (CH), 63.0 (CH), 54.7 ($2CH_2$), 51.2 ($CH_3$), 19.0 ($CH_3$); ***m/z*** (FAB, THIOG) 314.1 ($[M+H]^+$, 100%); **HRMS** (FAB, NOBA) $C_{19}H_{24}NO_3$ $[M+H]^+$ requires 314.1756, found 314.1756.

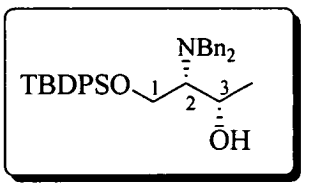
Spectroscopic data in good agreement with the literature.[115]

## (2*S*,3*S*)-2-*N*,*N*-Dibenzylamino-1,3-dihydroxybutane 118




To a solution of ester **117** (12.0 g, 38.3 mmol) in ether (120 $cm^3$) at 0 °C was added lithium borohydride (4.90 g, 0.230 mol) followed by methanol (12 $cm^3$). The mixture was stirred at 0 °C until effervescence ceased and then heated to reflux and held at reflux for 4 h. The reaction was quenched by the cautious addition of saturated aqueous $NH_4Cl$ (100 $cm^3$) and the aqueous phase was extracted with EtOAc (3 × 100 $cm^3$). The combined organic phases were washed with brine (100 $cm^3$), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed on silica gel [DCM:MeOH (30:1)] to give the title compound **118** (8.35 g, 92%) as a colourelss solid. $R_f$ [DCM:MeOH (10:1)] 0.55; $[\alpha]_D$ + 53.6 (c 0.69, $CHCl_3$); **mp** 92-94 °C; $\upsilon_{max}$ (neat)/$cm^{-1}$ 3372, 3021, 2854, 1455; $\delta_H$ (250 MHz, $CDCl_3$) 7.70-7.31 (10H, m, Ar*H*), 4.20 (2H, d, *J* 13.2, NC$H_x$$H_y$Ph × 2), 4.08 (1H, dq, *J* 9.3, 6.1, $C_3$*H*OH), 4.02 (2H, d, *J* 5.8, $C_1H_2$), 3.86 (2H, d, *J* 13.2, NC$H_x$$H_y$Ph × 2), 2.82 (1H, dt, *J* 9.3, 5.8, $C_2$*H*), 1.36 (3H, d, *J* 6.1, *Me*); $\delta_C$ (62.9 MHz, $CDCl_3$) 139.0 (2C), 129.1 (4CH), 128.4 (4CH), 127.2 (2CH), 65.3 (CH), 64.5 (CH), 59.2 ($CH_2$), 54.4 ($2CH_2$), 20.2 ($CH_3$); ***m/z*** (FAB, THIOG) 286 ($[M+H]^+$, 96%), 240 (72), 215 (13), 196 (23); **HRMS** (FAB, THIOG) $C_{18}H_{24}NO_2$ $[M+H]^+$ requires 286.1807, found 286.1808; **CHN** requires (%) C 75.76, H 8.12, N 4.91, found (%) C 75.47, H 7.96, N 4.69.

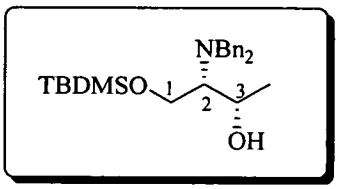
## (2*S*,3*S*)-1-*tert*-Butyldiphenylsilyloxy-2-*N*,*N*-dibenzylamino-3-hydroxy-butane 119




To a solution of (2*S*,3*S*)-2-*N*,*N*-dibenzylamino-1,3-dihydroxybutane **118** (5.00 g, 17.5 mmol) in anhydrous DMF (60 $cm^3$) was added *tert*-butyldiphenylsilylchloride (4.10 $cm^3$, 15.9 mmol) followed by imidazole (4.26 g, 61.3 mmol). The resultant mixture was stirred for 36 h at room temperature. Brine (30 $cm^3$) was added and the aqueous phase was extracted with EtOAc (3 × 50 $cm^3$). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (4:1)] to give the title compound **119** (6.68 g, 73%) as a colourless solid. $R_f$ [hexane:EtOAc (4:1)] 0.53; $[\alpha]_D$ + 53.04 (c 1.15, $CHCl_3$); **mp** 67-71 °C; $\upsilon_{max}$ **(Nujol)/cm$^{-1}$** 3412, 3023, 2854, 1462; $\delta_H$ (250 MHz, $CDCl_3$) 7.55-7.51 (4H, m, Ar*H*), 7.30-7.24 (6H, m, Ar*H*), 7.12-7.03 (10H, m, Ar*H*), 4.80 (1H, br s, O*H*), 3.80 (2H, d, *J* 13.3, NC$H_x$$H_y$Ph × 2), 3.67 (1H, dd, *J* 11.6, 4.0, $C_1H_AH_B$OTBDPS), 3.69 (1H, dd, *J* 11.6, 5.9, $C_1H_AH_B$OTBDPS), 3.63 (1H, dq, *J* 9.5, 6.0, $C_3H$OH), 3.43 (1H, d, *J* 13.3, NC$H_x$$H_y$Ph × 2), 2.39 (1H, ddd, *J* 9.5, 5.9, 3.9, $C_2H$), 0.91 (9H, s, $^tBu$), 0.82 (3H, d, *J* 6.0, *Me*); $\delta_C$ (62.9 MHz, $CDCl_3$) 139.2 (2C), 135.6 (2CH), 134.7 (CH), 132.9 (C), 132.8 (C), 129.8 (CH), 128.9 (4CH), 128.4 (4CH), 127.7 (4CH), 127.5 (CH), 127.1 (2CH), 121.8 (CH), 65.7 (CH), 63.3 (CH), 60.2 ($CH_2$), 54.5 ($2CH_2$), 26.8 ($3CH_3$), 19.6 ($CH_3$), 19.1 (C); ***m/z*** (FAB, THIOG) 524 ($[M+H]^+$, 80%), 478 (64), 254 (26), 199 (59); **HRMS** (FAB, THIOG) $C_{34}H_{42}NO_2Si$ $[M+H]^+$ requires 524.2985, found 524.2972; **CHN** requires (%) C 77.96, H 7.89, N 2.67, found (%) C 77.70, H 7.82, N 2.37.

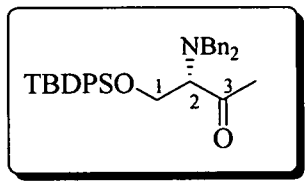
Spectroscopic data in good agreement with the literature.[115]

## (2*S*,3*S*)-1-*tert*-Butyldimethylsilyloxy-2-*N*,*N*-dibenzylamino-3-hydroxybutane 120




To a solution of (2*S*,3*S*)-2-*N*,*N*-dibenzylamino-1,3-dihydroxybutane **118** (8.50 g, 29.8 mmol) in anhydrous DMF (92 ml) was added *tert*-butyldimethylsilylchloride (4.60 g, 31.3 mmol) followed by imidazole (7.10 g, 104 mmol). The resultant mixture was stirred for 36 h at room temperature. Brine (100 $cm^3$) was added and the aqueous phase was extracted with EtOAc (3 × 100 $cm^3$). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed on silica gel [hexane:EtOAc (4:1)] to give a title compound **120** (7.50 g, 62%) as a colourless solid. $\mathbf{R_f}$ [hexane:EtOAc (4:1)] 0.53; $[\alpha]_D$ + 50.1 (c 2.5, $CHCl_3$); **mp** 54-56 °C; $\upsilon_{max}$ (neat)/$cm^{-1}$ 3400, 3025, 2854, 1457, 1084; $\delta_H$ (250 MHz, $CDCl_3$) 7.22-7.10 (10H, m, Ar*H*), 4.02 (1H, br s, O*H*), 3.87 (2H, d, *J* 13.3, NC$H_x$$H_y$Ph × 2), 3.78 (2H, dd, *J* 11.2, 3.3 $C_1H_AH_B$OTBDMS), 3.68 (2H, dd, *J* 11.2, 6.0, $C_1H_AH_B$OTBDMS), 3.75-3.65 (1H, m, $C_3H$OH), 3.48 (2H, d, *J* 13.3, NC$H_x$$H_y$Ph × 2), 2.38 (1H, ddd, *J* 9.1, 6.0, 3.3, $C_2H$), 0.96 (3H, d, *J* 6.0, *Me*), 0.82 (9H, s, *$^t$Bu*), 0.00 (3H, s, *Me*Si), -0.02 (3H, s, *Me*Si); $\delta_C$ (62.9 MHz, $CDCl_3$) 139.2 (2C), 129.0 (4CH), 128.3 (4CH), 127.0 (2CH), 65.3 (CH), 63.1 (CH), 59.0 ($CH_2$), 54.4 ($2CH_2$), 25.7 ($3CH_3$), 19.4 ($CH_3$), 17.9 (C), -5.7 ($2CH_3$); ***m/z*** (FAB, NOBA) 400 ($[M+H]^+$, 90%), 354 (98), 322 (48), 254 (66), 181 (43); **HRMS** (FAB, NOBA) $C_{24}H_{38}NO_2Si$ $[M+H]^+$ requires 400.2672, found 400.2667; **CHN** requires (%) C 72.13, H 9.33, N 3.50, found (%) C 71.02, H 9.50, N 3.36.

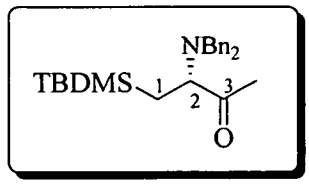
## (3*S*)-1-*tert*-Butyldiphenylsilyloxy-2-*N*,*N*-dibenzylaminobutan-3-one 8a




To a solution of oxalyl chloride (0.230 $cm^3$, 2.67 mmol) in DCM (9 $cm^3$) at -78 °C was added DMSO (0.280 $cm^3$, 4.01 mmol). The mixture was stirred for ca. 5 min when it became cloudy. A solution of alcohol **119** (1.00 g, 1.91 mmol) in DCM (10 $cm^3$) was added *via* cannula. The resulting clear solution was stirred at -78 °C for 1 h. Triethylamine (1.09 $cm^3$, 7.83 mmol) was added and the cloudy solution was allowed to warm to room temperature over ca. 15 min. Water (50 $cm^3$) was added and the aqueous phase was extracted with DCM (3 × 50 $cm^3$). The combined organic phases were washed with 1% HCl (50 $cm^3$), water (50 $cm^3$), $NaHCO_3$ (50 $cm^3$, sat. aq.) and brine (50 $cm^3$, sat.), then dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **8a** (1.00 g, 100%) as a very pale yellow oil that was used in subsequent stages without further purification. $\mathbf{R_f}$ [hexane:EtOAc (4:1)] 0.70; $\upsilon_{max}$ **(neat)/cm$^{-1}$** 3027, 2930, 2857, 1718, 1602, 1589, 1494; $\delta_H$ (360 MHz, $CHCl_3$) 7.64-7.62 (5H, m, Ar*H*), 7.37-7.20 (15H, m, Ar*H*), 4.07 (1H, dd, *J* 10.7, 6.0, $C_1H_AH_B$OTBDPS), 4.00 (1H, dd, *J* 10.7, 6.0, $C_1H_AH_B$OTBDPS), 3.78 (2H, d, *J* 13.7, $NCH_XH_YPh$ × 2), 3.72 (2H, d, *J* 13.7, $NCH_XH_YPh$ × 2), 3.50 (1H, t, *J* 6.0, $C_2H$), 2.09 (3H, s, *Me*), 1.02 (9H, s, $^tBu$); $\delta_C$ (62.9 MHz, $CHCl_3$) 208.7 (C), 139.5 (2C), 135.5 (4CH), 134.6 (CH), 132.9 (2C), 129.7 (CH), 129.6 (CH), 128.7 (4CH), 128.2 (4CH), 127.6 (3CH), 126.9 (2CH), 67.6 (CH), 60.7 ($CH_2$), 55.1 ($2CH_2$), 28.9 ($CH_3$), 26.7 ($3CH_3$), 19.0 (C).

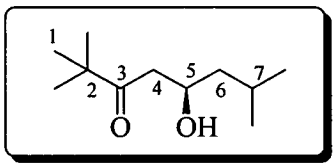
Spectroscopic data in good agreement with the literature.[115]

## (3*S*)-1-*tert*-Butyldimethylsilyloxy-2-*N*,*N*-dibenzylaminobutan-3-one 8b




To a solution of oxalyl chloride (0.160 $cm^3$, 1.82 mmol) in DCM (5 $cm^3$) at -78 °C was added DMSO (0.190 $cm^3$, 2.73 mmol). The mixture was stirred for ca. 5 min when it became cloudy. A solution of alcohol **120** (0.500 g, 1.30 mmol) in DCM (5 $cm^3$) was added *via* cannula. The resulting clear solution was stirred at -78 °C for 1 h. Triethylamine (0.740 $cm^3$, 5.33 mmol) was added and the cloudy solution was allowed to warm to room temperature over ca. 15 min. Water (30 $cm^3$) was added and the aqueous phase was extracted with DCM (3 × 50 $cm^3$). The combined organic phases were washed with 1% HCl (50 $cm^3$), water (50 $cm^3$), $NaHCO_3$ (50 $cm^3$, sat. aq.) and brine (50 $cm^3$, sat.), then dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **8b** (0.50 g, 100%) as a very pale yellow oil that was used in subsequent stages without further purification. $\mathbf{R_f}$ [hexane:EtOAc (4:1)] 0.56; $\upsilon_{max}$ **(neat)/cm$^{-1}$** 3029, 2856, 1735; $\delta_H$ (250 MHz, $CHCl_3$) 7.31-7.12 (10H, m, Ar*H*), 3.91 (2H, m, $C_4H_2$), 3.73 (4H, s, $NCH_2Ph$ × 2), 3.38 (1H, t, *J* 6.0, $C_3H$), 2.07 (3H, s, *Me*), 0.81 (9H, s, $^tBu$), 0.05 (6H, d, *J* 4.5, *Me*Si × 2); $\delta_C$ (62.9 MHz, $CHCl_3$) 209.3 (C), 139.6 (2C), 128.8 (4CH), 128.2 (4CH), 126.9 (2CH), 67.4 (CH), 60.4 ($CH_2$), 55.1 ($2CH_2$), 28.9 ($CH_3$), 25.8 ($3CH_3$), 18.1 (C), -5.7 ($2CH_3$).

## (5*RS*)-5-hydroxy-2,2,7-trimethyloctan-3-one[214] 153

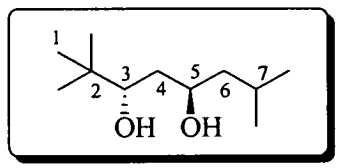



To a stirred solution of 3,3-dimethylbutan-2-one **149** (1.0 $cm^3$, 8.0 mmol) in DCM (5 $cm^3$) at -78 °C was added $Bu_2BOTf$ (3.5 $cm^3$, 1.0 M in hexane, 16 mmol) followed by $^iPr_2EtN$ (4.2 $cm^3$, 24 mmol). The mixture was stirred for 2 h at -78 °C and then isovaleraldehyde **150** (2.6 $cm^3$, 24 mmol) was added. The reaction mixture was stirred at -78 °C for 2 h and then was transferred to ice bath (0 °C) for 1 h. The mixture was quenched by addition of pH 7 buffer and MeOH (1:1, 0.5 $cm^3$) and diluted with MeOH (5 $cm^3$) to make a homogeneous solution. After careful addition of 30% $H_2O_2$ (0.25 $cm^3$) the reaction mixture was stirred at room temperature for 14 h. Brine (20 $cm^3$) was added and the mixture was extracted with DCM (3 × 20 $cm^3$). The combined organics were washed with brine (20 $cm^3$), dried ($MgSO_4$) and concentrated under the reduced pressure. The remaining residue was chromatographed on silica gel [Hexane : EtOAc (9 : 1)] to give the title compound **153** (1.40 g, 95%) as a pale yellow oil. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.40; $\nu_{max}$ (neat)/$cm^{-1}$ 3457, 2956, 2870, 1703; $\delta_H$ (250 MHz, $CDCl_3$) 4.03 (2H, tdd, *J* 9.0, 4.3, 3.0, $C_5H$), 3.30 (1H, br s, O*H*), 2.60 (1H, dd, *J* 17.9, 3.0, $C_4H_XH_Y$), 2.47 (1H, dd, *J* 17.9, 8.7, $C_4H_XH_Y$), 1.78-1.58 (1H, m, $C_6H_AH_B$), 1.42 (1H, ddd, *J* 13.8, 9.0, 5.4, $C_6H_AH_B$), 1.08 (9H, s, $^tBu$), 1.07 (1H, m, $C_7H$), 0.86 (6H, d, *J* 6.6, *Me*); $\delta_C$ (62.8 MHz, $CDCl_3$) 217.8 (C), 65.7 (CH), 45.5 ($CH_2$), 44.2 (C), 43.4 ($CH_2$), 26.1 ($3CH_3$), 24.2 (CH), 23.2 ($CH_3$), 21.9 ($CH_3$); ***m/z*** (FAB, THIOG) 187 ($[M+H]^+$, 38%), 169 (27), 111 (17), 109 (15); **HRMS** (FAB, THIOG) $C_{11}H_{23}O_2$ $[M + H]^+$ requires 187.1698, found 187.1698.

## General procedure A (*anti* reduction):

To a solution of $Me_4NHB(OAc)_3$ (1.53 mmol) in anhydrous acetonitrile (5.0 $cm^3$) and acetic acid (2.0 $cm^3$) at -40 °C was added the aldol adduct (0.31 mmol) in anhydrous acetonitrile (1 $cm^3$). The mixture was stirred overnight at -40 °C. The reaction mixture was quenched with saturated sodium-potassium tartrate (20 $cm^3$) and then allowed to warm to room temperature. The aqueous phase was extracted with DCM (3 × 20 $cm^3$). $NaHCO_3$ (20 $cm^3$, sat. aq.) was added slowly to the aqueous phase until effervescence ceased and the solution obtained was again extracted with DCM (3 × 20 $cm^3$). The combined organic phases were washed with $NaHCO_3$ (20 $cm^3$, sat. aq.), brine (20 $cm^3$), dried ($MgSO_4$) and concentrated under reduced pressure.

## (3*SR*,5*RS*)-2,2,7-trimethyloctane-3,5-diol 155




**General procedure A** was followed with $Me_4NHB(OAc)_3$ (1.13 g, 4.29 mmol), anhydrous acetonitrile (2.5 $cm^3$), acetic acid (1.5 $cm^3$) and (5*RS*)-5-hydroxy-2,2,7-trimethyloctan-3-one **153** (0.10 g, 0.54 mmol) thus providing the title compound **155** (0.084 g, 83%) as a clear oil as an inseparable mixture of diastereoisomers (90 : 10) after the chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (4 : 1)] 0.19; ***m/z*** (FAB, NOBA) 189 ($[M+H]^+$, 25%), 154 (60), 136 (56), 107 (43), 97 (60); **HRMS** (FAB, NOBA) $C_{11}H_{25}O_2$ $[M + H]^+$ requires 189.1855, found 187.1856.

