APPROACHES TOWARDS AMINOPYRUVATES AND *SYN*-AROMATIC AMINODIOLS

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For Lois Ellen and Kimberley-Jean

Declaration

I, Simon Faulkner, hereby state that the following is entirely my own work and has not been for any other degree or examination

Simon Faulkner

September 2007

Acknowledgement

First and foremost I would like to thank my wife and daughter for their perseverance whilst I have completed my Ph. D. Seeing "daddy" hard at work has not been easy for them and they have supported me every step of the way. In fact, had it not been for my wife's pearl of wisdom that 'each and every result *is* a result, regardless of being it the one you wanted or not', negative results would not have looked as good as they did.

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Abstract

The thesis describes synthetic approaches towards chiral aromatic aminodiols, an important structural motif that can also potentially be prepared *via* the sequential use of transketolase (TK) and transaminase (TA). Syntheses of aminopyruvates and analogues as novel TK donors are also described.

Initially, the aims of this Ph. D. are laid out in Chapter 1; a literature review of the uses of enzymes in chemoenzymatic syntheses is covered. In particular, carbon-carbon bond forming enzymes (TK) from a family of thiamine pyrophosphate enzymes are discussed. The latter part of Chapter 1 explores the use of TAs to convert ketone groups to amino groups stereoselectively. Chapter 2 is concerned with current synthetic strategies towards the synthesis of aminodiols and the Sharpless asymmetric aminohydroxylation (AA). This is a key reaction in establishing chiral aminoalcohol functionalities.

The results and discussion are described in Chapter 3 to Chapter 5. Chapter 3 outlines a systematic approach towards nitrogen containing pyruvate donors, *via* a number of synthetic protocols, for potential use with TK and TK mutants. Finally, a TK mimetic reaction is presented with the successful synthesis of 3-phthalimidopyruvate. Synthetic approaches towards aromatic aminodiols *via* a short reaction sequence for use as HPLC standards are outlined in Chapter 4. Initially, one-pot methodologies were explored to access both *syn* and *anti*-diastereomers and are described. Using 'masked' aromatic *syn*-aminodiols, the methodology for producing aromatic *syn*-aminodiols *via* a short reaction sequence is described along with the synthesis of cinnamate precursors for the Sharpless asymmetric aminohydroxylation (AA) reaction. Finally, the results of Sharpless AA reactions *via* homogeneous and heterogeneous catalytic conditions with cinnamate precursors are presented in Chapter 5.

An overall summary and future areas of research in the future are discussed in Chapter 6, whilst a formal description of the experimental methods and procedures employed in this thesis are presented in Chapter 7.

Contents

Contents of Figures, Schemes & Tables

Abbreviations

AA: asymmetric aminohydroxylation

AD: asymmetric dihydroxylation

AE: asymmetric epoxidation

Ala: alanine

β-Ala: β-alanine

APCI: atmospheric pressure chemical ionisation

atm: atmosphere

B: base

BBN: 9-Borabicyclo[3.3.1]nonane

BFD: benzyl formate decarboxylate

BiCE: Bioconversion-Chemistry-Engineering Interface Programme

Bn: benzyl

Boc: *tert*-butyloxycarbonyl

Cbz : benzyloxycarbonyl

CH_2Cl_2: dichloromethane

CI: chemical ionization

COSY: correlated spectroscopy

DBI: dibromoisocyanuric acid

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

DCC: *N*,*N*'-dicyclohexylcarbodiimide

DABCO: 1,4-diazabicyclo[2.2.2]octane

DEAD: diethyl azodicarboxylate

DIAD: diisopropyl azodicarboxylate

DIEA: diisopropyl ethylamine

(-)-DIPT: diisopropyl tartrate

DiTOX: dithiane oxide

DMAP: 4-dimethylaminopyridine

DMF: *N*,*N*'-dimethylformamide

DMM: Dimethoxymethane

DMSO: dimethylsulfoxide

DNA: deoxyribonucleic acid

EDCI: *N*-(3-dimethylaminopropyl)-3-ethylcarbodiimide

EI: electron impact

ESP: electrospray

Et: ethyl

FAB: fast atom bombardment

Gly: glycine

HPA: hydroxypyruvate

HPLC: high performance liquid chromatography

IBX: 2-iodoxybenzoic acid

I.R.: infrared

*i*Pr: isopropyl

Me: methyl

MOM: methoxymethyl

Mm Hg: millimetre mercury

MTBE methyl *tert* butyl ether

NBS: *N*-bromosuccinimide

NMM: *N*-methylmorpholine

NMO: N-methylmorpholine oxide

PAC: phenylacetylcarbinol

PCC: pyridinium chlorochromate

PDC: pyruvate decarboxylase

Pd/C: palladium on carbon

PLP: pyridoxal 5′-phosphate

PMP: pyridoxamine 5'phosphate

POX: pyruvate oxidase

RAMP: (*R*)-(+)-1-Amino-2-(methoxymethyl)pyrrolidine

SAMP: (*S*)-(+)-1-Amino-2-(methoxymethyl)pyrrolidine

TA: transaminase

TBDPS: *tert*-butyldiphenylsilyl

*t*Bu: *tert*-butyl

TEA: triethylamine

TES: Triethylsilyl

TFA: trifluoroacetic acid

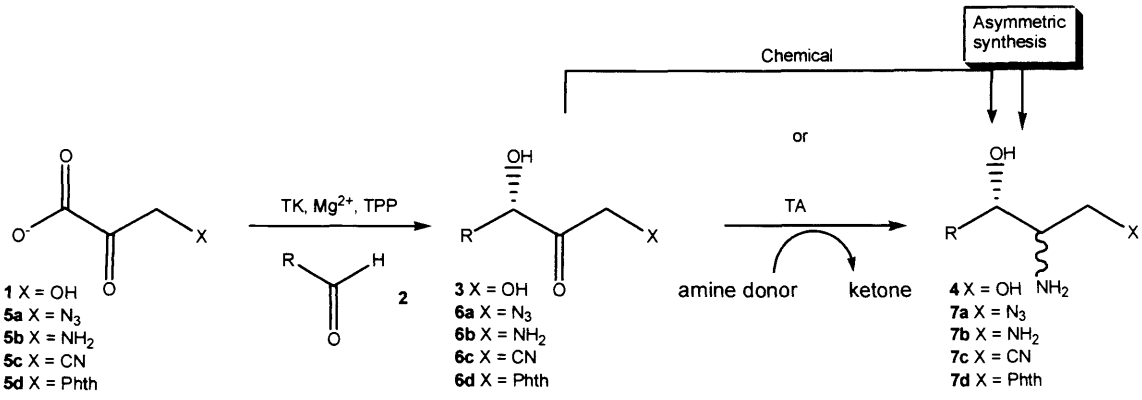
TK: transketolase

TLC: thin layer chromatrography

TPP: thiamine pyrophosphate

Aims & Objectives

The UCL-based Bioconversion-Chemistry-Engineering Interface Programme (BiCE) is interested in using transketolase (TK) to access enantiopure ketodiols for conversion into enantiopure aminodiols by transaminase (TA).[1] The project was split into two main themes (Scheme 1.1.1): transketolase and transaminase.



Scheme 1.1.1: Overall Project Scheme

An enantioselective carbon-carbon bond forming reaction (Scheme 1.1.1) is performed by transketolase (TK). A key constraint identified with the TK enzyme route is the lack of substituted pyruvate donors **1**. Hydroxypyruvate (**1**, X = OH) is the only pyruvate donor accepted by TK currently. TK, however, functions with many aldehyde acceptors **2**, where R can be alkyl, hydroxyalkyl and potentially aryl groups to give chiral ketodiols **3**. These are substrates for transamination using transaminases (TA) and could allow access to chiral aromatic aminodiols **4.**

Reaction of ketodiols **3** with transaminases (TAs) will generate aminodiols **4**. TAs are known to work with a number of aryl substrates.[1,2,3,4,5] In order to study the chemoselectivity of this reaction, synthetic routes to enantiopure aryl aminodiols were sought after for structural elucidation of the biocatalytic process by chiral HPLC and also to assess the efficacy of synthetic approaches versus biocatalytic strategies.