**Major diastereoisomer (*anti*):**

$\delta_H$ (250 MHz, $CDCl_3$) 4.11 (1H, ddd, *J* 10.6, 8.8, 4.4, $C_5H$), 3.69 (1H, dd, *J* 8.8, 4.0, $C_3H$), 2.60 (2H, br s, O*H*), 1.88-1.83 (1H, m, $C_7H$), 1.67-1.56 (3H, m, $C_4H_2$ and $C_6H_AH_B$), 1.36 (1H, ddd, *J* 13.0, 8.4, 4.7, $C_6H_AH_B$), 1.04 (3H, d, *J* 6.6, *Me*), 1.02 (3H, d, *J* 6.6, *Me*), 1.00 (9H, s, $^tBu$); $\delta_C$ (62.8 MHz, $CDCl_3$) 75.9 (CH), 67.5 (CH), 46.2 ($CH_2$), 37.4 ($CH_2$), 34.5 (C), 25.2 ($3CH_3$), 24.6 (CH), 23.2 ($CH_3$), 22.1 ($CH_3$);

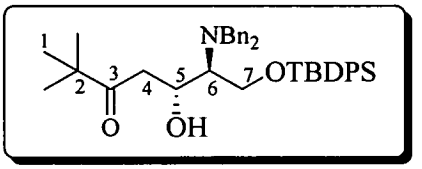
**Minor diastereoisomer (*syn*):**

$\delta_H$ (250 MHz, $CDCl_3$) 3.60 (1H, dd, *J* 9.0, 1.7, $C_5H$).

## General procedure B (Lithium enolate aldol reaction):

To a solution of LiHMDS (1.06 M in THF, 2.70 mmol) at -78 °C was added the ketone (1.80 mmol) in THF (6 $cm^3$) *via* cannula. The solution was stirred at -78 °C for 1 h. The aldehyde (2.16 mmol) was added and the solution was stirred for 10 min. The reaction was quenched by the addition of $NH_4Cl$ (20 $cm^3$, sat. aq.) at -78 °C and then the reaction mixture was transferred to an ice bath and allowed to warm up to 0 °C slowly. The reaction mixture was then warmed to room temperature. The aqueous phase was extracted with DCM (3 × 20 $cm^3$). The combined organic phases were washed with brine (20 $cm^3$, sat.), dried ($MgSO_4$) and concentrated under reduced pressure. The remaining residue was chromatographed on silica gel [Hexane : EtOAc (15:1)] to give the title compounds.

## (5*R*,6*S*)-7-*tert*-Butyldiphenylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one 157




**General procedure B** was followed with 3,3-dimethylbutan-2-one (0.220 $cm^3$, 1.75 mmol), LiHMDS (2.47 $cm^3$, 1.06 M in THF, 2.62 mmol) and the aldehyde **7a** (1.07 g, 2.10 mmol) thus providing the title compound **157** (0.710 g, 67%) as a clear oil as an inseparable mixture of diastereoisomers (97 : 3) after the chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.37; $\nu_{max}$ (neat)/$cm^{-1}$ 3521, 1694, 1602, 1589; ***m/z*** (FAB, THIOG) 608 ($[M+H]^+$, 73%), 530 (37), 479 (66), 338 (57), 252 (43); **HRMS** (FAB, THIOG) $C_{39}H_{49}NO_3Si$ $[M + H]^+$ requires 608.3560, found 608.3561.

**Major diastereoisomer (*anti*):**

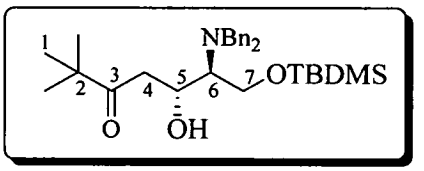
$\delta_H$ (360 MHz, $CDCl_3$) 7.78-7.75 (4H, m, Ar*H*), 7.47-7.42 (6H, m, Ar*H*), 7.30-7.24 (10H, m, Ar*H*), 4.27 (1H, tdd, *J* 9.8, 3.1, 1.8, $C_5H$), 4.19 (1H, dd, *J* 11.0, 4.2, $C_7H_AH_B$OTBDPS), 4.09 (1H, dd, *J* 11.0, 6.5, $C_7H_AH_B$OTBDPS), 3.91 (2H, d, *J* 13.6, $NCH_XH_YPh$), 3.73 (2H, d, *J* 13.6, $NCH_XH_YPh$), 3.20-3.15 (1H, dd, *J* 18.5, 1.8, $C_4H_CH_D$), 3.17 (1H, d, *J* 3.1, O*H*), 2.76 (1H, ddd, *J* 8.8, 6.5, 4.2, $C_6H$), 2.19 (1H, dd, *J* 18.5, 9.8, $C_4H_CH_D$), 1.14 (9H, s, *'Bu*), 1.10 (9H, s, *'Bu*); $\delta_C$ (90.6 MHz, $CDCl_3$) 217.8 (C), 140.1 (2C), 135.7 (2CH), 135.6 (2CH), 133.2 (C), 133.0 (C), 129.7 (CH), 129.6 (CH), 128.9 (4CH), 128.1 (4CH), 127.7 (2CH), 127.6 (2CH), 126.8 (2CH), 66.7 (2CH), 61.3 ($CH_2$), 55.2 ($2CH_2$), 44.0 (C), 41.7 ($CH_2$), 26.8 ($3CH_3$), 26.2 ($3CH_3$), 19.0 (C).

**Minor diastereoisomer (*syn*):**

$\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.35;

$\delta_H$ (360 MHz, $CDCl_3$) 3.57 (2H, d, *J* 13.4, $NCH_XH_YPh$).

## (5*R*,6*S*)-7-*tert*-Butyldimethylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one 158




**General procedure A** was followed with 3,3-dimethylbutan-2-one (0.10 $cm^3$, 0.79 mmol), LiHMDS (0.97 $cm^3$, 1.06 M in THF, 1.0 mmol) and the aldehyde **7b** (0.44 g, 1.19 mmol) thus providing the title compound **158** (0.34 g, 92%) as a clear oil as an inseparable mixture of diastereoisomers (95 : 5) after chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.35; $\nu_{max}$ **(neat)/cm$^{-1}$** 3522, 1693; ***m/z*** (FAB, NOBA) 484 ($[M+H]^+$, 51%), 355 (54), 210 (39); **HRMS** (FAB, NOBA) $C_{29}H_{46}NO_3Si$ $[M + H]^+$ requires 484.3247, found 484.3247.

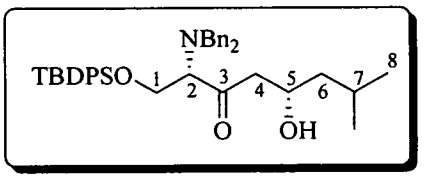
**Major diastereoisomer (*anti*):**

$\delta_H$ (360 MHz, $CDCl_3$) 7.31-7.21 (10H, m, Ar*H*), 4.29 (1H, tdd, *J* 9.8, 3.1, 1.8, $C_5H$), 4.13 (1H, dd, *J* 10.7, 4.1, $C_7H_AH_B$OTBDMS), 3.99 (1H, dd, *J* 10.7, 6.7, $C_7H_AH_B$OTBDMS), 3.87 (2H, d, *J* 13.6, $NCH_XH_Y$Ph), 3.69 (2H, d, *J* 13.6, $NCH_XH_Y$Ph), 3.19 (1H, d, *J* 3.1, O*H*), 3.16 (1H, dd, *J* 18.3, 1.8, $C_4H_CH_D$), 2.66 (1H, ddd, *J* 9.0, 6.7, 4.1, $C_6H$), 2.13 (1H, dd, *J* 18.3, 9.8, $C_4H_CH_D$), 1.08 (9H, s, $^tBu$), 0.95 (9H, s, $^tBu$), 0.12 (6H, s, *Me*Si × 2); $\delta_C$ (90.6 MHz, $CDCl_3$) 217.9 (C), 140.1 (2C), 129.0 (4CH), 128.1 (4CH), 126.7 (2CH), 66.8 (CH), 61.1 (CH), 60.5 ($CH_2$), 55.1 ($2CH_2$), 44.1 (C), 41.8 ($CH_2$), 26.2 ($3CH_3$), 25.9 ($3CH_3$), 18.1 (C), -5.5 ($2CH_3$).

**Minor diastereoisomer (*syn*):**

$\delta_H$ (360 MHz, $CDCl_3$) 3.60 (2H, d, *J* 13.2, $NCH_XH_Y$Ph).

## (2*S*,5*S*)-1-*tert*-Butyldiphenylsilyloxy-2-*N*,*N*-dibenzylamino-5-hydroxy-7-methyloctan-3-one 160




**General procedure B** was followed with (3*S*)-1-*tert*-butyldiphenylsilyloxy-2-*N*,*N*-dibenzylaminobutan-3-one **8a** (0.30 g, 0.58 mmol), LiHMDS (0.71 $cm^3$, 1.06 M in THF, 0.75 mmol) and isovaleraldehyde (0.10 g, 0.97 mmol) thus providing the title compound **160** (0.17 g, 50%) as a clear oil as an inseparable mixture of diastereoisomers (85 : 15) after chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (5 : 1)] 0.5; $\nu_{max}$ **(neat)/cm$^{-1}$** 3435, 2953, 2928, 2857, 1603, 1454.

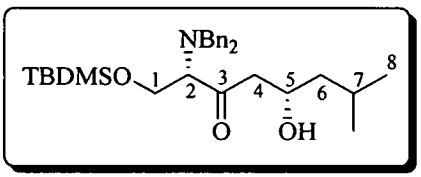
**Major diastereoisomer 160a (2,5-*syn*):**

$\delta_H$ (360 MHz, $CDCl_3$) 7.93-7.87 (4H, m, Ar*H*), 7.66-7.31 (16H, m, Ar*H*), 4.30 (1H, dd, *J*, 10.6, 6.4, $C_1H_AH_B$), 4.29-4.19 (1H, m, $C_5H$), 4.25 (1H, dd, *J* 10.6, 5.6, $C_1H_AH_B$), 4.07 (2H, d, *J* 13.7, $NCH_XH_YPh$), 3.96 (2H, d, *J* 13.7, $NCH_XH_YPh$), 3.79 (1H, t, *J* 6.0, $C_2H$), 3.20 (1H, br s, O*H*), 2.83 (1H, dd, *J* 17.4, 7.7, $C_4H_CH_D$), 2.74 (1H, dd, *J* 17.4, 3.2, $C_4H_CH_D$), 2.06-1.83 (1H, m, $C_6H_CH_D$), 1.73 (1H, ddd, *J* 13.7, 8.8, 5.4, $C_6H_CH_D$), 1.35-1.20 (1H, m, $C_7H$), 1.29 (9H, s, $^tBu$), 1.10 (3H, d, *J* 7.0, $CH_3$), 1.09 (3H, d, *J* 6.6, $CH_3$); $\delta_C$ (90.6 MHz, $CDCl_3$) 211.9 (C), 139.3 (2C), 135.5 (4CH), 134.7 (2CH), 132. 8 (C), 129.7 (CH), 129.6 (C), 128.7 (4CH), 128.2 (4CH), 127.5 (4CH), 127.0 (CH), 67.1 (CH), 65.6 (CH), 60.6 ($CH_2$), 55.1 ($2CH_2$), 48.6 ($CH_2$), 45.5 ($CH_2$), 26.4 ($3CH_3$), 24.2 (CH), 23.2 ($CH_3$), 22.0 ($CH_3$), 18.9 (C).

**Minor diastereoisomer 160b (2,5-*anti*):**

$\delta_H$ (360 MHz, $CDCl_3$) 2.92 (1H, dd, *J* 17.4, 2.5, $C_4H_CH_D$), 2.58 (1H, dd, *J* 17.4, 9.1 $C_4H_CH_D$).

## (2*S*,5*S*)-1-*tert*-Butyldimethylsilyloxy-2-*N*,*N*-dibenzylamino-5-hydroxy-7-methyloctan-3-one 161




**General procedure B** was followed with (3*S*)-1-*tert*-butyldimethyllsilyloxy-2-*N*,*N*-dibenzylaminobutan-3-one **8b** (0.35 g, 0.89 mmol), LiHMDS (1.25 $cm^3$, 1.06 M in THF, 1.33 mmol) and isovaleraldehyde (0.11 g, 1.07 mmol) thus providing the title compound **161** (0.41 g, 99%) as a clear oil as an inseparable mixture of diastereoisomers (87 : 13) after chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.34; $\nu_{max}$ **(neat)/cm$^{-1}$** 3435, 2953, 2928, 2857, 1603, 1454; ***m/z*** (FAB, NOBA) 484 ($[M+H]^+$, 15%), 355 (92), 350 (10), 196 (5), 181 (4).

**Major diastereoisomer 161a (2,5-*syn*):**

$\delta_H$ (360 MHz, $CDCl_3$) 7.37-7.18 (10H, m, Ar*H*), 4.04 (2H, d, *J* 6.1, $C_1H_2$), 4.03-3.98 (1H, m, $C_5H$), 3.84 (2H, d, *J* 13.5, $NCH_XH_YPh$), 3.79 (2H, d, *J* 13.5, $NCH_XH_YPh$), 3.50 (1H, t, *J* 6.1, $C_2H$), 2.97 (1H, d, *J* 3.0, O*H*), 2.64-2.52 (2H, m, $C_4H_2$), 1.79-1.70 (1H, m, $C_7H$), 1.45 (1H, ddd, *J* 13.7, 8.9, 5.5, $C_6H_AH_B$), 1.09 (1H, ddd, *J* 13.7, 8.5, 4.4, $C_6H_AH_B$), 0.96-0.94 (6H, m, $CH_3$ x 2), 0.90 (9H, s, $^tBu$), 0.09 (3H, s, *Me*Si), 0.07 (3H, s, *Me*Si); $\delta_C$ (90.6 MHz, $CDCl_3$) 212.5 (C), 139.4 (2C), 129.9 (4CH), 129.4 (4CH), 128.2 (2CH), 66.8 (CH), 65.6 (CH), 59.8 ($CH_2$), 55.1 ($2CH_2$), 48.7 ($CH_2$), 45.6 ($CH_2$), 25.2 ($3CH_3$), 24.2 (CH), 23.2 ($CH_3$), 22.5 ($CH_3$), 18.1 (C), -4.5 ($2CH_3$).

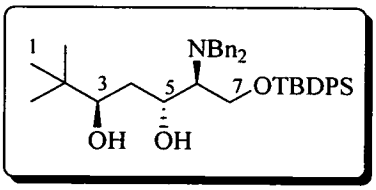
**Minor diastereoisomer 161b (2,5-*anti*):**

$\delta_H$ (360 MHz, $CDCl_3$) 4.05 (2H, d, *J* 6.1, $C_1H_2$), 2.76 (1H, dd, *J* 17.3, 2.6, $C_4H_CH_D$), 2.38 (1H, dd, *J* 17.3, 9.4, $C_4H_CH_D$), 1.42 (1H, ddd, *J* 13.8, 8.6, 5.7, $C_6H_AH_B$).

## General procedure E:

To a stirred solution of Evans-Tishchenko coupling product (0.25 mmol) in MeOH (5 $cm^3$) and water (0.5 $cm^3$) was added $K_2CO_3$ (0.50 mmol). The resulting mixture was stirred at r.t. for 16 h. the mixture was diluted with water (20 $cm^3$) and extracted with DCM (3 x 50 $cm^3$). The combined organics were dried ($MgSO_4$), concentrated in *vacuo* and purified by column chromatography.

## (3*R*,5*R*,6*S*)-7-*tert*-Butyldiphenylsilyloxy-6-dibenzylamino-2,2-dimethylheptane-3,5-diol 179




**General procedure A** was followed with $Me_4NHB(OAc)_3$ (0.58 g, 2.20 mmol) and (5*R*,6*S*)-7-*tert*-butyldiphenylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one **157** (0.27 g, 0.44 mmol) thus providing the title compound **179** (0.19 g, 70%) as a clear oil as an inseparable mixture of diastereoisomers (94:6) after chromatography.

**General procedure E** was followed with (3*S*,5*R*,6*R*)-7-(tert-butyldiphenylsilyloxy)-2-(dibenzylamino)-3-hydroxy-2,2-dimethylheptan-5-yl propionate **181** (0.11 g, 0.25 mmol), $K_2CO_3$ (0.050 g, 0.50 mmol) thus providing the title compound **179** (0.070 g, 70%) as a clear oil as an inseparable mixture of diastereoisomers (>97:3) after the chromatography.

**$R_f$** [Hexane : EtOAc (9 : 1)] 0.2; **$\upsilon_{max}$ (neat)/cm$^{-1}$** 3445, 1602, 1589, 1494; ***m/z*** (FAB, NOBA) 610 ([M+H$^+$]$^+$, 63%), 552 (10), 532 (15 ), 478 (100); **HRMS** (FAB, NOBA) $C_{39}H_{52}NO_3Si$ [M-H$^+$]$^-$ requires 610.3717, found 610.3718.

**Major diastereoisomer (3,5-*anti*):**

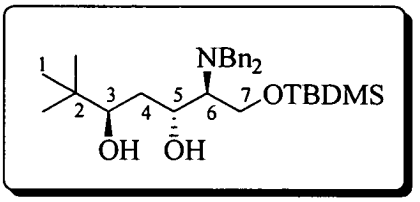
**$\delta_H$** (360 MHz, $CHCl_3$) 7.74-7.71 (4H, m, Ar*H*), 7.48-7.44 (6H, m, Ar*H*), 7.29-7.22 (10H, m, Ar*H*), 4.28 (1H, td, *J* 8.4, 2.7, $C_5H$), 4.13 (2H, d, *J* 5.4, $C_7H$), 3.85 (2H, d, *J* 13.6, $NCH_AH_BPh$), 3.50 (2H, d, *J* 13.6, $NCH_AH_BPh$), 3.44 (1H, dd, *J* 11.2, 2.0, $C_3H$), 2.84 (1H, dt, *J* 8.4, 5.4, $C_6H$), 1.83 (1H, ddd, *J* 14.1, 11.2, 2.7, $C_4H_XH_Y$), 1.49 (1H, ddd, *J* 14.1, 8.4, 2.0, $C_4H_XH_Y$), 1.13 (9H, s, $^tBu$), 0.88 (9H, s, $^tBu$); **$\delta_C$** (90.6 MHz, $CHCl_3$) 140.8 (2C), 136.7 (4CH), 133.7 (2C), 131.1 (2CH), 130.0 (4CH), 129.3 (4CH), 128.9 (4CH), 128.0 (2CH), 76.6 (CH), 71.1 (CH), 63.2 ($CH_2$), 62.5 (CH), 56.1 ($2CH_2$), 36.2

(C), 35.7 ($CH_2$), 27.9 ($3CH_3$), 26.7 ($3CH_3$), 20.1 (C).

**Minor diastereoisomer:**

$\delta_H$ (360 MHz, $CHCl_3$) 7.83-7.81 (4H, m, Ar*H*).

## (3*R*,5*R*,6*S*)-7-*tert*-Butyldimethylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethylheptane-3,5-diol 180




**General procedure A** was followed with $Me_4NHB(OAc)_3$ (1.27 g, 4.83 mmol), anhydrous acetonitrile (2.5 $cm^3$), acetic acid (1.5 $cm^3$) and (5*R*,6*S*)-7-*tert*-butyldimethylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one **158** (117 mg, 0.366 mmol) in anhydrous acetonitrile (1 $cm^3$) thus providing the title compound **180** (100 mg, 85%) as a clear oil as an inseparable mixture of diastereoisomers (>97:3) after the chromatography.

**General procedure E** was followed with (3*S*, 5*R*, 6*R*)-7-(tert-butyldimethylsilyloxy)-2-(dibenzylamino)-3-hydroxy-2,2-dimethylheptan-5-yl propionate **182** (0.12 g, 0.24 mmol), $K_2CO_3$ (0.07 g, 0.50 mmol) thus providing the title compound **180** (0.09 g, 78%) as a clear oil as an inseparable mixture of diastereoisomers (>97:3) after the chromatography.

$R_f$ [Hexane : EtOAc (4 : 1)] 0.63; $\nu_{max}$ ($CDCl_3$)/$cm^{-1}$ 3455, 1602, 1586; $\delta_H$ (250 MHz, $CDCl_3$) 7.20-7.08 (10H, m, Ar*H*), 4.09 (1H, td, *J* 8.3, 2.8, $C_5H$), 3.95 (2H, d, *J* 5.3, $C_7H_2$), 3.77 (2H, d, *J* 13.6, $NCH_AH_BPh$), 3.47 (2H, d, *J* 13.6, $NCH_AH_BPh$), 3.29 (1H, dd, *J* 11.0, 2.0, $C_3H$), 2.65 (1H, dt, *J* 8.3, 5.3, $C_6H$), 2.20 (1H, br s, O*H*), 1.67 (1H, ddd, *J* 14.1, 11.0, 2.8, $C_4H_CH_D$), 1.37 (1H, ddd, *J* 14.1, 8.3, 2.0, $C_4H_CH_D$), 0.80 (9H, s, $^tBu$), 0.75 (9H, s, $^tBu$), 0.01 (3H, s, *Me*Si), 0.00 (3H, s, *Me*Si); $\delta_C$ (62.8 MHz, $CDCl_3$) 139.7 (2C), 128.9 (4CH), 128.1 (4CH), 126.9 (2CH), 75.5 (CH), 70.0 (CH), 61.2 ($CH_2$), 61.1

(CH), 55.0 ($2CH_2$), 35.1 ($CH_2$), 34.6 (C), 25.8 ($3CH_3$), 25.5 ($3CH_3$), 18.0 (C), -5.6 ($2CH_3$); ***m/z*** (FAB, THIOG) 486 ($[M+H]^+$, 50%), 354 (67), 340 (42), 264 (33), 210 (41), 196 (39), 181 (41); **HRMS** (FAB, THIOG) $C_{29}H_{48}NO_3Si$ $[M + H]^+$ requires 486.3404, found 486.3396.

## General procedure C: Preparation of Samarium (II) iodide.

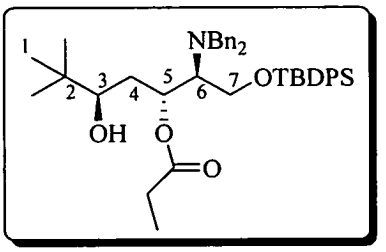
A suspension of iodide (259 mg, 1.00 mmol) and samarium (207 mg, 1.40 mmol, ~ 40 mesh) in THF (10 $cm^3$) was heated under reflux in the absence of light[vi] for 1 h after which time a deep blue solution of $SmI_2$ (0.1 M in THF) had formed. The solution was then cooled to room temperature where it could be stored argon before oxidation to a yellow Sm (III) species occurred.

## General procedure D: Evans-Tishchenko coupling

To a stirred solution of hydroxyketone (0.415 mmol) and propionaldehyde (1.66 mmol) in THF (10 $cm^3$) at – 10 °C was added dropwise, a solution of $SmI_2$ (0.1 M, 0.08 mmol), such that the deep blue $SmI_2$ solution was decolourised to pale yellow. After 1.5 h at – 10 °C, the reaction mixture was portioned between $NaHCO_3$ (15 $cm^3$, sat.) and $Et_2O$ (3 x 20 $cm^3$). The combined organics were dried ($MgSO_4$), evaporated *in vacuo* and purified by column chromatography.

[vi] This is not critical but $SmI_2$ is known to be light sensitive and should be treated accordingly.

## (3*R*,5*R*,6S)-7-*tert*-Butyldiphenylsilyloxy-2-dibenzylamino-3-hydroxy-2,2-dimethylheptan-5-yl propionate 181




**General procedure D** was followed with (5*R*,6*S*)-7-*tert*-butyldiphenylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one **157** (0.240 g, 0.388 mmol), propionaldehyde (0.110 $cm^3$, 1.55 mmol) and a solution of $SmI_2$ (0.80 $cm^3$, 0.1 M in THF, 0.08 mmol) thus providing the title compound **181** (0.221 g, 85%) as a clear oil as an inseparable mixture of diastereoisomers (97 : 3) after chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.31; $\upsilon_{max}$ **(neat)/cm$^{-1}$** 3499, 1715, 1602, 1589, 1493; ***m/z*** (FAB, THIOG) 666 ($[M+H]^+$, 27%), 478 (45), 396 (42), 210 (26), 199 (56), 181 (48); **HRMS** (FAB, THIOG) $C_{42}H_{56}NO_4Si$ $[M+H]^+$ requires 666.3979, found 666.3978.

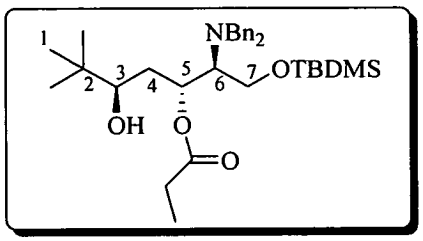
**Major diastereoisomer (3,5-*anti*):**

$\delta_H$ (360 MHz, $CHCl_3$) 7.70-7.68 (4H, m, Ar*H*), 7.44-7.40 (6H, m, Ar*H*), 7.32-7.23 (10H, m, Ar*H*), 5.35 (1H, ddd, *J* 11.0, 7.8, 2.0, $C_5H$), 3.93-3.91 (2H, m, $C_7H_2$), 3.86 (2H, d, *J* 13.5, $NCH_AH_BPh$), 3.75 (2H, d, *J* 13.5, $NCH_AH_BPh$), 2.94 (1H, ddd, *J* 7.8, 6.1, 4.5, $C_6H$), 2.92-2.85 (2H, m, $C_3H$+O*H*), 2.15 (1H, dq, *J* 16.4, 7.5, $CH_AH_B$), 2.04 (1H, dq, *J* 16.4, 6.9, $CH_AH_B$), 1.94 (1H, ddd, *J* 14.6, 11.0, 2.0, $C_4H_CH_D$), 1.23 (1H, ddd, *J* 14.6, 11.0, 2.0, $C_4H_CH_D$), 1.13 (9H, s, $^tBu$), 0.97 (3H, t, *J* 7.6, $CH_3$), 0.85 (9H, s, $^tBu$); $\delta_C$ (62.9 MHz, $CHCl_3$) 175.3 (C), 139.2 (2C), 135.6 (2CH), 135.0 (C), 134.6 (2CH), 133.1 (C), 129.6 (2CH), 129.5 (2CH), 129.0 (2CH), 128.0 (4CH), 127.5 (4CH), 126.7 (2CH), 74.1 (CH), 70.2 (CH), 61.2 (CH), 60.9 ($CH_2$), 54.9 ($2CH_2$), 35.2 (C), 34.8 ($CH_2$), 27.5 ($CH_2$), 26.8 ($3CH_3$), 25.8 ($3CH_3$), 18.9 (C), 8.9 ($CH_3$).

**Minor diastereoisomer:**

$\delta_H$ (360 MHz, $CHCl_3$) 3.54 (2H, d, *J* 13.4, $NCH_AH_BPh$).

## (3*R*,5*R*,6S)-7-*tert*-Butyldimethylsilyloxy-2-dibenzylamino-3-hydroxy-2,2-dimethylheptan-5-yl propionate 182




**General procedure D** was followed with (5*R*,6*S*)-7-*tert*-butyldimethylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one **158** (0.201 g, 0.415 mmol), propionaldehyde (0.120 $cm^3$, 1.66 mmol) and a solution of $SmI_2$ (0.83 $cm^3$, 0.1 M in THF, 0.08 mmol) thus providing the title compound **182** (0.137 g, 61%) as a clear oil as an inseparable mixture of diastereoisomers (93 : 7) after chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.43; $\upsilon_{max}$ **(neat)/cm$^{-1}$** 3532, 1721, 1602, 1586, 1494; ***m/z*** (FAB, NOBA) 541 ($[M]^+$, 100%), 484 (38), 468 (52), 396 (77), 354 (80), 210 (45), 194 (50); **HRMS** (FAB, NOBA) $C_{32}H_{52}NO_4Si$ $[M+H]^+$ requires 542.3666, found 542.3656.

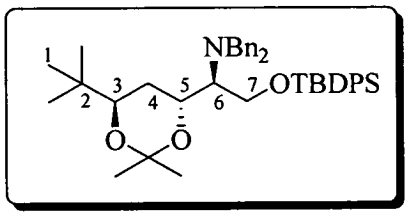
**Major diastereoisomer (3,5-*anti*):**

$\delta_H$ (360 MHz, $CHCl_3$) 7.32-7.17 (10H, m, Ar*H*), 5.36 (1H, ddd, *J* 10.7, 8.5, 2.0, $C_5H$), 3.94 (1H, dd, *J* 10.7, 3.2, $C_7H_XH_Y$), 3.88 (2H, d, *J* 13.5, $NCH_AH_BPh$), 3.80 (1H, dd, *J* 10.7, 6.2, $C_7H_XH_Y$), 3.64 (2H, d, *J* 13.5, $NCH_AH_BPh$), 2.94 (1H, d, *J* 4.2, O*H*), 2.91 (1H, ddd, *J* 11.1, 4.2, 1.7, $C_3H$), 2.82 (1H, ddd, *J* 8.5, 6.2, 3.2, $C_6H$), 2.30 (1H, dq, *J* 16.4, 7.6, $CH_AH_B$), 2.24 (1H, dq, *J* 16.4, 7.5, $CH_AH_B$), 2.07 (1H, ddd, *J* 14.5, 11.1, 2.0, $C_4H_CH_D$), 1.24 (1H, ddd, *J* 14.5, 10.7, 1.7, $C_4H_CH_D$), 1.10 (3H, t, *J* 7.5, $CH_3$), 0.92 (9H, s, *'Bu*), 0.86 (9H, s, *'Bu*), 0.10 (3H, s, *Me*), 0.07 (3H, s, *Me*); $\delta_C$ (90.6 MHz, $CHCl_3$) 175.2 (C), 139.9 (2C), 128.9 (4CH), 128.0 (4CH), 126.7 (2CH), 74.0 (CH), 70.1 (CH), 60.8 (CH), 59.5 ($CH_2$), 55.0 ($2CH_2$), 35.1 ($CH_2$), 34.3 (C), 27.7 ($CH_2$), 25.8 ($3CH_3$), 25.7 ($3CH_3$), 17.9 (C), 9.1 ($CH_3$), -5.7 ($2CH_3$).

**Minor diastereoisomer:**

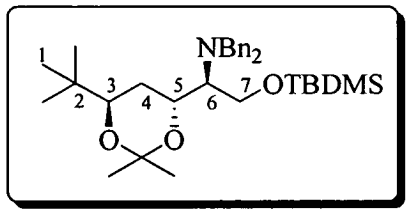
$\delta_H$ (360 MHz, $CHCl_3$) 3.62 (2H, d, *J* 13.4, $NCH_AH_BPh$).

## (3*R*,5*R*,6*S*)-7-*tert*-Butyldiphenylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethyl-3,5-isopropylidinedioxyheptane 179A



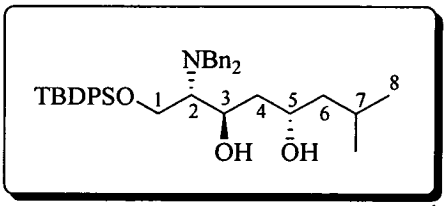

To a stirred solution of (3*R*,5*R*,6*S*)-7-*tert*-butyldiphenylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethylheptane-3,5-diol **179** (0.025 g, 0.041 mmol) in DCM (3 $cm^3$) and 2,2-dimethoxypropane (0.41 $cm^3$) at room temperature was added a few crystals of camphor sulphonic acid. The reaction was stirred at room temperature for 20 h. The reaction mixture was quenched with $NaHCO_3$ (20 $cm^3$, sat. aq.) and extracted with DCM (3 × 20 $cm^3$). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **179A** (27 mg, 52%) as a colourless oil. $R_f$ [Hexane : EtOAc (9 : 1)] 0.80; $\nu_{max}$ (neat)/$cm^{-1}$ 2956, 2931, 1604, 1494; $\delta_H$ (360 MHz, $CDCl_3$) 7.75-7.73 (4H, m, Ar*H*), 7.44-7.42 (6H, m, Ar*H*), 7.40-7.27 (10H, m, Ar*H*), 4.05-3.98 (2H, m, $C_7H_AH_B$ + $C_5H$), 3.95 (1H, dd, *J* 11.1, 5.9, $C_7H_AH_B$), 3.87 (2H, d, *J* 13.9, $NCH_AH_BPh$), 3.76 (2H, d, *J* 13.9, $NCH_AH_BPh$), 3.22 (1H, dd, *J* 10.4, 5.9, $C_3H$), 2.73 (1H, ddd, *J* 6.8, 5.9, 3.5, $C_6H$), 1.75 (1H, ddd, *J* 13.1, 10.4, 6.4, $C_4H_CH_D$), 1.44 (1H, ddd, *J* 13.1, 9.5, 5.9, $C_4H_CH_D$), 1.22 (3H, s, *Me*), 1.19 (3H, s, *Me*), 1.12 (9H, s, $^t$Bu), 0.82 (9H, s, $^t$Bu); $\delta_C$ (90.6 MHz, $CDCl_3$) 140.5 (2C), 135.7 (2CH), 135.7 (2CH), 133.4 (2C), 129.6 (CH), 129.5 (CH), 128.7 (4CH), 128.0 (4CH), 127.6 (2CH), 127.5 (2CH), 126.6 (2CH), 99.9 (C), 73.6 (CH), 65.6 (CH), 62.3 (CH), 60.3 ($CH_2$), 55.3 ($2CH_2$), 33.1 (C), 31.9 ($CH_2$), 29.6 ($CH_2$), 26.9 ($3CH_3$), 25.2 ($3CH_3$), 24.4 ($CH_3$), 24.1 ($CH_3$), 19.0 (C); ***m/z*** (FAB, NOBA) 650 ($[M+H]^+$, 57%), 592 (23), 478 (61), 380 (44), 252 (34), 223 (36), 210 (49); **HRMS** (FAB, NOBA) $C_{42}H_{56}NO_3Si$ $[M+H]^+$ requires 650.4029, found 650.4021.

## (3*R*,5*R*,6*S*)-7-*tert*-Butyldimethylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethyl-3,5-isopropylidonedioxyheptane 180A




To a stirred solution of (3*R*,5*R*,6*S*)-7-*tert*-butyldimethylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethylheptane-3,5-diol **180** (75 mg, 0.15 mmol) in DCM (3 $cm^3$) and 2,2-dimethoxypropane (1.5 $cm^3$) at room temperature was added a few crystals of camphor sulphonic acid. The reaction was stirred at room temperature for 20 h. The reaction mixture was quenched with $NaHCO_3$ (20 $cm^3$, sat. aq.) and extracted with DCM (3 × 20 $cm^3$). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **180A** (65 mg, 80%) as a colourless oil. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.43; $\nu_{max}$ (neat)/$cm^{-1}$ 2955, 2929, 1642, 1493; $\delta_H$ (360 MHz, $CDCl_3$) 7.37-7.21 (10H, m, Ar*H*), 4.01 (1H, dd, *J* 10.7, 3.0, $C_7H_XH_Y$), 3.96 (1H, ddd, *J* 9.5, 8.2, 6.3, $C_5H$), 3.89 (2H, d, *J* 13.9, $NCH_AH_BPh$), 3.75 (1H, dd, *J* 10.7, 6.1, $C_7H_XH_Y$), 3.66 (2H, d, *J* 13.9, $NCH_AH_BPh$), 3.16 (1H, dd, *J* 6.1, 10.3, $C_3H$), 2.57 (1H, ddd, *J* 8.2, 6.1, 3.0, $C_6H$), 1.83 (1H, ddd, *J* 13.1, 10.3, 6.3, $C_4H_CH_D$), 1.45 (1H, ddd, *J* 13.1, 9.5, 6.1, $C_4H_CH_D$), 1.16 (3H, s, *Me*), 1.11 (3H, s, *Me*), 0.86 (9H, s, $^t$Bu), 0.73 (9H, s, $^t$Bu), 0.01 (3H, s, *Me*Si ), 0.00 (3H, s, *Me*Si ); $\delta_C$ (62.8 MHz, $CDCl_3$) 140.6 (2C), 129.2 (4CH), 128.0 (4CH), 126.6 (2CH), 99.9 (C), 73.7 (CH), 65.4 (CH), 62.0 (CH), 59.5 ($CH_2$), 55.4 ($2CH_2$), 33.1 (C), 32.0 ($CH_2$), 25.9 ($3CH_3$), 25.2 ($3CH_3$), 24.5 ($CH_3$), 24.1 ($CH_3$), 18.1 (C), -5.6 ($2CH_3$); ***m/z*** (FAB, THIOG) 524 ($[M-H]^+$, 32%), 380 (39), 355 (49), 210 (36); **HRMS** (FAB, NOBA) $C_{32}H_{50}NO_3Si$ $[M-H]^+$ requires 524.3560, found 524.3566.

## (2*S*,3*R*,5*S*)-1-*tert*-Butyldiphenylsilyloxy-2-*N*,*N*-dibenzylamino-7-methyl-octane-3,5-diol 185




**General procedure A** was followed with $Me_4NHB(OAc)_3$ (0.990 g, 3.75 mmol) and (2*S*,5*S*)-1-*tert*-Butyldiphenylsilyloxy-2-*N*,*N*-dibenzylamino-5-hydroxy-7-methyloctan-3-one **160** (0.460 g, 0.75 mmol) thus providing the title compounds (0.085 g, 19%) as a mixture of diastereoisomers (82:18) after chromatography. The mixture was separated by HPLC [Hexane : EtOAc (4 :1)] to afford the title compounds **185** (0.045 g, 10%) and a minor diastereoisomer (0.013 g, 3%) as colourless oils. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.13; $\nu_{max}$ **(neat)/cm$^{-1}$** 3434, 1603, 1589, 1494; ***m/z*** (FAB, NOBA) 611 ($[M+H]^+$, 25%), 479 (45), 354 (8), 340 (9), 199 (11), 197 (14); **HRMS** (FAB, NOBA) $C_{39}H_{52}NO_3Si$ $[M+H]^+$ requires 610.37165, found 610.36967.