To further enhance the synthetic utility of this important C-C bond forming reaction, the aim was to investigate the chemoselectivity of TK mutants by synthesising nitrogen-containing pyruvates, where X contained an azide (**5a**), amino (**5b**), cyano (**5c**) or phthalimido group (**5d**). From this we aimed to generate enantiopure α-hydroxyketones

(**6a-d**) and the subsequent aminoalcohols (**7a-d**) by transamination or a chemical method.

If no chemoselectivity was expressed towards the nitrogen containing pyruvates **5a-d** by TK or its mutants, a recently discovered a mimetic TK reaction[6] could be used to study the formation of racemic products (**6a-d**), and still allow access, through transaminase (TA), or by reductive amination, to an interesting pool of synthons **7a-d**. In particular, if X contained an amino group **(6b)**, reaction with TA could provide chiral diamino alcohols (**7b**). These are structural motifs found in a number of natural products and anti depressant and anti psychotic drugs.

There are several synthetic methods that could have been explored, however, we were keen to establish a general approach with a broad substrate range and versatility. Investigations into the Sharpless aminohydroxylation (Sharpless AA) were carried out, as the literature predominantly reported the syntheses of *syn* aminoalcohol moieties where, aryl β-hydroxy-α-amino esters resulted (*i.e.* undesired 3-phenylisoserine enantiomers). We aimed to carry out an in-depth study, using homogeneous and heterogeneous Sharpless AA conditions with *N*-bromoacetamide and *N*-chloro, *N*-sodio-carbamate salts to allow rapid access to *syn* aryl α-hydroxy-β-amino esters (*i.e.* 3-phenylserine enantiomers).[7,8] These 3-phenylserine enantiomers could be then readily converted into enantiopure aryl aminodiols (**4**, X = OH). To enhance this study, we aimed to make the process more environmentally benign, by extending the study to include the use of solid supported catalysts. This was to minimise the danger of using osmium tetroxide and to investigate the effects of catalyst structure on the regio- and enantioselectivities of this reaction.

Within this study, we aimed to synthesise cinnamate substrates by aqueous Wittig reactions and by conventional organic syntheses in organic solvents. These cinnamate substrates would then be used for subsequent Sharpless AA reactions, initially to investigate the ease of forming enantiopure aryl *syn*-aminodiols **4**, before potentially considering alkyl substrates.

We aimed to also explore other one-pot syntheses towards aryl β-hydroxy-α-amino esters (3-phenylserine enantiomers), providing HPLC standards for both *syn* and/or *anti* β-hydroxy-α-amino esters.

Introduction

Chapter 1

1.1 Enzymatic and Chemoenzymatic syntheses

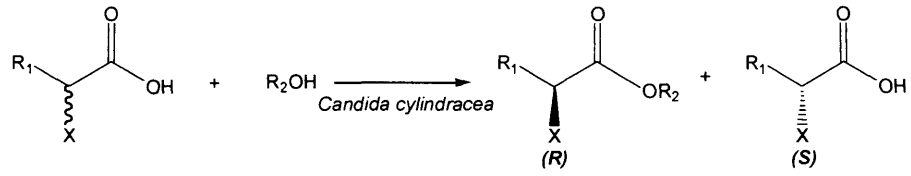
"Biotransformations involve the use of biological systems to bring about chemical changes on systems that would not otherwise occur in nature."[9] These reactions are performed using enzymes, which help to satisfy the increasingly stringent environmental constraints placed upon industry to replace 'harmful' chemical reagents and avoid the generation of harmful waste.

Enzymes have been used in organic synthesis for several decades. Since the 1960s, glucoamylase has been used for the hydrolysis of starch and the use of encapsulated enzymes for detergents was introduced in the 1970s.[10] Recently, the use of enzymes in chemical synthesis has increased due to advances in recombinant DNA technology, protein engineering and metabolic engineering. The enzyme itself may only represent one part of an overall synthesis as a biocatalytic conversion, but when coupled with other chemical steps, the whole process is referred to as a chemoenzymatic synthesis.

The main advantages of using enzymes in organic syntheses are that they usually operate at ambient temperatures and under neutral aqueous conditions. Furthermore, conversions can normally occur in the absence of functional group protection.[11] Although this is potentially beneficial, there is a disadvantage: enzymes have a tendency to denature in organic solvents due to the removal of water molecules from around the enzyme structure. This can limit their usefulness in organic synthesis where a large number of reactions are performed exclusively in organic solvents. For enzymes, water can act like a "molecular lubricant" keeping the enzyme in a rigid conformation and allowing them to catalyse reactions.[12,13] Despite this, studies over the past 15 years have shown that enzymes can function in organic solvents with surprisingly high catalytic efficiencies.[14,15] The use of hydrophobic solvents can be more effective than the use of

hydrophilic solvents due to the former removing tightly bound water molecules from the enzymes active site. Additional advantages of using organic solvents with enzymes include increased solubility of substrates in hydrophobic solvents and a favourable shift of equilibrium to favour the formation of the desired product.[16]

Enantiopure 2-chloro- and 2-bromo propionic acids (entries **1-4**, Table 1.1.1) are used as intermediates in the synthesis of the phenoxypropionic herbicides. They have been successfully generated from a yeast-lipase (*Candida cylindracea*) catalysed enantioselective butanolysis in solvents containing as little as 0.1% water.[17] This reaction is of interest as it is thermodynamically impractical in water. This is because water promotes racemisation and hinders resolution of the acid.[18]



Entry	R_1	X	R_2OH	solvent	Reaction time, h	Yield (%)	isomer	*Ee*, %
1	CH_3	Br	*n*-butyl alcohol	Hexane	14.5	96	*S*	99
2	CH_3	Cl	*n*-butyl alcohol	Hexane	14.5	96	*S*	95
3	$CH_3(CH_2)_3$	Br	*n*-butyl alcohol	Hexane[1]	48	89	S	73
4	$CH_3(CH_2)_{13}$	Br	*n*-butyl alcohol	Hexane[1]	32	67	S	Unknown

[1]Distilled water (0.1%) was added.

Table 1.1.1: Production of optically active acids from racemates *via* yeast lipase in organic solvents[17]

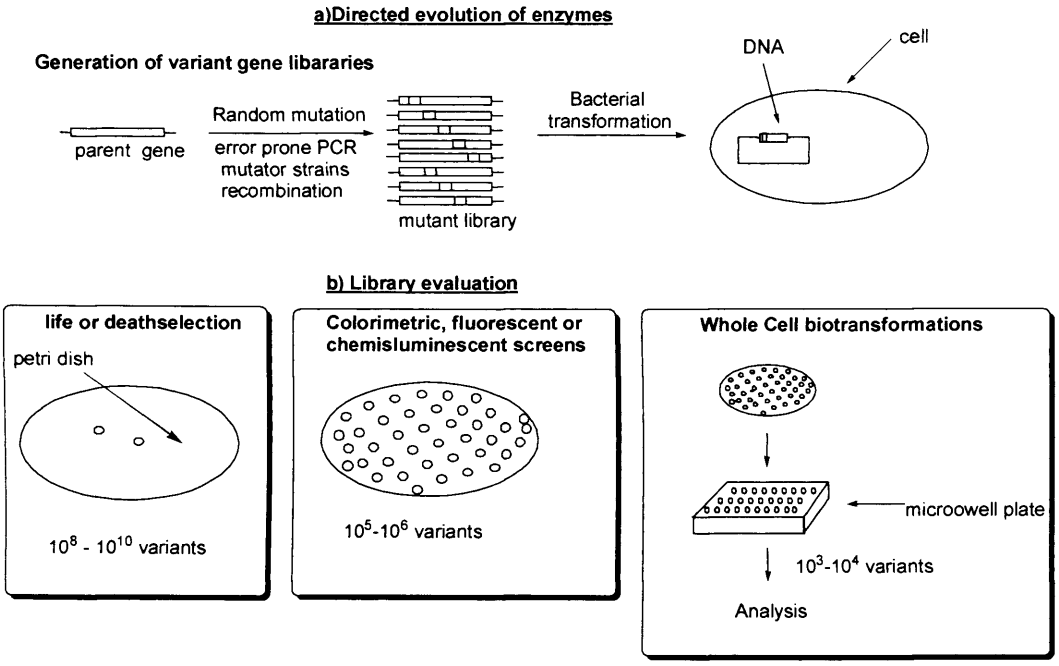
As shown in Table 1.1.1, good yields and enantioselectivities were attained (entries **1-4**), making this bioconversion an attractive strategy for the generation of 2-chloro- and 2-bromo propionic acids.