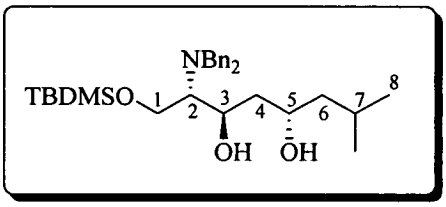
**Major diastereoisomer 185a (3,5-*anti*):**

$\delta_H$ (360 MHz, $CDCl_3$) 7.70-7.68 (4H, m, Ar*H*), 7.49-7.43 (6H, m, Ar*H*), 7.29-7.21 (10H, m, Ar*H*), 4.30 (1H, td, *J* 8.6, 2.9, $C_3H$), 4.14-4.05 (2H, d, *J* 5.7, $C_1H_2$), 3.92-3.83 (1H, m, $C_5H$), 3.82 (2H, d, *J* 13.7, $NCH_AH_B$), 3.47 (2H, d, *J* 13.7, $NCH_AH_B$), 2.83 (1H, dt, *J* 7.9, 5.7, $C_2H$), 2.63 (1H, br s, O*H*), 1.82 (1H, ddd, *J* 14.5, 8.2, 2.9, $C_4H_CH_D$), 1.80-1.68 (1H, m, $C_7H$), 1.61 (1H, ddd, *J* 14.5, 8.6, 2.5, $C_4H_CH_D$), 1.51-1.43 (1H, m, $C_6H_DH_F$), 1.19-1.10 (1H, m, $C_6H_DH_F$), 1.09 (9H, s, Si*t-Bu*), 0.92 (3H, d, *J* 6.6, *Me*), 0.89 (3H, d, *J* 6.6, *Me*); $\delta_C$ (90.6 MHz, $CDCl_3$) 139.5 (2C), 135.6 (4CH), 132.5 (2C), 129.9 (2CH), 128.7 (4CH), 128.2 (4CH), 127.8 (4CH), 127.0 (2CH), 69.9 (CH), 67.1 (CH), 61.9 ($CH_2$), 61.3 (CH), 55.0 ($2CH_2$), 46.4 ($CH_2$), 40.3 ($CH_2$), 26.8 ($3CH_3$), 24.5 (CH), 23.4 ($CH_3$), 22.1 ($CH_3$), 19.0 (C).

**Minor diastereoisomer 185b:**

$\delta_H$ (360 MHz, $CDCl_3$) 7.72-7.68 (4H, m, Ar*H*), 7.47-7.43 (6H, m, Ar*H*), 7.31-7.21 (10H, m, Ar*H*), 3.99 (2H, d, *J* 13.2, $NCH_AH_B$), 3.89 (1H, ddd, *J* 9.6, 7.6, 3.3, $C_3H$), 3.87-3.83 (2H, m, $C_1H_2$), 3.75-3.68 (1H, m, $C_5H$), 3.64 (2H, d, *J* 13.2, $NCH_AH_B$), 2.85-2.82 (1H, br d, O*H*), 2.80 (1H, dt, *J* 9.6, 4.6, $C_2H$), 1.68-1.61 (1H, m, $C_7H$), 1.50 (1H, ddd, *J* 14.3, 8.9, 3.3, $C_4H_CH_D$), 1.34 (1H, ddd, *J* 13.5, 8.8, 5.6, $C_6H_DH_F$), 1.24 (1H, ddd, *J* 14.3, 7.6, 2.0, $C_4H_CH_D$), 1.13 (9H, s, Si$^t$*Bu*), 1.00 (1H, ddd, *J* 13.5, 8.4, 4.6, $C_6H_DH_F$), 0.84 (3H, d, *J* 6.6, *Me*), 0.82 (3H, d, *J* 6.5, *Me*); $\delta_C$ (90.6 MHz, $CDCl_3$) 138.8 (2C), 135.7 (2CH), 135.5 (2CH), 129.8 (2CH), 128.9 (4CH), 128.4 (4CH), 127.8 (4CH), 127.2 (2CH), 66.6 (CH), 63.0 ($CH_2$), 60.2 (CH), 54.4 ($2CH_2$), 46.3 ($CH_2$), 39.6 ($CH_2$), 26.9 ($3CH_3$), 24.3 (CH), 23.2 ($CH_3$), 22.0 ($CH_3$), 19.0 (C).

## (2*S*,3*R*,5*S*)-1-*tert*-butyldimethylsilyloxy-2-*N*,*N*-dibenzylamino-7-methyl-octane-3,5-diol 186




**General procedure A** was followed with $Me_4NHB(OAc)_3$ (0.98 g, 3.75 mmol) and (3*S*)-4-*tert*-butyldimethylsilyloxy-3-*N*,*N*-dibenzylaminobutan-2-one **161** (0.25 g, 0.75 mmol) thus providing the title compounds **186** (0.11 g, 45%) as a clear oil as a mixture of diastereoisomers (85:15) after the chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.13; $\nu_{max}$ **(neat)/cm$^{-1}$** 3435, 2953, 2928, 1603, 1495, 1454; ***m/z*** (FAB, NOBA) 486 ($[M+H]^+$, 19%), 354 (57), 340 (10), 196 (4), 91 (100); **HRMS** (FAB, THIOG) $C_{29}H_{48}NO_3Si$ $[M+H]^+$ requires 486.34035, found 486.3395.

**Major diastereoisomer 186a (*anti*):**

$\delta_H$ (360 MHz, $CDCl_3$) 7.30-7.17 (10H, m, Ar*H*), 4.23 (1H, td, *J* 8.4, 3.1, $C_3H$), 4.02 (2H, dd, *J* 5.0, 1.1, $C_1H_2$), 3.85 (2H, d, *J* 13.7, $NCH_AH_B$), 3.82-3.79 (1H, m, $C_5H$), 3.57 (2H, d, *J* 13.7, $NCH_AH_B$), 2.95 (1H, br s, O*H*), 2.72 (1H, dt, *J* 8.1, 5.3, $C_2H$), 1.79 (1H, ddd, *J* 14.4, 8.1, 3.1, $C_4H_CH_D$), 1.73-1.68 (1H, m, $C_7H$), 1.54 (1H, ddd, *J* 14.4, 8.4, 2.7, $C_4H_CH_D$), 1.44 (1H, ddd, *J* 13.6, 9.0, 5.4, $C_6H_DH_F$), 1.10 (1H, ddd, *J* 13.6, 9.0, 4.5, $C_6H_DH_F$), 0.89 (9H, s, $^tBu$), 0.87 (3H, d, *J* 5.6, $CH_3$), 0.85 (3H, d, *J* 6.6, $CH_3$), 0.10 (3H, s, Si*Me*), 0.09 (3H, s, Si*Me*); $\delta_C$ (90.6 MHz, $CDCl_3$) 139.6 (2C), 128.7 (4CH), 128.4 (4CH), 126.9 (2CH), 70.0 (CH), 67.1 (CH), 61.2 ($CH_2$), 61.1 (CH), 55.1 ($2CH_2$), 46.4 ($CH_2$), 40.4 ($CH_2$), 25.7 ($3CH_3$), 24.4 (CH), 23.4 ($CH_3$), 22.0 ($CH_3$), 17.9 (C), -5.6 ($CH_3$), -5.7 ($CH_3$).

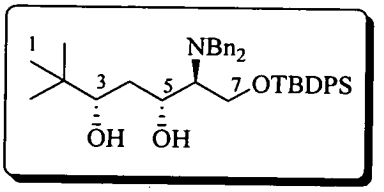
**Minor diastereoisomer 186b:**

$\delta_H$ (360 MHz, $CDCl_3$) 3.96 (2H, d, *J* 13.2, NC*H*$_A$H$_B$); $\delta_C$ (90.6 MHz, $CDCl_3$) 138.9 (2C), 129.0 (4CH), 128.4 (4CH), 127.2 (2CH), 66.7 (CH), 64.7 (CH), 62.8 (CH), 59.2 ($CH_2$), 54.3 ($2CH_2$), 46.3 ($CH_2$), 39.6 ($CH_2$), 25.8 ($3CH_3$), 24.3 (CH), 23.1 ($CH_3$), 22.0 ($CH_3$), 18.0 (C), -5.6 ($CH_3$), -5.7 ($CH_3$).

## General procedure F (*syn* reduction):

To a solution of triethylborane (1M in THF, 0.36 mmol) and pivalic acid (0.012 mmol) dissolved in THF (0.5 $cm^3$) and MeOH (1 $cm^3$) was stirred at r.t. for 1 h and was then cooled to – 70 °C. Ketone (0.24 mmol) in THF (1.0 $cm^3$) was added *via can nula* followed by sodium borohydride (0.72 mmol) and the resulting mixture was stirred at – 70 °C for 1 h. Hydrogen peroxide (3 $cm^3$) was added followed by water (5 $cm^3$) at – 70 °C and the reaction mixture allowed to warm to r.t. Then DCM (15 $cm^3$) and water (15 $cm^3$) was added and the aqueous phase extracted with DCM (3 x 30 $cm^3$). Combined organic phases were washed with water (3 x 30 $cm^3$), dried ($Na_2SO_4$), concentrated in *vacuo* and purified by column chromatography.

## (3*S*,5*R*,6*S*)-7-*tert*-Butyldiphenylsilyloxy-6-dibenzylamino-2,2-dimethylheptane-3,5-diol 195




**General procedure F** was followed with triethylborane ($Et_3B$) (0.47 $cm^3$, 1.0 M in THF, 0.31 mmol) and pivalic acid (1.6 mg, 0.016 mmol), (5*R*,6*S*)-7-*tert*-butyldiphenylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one **157** (0.19 g, 0.31 mmol), sodium borohydride (0.04 g, 0.93 mmol) to afford the title compound **195** (0.19 g, 65%) as a pale oil as an inseparable mixture of diastereoisomers (95:5) after chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.32; $\upsilon_{max}$ **(neat)/cm$^{-1}$** 3454, 2957, 1602, 1589, 1494; ***m/z*** (FAB, THIOG) 610 ($[M-H]^+$, 51%), 608 ($[M+H^+]^-$, 44), 520 (38), 478 (45),340 (30), 199 (51), 197 (57); **HRMS** (FAB, THIOG) $C_{39}H_{50}NO_3Si$ $[M-H^+]^-$ requires 608.3560, found 608.3557.

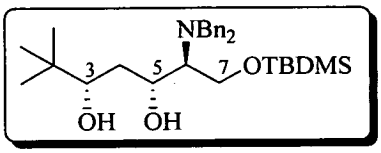
**Major diastereoisomer (3,5-*syn*):**

$\delta_H$ (360 MHz, $CHCl_3$) 7.79-7.74 (4H, m, Ar*H*), 7.56-7.46 (6H, m, Ar*H*), 7.35-7.25 (10H, m, Ar*H*), 4.19-4.09 (3H, m, $C_5H + C_7H_2$), 3.89 (2H, d, *J* 13.7, $NCH_AH_BPh$), 3.54 (2H, d, *J* 13.7, $NCH_AH_BPh$), 3.50 (1H, d, *J* 12.0, $C_3H$), 2.77 (1H, dt, *J* 13.6, 5.5, $C_6H$), 2.24 (1H, d, *J* 14.7, $C_4H_XH_Y$), 1.14 (9H, s, $^tBu$), 1.08 (1H, dt, *J* 14.7, 10.8, $C_4H_XH_Y$), 0.95 (9H, s, $^tBu$); $\delta_C$ (90.6 MHz, $CHCl_3$) 139.6 (2C), 135.5 (4CH), 132.5 (2C), 129.9 (2CH), 128.8 (4CH), 128.1 (4CH), 127.8 (4CH), 126.9 (2CH), 80.9 (CH), 74.0 (CH), 62.0 (CH), 61.6 ($CH_2$), 55.1 ($CH_2$), 35.1 ($CH_2$), 34.7 (C), 26.8 ($3CH_3$), 25.5 ($3CH_3$), 18.9 (C).

**Minor diastereoisomer:**

$\delta_H$ (360 MHz, $CHCl_3$) 1.94-1.88 (1H, m, $C_4H_XH_Y$).

## (3S,5*R*,6*S*)-7-*tert*-Butyldimethylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethylheptane-3,5-diol 196




**General procedure F** was followed with triethylborane ($Et_3B$) (0.43 $cm^3$, 1.0 M in THF, 0.43 mmol) and pivalic acid (1.4 mg, 0.014 mmol), (5*R*,6*S*)-7-*tert*-butyldimethylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one **158** (0.14 g, 0.29 mmol), sodium borohydride (0.030 g, 0.86 mmol) to afford the title compound **196** (0.11 g, 74%) as a pale oil as an inseparable mixture of diastereoisomers (98:2) after chromatography. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.24; $\nu_{max}$ ($CDCl_3$)/$cm^{-1}$ 3456, 1603, 1586; ***m/z*** (FAB, THIOG) 486 ($[M+H]^+$, 50%), 468 (36), 408 (41), 354 (78), 340 (59), 196 (45); **HRMS** (FAB, THIOG) $C_{29}H_{48}NO_3Si$ $[M+H]^+$ requires 486.3404, found 486.3414.

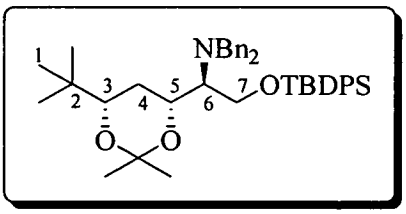
**Major diastereoisomer (3,5-*syn*):**

$\delta_H$ (360 MHz, $CDCl_3$) 7.31-7.19 (10H, m, Ar*H*), 4.09 (1H, dd, *J* 10.6, 5.3, $C_7H_AH_B$), 4.01 (1H, dd, *J* 10.6, 5.3, $C_7H_AH_B$) 4.00-3.98 (1H, m, $C_5H$), 3.88 (2H, d, *J* 13.6, $NCH_AH_BPh$), 3.57 (2H, d, *J* 13.6, $NCH_AH_BPh$), 3.43 (1H, dd, *J* 10.5, 1.2, $C_3H$), 2.63 (1H, dt, *J* 8.5, 5.3, $C_6H$), 2.18 (1H, br d, *J* 14.5, $C_4H_CH_D$), 0.99 (1H, dt, *J* 14.5, 10.5, $C_4H_CH_D$), 0.91 (9H, s, $^tBu$), 0.89 (9H, s, $^tBu$), 0.13 (3H, s, *Me*Si), 0.10 (3H, s, *Me*Si); $\delta_C$ (90.6 MHz, $CDCl_3$) 139.7 (2C), 128.8 (4CH), 128.1 (4CH), 126.9 (2CH), 80.9 (CH), 74.0 (CH), 61.9 (CH), 60.6 ($CH_2$), 55.2 ($2CH_2$), 35.1 ($CH_2$), 34.7 (C), 25.7 ($3CH_3$), 25.5 ($3CH_3$), 17.9 (C), -5.6 ($CH_3$), -5.7 ($CH_3$).

**Minor diastereoisomer:**

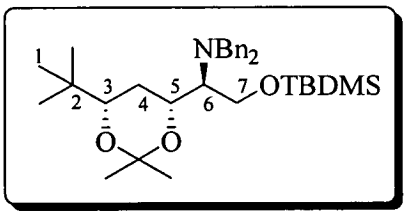
$\delta_H$ (360 MHz, $CHCl_3$) 1.90-1.85 (1H, m, $C_4H_XH_Y$).

## (3*S*,5*R*,6*S*)-7-*tert*-Butyldiphenylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethyl-3,5-isopropylidinedioxyheptane 198



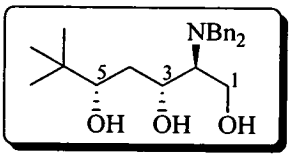

To a stirred solution of (3*S*,5*R*,6*S*)-7-*tert*-butyldiphenylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethylheptane-3,5-diol **195** (67 mg, 0.11 mmol) in DCM (3 $cm^3$) and 2,2-dimethoxypropane (1.1 $cm^3$) at room temperature was added a few crystals of camphor sulphonic acid. The reaction was stirred at room temperature for 20 h. The reaction mixture was quenched with $NaHCO_3$ (20 $cm^3$, sat. aq.) and extracted with DCM (3 × 20 $cm^3$). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **198** (57 mg, 80%) as a colourless oil. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.80; $\nu_{max}$ (neat)/$cm^{-1}$ 2956, 2931, 1604, 1494; $\delta_H$ (360 MHz, $CDCl_3$) 7.76-7.74 (4H, m, Ar*H*), 7.43-7.40 (6H, m, Ar*H*), 7.40-7.21 (10H, m, Ar*H*), 4.12 (1H, ddd, *J* 11.5, 7.9, 2.4, $C_5H$), 4.04 (1H, dd, *J* 11.0, 2.8, $C_7H_AH_B$), 3.97 (2H, d, *J* 13.8, $NCH_AH_BPh$), 3.94 (1H, dd, *J* 11.0, 5.3, $C_7H_AH_B$), 3.71 (2H, d, *J* 13.8, $NCH_AH_BPh$), 3.36 (1H, dd, *J* 11.8, 2.4, $C_3H$), 2.62 (1H, ddd, *J* 7.9, 5.3, 2.8, $C_6H$), 1.89-1.77 (2H, m, $C_4H_2$), 1.32 (3H, s, *Me*), 1.25 (3H, s, *Me*), 1.09 (9H, s, $^t$Bu), 0.81 (9H, s, $^t$Bu); $\delta_C$ (90.6 MHz, $CDCl_3$) 140.5 (2C), 135.7 (2CH), 135.6 (2CH), 133.4 (2C), 129.6 (CH), 129.5 (CH), 128.6 (4CH), 128.1 (4CH), 127.6 (2CH), 127.5 (2CH), 126.7 (2CH), 98.1 (C), 76.4 (CH), 67.4 (CH), 62.9 (CH), 59.3 ($CH_2$), 55.7 ($2CH_2$), 33.5 (C), 29.9 ($CH_3$), 29.6 ($CH_2$), 26.9 ($3CH_3$), 25.4 ($3CH_3$), 19.6 (C), 19.0 ($CH_3$); ***m/z*** (FAB, NOBA) 650 ($[M+H]^+$, 2%), 479 (22), 380 (5), 323 (3), 135 (22); **HRMS** (FAB, NOBA) $C_{42}H_{56}NO_3Si$ $[M+H]^+$ requires 650.4029, found 650.4021

## (3*S*,5*R*,6*S*)-7-*tert*-Butyldimethylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethyl-3,5-isopropylidonedioxyheptane 199




To a stirred solution of (3*S*,5*R*,6*S*)-7-*tert*-butyldimethylsilyloxy-6-*N*,*N*-dibenzylamino-2,2-dimethylheptane-3,5-diol **196** (0.11 g, 0.22 mmol) in DCM (3 $cm^3$) and 2,2-dimethoxypropane (2.2 $cm^3$) at room temperature was added a few crystals of camphor sulphonic acid. The reaction was stirred at room temperature for 20 h. The reaction mixture was quenched with $NaHCO_3$ (20 $cm^3$, sat. aq.) and extracted with DCM (3 × 20 $cm^3$). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound **199** (93 mg, 80%) as a colourless oil. $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.84; $\nu_{max}$ (neat)/$cm^{-1}$ 2954, 2930, 1493; $\delta_H$ (360 MHz, $CDCl_3$) 7.25-7.06 (10H, m, Ar*H*), 3.90 (1H, dd, *J* 10.7, 2.9, $C_7H_AH_B$), 3.89-3.83 (1H, m, $C_5H$), 3.82 (2H, d, *J* 13.9, $NCH_AH_BPh$), 3.74 (1H, dd, *J* 10.7, 5.9, $C_7H_AH_B$), 3.61 (2H, d, *J* 13.9, $NCH_AH_BPh$), 3.23 (1H, dd, *J* 12.3, 2.3, $C_3H$), 2.48 (1H, ddd, *J* 8.6, 5.9, 2.9, $C_6H$), 1.71 (1H, dt, *J* 12.6, 2.3, $C_4H_CH_D$), 1.22 1.16 (3H, s, *Me*), 1.18-1.14 (1H, m, $C_4H_CH_D$), 1.15 (3H, s, *Me*), 0.84 (9H, s, $^t$Bu), 0.72 (9H, s, $^t$Bu), 0.00 (3H, s, *Me*Si ), -0.01 (3H, s, *Me*Si ); $\delta_C$ (62.8 MHz, $CDCl_3$) 141.2 (2C), 129.3 (4CH), 128.5 (4CH), 127.1 (2CH), 98.5 (C), 76.9 (CH), 67.9 (CH), 63.1 (CH), 59.4 ($CH_2$), 56.1 ($2CH_2$), 34.0 (C), 30.5 ($CH_3$), 30.1 ($CH_2$), 26.4 ($3CH_3$), 25.9 ($3CH_3$), 20.1 ($CH_3$), 18.6 (C), -5.1 ($CH_3$), -5.2 ($CH_3$); ***m/z*** (FAB, THIOG) 524 ($[M-H]^+$, 42%), 380 (45), 354 (62), 210 (41); **HRMS** (FAB, NOBA) $C_{32}H_{50}NO_3Si$ $[M-H]^+$ requires 524.3560, found 524.3566.