The advantages of enzymes over traditional chemical synthesis and the disadvantage of using enzymes exclusively in aqueous media have been addressed by some enzymes being able to function in organic solvents. Despite this, however, genetic manipulation of the enzyme itself is a far more valuable tool as it allows a deeper understanding of enzyme mechanism (through site-directed mutagenesis) and the acceptance of unnatural substrates (through directed evolution). In addition, enzymes can be genetically engineered to increase their structural and thermostability.

1.2 Genetic manipulation of enzymes

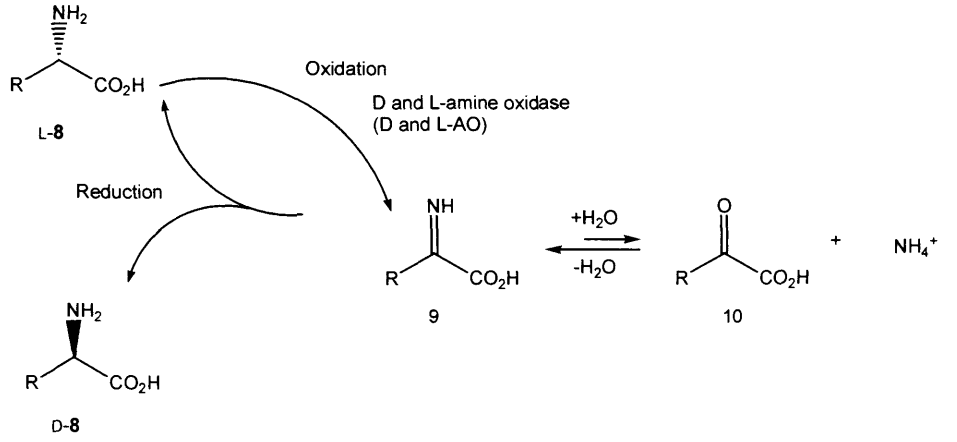
Once an enzyme is identified as having potential catalytic ability, cloning and over-expression allows for its production on a multi-gram scale, which before the advent of recombinant DNA technology was not possible. There are two methods for improving enzymes turnover of substrate or for broadening the enzymes substrate specificity. Firstly, site-directed mutagenesis (1980s), a forerunner to directed evolution (1990s) succeeds in providing a good understanding of protein structure and function. Whilst this is a successful method, previous knowledge of the enzymes structure is required, which is not always easily obtained. Despite this, site-directed mutagenesis is a valuable tool, particularly when successful mutants are identified and can then be implemented in analysing the enzymes function, however this technique is much too time-intensive for the development of high throughput protocols.[19]

The second method is directed evolution, which was designed to generate enzymes with specific characteristics in a random, selection-driven manner. The main advantage of directed evolution over site-directed mutagenesis, is that prior knowledge of the enzymes structure is not required and its compatibility with high throughput protocols. Directed evolution involves two independent but powerful technologies: methods for the generation of random genetic libraries, and strategies for the selection of variant enzymes that possesses specific characteristics. Such characteristics include increased catalytic activity, enhanced selectivity for a substrate, or an improved stability of the enzyme.[20] The genetic libraries are then run through a high-throughput screen that is capable of dealing with large numbers of variants based on *in vivo* selection (10^8-10^{10} variants) or *in vitro* selection (10^5-10^6 variants). This is achieved by detection of colonies on agar plates using, for example, fluorescence or colorimetric product formation. Alternatively, individual clones can be assayed on 96-well microtitre plates (10^3-10^4 variants) for a more accurate determination of activity (Scheme 1.2.1).[21]



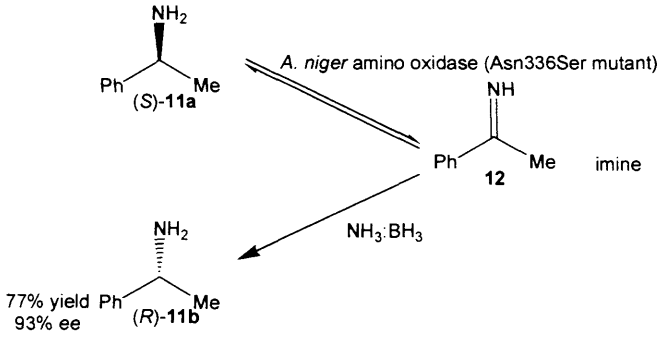
Scheme 1.2.1: Strategies for the directed evolution of enzymes involving (a) generation of libraries of variant genes and (b) high-throughput screening of the libraries using different analytical methods[21]

One example of the development of directed evolution using enzymes was to allow ease of access to enantiopure amines by deracemisation. This was based on initial work involving the deracemisation of natural and unnatural DL-amino acids, using a chemoenzymatic process (Scheme 1.2.2). L-Amino acid oxidases promote stereoinversion by enantioselective oxidation of a DL-amino acid (L-**8**) to imino acid (**9**), followed by reduction with non-selective reducing agent. An array of non-selective chemical reducing agents (sodium cyanoborohydride; sodium borohydride; amine–boranes and Pd/C-ammonium formate) was considered. Alternatively, keto acid **10** could be obtained through hydrolysis of the imine. Various experiments by Turner *et al.*, showed reducing agents, such as amine-boranes could be implemented for use in water, whereas sodium cyanoborohydride and sodium borohydride were less successful.[22,23]



Scheme 1.2.2: Deracemisation of α-amino acids using an enantioselective D and L-amine oxidases in combination with a non-selective chemical reducing agents[22]

Based on this platform methodology, directed evolution techniques were then used with the amine oxidase (*Aspergillus niger*) to catalyse the enantioselective oxidation of α-methylbenzylamine (Scheme 1.2.3), starting with (*S*)-α-methylbenzylamine (**11a**) to imine (**12**), which was converted to (*R*)-α-methylbenzylamine (**11b**) by ammonia-borane. From approximately 150,000 clones, one mutant (Asn336Ser), showed 50-fold greater activity with (*S*)-**11a** and a six fold greater enantioselectivity compared with the wild-type enzyme, resulting in (*R*)-**11b** formation in 77 % yield and 93 % *ee*.[24,25]

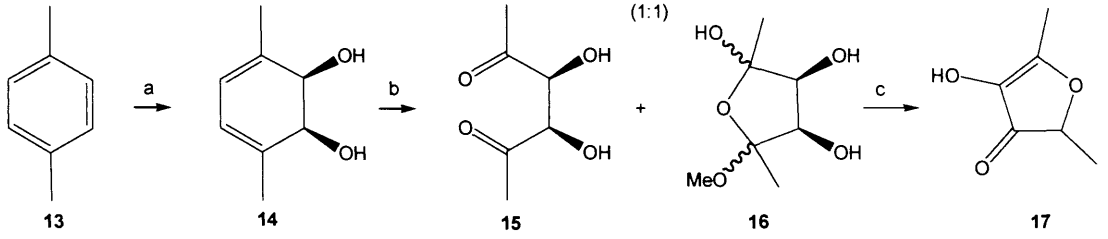


Scheme 1.2.3: Deracemisation of α-methylbenzylamine using an Asn336Ser mutant of *A. niger* amine oxidase[24,25]

Newman *et al.*, reported a further example of the use of directed evolution in the production of furanone flavour compounds (Scheme 1.2.4). Microorganisms containing aryl dioxygenases converted aromatic hydrocarbons to the corresponding *cis*-diols, important fragments for the manufacture of a wide range of fine chemicals.[26] Multigene DNA shuffling was applied to toluene dioxygenase (*tod*) and tetrachlorobenzene dioxygenase (*tec*) operons to improve the biotransformation of *cis*-dihydroxylated aromatic compounds. Four gene operons, *tod*C1C2BA and *tec*A1A2A3A4 were shuffled and were pre-screened. A high-throughput screen was then developed where

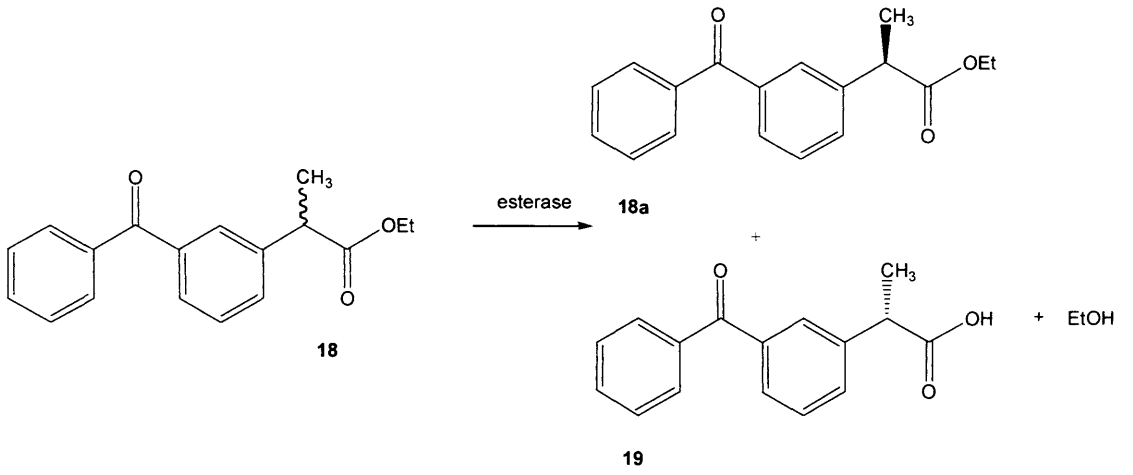
slow release of the substrate circumvented substrate toxicity to the bacterial host. As a result, nine clones were selected and tested. Clone C10 was found to have the best activity and accepted a number of substrates including *p*-xylene, *m*-xylene, 1,2,3-trimethylbenzene and naphthalene.

Exploiting the use of the inexpensive starting material (Scheme 1.2.4) *p*-xylene (**13**) underwent a whole cell biotransformation with "directly evolved" bacterial toluene dioxygenase (*E. coli* JM 109 (pTrocdNK1)), which led to *cis*-1,2-dihydroxy-3,6-dimethyl-cyclohexa-3,5-diene (**14**) on an 88 g scale. Ozonolysis of this *meso* diol **14** gave a 1:1 diastereomeric mixture of diketone (**15**) and o-methylketal-hemiketal (**16**). These diastereoisomers were dissolved in *n*-butyl acetate containing a phosphate buffer and heated at 95 °C for 5 h. The reaction mixture was then left to stand for 12 h and gave furanone (**17**). This compound is normally synthesized from the expensive 2,5-hex-3-ynediol, so using *p*-xylene **13** made this process more economically feasible on an industrial scale.[26,27]



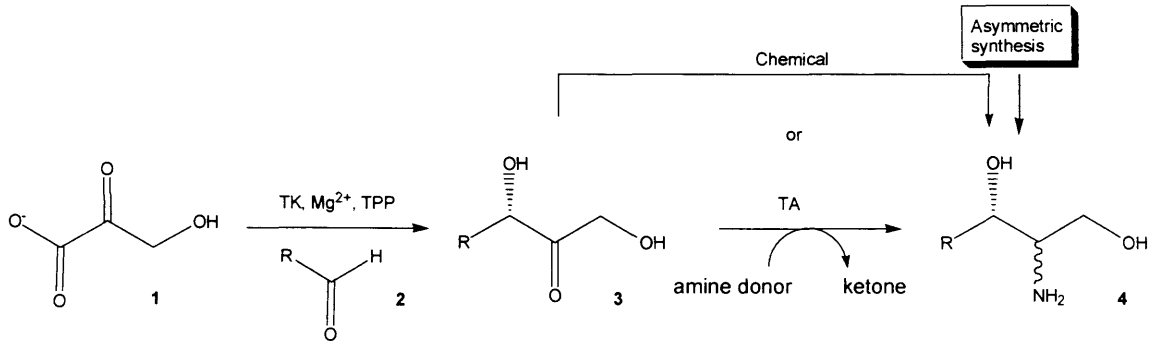
Scheme 1.2.4: (a) *E. coli* JM109 (pTrcodNK1); (b) O_3, MeOH; then aq. $Na_2S_2O_3$, $NaHCO_3$ (84-92%): (c) *n*BuOAc, H_2O, phosphate buffer, 95 °C, 5 h (39-40%)[26]

Directed evolution can also be used to enhance the thermostability of enzymes. For example, a newly isolated enantioselective esterase from *Pseudomonas fluorescens* KCTC 1767 had been identified as a possible biocatalyst, which converted racemic ketoprofen ethyl ester (**18**) to (*R*)-ketoprofen ethyl ester (**18a**) and (*S*)-ketoprofen (**19**). The only problem was that *Pseudomonas fluorescens* KCTC 1767 was reported to have low structural and thermal stability.[28] In order to overcome this, Kim *et al.*, performed directed evolution on this enantioselective esterase by successive steps of error prone and staggered extension polymerase chain reactions (PCR). After two rounds of *in vitro* directed evolution, a mutant 6-52 that had 5-point mutations was identified. Three of these mutations were found to affect the amino acid residues (L120P, I208V and T249A) whilst the other two mutations were silent. Mutant 6-52 was identified with enhanced thermal stability (12.8 % conversion *c.f.* wild type 0.2%).[29]



Scheme 1.2.5: Schematic representation for the enzymatic resolution of ketoprofen ethyl ester (**18**) to (*R*)-ketoprofen ethyl ester (**18a**) and (*S*)- ketoprofen (**19**) using an esterase

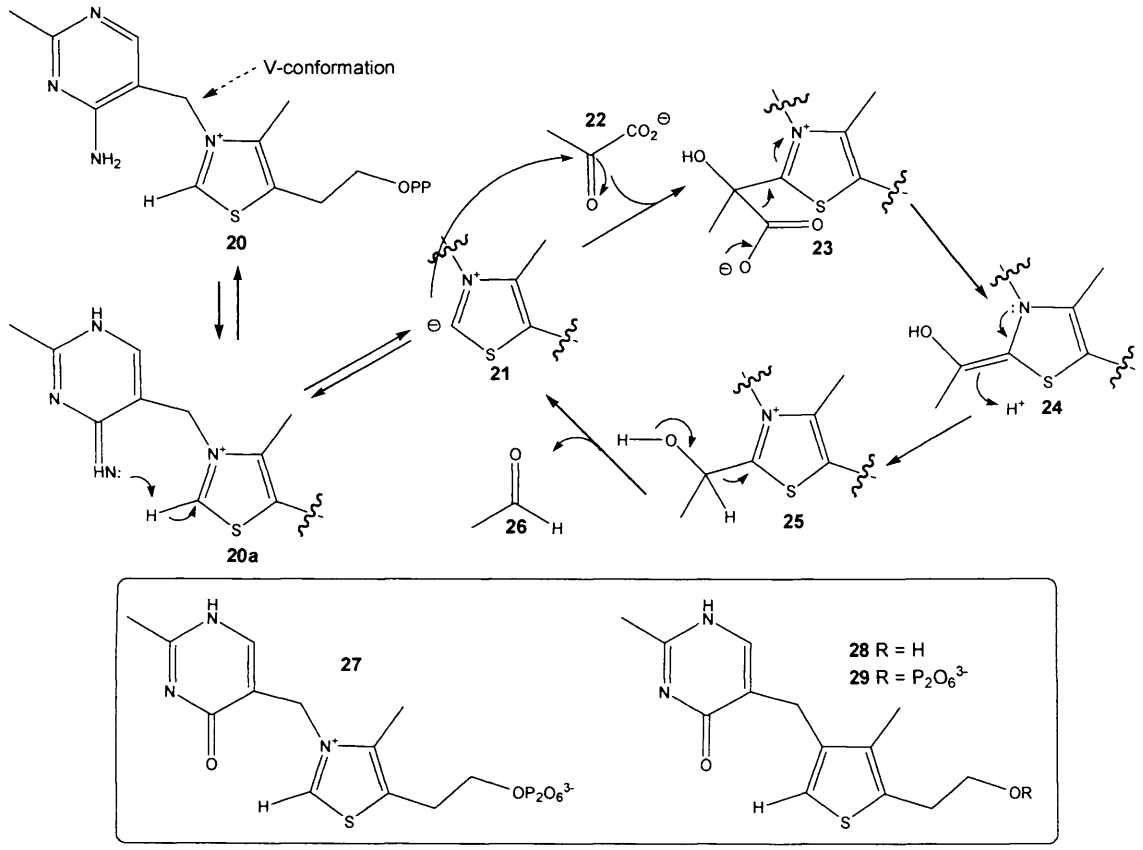
This general preamble of genetic manipulation techniques used to improve the enzymes chemoselectivity towards unnatural substrates introduced some of the methods that are currently employed by the BiCE collaboration. The BiCE collaborators are interested in two enzymes as part of a dual enzymatic or chemoenzymatic pathway (Scheme 1.2.6). These are transketolase (TK), a thiamine pyrophosphate dependentenzyme and transaminase (TA) to access enantiopure aminodiols **4**.