## (2*S*,3*R*,5*S*)-2-(Dibenzylamino)-6,6-dimethylheptane-1,3,5-triol 197




To a solution of (5*R*,6*S*)-7-*tert*-butyldiphenylsilyloxy-6-dibenzylamino-5-hydroxy-2,2-dimethylheptan-3-one **157** (0.14 g, 0.23 mmol) in dry THF (10 $cm^3$) at r.t. under nitrogen was added lithium iodide (0.31 g, 2.3 mmol). The mixture was cooled to -78 °C over 20 min. Lithium aluminum hydride (0.090 g, 2.3 mmol) was then added and the reaction mixture was stirred at -78 °C for 1.5 hour. The solution was warmed up to 0 °C (ice bath), quenched by slow addition (dropwise) of NaOH (1M, aq.) over 30 min. Potassium-sodium tartrate was added and the mixture was stirred at room temperature overnight. The resulting mixture was extracted with DCM (20 $cm^3$ x 3). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography to give the title compound **197** as an inseparable mixture of diastereoisomers (95 : 5) as a clear yellow oil (0.050 g, 60%). $\mathbf{R_f}$ [hexane:EtOAc (1:1)] 0.42; $\upsilon_{max}$ **(neat)/cm$^{-1}$** 3397, 2958, 2868, 1602, 1586, 1493; **HRMS** (FAB, NOBA) $C_{23}H_{34}NO_3$ $[M+H]^+$ requires 372.2539, found 372.2539.

**Major diastereoisomer (*syn*):**

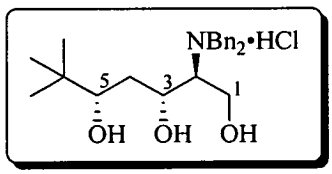
$\delta_H$ (360 MHz, $CHCl_3$) 7.31-7.20 (10H, m, Ar*H*), 4.04 (1H, t, *J* 8.7, $C_3H$), 3.99 (1H, dd, *J* 11.5, 4.8, $C_1H_AH_B$), 3.93 (1H, dd, *J* 11.5, 6.5, $C_1H_AH_B$), 3.79 (2H, d, *J* 13.6, $NCH_AH_BPh$),

3.59 (2H, d, *J* 13.6, $NCH_AH_BPh$), 3.47 (1H, dd, *J* 10.8, 1.4, $C_5H$), 2.57 (1H, ddd, *J* 8.7, 6.5, 4.8, $C_2H$), 2.52 (1H, br d, *J* 14.6, $C_4H_CH_D$), 1.01 (1H, dt, *J* 14.6, 10.5, $C_4H_CH_D$), 0.88 (9H, s, *'Bu*); $\delta_C$ (90.6 MHz, $CHCl_3$) 139.6 (2C), 128.9 (4CH), 128.1 (4CH), 126.9 (2CH), 82.0 (CH), 73.9 (CH), 62.5 (CH), 59.5 ($CH_2$), 54.7 ($2CH_2$), 35.0 ($CH_2$), 34.9 (C), 20.9 ($3CH_3$); *m/z* (FAB, NOBA) 372 ($[M+H]^+$, 70%), 340 (54), 241 (49), 91 (100).

**Minor diastereoisomer (*anti*):**

$\delta_H$ (360 MHz, $CHCl_3$) 3.70 (2H, d, *J* 13.2, $NCH_AH_BPh$).

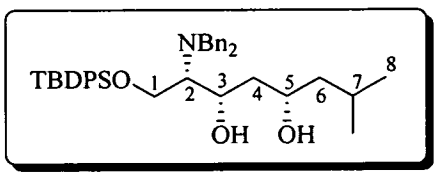
## (2*S*,3*R*,5*S*)-2-(Dibenzylamino)-6,6-dimethylheptane-1,3,5-triol hydrochloride salt 200




To a solution of (2*S*,3*R*,5*S*)-2-(dibenzylamino)-6,6-dimethylheptane-1,3,5-triol **197** (0.050 g, 0.13 mmol) in dry DCM (10 $cm^3$) was added 1M HCl in $Et_2O$ (0.67 $cm^3$, 0.67 mmol). The resulting mixture was stirred at room temperature for 2 hours and the resultant precipitate was remove by filtration and dried under reduced pressure to give a title compound **200** as a clourless solid (0.053 g, 95%). $\delta_H$ (360 MHz, $CHCl_3$) 7.27-7.19 (10H, m, Ar*H*), 4.29 (1H, br t, *J* 6.2, $C_3H$), 4.02 (2H, dd, *J* 13.0, 9.2, $C_1H_AH_B$), 3.85 (1H, dd, *J* 13.0, 4.4, $C_1H_AH_B$), 3.25 (1H, ddd, *J* 9.2, 4.4, 1.3, $C_2H$), 2.77 (1H, dd, *J* 10.4, 1.7, $C_5H$), 1.49 (1H, ddd, *J* 14.7, 7.2, 1.7, $C_4H_CH_D$), 1.30 (1H, ddd, *J* 14.7, 10.4, 6.2, $C_4H_CH_D$), 0.54 (9H, s, *'Bu*)[vii]; $\delta_C$ (90.6 MHz, $CHCl_3$) 132.1 (CH), 132.8 (2C), 131.5 (CH), 131.2 (4CH), 130.5 (4CH), 78.7 (CH), 67.6 (CH), 65.9 (CH), 56.8 ($2CH_2$), 55.6 ($CH_2$), 37.2 ($CH_2$), 35.4 (C), 25.7 ($3CH_3$). Crystal structure in **Appendix 6.**

[vii] Benzyl $CH_2$ missing.

## (2*S*,3*S*,5*S*)-1-*tert*-Butyldiphenylsilyloxy-2-*N*,*N*-dibenzylamino-7-methyl-octane-3,5-diol 201




**General procedure F** was followed with triethylborane ($Et_3B$) (1.59 $cm^3$, 1M in THF, 1.59 mmol) and pivalic acid (3.8 mg, 0.04 mmol), (2*S*,5*S*)-1-(*tert*-butyldimethylsilyloxy)-2-(dibenzylamino)-5-hydroxy-7-methyloctan-3-one **160** (0.65 g, 1.06 mmol), sodium borohydride (0.12 g, 3.2 mmol) to afford a title compound **201** (0.26 g, 40%) as a pale oil as an inseparable mixture of diastereoisomers (58 : 42) after chromatography.

**$R_f$** [Hexane : EtOAc (9 : 1)] 0.10; **$\upsilon_{max}$ (neat)/$cm^{-1}$** 3434, 2954, 2930, 2858, 1603;

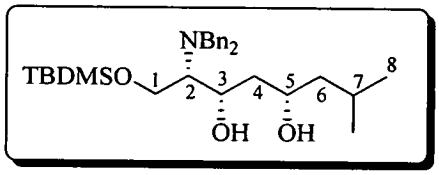
**Major diastereoisomer 201a (3,5-*syn*):**

**$\delta_H$** (360 MHz, $CHCl_3$) 7.72-7.71 (4H, m, Ar*H*), 7.47-7.44 (6H, m, Ar*H*), 7.32-7.22 (10H, m, Ar*H*), 3.99 (2H, d, *J* 13.2, NC$H_A$$H_B$Ph), 3.91 (1H, ddd, *J* 9.6, 7.4, 3.3, $C_3H$), 3.87-3.81 (2H, m, $C_1H_2$), 3.74-3.71 (1H, m, $C_5H$), 3.65 (2H, d, *J* 13.2, NC$H_A$$H_B$Ph), 2.84-2.82 (1H, br s, O*H*), 2.79 (1H, dt, *J* 9.6, 4.6, $C_2H$), 1.68-1.60 (1H, m, $C_7H$), 1.51 (1H, ddd, *J* 14.5, 8.8, 3.3, $C_4H_XH_Y$), 1.34 (1H, ddd, J 13.6, 8.5, 5.7, $C_6H_EH_F$), 1.26-1.21 (1H, m, $C_4H_XH_Y$), 1.12 (9H, s, $^tBu$), 1.02 (1H, ddd, *J* 13.6, 8.5, 4.6, $C_6H_EH_F$), 0.84 (3H, d, *J* 4.6, Si*Me*), 0.82 (3H, d, *J* 4.5, Si*Me*); **$\delta_C$** (90.6 MHz, $CHCl_3$) 138.8 (2C), 135.6 (4CH), 132.8 (C), 132.7 (C), 129.9 (2CH), 128.4 (4CH), 127.7 (4CH), 127.2 (2CH), 66.6 (CH), 64.8 (CH), 63.1 (CH), 60.3 ($CH_2$), 54.4 ($2CH_2$), 46.3 ($CH_2$), 39.6 ($CH_2$), 26.9 ($3CH_3$), 24.3 (CH), 23.2 ($CH_3$), 22.0 ($CH_3$), 19.0 (C).

**Minor diastereoisomer 201b:**

$\delta_H$ (360 MHz, $CHCl_3$) 3.47 (2H, d, *J* 13.6, NC$H_XH_Y$Ph), 1.16 (9H, s, $^tBu$), 0.95 (3H, d, *J* 4.3, Si*Me*), 0.93 (3H, d, *J* 4.3, Si*Me*); $\delta_C$ (90.6 MHz, $CHCl_3$) 139.5 (2C), 135.7 (4CH), 132.5 (C), 132.4 (C), 129.8 (2CH), 128.7 (4CH), 128.2 (4CH), 127.8 (4CH), 127.0 (2CH), 73.7 (CH), 70.8 (CH), 61.9 (CH), 61.5 ($CH_2$), 55.2 ($2CH_2$), 47.1 ($CH_2$), 40.9 ($CH_2$), 26.8 ($3CH_3$), 24.2 (CH), 23.1 ($CH_3$), 22.3 ($CH_3$), 18.9 (C).

## (2*S*,3*S*,5*S*)-1-*tert*-Butyldimethylsilyloxy-2-*N*,*N*-dibenzylamino-7-methyl-octane-3,5-diol 202




**General procedure F** was followed with triethylborane ($Et_3B$) (1.1 $cm^3$, 1M in THF, 1.1 mmol) and pivalic acid (4.0 mg, 0.04 mmol), (2*S*,5*S*)-1-(*tert*-butyldimethylsilyloxy)-2-(dibenzylamino)-5-hydroxy-7-methyloctan-3-one **161** (0.36 g, 0.74 mmol), sodium borohydride (0.084 g, 2.2 mmol) to afford a title compound **202** (0.10 g, 29%) as a pale oil as an inseparable mixture of diastereoisomers (50 : 25 : 25) after chromatography. Unreacted starting material **161** was also recovered (0.12 g, 33%). $\mathbf{R_f}$ [Hexane : EtOAc (9 : 1)] 0.13; $\upsilon_{max}$ **(neat)/cm$^{-1}$** 3434, 2954, 2930, 2858, 1603; ***m/z*** (FAB, NOBA) 487 ($[M+H]^+$, 16%), 355 (37), 351 (9), 197 (3), 92 (100); **HRMS** (FAB, THIOG) $C_{29}H_{48}NO_3Si$ $[M+H]^+$ requires 486.34035, found 486.34071.

**Major diastereoisomer 202a (3,5-*syn*):**

$\delta_H$ (360 MHz, $CHCl_3$) 7.33-7.25 (10H, m, Ar*H*), 4.02 (2H, d, *J* 13.3, $NCH_AH_BPh$), 3.97-3.89 (2H, m, $C_5H$ + $C_3H$), 3.93 (1H, dd, *J* 11.2, 3.3, $C_1H_CH_D$), 3.82 (1H, dd, *J* 11.2, 5.7, $C_1H_CH_D$), 3.58 (2H, d, *J* 13.3, $NCH_AH_BPh$), 2.59 (1H, ddd, *J* 9.3, 5.7, 3.3, $C_2H$), 1.83-1.69 (1H, m, $C_7H$), 1.62 (1H, dt, *J* 13.8, 2.1, $C_4H_XH_Y$), 1.44 (1H, ddd, J 13.7, 8.1, 5.7, $C_6H_EH_F$), 1.27-1.09 (2H, m, $C_4H_XH_Y$ + $C_6H_EH_F$), 0.97 (9H, s, *'Bu*), 0.92 (3H, d, *J* 6.6, $CH_3$), 0.91 (3H, d, *J* 6.6, $CH_3$), 0.15 (3H, s, Si*Me*), 0.12 (3H, s, Si*Me*); $\delta_C$ (90.6 MHz, $CHCl_3$) 138.8 (2C), 128.9 (4CH), 128.4 (4CH), 127.2 (2CH), 69.8 (CH), 68.1 (CH), 63.7 (CH), 58.6 ($CH_2$), 54.3 ($2CH_2$), 46.9 ($CH_2$), 40.7 ($CH_2$), 25.7 ($3CH_3$), 24.2 (CH), 23.2 ($CH_3$), 22.1 ($CH_3$), 17.9 (C), -5.7 ($CH_3$), -5.8 ($CH_3$).

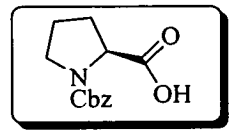
**Minor diastereoisomer 202b:**

$\delta_H$ (360 MHz, $CHCl_3$) 4.10 (1H, ddd, *J* 10.4, 7.8, 2.2, $C_3H$), 4.03 (2H, dd, *J* 5.4, 0.9, $C_1H_2$), 3.90 (2H, d, *J* 13.7, $NCH_XH_YPh$), 3,61 (2H, d, *J* 13.7, $NCH_XH_YPh$), 0.93 (9H, s, *'Bu*), 0.88 (3H, d, *J* 5.8, $CH_3$), 0.86 (3H, d, *J* 5.8, $CH_3$), 0.14 (3H, s, Si*Me*), 0.13 (3H, s, Si*Me*); $\delta_C$ (90.6 MHz, $CHCl_3$) 139.6 (2C), 128.7 (4CH), 128.2 (4CH), 127.0 (2CH), 73.8 (CH), 70.8 (CH), 61.8 (CH), 60.8 ($CH_2$), 55.2 ($2CH_2$), 47.1 ($CH_2$), 41.1 ($CH_2$), 25.7 ($3CH_3$), 24.3 (CH), 23.1 ($CH_3$), 22.4 ($CH_3$), 18.0 (C), -5.6 ($2CH_3$).

**Minor diastereoisomer 186a:**

$\delta_C$ (90.6 MHz, $CHCl_3$) 138.9 (2C), 129.0 (4CH), 128.4 (4CH), 127.2 (2CH), 66.8 (CH), 64.8 (CH), 62.9 (CH), 59.3 ($CH_2$), 54.4 ($2CH_2$), 46.4 ($CH_2$), 39.6 ($CH_2$), 25.8 ($3CH_3$), 24.4 (CH), 23.2 ($CH_3$), 22.1 ($CH_3$), 18.0 (C), -5.6 ($2CH_3$).

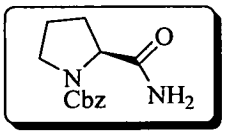
## (2*S*)-2-Carboxy-pyrrolidine-1-carboxylic acid benzyl ester 237




To a solution of L-proline **227** (4.86 g, 42.2 mmol) in THF (60 $cm^3$) and saturated sodium bicarbonate solution (60 $cm^3$) at 5 °C was added benzyl chloroformate (12 $cm^3$, 84.1 mmol) dropwise. The mixture was stirred at room temperature overnight. The aqueous layer was extracted with DCM (3 x 100 $cm^3$) and the combined organic layers dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel, eluting with 50% ethyl acetate in hexane then ethyl acetate to yield compound **237** as a clear oil (8.27 g, 79%). $\mathbf{R_f}$ (EtOAc) 0.33; $\delta_H$ (250 MHz, $CDCl_3$) 9.70 (1H, br s, O*H*), 7.55-7.37 (5H, m, Ar*H*), 5.35-5.25 (2H, m, Ar$CH_2$), 4.63-4.48 (1H, m, $CHCO_2H$), 3.84-3.55 (2H, m, N$CH_2$), 2.38-1.97 (4H, m, $CH_2CH_2$); $\delta_C$ (62.9 MHz, $CDCl_3$) 177.0 (C), 154.9 (C), 136.7 (C), 128.9 (CH), 128.5 (CH), 128.03 (CH), 67.5 ($CH_2$), 59.0 (CH), 46.9 ($CH_2$), 29.9 ($CH_2$), 23.8 ($CH_2$).