Scheme 1.2.6: Overall Project Scheme

1.3 Thiamine pyrophosphate dependent(TPP) enzymes

Thiamine pyrophosphate dependentenzymes include many enzymes, but for this section, the focus was on pyruvate decarboxylase (PDC), benzoylformate decarboxylase (BFD) and transketolase (TK). Thiamine (vitamin B_1) is required for the production of thiamine pyrophosphate (TPP) (**20**). TPP dependent enzymes can catalyse either the cleavage or formation of bonds to the carbon atom of a carbonyl group. The mechanism shown (Scheme 1.3.1) is pyruvate decarboxylation to give acetaldehyde (**26**).



Scheme 1.3.1: Structure of TPP (**20**), mechanism of PDC and inactive analogues deazaTPP (**27-29**)

Deprotonation of the C-2 in the thiazolium ring **20** gives the ylid (**21**). The ylid subsequently attacks the carbonyl group of pyruvate (**22**) and gives the 2-lactylTPP species (**23**). The positively charged thiazolium ring then facilitates decarboxylation and affords the enamine intermediate (**24**). The mechanisms of TPP dependent enzymes are very similar, going through the enamine intermediate **24** (Scheme 1.3.1), whether for C-C bond formation or bond cleavage. In the case of PDC, protonation of the enamine **23** yields 2-(1-hydroxyethyl) TPP (**25**). Release of acetaldehyde **26** from the catalytic cycle regenerates the ylid **21** and thus completes the catalytic cycle.[30]

How the substrate binds to TPP-dependent enzymes and which acid or base groups are involved in the C-2 deprotonation at the C-2 of the thiazolium ring, remains unknown. This is in spite of the number of crystal structures solved for TPP-dependent enzymes.[31] It is suggested that mobile loops close over the active site when the substrate binds and masks the essential catalytic groups. The only exception to this is transketolase (TK), which is known to catalyse the formation of enamine intermediate **24** and was characterised by X-ray crystallography.[32]

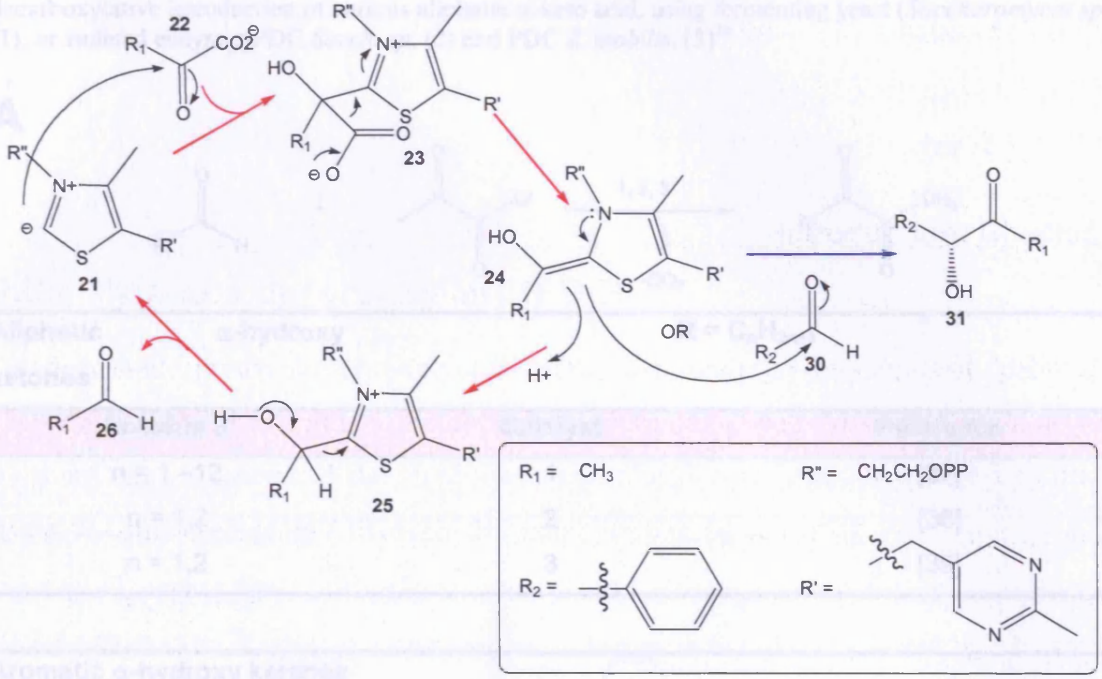
Invariably, many TPP-dependent enzymes exist as dimers, with the active sites at the interfaces between the two subunits.[31] TPP is usually bound in the V-conformation **20** (Scheme 1.3.1), which is not the lowest energy state. This conformation was characterised by the close proximity of the 4′-amino groups on the pyrimidine ring to the hydrogen atom on the C-2 atom of the thiazolium ring heading to form ylid **21**. At the centre of much controversy is the deprotonation of the hydrogen atom at C-2 of the thiazolium ring. It is believed the aminopyrimidine unit is responsible for this deprotonation. In its usual form, the amino group exists with its lone pairs in conjugation with the π-system of the pyrimidine ring. Tautomerisation to the less stable imino group (**20a**) (Scheme 1.3.1) allows the iminopyrimidine nitrogen to act as the base. Evidence for this hypothesis came from the studies on oxythamine diphosphate (**27**) (Scheme 1.3.1). This was completely inactive when bound instead of TPP **20**. TPP hydrogen exchange at C-2 was measured using NMR experiments with PDC and was 10^5 times faster than when oxythiamine diphosphate **27** was bound, which retarded the exchange of hydrogen.[33]

One method to overcome the problem of masking essential catalytic groups was explored by combining an inhibitor and crystallographic studies. Deazathiamine (Deaza) (**28**) (Scheme 1.3.1) was converted into DeazaTPP (**29**) using concentrated phosphoric acid and is isoelectronic with TPP **20**.[30] The lack of a positive charge on DeazaTPP **29** was expected to prevent the formation of C-2 ylid **21**. A deazaTPP crystal structure had been solved, but in proteolysis studies with chymotrypsin, where **29** existed in an identical position to TPP. The Enz-deazaTPP complex was observed to protect both the α-chains of the tetramer (enzyme subunit is an $\alpha_2\beta_2$ tetramer) from proteolysis. The similarity between the ylid **21** and deazaTPP **29** complex suggested that the loop closed once the ylid was formed. Therefore in the Enz-TPP complex, one of the two TPP molecules was present as the ylid **21**, whilst the other was the protonated TPP **20**.

TK is known not to accept pyruvate **22**, but will accept hydroxypyruvate **1**. Whereas conversely, PDC does not accept hydroxypyruvate **1**, but accepts pyruvate **22**. If successful amino pyruvate analogues **5a-d** were synthesised there is a possibility of testing these substrates with α-keto decarboxylases, TK and TK mutants. Furthermore, the possibility of novel biotransformations could enable the production of novel and interesting chiral synthons through carbon-carbon bond forming reactions.

1.3.1 Pyruvate decarboxylase (PDC)

As discussed, pyruvate decarboxylase (PDC) can undergo two reaction pathways (Scheme 1.3.2). One allows the formation of acetaldehyde **26** and ethanol, whilst Neuberg *et al.*, discovered the other was a carboligation reaction that occurred during 'phytochemical reductions' of aldehydes using fermenting yeast.[34,35]



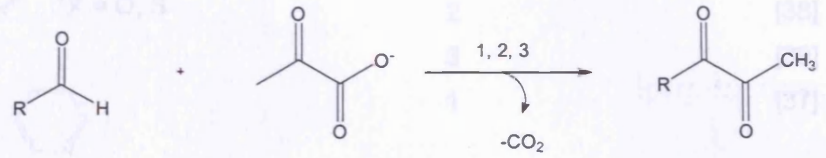
Scheme 1.3.2: Reaction pathways of enzymatic a-keto decarboxylase (red arrows) and formation of α-hydroxyketones by PDC (blue arrows)

The mechanism of decarboxylation depends on two properties of the thiazolium ring. The first is its capacity to ionise and form a nucleophilic anion and thus bind the α-carbonyl group of α-keto acids; and second was its ability to stabilize the negative charge upon decarboxylation of the substrate. The reaction cycle is initiated by the activation of TPP **20** by the enzyme. The C2-TPP ylid **21** then performs a nucleophilic attack on pyruvate **22**. Proton transfer gives 2-lactylTPP **23**, which through decarboxylation stabilizes the resulting doubly negatively charged species **24**. This 'active aldehyde **24**' leads to the formation of chiral α-hydroxy ketones *via* a reversible reaction. This is achieved by addition of a second aldehyde, such as benzaldehyde (**30**) and gives (*R*)-phenylacetylcarbinol (PAC) (**31**) through an acyloin-type condensation reaction. Unequivocal proof of the ability of PDC to undergo both reaction pathways was discovered by Hanc and Kakac[36], who incubated an enriched fraction of PDC from yeast with pyruvate **22** and benzaldehyde **30** and afforded (*R*)-phenylacetylcarbinol (PAC) **31**.

Following this work, a wide range of aldehydes (aliphatic, aromatic, heteroaromatic and α,β-unsaturated aldehydes) have been condensed with pyruvate **22** (Table 1.3.1 A and B). The majority of these reactions were reported using fermenting yeasts and only a few with isolated enzymes.[34]

Table 1.3.1: α-Hydroxyketones obtained by (**A**) decarboxylative incorporation of pyruvate and (**B**) decarboxylative introduction of various aliphatic α-keto acid, using fermenting yeast (*Saccharomyces sp.*) (1), or isolated enzymes PDC *Sacch. sp.* (2) and PDC *Z. mobilis*. (3)[34]

A

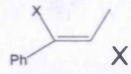
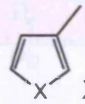
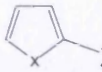
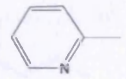
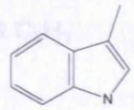
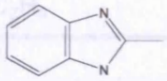


Aliphatic ketones	**α-hydroxy**	**R = C_nH_{2n+1}**
Residue R	**Catalyst**	**Reference**
n = 1 –12	1	[37]
n = 1,2	2	[38]
n = 1,2	3	[39]

Aromatic α-hydroxy ketones	**R =**  **(Benzaldehyde derivatives)**	
Residue	**Catalyst**	**Reference**
X, Y, Z = -H	1	[40]
	2	[41]
	3	[39]

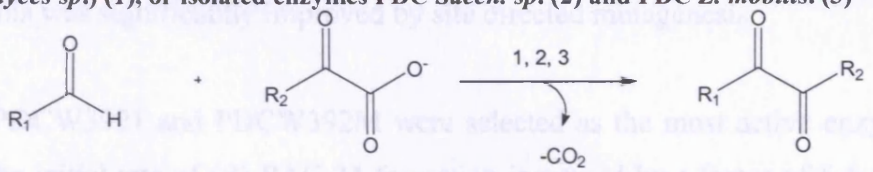
Monosubstituted derivatives		
X, Y, Z = -F, -Cl, -Br	1	[42]
	2	[41]
	3	[38]
X, Y, Z = -OH, CH_3, OCH_3, $-CF_3$	1	[37]
Z = $-CH(CH_3)_2$, $-N(CH_3)_2$, $-C_6H_5$	1	[37]
Disubstituted derivatives		
X, Y = -di-F	1,2	[41]
X, X′ = - di-F	1,2	[41]

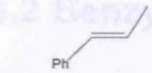
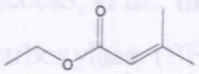
Aromatic and heterocyclic α-hydroxy ketones

Residue	Catalyst	Reference
R = $Ph(CH_2)_n$, n = 1,2	1	[37]
 X = H, $-CH_3$	1	[43]
 X = O, S	2	[38]
	3	[39]
 X = O, S	1	[37]
	2	[38]
	3	[39]
	1	[37]
	1	[37]
	1	[37]

Despite reports of PDC's chemoselectivity to a broad range of substrates, allowing the introduction of a C2 unit *via* decarboxylative incorporation of pyruvate **22**, only a few examples of decarboxylative incorporation of alternative pyruvate donors have been reported (Table 1.3.1, B).

B **decarboxylative introduction of various aliphatic α-keto acid, using fermenting yeast (*Saccharomyces sp.*) (1), or isolated enzymes PDC *Sacch. sp.* (2) and PDC *Z. mobilis.* (3)[34]**



R_1	R_2	Catalyst	Reference
Ph	$-CH_3$	1	[43]
	$-C_2H_5$		
	$-C_3H_7$		
	$-CH_3$ & $-C_2H_5$	1	[43]
	$-CH_3$ & $-C_2H_5$	1	[43]
$-CH_3$ & C_2H_5	$-CH_3$ & $-C_2H_5$	1	[44]
		2	[38]
$-C_2H_5$	$-CH_3$ & $-C_2H_5$	3	[39]

It is notable that the majority of the α-hydroxy ketones shown in Table 1.3.1 (A and B) were synthesized on an analytical scale, rather than preparative. The most extensively studied reaction with regards to improvement in yields and stability of the microbial catalyst, particularly in the presence of benzaldehyde **30**, was the formation of (*R*)-phenylacetylcarbinol (PAC) **31**. Various yeasts from *Saccharomyces sp.* and *Candida sp.* were at the forefront of these investigations.

One of the main problems encountered was that whole cell reactions with yeasts also contained alcohol dehydrogenases, which converted benzaldehyde-like substrates to benzylic alcohols, lowering the efficacy of the reaction. Other by-products included acetoin, 2-hydroxypropiophenone (2-HPP), benzoin, benzoic acid, butane-2,3-dione (diketone), 1-phenyl-propan-2,3-dione (acetylbenzoyl) and *trans*-cinnamaldehyde. Additionally (*R*)-PAC **31** could be reduced to (1*R*,2*S*)-1-phenyl-1,2-propanediol.[34]

The best way of counteracting these unwanted by-products was to use isolated pyruvate decarboxylases for the production of (*R*)-PAC-**31**. Although benzaldehyde **30** had the ability to inhibit PDC, concentrations of up to 70 mM have been achieved with PDC from *Z. mobilis.* A comparison of *S. cerevisiae* and *C. utilis* PDC to PDC from *Z. mobilis* showed the former were used at ambient temperatures, whereas the latter would