Spectroscopic data in good agreement with literature.[215]

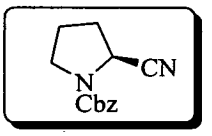
## (2*S*)-2-Carboamoyl-pyrrolidine-1-carboxylic acid benzyl ester 238




To a solution of (2*S*)-2-carboxy-pyrrolidine-1-carboxylic acid benzyl ester **237** (8.27 g, 33.2 mmol) in dry THF (130 $cm^3$) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (6.36 g, 33.2 mmol), 1-hydroxybenzotriazole (6.72 g, 49.8 mmol) and the resulting mixture stirred at room temperature for 90 min. Aqueous ammonia solution (23 $cm^3$) was added slowly and the mixture allowed to stir for 36 h. Saturated ammonium chloride solution (120 $cm^3$) was added and the mixture extracted with ethyl acetate (3 x 50 $cm^3$). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel, eluting with 10-30% ethyl acetate in petroleum ether (40-60 °C) then ethyl acetate to yield compound **238** as a white foam (5.70 g, 69%). $\mathbf{R_f}$ (EtOAc) 0.35; $\delta_H$ (250 MHz, $CDCl_3$) 7.59-7.45 (5H, m, Ar*H*), 7.10-6.05 (2H, N$H_2$ rotamers), 5.35-5.25 (2H, m, Ar$CH_2$), 4.62-4.45 (1H, m, $CH$CONH$_2$), 3.81-3.55 (2H, m, N$CH_2$), 2.55-2.00 (4H, m, $CH_2CH_2$); $\delta_C$ (62.9 MHz, $CDCl_3$) $\delta_C$ 174.9 (C), 156.4 (C), 136.7 (C), 128.9 (CH), 128.5 (CH), 128.2 (CH), 67.7 ($CH_2$), 60.6 (CH), 47.4 ($CH_2$), 28.9 ($CH_2$), 24.0 ($CH_2$).

Spectroscopic data in good agreement with literature.[76]

## (2*S*)-2-Cyano-pyrrolidine-1-carboxylic acid benzyl ester 239

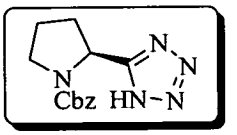



To a solution of (2*S*)-2-carboamoyl-pyrrolidine-1-carboxylic acid benzyl ester **238** (5.70 g, 23.0 mmol) in DCM (60 $cm^3$) was added pyridine (9.3 $cm^3$, 115 mmol) and tosyl chloride (8.78 g, 46.0 mmol) and the resulting mixture stirred at room temperature for 72 hours. The reaction mixture was treated with saturated ammonium chloride solution (45 $cm^3$) and water (30 $cm^3$) and extracted with ethyl acetate (3 x 60 $cm^3$). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel, eluting with 70% ethyl acetate in petroleum ether (40-60 °C) to yield compound **239** as a clear yellow oil (4.10 g, 77%). $\mathbf{R_f}$ (EtOAc) 0.72; $\delta_H$ (250 MHz, $CDCl_3$) 7.47-7.66 (5H, m, Ar*H*), 5.46-5.35 (2H, m, Ar$CH_2$), 4.83-4.74 (1H, m, C*H*CN), 3.82-3.75 (2H, m, N$CH_2$), 2.53-2.20 (4H, m, $CH_2CH_2$); $\delta_C$ (62.9 MHz, $CDCl_3$) 154.7 (C), 136.3 (C), 128.9 (CH), 128.6 (CH), 128.5 (CH), 119.1 (C), 67.9 ($CH_2$), 47.9 (CH), 46.3 ($CH_2$), 31.1 ($CH_2$), 24.1 ($CH_2$).

Spectroscopic data in good agreement with literature.[76]

## (2*S*)-2-(1*H*-Tetrazol-5-yl)-pyrrolidine-1-carboxylic acid benzyl ester

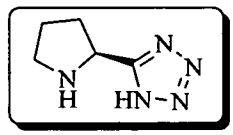
## 240




To a solution of (2*S*)-2-cyano-pyrrolidine-1-carboxylic acid benzyl ester **239** (1.52 g, 6.59 mmol) in DMF (15 $cm^3$) was added sodium azide (638 mg, 9.81 mmol) and ammonium chloride (380 mg, 7.10 mmol) and the resulting mixture heated to 90 °C overnight. The mixture was cooled and acidified to approximately pH 1 with 1N hydrochloric acid. The aqueous layer was extracted with chloroform (3 x 25 $cm^3$) and the combined organic layers washed with saturated lithium chloride (50 $cm^3$), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel, eluting with 50% ethyl acetate in hexane. In order to remove excess remaining DMF, the resulting material was passed through a silica plug, eluting with ethyl acetate, then dried on a high vacuum to yield compound **240** as a clear oil (1.36 g, 76%). $\mathbf{R_f}$ (EtOAc) 0.45; $\delta_H$ (250 MHz, $CDCl_3$) 7.40-7.31 (5H, m, Ar*H*), 7.12 (1H, m, N*H*), 5.31-5.10 (3H, m, Ar$CH_2$ and $CH$CNN), 3.70-3.61 (2H, m, N$CH_2$), 2.40-2.00 (4H, m, $CH_2CH_2$); $\delta_C$ (62.9 MHz, $CDCl_3$) 163.4 (C), 156.6 (C), 136.1 (C), 128.9 (CH), 128.7 (CH), 128.2 (CH), 67.9 ($CH_2$), 51.8 (CH), 47.4 ($CH_2$), 32.0 ($CH_2$), 24.9 ($CH_2$).

Spectroscopic data in good agreement with literature.[76]

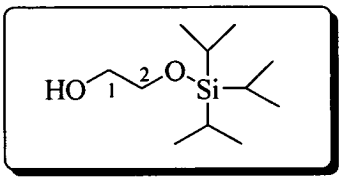
## (2*S*)-5-Pyrrolidin-1*H*-tetrazole 234



A solution of (2S)-2-(1*H*-Tetrazol-5-yl)-pyrrolidine-1-carboxylic acid benzyl ester **240** (1.36 g, 4.98 mmol) and 10% Pd/C (268 mg) in acetic acid-water (9:1, 75 $cm^3$) was stirred under an atmosphere of hydrogen for 3 h. The mixture was filtered through Celite, washing extensively with methanol and the filtrate evaporated *in vacuo*. The residue was azeotroped with toluene to aid removal of acetic acid. The resulting solid was recrystallised from a mixture of toluene and methanol to yield compound **234** as a beige solid (481 mg, 69%). $\mathbf{R_f}$ (EtOAc) 0.03; $\delta_H$ (250 MHz, DMSO) 5.31-5.10 (1H, t, *J* 7.6, C*H*CNN), 3.24-3.07 (2H, m, NC$H_2$), 2.23-1.79 (4H, m, C$H_2$C$H_2$); $\delta_C$ (62.9 MHz, DMSO) 158.1 (C), 55.2 (CH), 44.9 ($CH_2$), 30.3 ($CH_2$), 23.5 ($CH_2$).

Spectroscopic data in good agreement with literature.[76]

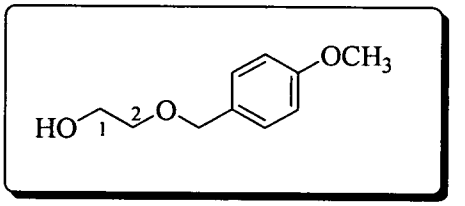
## 2-(Triisopropylsilyloxy)-ethanol 242




A solution of potassium hydroxide (85% pure, 7.69 g, 0.110 mmol) in ethylene glycol **241** (13.5 $cm^3$) was stirred until full dissolution occurred. The temperature was raised to 145 °C and maintained at this temperature for 2 days until water stopped collecting in the air condenser and the solution changed from colourless to brown. The reaction was then cooled to room temperature and triisopropylsilyl chloride (23.5 $cm^3$, 0.110 mmol) added. The temperature was raised to 35 °C and the mixture stirred overnight. The reaction was allowed to cool, diluted with water (50 $cm^3$) and extracted with diethyl ether (3 x 50 $cm^3$). The combined organic layers were washed with water (100 $cm^3$), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography to give the title compound **242** as a clear yellow oil (5.76 g, 35%). $\mathbf{R_f}$ [hexane:EtOAc (4 : 1)] 0.53; $\upsilon_{max}$ **(neat)/cm$^{-1}$** 3367, 2943, 2867, 1464; $\delta_H$ (250 MHz, $CHCl_3$) 3.98-3.94 (2H, m, $C_2H_2$), 3.84-3.80 (2H, m, $C_1H_2$), 2.54 (1H, br s, O*H*), 1.25 (21H, s, $^iPr$ × 3); $\delta_C$ (62.9 MHz, $CHCl_3$) 64.2 ($CH_2$), 63.9 ($CH_2$), 17.5 ($CH_3$), 11.7 ($CH_3$); ***m/z*** (FAB, NOBA) 219 ($[M+H]^+$, 100%), 205 (40), 201 (47); **HRMS** (FAB, NOBA) $C_{11}H_{27}O_2Si$ $[M+H]^+$ requires 219.1780, found 219.1773.

Spectroscopic data in good agreement with literature.[216]

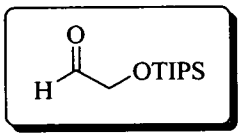
## 2-(4-Methoxybenzyloxy)-ethanol 243




A solution of potassium hydroxide (85% pure, 0.530 g, 8.06 mmol) in ethylene glycol **241** (4.5 $cm^3$) was stirred till full dissolution occurred. The temperature was raised to 145 °C and maintained for 18 hours until water had stopped collecting in the air condenser and the solution had changed from colourless to brown. The reaction was then cooled to room temperature and *p*-methoxybenzyl chloride (1.10 $cm^3$, 8.06 mmol) added. The temperature was raised to 35 °C and the mixture stirred overnight. The reaction was allowed to cool, diluted with water (50 $cm^3$) and extracted with diethyl ether (3 x 50 $cm^3$). The combined organic layers were washed with water (100 $cm^3$), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel to give a title compound **243** (1.10 g, 75%) as a clear yellow oil. $\mathbf{R_f}$ [hexane:EtOAc (1:1)] 0.28; $\delta_H$ (250 MHz, $CHCl_3$) 7.08-6.99 (2H, m, Ar*H*), 6.69-6.64 (2H, m, Ar*H*), 4.26 (2H, s, $OCH_2$), 3.58 (3H, s, O*Me*), 3.52-3.48 (2H, m, $C_2H_2$), 3.35-3.31 (2H, $C_1H_2$), 2.56 (1H, br s, O*H*); $\delta_C$ (62.9 MHz, $CHCl_3$) 158.9 (C), 129.8 (CH), 129.2 (2CH), 113.5 (2CH), 72.6 ($CH_2$), 70.9 ($CH_2$), 61.5 ($CH_2$), 54.9 ($CH_3$); ***m/z*** (FAB, NOBA) 182 ($[M]^+$, 77%), 154 (23), 137 (70), 121 (100); **HRMS** (FAB, NOBA) $C_{10}H_{14}O_3$ $[M]^+$ requires 182.0943, found 182.0943.

Spectroscopic data in good agreement with literature.[212]

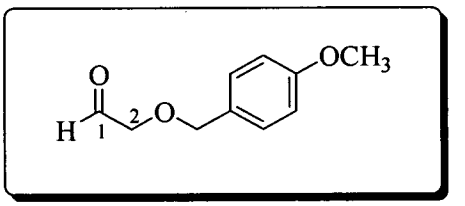
## Triisopropylsilyloxyacetaldehyde 244




To a solution of oxalyl chloride (0.280 $cm^3$, 3.25 mmol) in DCM (10 $cm^3$) at -78 °C was added DMSO (0.460 $cm^3$, 6.51 mmol) and the mixture stirred for 10 min. A solution of 2-triisopropylsilyloxyethanol **242** (0.355 g, 1.63 mmol) in DCM (5 $cm^3$) was added via cannula and the resulting mixture stirred for 1 h. Triethylamine (1.13 $cm^3$, 8.14 mmol) was added, the mixture allowed to warm to room temperature and stirred for a further hour. The mixture was diluted with water (30 $cm^3$) and extracted with DCM (3 x 30 $cm^3$). The combined organic layers were washed with HCl (50 $cm^3$, 1N aq.), water (50 $cm^3$), $NaHCO_3$ (50 $cm^3$, sat. aq.), brine (50 $cm^3$, sat.), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel, eluting with DCM to yield compound **244** as a colourless oil (0.24 g, 67%). $\mathbf{R_f}$ [hexane:EtOAc (9 : 1)] 0.45; $\delta_H$ (250 MHz, $CHCl_3$) 9.93 (1H, s, C*H*O), 4.47 (2H, s, C$H_2$), 1.37-1.27 (21H, m, 3xC*H*($CH_3$)$_2$); $\delta_C$ (62.9 MHz, $CHCl_3$) 203.3 (C), 70.1 ($CH_2$), 18.2 ($3CH_3$), 12.2 ($3CH_3$); ***m/z*** (FAB, NOBA) 217 ($[M+H]^+$, 28%), 204 (33), 202 (35), 199 (23), 173 (53); **HRMS** (FAB, NOBA) $C_{11}H_{25}O_2Si$ $[M+H]^+$ requires 217.1624, found 217.1624.

Spectroscopic data in good agreement with literature.[193]

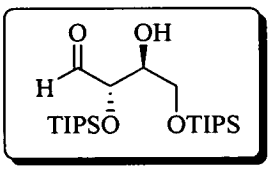
## 2-(4-Methoxybenzyloxy)-acetaldehyde 245




To a solution of oxalyl chloride (0.630 $cm^3$, 7.25 mmol) in DCM (10 $cm^3$) at -78 °C was added DMSO (1.10 $cm^3$, 15.1 mmol) and the mixture stirred for 10 min. A solution of 2-*p*-methoxybenzyloxyethanol **243** (1.10 g, 6.04 mmol) in DCM (10 $cm^3$) was added via cannula and the resulting mixture stirred for 1 h. Triethylamine (3.40 $cm^3$, 24.2 mmol) was added, the mixture allowed to warm to room temperature and stirred for a further hour. The mixture was diluted with water (50 $cm^3$), stirred for 10 min and then extracted with DCM (3 x 50 $cm^3$). The combined organic layers were washed with 1N HCl (50 $cm^3$), water (50 $cm^3$), saturated aqueous $NaHCO_3$ (50 $cm^3$), brine (50 $cm^3$), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was purified by flash chromatography to give a title compound **245** (0.890 g, 82%) as a pale yellow oil. $\mathbf{R_f}$ [hexane:EtOAc (1:1)] 0.4; $\delta_H$ (250 MHz, $CHCl_3$) 9.96 (1H, s, $C_1H$), 7.57-7.49 (2H, m, Ar*H*), 7.18-7.10 (2H, m, Ar*H*), 4.82 (2H, s, $C_2H_2$), 4.33 (2H, s, $OCH_2$), 4.07 (3H, s, O*Me*); $\delta_C$ (62.9 MHz, $CHCl_3$) 199.9 (C), 158.9 (C), 129.1 (2CH), 128.8 (C), 113.3 (2CH), 74.3 ($CH_2$), 72.6 ($CH_2$), 54.6 ($CH_3$); ***m/z*** (FAB, NOBA) 179 ($[M-H]^+$, 25%), 137 (29), 121 (100); **HRMS** (FAB, NOBA) $C_{10}H_{11}O_3$ $[M-H]^+$ requires 179.0708, found 179.0704.

Spectroscopic data in good agreement with literature.[217]

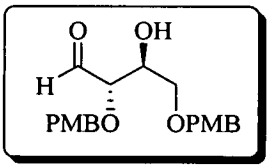
## (2*S*,3*S*)-3-Hydroxy-2,3-bis-triisopropylsilyloxy-butanal 246




To a solution of triisopropylsilyloxyacetaldehyde **244** (150 mg, 0.69 mmol) in deuteriated DMSO (3.0 $cm^3$) was added catalyst **227** or **234** (10 mol%) and the resulting mixture monitored by $^1H$ NMR. The mixture was diluted with water (15 $cm^3$) and extracted with ethyl acetate (3 x 15 $cm^3$). The combined organic layers were washed with brine (30 $cm^3$), dried ($MgSO_4$) and concentrated *in vacuo* to afford the title compound **246** as an pale oil.

Diagnostic Peaks: **$^1H$ NMR** (250 MHz, DMSO) $\delta_H$ 9.28 (1H, s, compound **248** C*H*O), 4.40 (2H, s, compound **244** C$H_2$), 4.25 (1H, dd, *J* 1.0, 4.2, compound **246** (*syn*) C*H*OH), 4.21 (1H, t, *J* 1.8, compound **246** (*anti*) C*H*OH).

## (2*S*,3*S*)-3-Hydroxy-2,3-bis-(4-methoxybenzyloxy)-butanal 249




To a solution of *p*-methoxybenzyloxyacetaldehyde **245** (90 mg, 0.50 mmol) in deuteriated solvent (0.7 $cm^3$) was added catalyst **227** or **234** (10 mol%) and the resulting mixture monitored by $^1H$ NMR. The mixture was diluted with water (15 $cm^3$) and extracted with ethyl acetate (3 x 15 $cm^3$). The combined organic layers were washed with brine (30 $cm^3$), dried ($MgSO_4$) and concentrated *in vacuo* afford the title compound **249** as an pale oil.

Diagnostic Peaks: **$^1H$ NMR** (360 MHz, DMF) $\delta_H$ 9.80 (1H, d, *J* 0.9, compound **249** (*syn*) C*H*O), 9.70 (1H, d, *J* 1.8, compound **249** (*anti*) C*H*O), 9.41 (1H, s, compound elimination product C*H*O); **$^1H$ NMR** (250 MHz, DMSO) $\delta_H$ 6.33 (1H, t, *J* 5.9, elimination product C=C*H*), 4.40 (4H, m, compound **249** 2x$CH_2$Ar), 4.17 (2H, s, compound **245** O$CH_2$CHO).