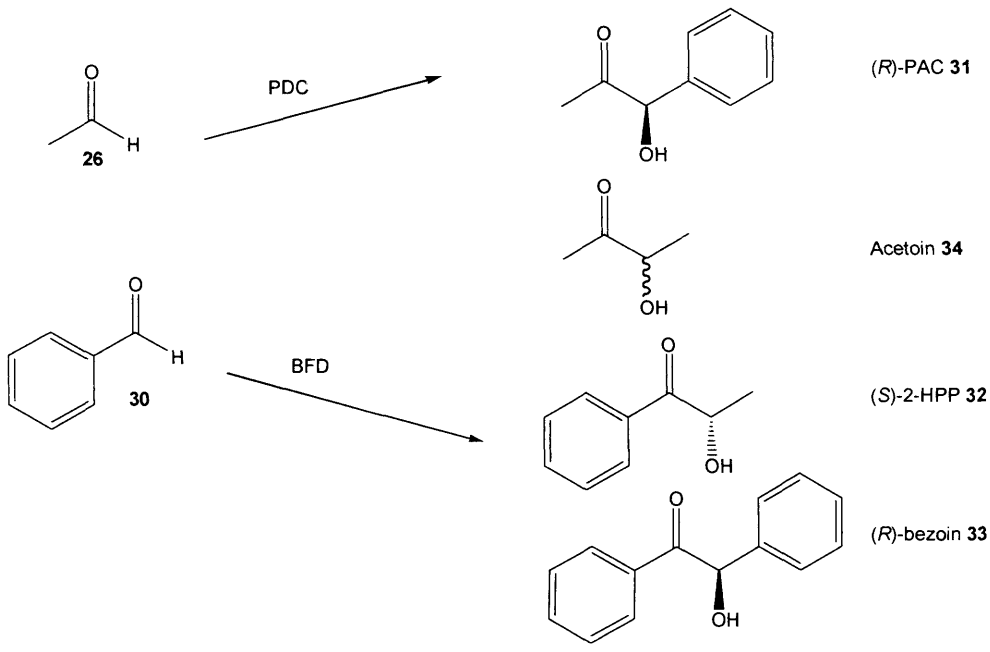
only function at 4 °C. Although, wild-type *Z. mobilis* enzymes had lower carboligase activity, this was significantly improved by site directed mutagenesis.

Mutants PDCW3921 and PDCW392M were selected as the most active enzymes in a series. The initial rate of (*R*)-PAC-**31** formation increased by a factor of 5-6 compared with the wild-type enzyme. Another enzyme capable of producing (*R*)-PAC **31** was benzyl formate decarboxylase.

1.3.2 Benzylformate decarboxylase (BFD)

Wilcocks, *et al.*, first described the formation of α-hydroxyketones using benzylformate decarboxylase (BFD) from *Pseudomonas putida* whole cells and cell extracts. In particular, the formation of a tautomer of (*R*)-PAC **31**, (*S*)-2-hydroxypropiophenone (2-HPP) **32**, was obtained from acetaldehyde **26** and benzoylformate (Scheme 1.3.3).[45] The enantiomeric excess of the reaction was determined to be 91-92%. Initially, it was found that benzaldehyde **30** was produced at a higher rate than (*S*)-2-HPP **32**, but this rate declined after reaching a maximum. This was attributed to the reversibility in the last step of the catalytic cycle, (which was also the case with the PDC catalytic cycle) (Scheme 1.3.2), and allowed the binding of the released aldehyde to TPP **20**, to form the 'active aldehyde' **24** and subsequent reaction with acetaldehyde **26**.

Several investigations have revealed similar reaction conditions (pH 6; 30°C) are required for the synthesis of (*R*)-PAC-**31**, as are required for the synthesis of (*S*)-2-HPP-**32**. BFD (*Ps. p.*) biotransformations were investigated by Wilcocks and Ward, and BFD tolerated a broad pH range (5-8) and temperature optima (20-40°C).[45] BFD (*Ps. p.*) was also identified to produce (*R*)-benzoin (**33**) in high enantiomeric excess. Ideally, BFD and PDC could complement each other as biocatalysts for the production of symmetric and mixed α-hydroxy ketones as seen (Scheme 1.3.3).



Scheme 1.3.3: Reaction products of the biotransformation using pyruvate **22** and benzaldehyde **30** with PDC and benzoylformate and acetaldehyde **26** with BFD, respectively

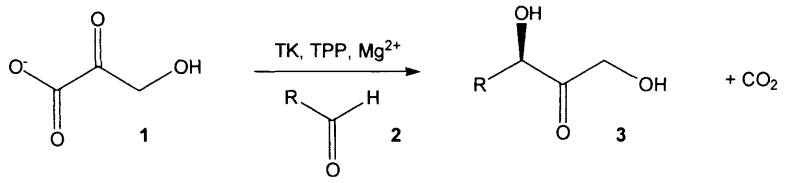
Pyruvate **22** and acetaldehyde **26**, when incubated with PDC formed acetoin (**34**) as a racemate. In the presence of acetaldehyde **26** and benzaldehyde **30**, however, formation of symmetric acetoin **34** was reduced and formation of (*R*)-PAC-**30** was predominant.

In the case of BFD, the formation of (*R*)-benzoin **33** occurred either in the presence of benzylformate or benzaldehyde **30** as substrates. Whereas, the mixed product (*S*)-2-HPP **32**, resulted by generating an "active benzaldehyde" at TPP (Scheme 1.3.2), which was followed by the addition of acetaldehyde **26**.[34]

The last carboligation study considered with TK is shown next and its mechanistic details are illustrated, complete with an analysis of which amino acid residues within TKs active site are responsible for these inferences.

1.3.3 Transketolase (TK)

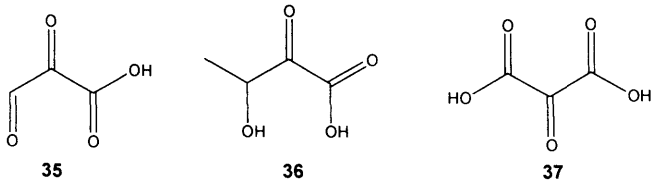
Transketolase (TK) catalyses (Scheme 1.3.4) the asymmetric transfer of a two-carbon unit from, typically, 3-hydroxypyruvate **1** to an aldehyde acceptor **2** in the presence of thiamine pyrophosphate (TPP **20**) and magnesium (II) ions. This reaction was made irreversible through the loss of carbon dioxide.[46,47] One of the aims of the Bioconversion Chemistry Engineering Interface Programme (BiCE) was to use TK to access enantiopure ketodiols (**3**) for conversion into enantiopure aminodiols **4** by transaminase (TA).[1]



Scheme 1.3.4: Formation of ketodiol **3**

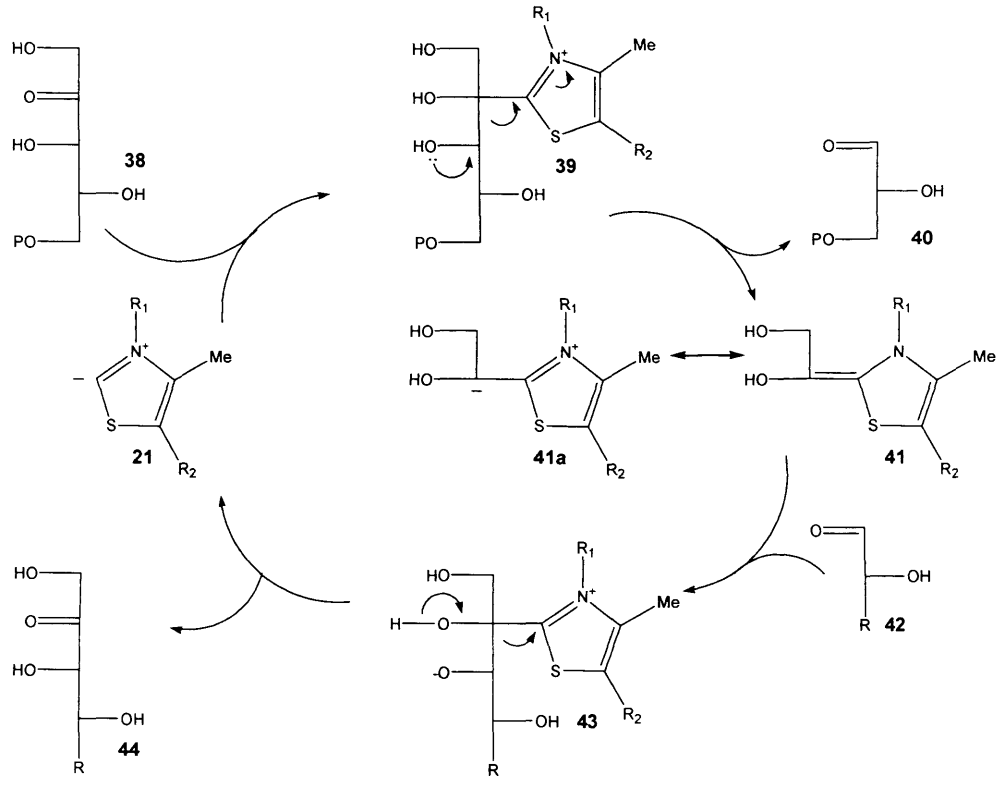
TK was first identified by Racker *et al.*, in 1953[48] in *Saccharomyces cerevisiae* (yeast) and later in spinach leaves.[46] Investigations into the use of TK in synthesis have, however, mainly been reported using over-expressed TK from *Escherichia coli*[49] and *S. cerevisiae*.[50]