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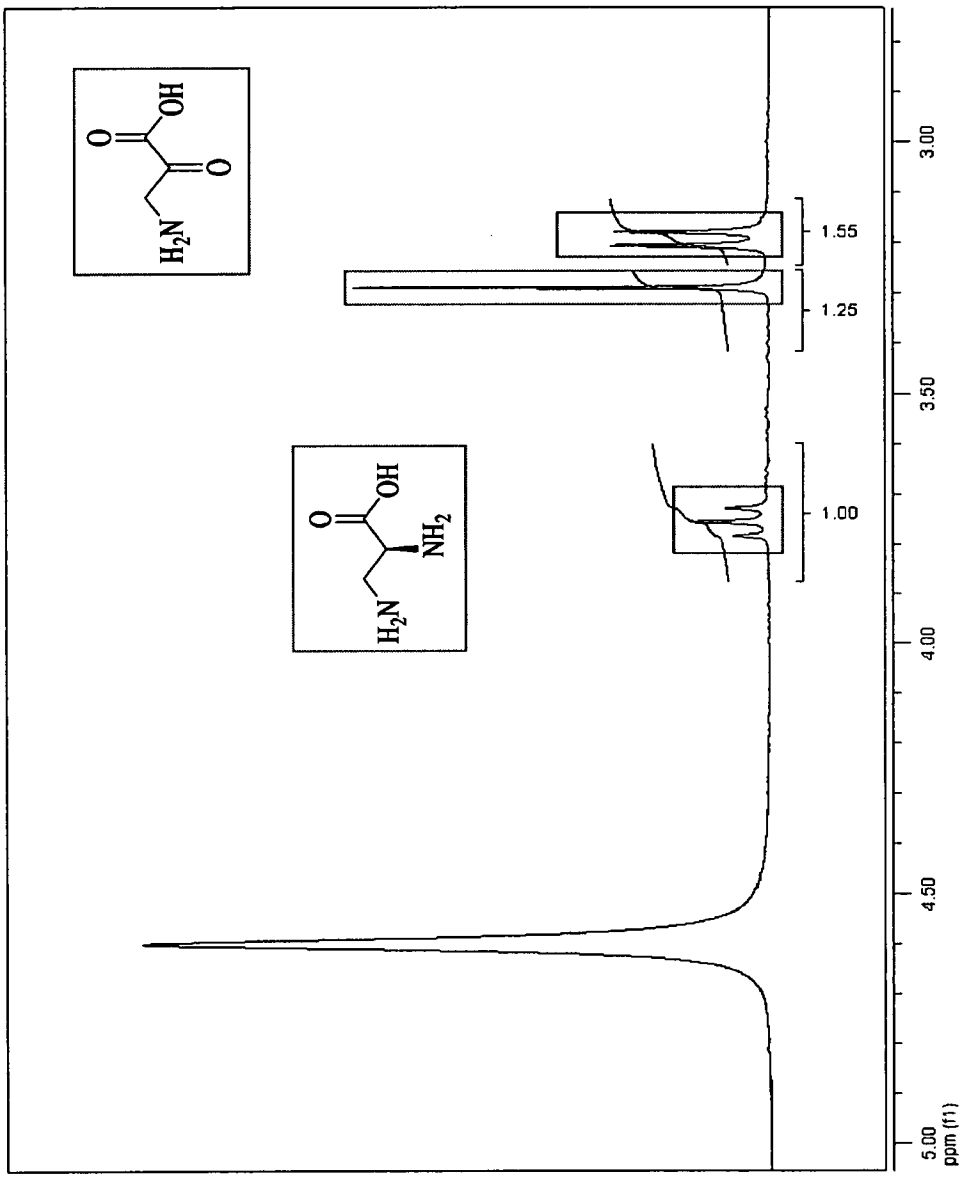
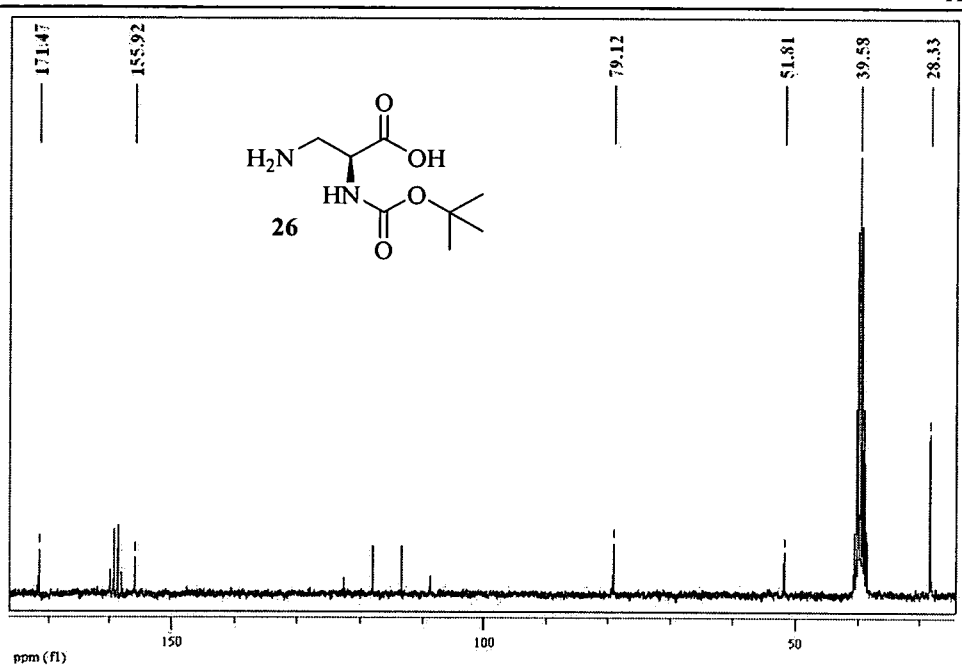
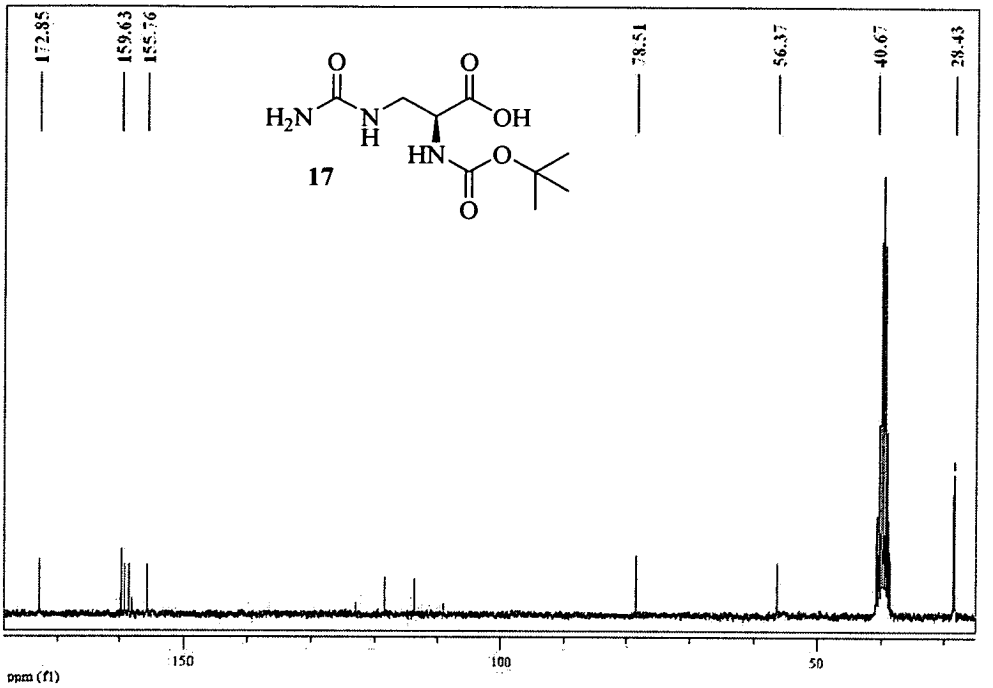
# Appendix:



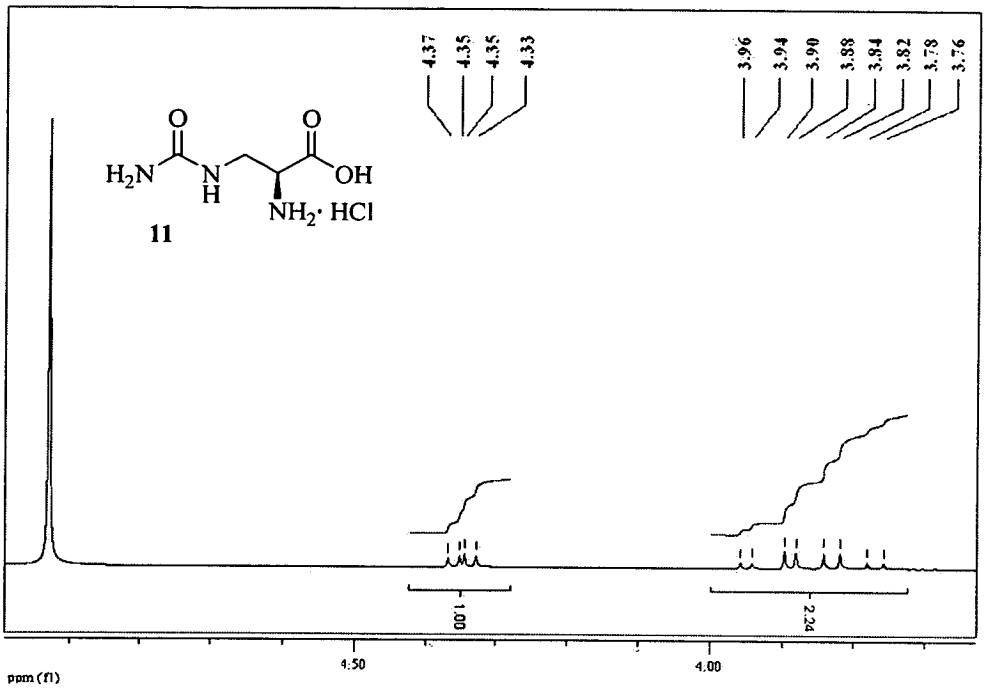

**Appendix 1:** $^{1}$H NMR spectra of the reaction products of enzymatic separation of *D,L*-2,3-diaminopropionic acid using DAAO.

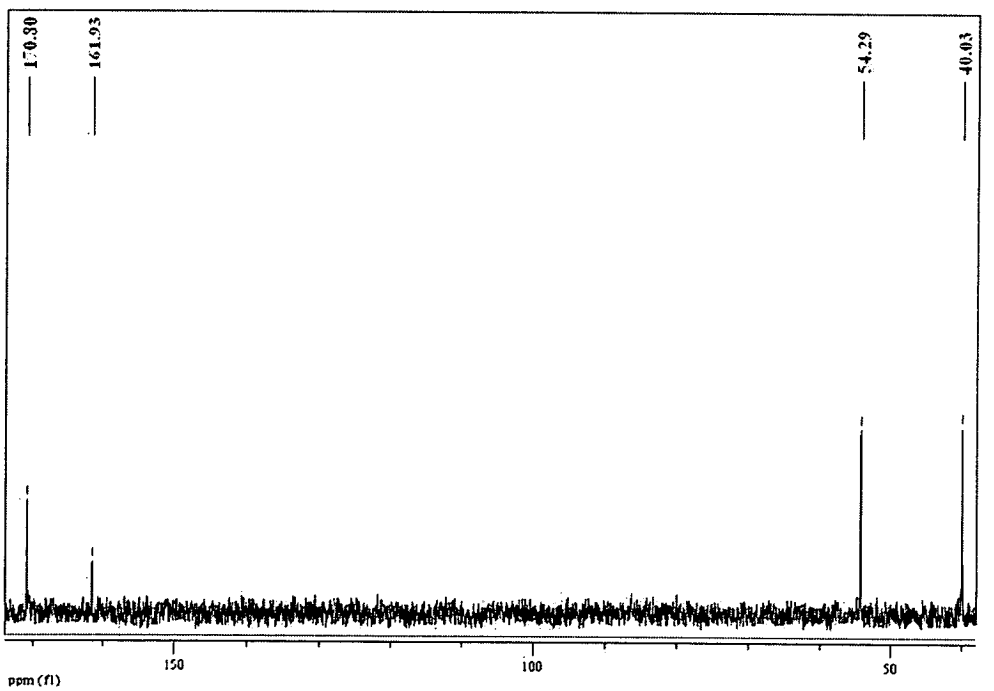



**Appendix 2:** $^{13}C$ spectra of ((2*S*)-3-amino-2-*tert*-butoxycarbonylamino-propanoic acid **26** (in DSMO/TFA).

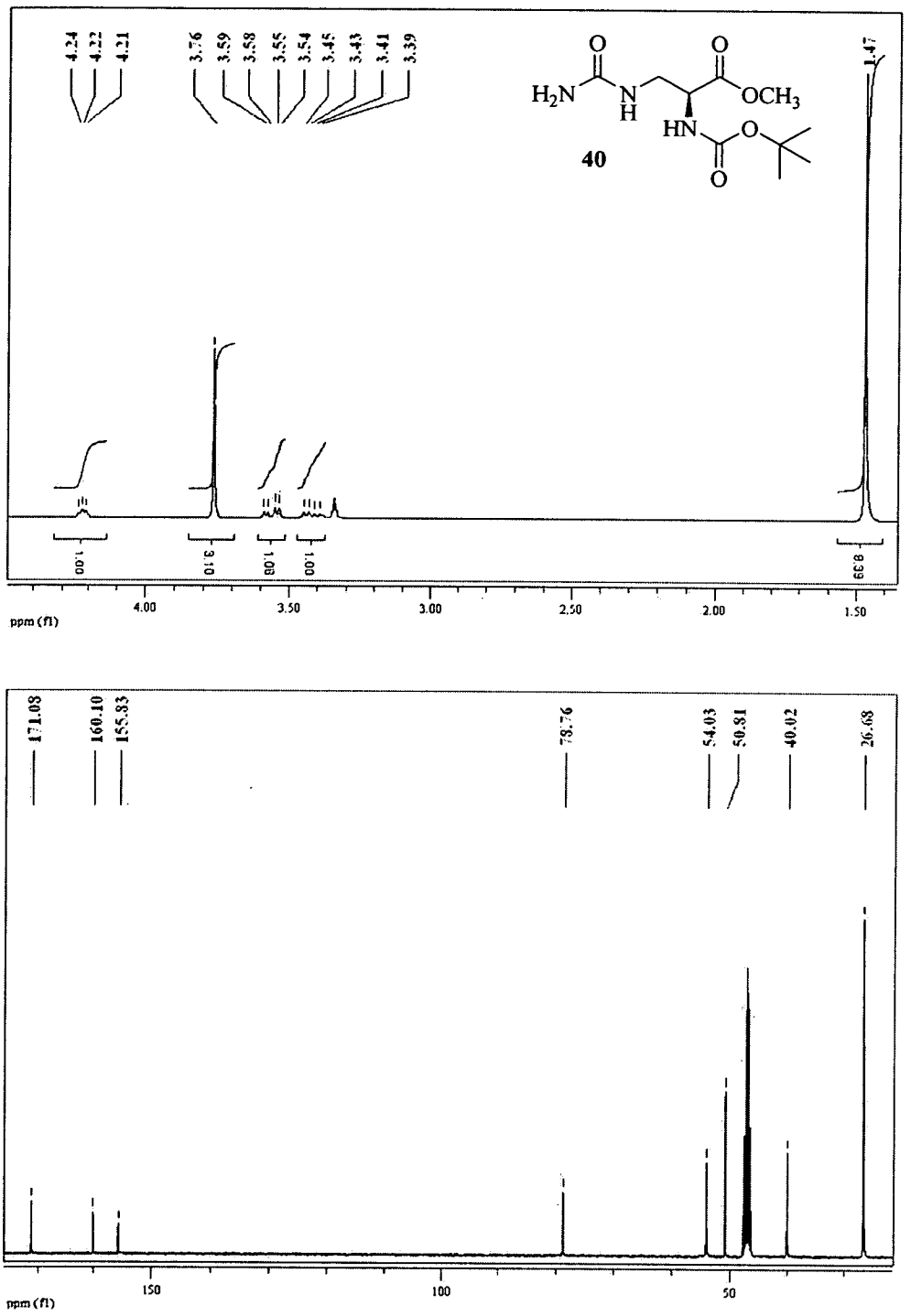



**Appendix 3:** (2*S*)-2-*tert*-butoxycarbonylamino-3-ureidopropanoic acid **17** (in DSMO/TFA).




**Appendix 4:** $^{1}H$ and $^{13}C$ NMR spectra of L-albizziine **11** (in $D_2O$).




**Appendix 5:** $^{1}H$ and $^{13}C$ NMR spectra of $N_{\alpha}$-Boc protected albizziine methyl ester **40** (in MeOH).

**Appendix 6:** Supportive information for crystal structure **200**.

**Table 1:** Crystal data and structure refinement for **200**.

Contact R.D.L.Johnstone, R.D.L.Johnstone@sms.ed.ac.uk

| **PART A. Crystal Data** | |
|---|---|
| Empirical formula | $C_{23}$ $H_{33}$ $Cl_1$ $N_1$ $O_3$ |
| Formula weight | 406.97 |
| Wavelength | 0.71073 A |
| Temperature | 150 K |
| Crystal system | Monoclinic |
| Space group | P 1 21 1 |
| Unit cell dimensions | a = 12.2144(9) A alpha = 90 deg.<br>b = 7.1526(5) A beta = 106.052(5) deg.<br>c = 13.1688(9) A gamma = 90 deg. |
| Volume | 1105.63(14) A^3 |
| Number of reflections for cell | 1659 (3 < theta < 22 deg.) |
| Z | 2 |
| Density (calculated) | 1.222 Mg/m^3 |
| Absorption coefficient | 0.195 mm^-1 |
| F(000) | 438 |
| **PART A. Data Collection** | |
| Crystal description | colourless block |
| Crystal size | 0.80 x 0.12 x 0.12 mm |
| Instrument | Bruker SMART |
| Theta range for data collection | 1.609 to 28.858 deg |

| | |
|---|---|
| Index ranges | -15<=h<=16, -9<=k<=9, -17<=l<=17 |
| Reflections collected | 12992 |
| Independent reflections | 5209 [R(int) = 0.061] |
| Scan type | \w |
| Absorption correction | Semi-empirical from equivalents (Tmin= 0.49, Tmax=0.98) |
| **PART C. Solution and Refinement** | |
| Solution | direct (SIR92 (Altomare et al., 1994)) |
| Refinement type | Full-matrix least-squares on F^2 |
| Program used for refinement | CRYSTALS |
| Hydrogen atom placement | geom |
| Hydrogen atom treatment | constr |
| Data | 5209 |
| Restraints | 1 |
| Parameters | 254 |
| Goodness-of-fit on F^2 | 0.9564 |
| Conventional R [F>4sigma(F)] | R1 = 0.0551 [2908 data] |
| Rw | 0.1471 |
| Absolute structure parameter | 0.05(10) |
| Final maximum delta/sigma | 0.000155 |
| Weighting scheme | Sheldrick Weights |
| Largest diff. peak and hole | 0.72 and -0.54 e.A^-3 |

**Table 2:** Atomic coordinates ( x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for **200**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| C(1) | 8266(3) | 11757(6) | 5410(3) | 35 |
| C(2) | 8093(5) | 10726(8) | 4366(4) | 57 |
| C(3) | 8250(4) | 13867(7) | 5234(4) | 51 |
| C(4) | 9419(4) | 11243(7) | 6163(4) | 46 |
| C(5) | 7303(3) | 11240(5) | 5916(3) | 28 |
| O(6) | 6226(2) | 11888(4) | 5255(2) | 36 |
| C(7) | 7178(3) | 9164(5) | 6121(3) | 29 |
| C(8) | 6817(3) | 8823(5) | 7137(3) | 26 |
| O(9) | 5735(2) | 9639(4) | 7054(2) | 36 |
| C(10) | 6800(3) | 6737(5) | 7416(3) | 24 |
| C(11) | 5717(4) | 5698(6) | 6843(3) | 32 |
| O(12) | 5750(2) | 5385(4) | 5780(2) | 32 |
| N(13) | 7018(2) | 6425(4) | 8606(2) | 25 |
| C(14) | 6020(3) | 7041(6) | 9016(3) | 29 |
| C(15) | 6249(3) | 6922(6) | 10207(3) | 29 |
| C(16) | 6256(4) | 5224(6) | 10708(3) | 33 |
| C(17) | 6419(4) | 5170(6) | 11800(4) | 37 |
| C(18) | 6549(3) | 6803(7) | 12370(3) | 39 |
| C(19) | 6546(4) | 8502(7) | 11879(4) | 40 |
| C(20) | 6400(4) | 8562(6) | 10784(3) | 34 |
| C(21) | 8107(3) | 7290(6) | 9283(3) | 26 |
| C(22) | 9130(3) | 7070(6) | 8861(2) | 28 |
| C(23) | 9715(4) | 8654(6) | 8709(3) | 33 |

| | | | | |
|---|---|---|---|---|
| C(24) | 10706(4) | 8513(7) | 8396(3) | 40 |
| C(25) | 11104(3) | 6775(7) | 8230(3) | 44 |
| C(26) | 10531(4) | 5184(7) | 8366(4) | 42 |
| C(27) | 9535(4) | 5308(6) | 8689(3) | 33 |
| Cl(1) | 2906(1) | 7236(2) | 1083(1) | 40 |

**Table 3:** Bond lengths [A] and angles [deg] for **200**.

| | |
|---|---|
| C(1)-C(2) | 1.523(6) |
| C(1)-C(3) | 1.526(6) |
| C(1)-C(4) | 1.526(6) |
| C(1)-C(5) | 1.549(5) |
| C(2)-H(231) | 0.942 |
| C(2)-H(232) | 0.956 |
| C(2)-H(233) | 0.967 |
| C(3)-H(221) | 0.980 |
| C(3)-H(222) | 0.988 |
| C(3)-H(223) | 0.952 |
| C(4)-H(211) | 0.959 |
| C(4)-H(212) | 0.986 |
| C(4)-H(213) | 0.970 |
| C(5)-O(6) | 1.440(5) |
| C(5)-C(7) | 1.524(5) |
| C(5)-H(191) | 1.007 |
| O(6)-H(263) | 0.855 |
| C(7)-C(8) | 1.540(5) |
| C(7)-H(181) | 0.961 |
| C(7)-H(182) | 1.010 |

| | |
|---|---|
| C(8)-O(9) | 1.421(4) |
| C(8)-C(10) | 1.538(5) |
| C(8)-H(171) | 1.012 |
| O(9)-H(22) | 0.846 |
| C(10)-C(11) | 1.523(5) |
| C(10)-N(13) | 1.531(4) |
| C(10)-H(11) | 1.010 |
| C(11)-O(12) | 1.430(5) |
| C(11)-H(261) | 0.994 |
| C(11)-H(262) | 0.994 |
| O(12)-H(23) | 0.850 |
| N(13)-C(14) | 1.528(4) |
| N(13)-C(21) | 1.514(5) |
| C(14)-C(15) | 1.517(5) |
| C(14)-H(31) | 0.999 |
| C(14)-H(32) | 0.990 |
| C(15)-C(16) | 1.382(6) |
| C(15)-C(20) | 1.382(6) |
| C(16)-C(17) | 1.396(6) |
| C(16)-H(51) | 0.957 |
| C(17)-C(18) | 1.374(6) |
| C(17)-H(61) | 0.975 |
| C(18)-C(19) | 1.376(7) |
| C(18)-H(71) | 0.962 |
| C(19)-C(20) | 1.404(6) |
| C(19)-H(81) | 0.980 |
| C(20)-H(91) | 0.956 |
| C(21)-C(22) | 1.509(5) |
| C(21)-H(101) | 0.998 |
| C(21)-H(102) | 0.969 |

| | |
|---|---|
| C(22)-C(23) | 1.383(6) |
| C(22)-C(27) | 1.395(6) |
| C(23)-C(24) | 1.386(6) |
| C(23)-H(121) | 0.974 |
| C(24)-C(25) | 1.374(7) |
| C(24)-H(131) | 0.980 |
| C(25)-C(26) | 1.374(7) |
| C(25)-H(141) | 0.941 |
| C(26)-C(27) | 1.398(6) |
| C(26)-H(151) | 0.962 |
| C(27)-H(161) | 0.960 |

| | |
|---|---|
| C(2)-C(1)-C(3) | 110.4(4) |
| C(2)-C(1)-C(4) | 110.0(4) |
| C(3)-C(1)-C(4) | 107.9(4) |
| C(2)-C(1)-C(5) | 109.9(3) |
| C(3)-C(1)-C(5) | 108.8(3) |
| C(4)-C(1)-C(5) | 109.7(3) |
| C(1)-C(2)-H(231) | 109.9 |
| C(1)-C(2)-H(232) | 110.6 |
| H(231)-C(2)-H(232) | 109.3 |
| C(1)-C(2)-H(233) | 108.2 |
| H(231)-C(2)-H(233) | 109.7 |
| H(232)-C(2)-H(233) | 109.3 |
| C(1)-C(3)-H(221) | 109.7 |
| C(1)-C(3)-H(222) | 110.5 |
| H(221)-C(3)-H(222) | 107.3 |
| C(1)-C(3)-H(223) | 111.0 |
| H(221)-C(3)-H(223) | 108.6 |
| H(222)-C(3)-H(223) | 109.6 |

| | |
|---|---|
| C(1)-C(4)-H(211) | 112.2 |
| C(1)-C(4)-H(212) | 110.9 |
| H(211)-C(4)-H(212) | 107.4 |
| C(1)-C(4)-H(213) | 112.1 |
| H(211)-C(4)-H(213) | 107.7 |
| H(212)-C(4)-H(213) | 106.3 |
| C(1)-C(5)-O(6) | 109.8(3) |
| C(1)-C(5)-C(7) | 115.7(3) |
| O(6)-C(5)-C(7) | 107.5(3) |
| C(1)-C(5)-H(191) | 106.5 |
| O(6)-C(5)-H(191) | 108.4 |
| C(7)-C(5)-H(191) | 108.7 |
| C(5)-O(6)-H(263) | 109.5 |
| C(5)-C(7)-C(8) | 112.0(3) |
| C(5)-C(7)-H(181) | 109.9 |
| C(8)-C(7)-H(181) | 108.5 |
| C(5)-C(7)-H(182) | 109.4 |
| C(8)-C(7)-H(182) | 108.1 |
| H(181)-C(7)-H(182) | 108.9 |
| C(7)-C(8)-O(9) | 110.6(3) |
| C(7)-C(8)-C(10) | 112.8(3) |
| O(9)-C(8)-C(10) | 110.1(3) |
| C(7)-C(8)-H(171) | 107.1 |
| O(9)-C(8)-H(171) | 109.0 |
| C(10)-C(8)-H(171) | 107.2 |
| C(8)-O(9)-H(22) | 102.7 |
| C(8)-C(10)-C(11) | 115.0(3) |
| C(8)-C(10)-N(13) | 112.1(3) |
| C(11)-C(10)-N(13) | 108.5(3) |
| C(8)-C(10)-H(11) | 108.4 |

| | |
|---|---|
| C(11)-C(10)-H(11) | 106.5 |
| N(13)-C(10)-H(11) | 105.9 |
| C(10)-C(11)-O(12) | 107.8(3) |
| C(10)-C(11)-H(261) | 110.0 |
| O(12)-C(11)-H(261) | 110.2 |
| C(10)-C(11)-H(262) | 109.6 |
| O(12)-C(11)-H(262) | 110.5 |
| H(261)-C(11)-H(262) | 108.8 |
| C(11)-O(12)-H(23) | 106.5 |
| C(10)-N(13)-C(14) | 113.0(3) |
| C(10)-N(13)-C(21) | 114.7(3) |
| C(14)-N(13)-C(21) | 109.3(3) |
| N(13)-C(14)-C(15) | 114.4(3) |
| N(13)-C(14)-H(31) | 108.8 |
| C(15)-C(14)-H(31) | 109.7 |
| N(13)-C(14)-H(32) | 108.4 |
| C(15)-C(14)-H(32) | 107.5 |
| H(31)-C(14)-H(32) | 108.0 |
| C(14)-C(15)-C(16) | 121.2(4) |
| C(14)-C(15)-C(20) | 118.6(4) |
| C(16)-C(15)-C(20) | 120.1(3) |
| C(15)-C(16)-C(17) | 119.8(4) |
| C(15)-C(16)-H(51) | 119.2 |
| C(17)-C(16)-H(51) | 121.0 |
| C(16)-C(17)-C(18) | 120.1(4) |
| C(16)-C(17)-H(61) | 118.5 |
| C(18)-C(17)-H(61) | 121.4 |
| C(17)-C(18)-C(19) | 120.6(4) |
| C(17)-C(18)-H(71) | 118.9 |
| C(19)-C(18)-H(71) | 120.5 |

| | |
|---|---|
| C(18)-C(19)-C(20) | 119.6(4) |
| C(18)-C(19)-H(81) | 121.4 |
| C(20)-C(19)-H(81) | 119.1 |
| C(19)-C(20)-C(15) | 119.9(4) |
| C(19)-C(20)-H(91) | 119.4 |
| C(15)-C(20)-H(91) | 120.7 |
| N(13)-C(21)-C(22) | 115.0(3) |
| N(13)-C(21)-H(101) | 107.0 |
| C(22)-C(21)-H(101) | 108.4 |
| N(13)-C(21)-H(102) | 108.7 |
| C(22)-C(21)-H(102) | 108.5 |
| H(101)-C(21)-H(102) | 109.1 |
| C(21)-C(22)-C(23) | 118.8(4) |
| C(21)-C(22)-C(27) | 121.4(4) |
| C(23)-C(22)-C(27) | 119.7(3) |
| C(22)-C(23)-C(24) | 120.8(4) |
| C(22)-C(23)-H(121) | 119.5 |
| C(24)-C(23)-H(121) | 119.7 |
| C(23)-C(24)-C(25) | 119.3(4) |
| C(23)-C(24)-H(131) | 122.1 |
| C(25)-C(24)-H(131) | 118.6 |
| C(24)-C(25)-C(26) | 120.9(4) |
| C(24)-C(25)-H(141) | 119.7 |
| C(26)-C(25)-H(141) | 119.3 |
| C(25)-C(26)-C(27) | 120.3(5) |
| C(25)-C(26)-H(151) | 119.6 |
| C(27)-C(26)-H(151) | 120.1 |
| C(26)-C(27)-C(22) | 119.0(4) |
| C(26)-C(27)-H(161) | 121.6 |
| C(22)-C(27)-H(161) | 119.5 |

Symmetry transformations used to generate equivalent atoms:

**Table 4:** Anisotropic displacement parameters (A^2 x 10^3) for **200**.
The anisotropic displacement factor exponent takes the form:
-2 pi^2 [ h^2 a*^2 U11 + ... + 2 h k a* b* U12 ]

| | U11 | U22 | U33 | U23 | U13 | U12 |
|---|---|---|---|---|---|---|
| C(1) | 40(2) | 38(3) | 31(2) | -2(2) | 18(2) | -3(2) |
| C(2) | 65(4) | 77(4) | 35(3) | -5(2) | 26(2) | 1(3) |
| C(3) | 53(3) | 51(3) | 58(3) | 11(2) | 29(3) | -4(2) |
| C(4) | 39(3) | 57(3) | 46(3) | -2(2) | 19(2) | -2(2) |
| C(5) | 26(2) | 35(2) | 23(2) | -4(2) | 8(2) | 1(2) |
| O(6) | 32(1) | 32(2) | 39(1) | 0(1) | 1(1) | 1(1) |
| C(7) | 31(2) | 35(2) | 24(2) | -1(2) | 12(2) | 5(2) |
| C(8) | 26(2) | 30(2) | 23(2) | -1(2) | 8(2) | 3(2) |
| O(9) | 33(2) | 39(2) | 39(2) | 6(1) | 15(1) | 12(1) |
| C(10) | 24(2) | 26(2) | 21(2) | 1(1) | 6(1) | 4(2) |
| C(11) | 33(2) | 32(2) | 29(2) | 0(2) | 7(2) | -4(2) |
| O(12) | 33(2) | 34(2) | 24(1) | -4(1) | 2(1) | 4(1) |
| N(13) | 20(2) | 33(2) | 24(2) | 1(1) | 7(1) | 0(1) |
| C(14) | 26(2) | 37(2) | 26(2) | 1(2) | 10(1) | 1(2) |
| C(15) | 24(2) | 35(2) | 31(2) | -1(2) | 12(1) | 0(2) |
| C(16) | 31(2) | 38(2) | 34(2) | -1(2) | 17(2) | -2(2) |
| C(17) | 31(2) | 47(3) | 37(2) | 8(2) | 14(2) | -2(2) |

| | | | | | | |
|---|---|---|---|---|---|---|
| C(18) | 34(2) | 58(3) | 28(2) | -2(2) | 13(2) | -5(2) |
| C(19) | 37(3) | 48(3) | 36(3) | -12(2) | 15(2) | -10(2) |
| C(20) | 34(2) | 34(2) | 38(2) | 4(2) | 13(2) | -2(2) |
| C(21) | 29(2) | 30(2) | 22(2) | -2(2) | 7(1) | -2(2) |
| C(22) | 24(2) | 39(2) | 19(2) | 1(2) | 2(1) | 2(2) |
| C(23) | 26(2) | 44(2) | 27(2) | -1(2) | 7(2) | 2(2) |
| C(24) | 27(2) | 59(3) | 31(2) | -3(2) | 5(2) | -9(2) |
| C(25) | 26(2) | 75(4) | 32(2) | -4(2) | 10(2) | 5(2) |
| C(26) | 34(3) | 56(3) | 36(3) | -9(2) | 9(2) | 13(2) |
| C(27) | 31(2) | 39(2) | 27(2) | 1(2) | 3(2) | 9(2) |
| Cl(1) | 62(1) | 26(1) | 33(1) | 1(1) | 15(1) | -2(1) |

**Table 5:** Hydrogen coordinates ( x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **200**.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| H(11) | 7447 | 6096 | 7216 | 29 |
| H(31) | 5807 | 8350 | 8776 | 39 |
| H(32) | 5361 | 6225 | 8698 | 39 |
| H(51) | 6131 | 4101 | 10297 | 41 |
| H(61) | 6446 | 3955 | 12142 | 49 |
| H(71) | 6648 | 6739 | 13120 | 52 |
| H(81) | 6645 | 9676 | 12278 | 51 |
| H(91) | 6420 | 9742 | 10448 | 41 |
| H(101) | 8278 | 6675 | 9990 | 36 |
| H(102) | 7978 | 8613 | 9362 | 36 |

| | | | | |
|---|---|---|---|---|
| H(121) | 9423 | 9882 | 8819 | 46 |
| H(131) | 11145 | 9615 | 8304 | 48 |
| H(141) | 11776 | 6670 | 8017 | 50 |
| H(151) | 10822 | 3980 | 8244 | 51 |
| H(161) | 9129 | 4212 | 8799 | 39 |
| H(171) | 7407 | 9446 | 7735 | 34 |
| H(181) | 7889 | 8535 | 6187 | 37 |
| H(182) | 6578 | 8602 | 5510 | 37 |
| H(191) | 7472 | 11928 | 6610 | 34 |
| H(211) | 10042 | 11679 | 5915 | 70 |
| H(212) | 9508 | 11795 | 6867 | 70 |
| H(213) | 9506 | 9903 | 6269 | 70 |
| H(221) | 8870 | 14218 | 4932 | 88 |
| H(222) | 8376 | 14537 | 5913 | 88 |
| H(223) | 7547 | 14255 | 4762 | 88 |
| H(231) | 8665 | 11073 | 4047 | 92 |
| H(232) | 8119 | 9403 | 4476 | 92 |
| H(233) | 7354 | 11068 | 3909 | 92 |
| H(261) | 5676 | 4486 | 7199 | 41 |
| H(262) | 5041 | 6463 | 6855 | 41 |
| H(263) | 6112 | 13012 | 5422 | 52 |
| H(23) | 5131 | 5823 | 5383 | 49 |
| H(22) | 5903 | 10586 | 7451 | 57 |

## Abbreviations:

| | |
|---|---|
| Ac | acetyl |
| aq. | aqueous |
| Ar | aryl |
| atm. | atmosphere |
| Bn | benzyl |
| Boc | *N-tert*-butoxycarbonyl |
| Cbz | *N*-benzyloxycarbonyl |
| CSA | camphor sulphonic acid |
| DAAO | *D*-amino acid oxidase |
| DAPA | diaminopropionic acid |
| DCC | *N,N*-dicyclohexylcarbodiimide |
| DCM | dichloromethane |
| DIBAL-H | Diisobutylaluminium hydride |
| DIC | *N,N*-diisopropylcarbodiimide |
| DMAP | 4-dimethylaminopyridine |
| DMF | *N,N*-dimethylformamide |
| DMP | Dess-Martin periodinane |
| DMSO | dimethylsulfoxide |
| DMT-MM | 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride |
| EDCI | 1-(3,3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride |
| ee | enantiomeric excess (i.e. % of major diastereomer - % of minor diastereomer |
| Et | ethyl |
| FAB | fast atom bombardment |
| HMBC | heteronuclear multiple bond correlation |
| HOBt | 1-hydroxybenzotriazole monohydrate |

| | |
|---|---|
| HPLC | high performance liquid chromatography |
| HRMC | high resolution mass spectrum |
| Hz | hertz |
| IBC | isobutyl chloroformate |
| IBLC | *N*-isobutyryl-*L*-cysteine |
| IBX | 2-iodoxybenzoic acid |
| $^{i}$Pr | *iso*-propyl |
| IR | infra red |
| LDA | Lithium diisopropylamide |
| LiHMDS | Lithium bis(trimethylsilyl)amide |
| M | mol $dm^{-3}$ |
| Me | methyl |
| NaHMDS | Sodium bis(trimethylsilyl)amide |
| NMM | *N*-methylmorpholine |
| NMR | nuclear magnetic resonance |
| NRPS | nonribosomal peptide synthase |
| Nu | nucleophile |
| OPA | *o*-phthalaldehyde |
| P | unspecified protecting group |
| Ph | phenyl |
| PIDA | iodosobenzene diacetate |
| PIFA | [*I,I*-bis(trifluoroacetoxy)-iodo]benzene |
| PKS | polyketide synthase |
| PMB | *p*-methoxybenzyl |
| Poc | *N*-isopropoxycarbonyl |
| ppm | parts per million |
| *p*-TsCl | *para*-toluene sulphonyl chloride |
| SNAC | *N*-acetylcysteamine thioester |
| t.l.c. | thin layer chromatography |
| TBDPS | *tert*-butyldiphenylsilyl |

| | |
|---|---|
| TBS/TBDMS | *tert*-butyldimethylsilyl |
| $^t$Bu | *tert*-butyl |
| THF | tetrahydrofuran |
| TIPS | triisopropylsilyl |
| UV | ultraviolet |
| μw | microwave |
| (-)-$(Ipc)_2$BOTf | (-)-diisopinocampheylboron triflate |
| $(Boc)_2O$ | di-*tert*-butyldicarbonate |
| 9-BBN | 9-borabicyclo[3,3,1]nonane |