TK catalysed condensation can occur with a wide range of aldehydes, particularly those having (*R*)-stereogenic hydroxyl centres at C2 (**2**, R = AlkylCH(OH)) and this was seen as particularly useful for synthesising novel carbohydrates.[49] The ketol pyruvate donors (Scheme 1.3.5), 2,3-dioxopropionoic acid (**35**), 2-oxo-3-hydroxybutyric acid (**36**) and 2-oxomalonic acid (**37**) were known, but did not to undergo conversion to α-hydroxyketones using TK.[51] This meant, β-hydroxypyruvic acid (**1a** = H) or salts of β-hydroxypyruvate (**1b** = K^+ or **1c** = Li^+) were the only known pyruvate donors for this reaction currently known.



Scheme 1.3.5: Other potential pyruvate donors for TK[51]

In vivo TK catalyses a step in the non-oxidative pentose phosphate pathway and this involved the coenzyme, TPP **20** (Scheme 1.3.6).



Scheme 1.3.6: Ketol transfer reactions in the non oxidative pentose phosphate pathway catalysed by TK[52]

TPP was deprotonated at C-2 to give carbanion **21**. From there, attack on the carbonyl unit of D-xylulose-5-phosphate (**38**), afforded the activated TPP-D-xylulose-5-phosphate complex (**39**). Subsequent release of glyceraldehyde phosphate (**40**) gave species (**41**) and (**41a**), which reacted with R-substituted glycolaldehydes (**42**) and formed complex (**43**). Subsequent rearrangement promoted by the positive charge of the thiazolium ring, gave ketose **44** and released the TPP ylid **21**, thus completing the catalytic cycle.[52]

An understanding of this mechanism, along with the known crystal structures of TK (*E. coli* and *S. cerevisiae*) has identified the amino acid residues involved in catalysis of this reaction. In particular, the X-ray crystallographic study on *S. cerevisiae* TK (Figure 1.3.1), by Schneider and Lindqvist showed the enzyme subunit consisted of three domains, all of the α/β type.[53]

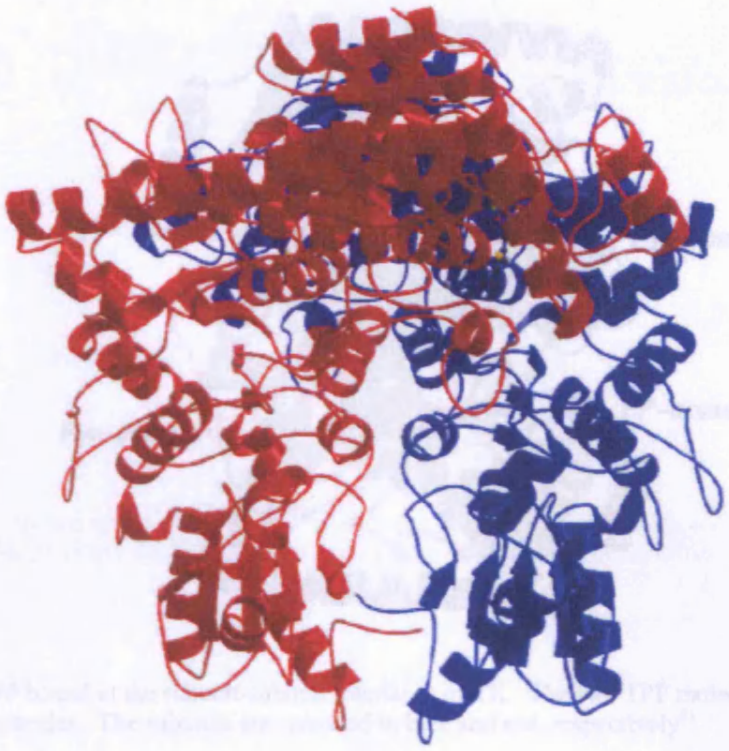


Figure 1.3.1: Schematic view of the dimer of transketolase. The subunits are coloured in blue and red, respectively[41]

The amino-terminal or PP domain (Figure 1.3.2) consisted of 320 amino acids and a five-stranded parallel β-sheet with helices on both sides. The second domain, the Pyr domain (residues 323-538) contained a six-stranded parallel β-sheet, sandwiched between α-helices. The third domain, the C-domain (omitted from Figure 1.3.2) was the carboxy-terminal domain (residues 539-680); and though it was far away from the active site of the enzyme, its catalytic function, if any, was unresolved. The TK dimer was formed through tight interactions between the PP- and Pyr domains, where the cofactor was bound at the interface between the subunits and the Pyr domain of the second subunit (Figure 1.3.2).[53]

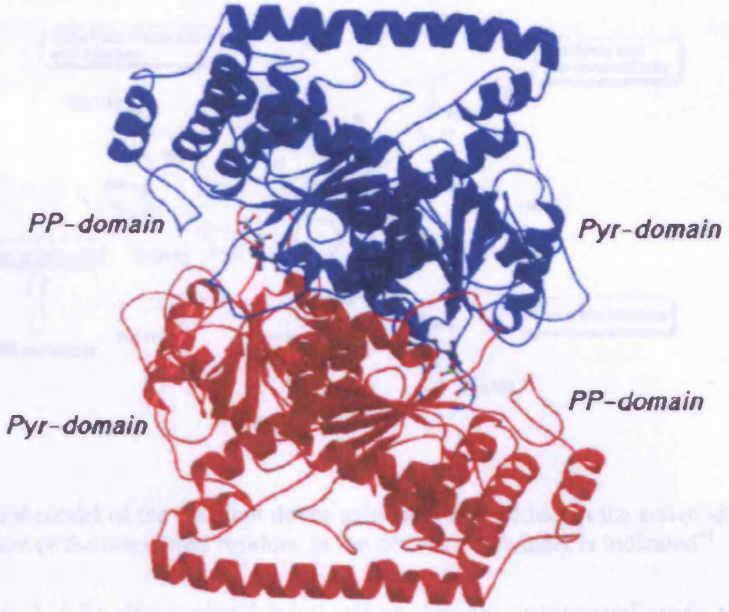


Figure 1.3.2: TPP bound at the subunit-subunit interfaces of TK. The two TPP molecules are shown as ball and stick molecules. The subunits are coloured in blue and red, respectively[41]

The cofactor TPP was deeply buried within the active site of TK with the only atom accessible being the C-2 atom of the thiazolium ring. The conformation of TPP binding with TK haloenzyme differed from that in of the free crystal structure of TPP **20**, which existed in a high-energy conformation (the V-conformation (Scheme 1.3.1)). In this conformation the C-2 carbon atom of the thiazolium ring was brought close (3.1Å) to the 4-amino group of the pyrimidine ring.[53] Yet, in the crystal structure of TK, there was no enzymic base suitably located to abstract the proton at C-2 of the thiazolium ring of TPP **20**. It was believed that TPP assisted deprotonation occurred through the observed hydrogen bond between the side chain of the conserved residue Glu418 and N1′-nitrogen of TPP **20** (Figure 1.3.3).

The molecular basis of substrate binding in TK was identified using a crystallographic structure with the acceptor substrate erythrose-4-phosphate (Figure 1.3.3). Figure 1.3.3 highlighted the possible functions of the conserved amino acids in substrate binding/recognition and in catalysis, as deduced from various crystallographic and mutagenesis studies.[53]

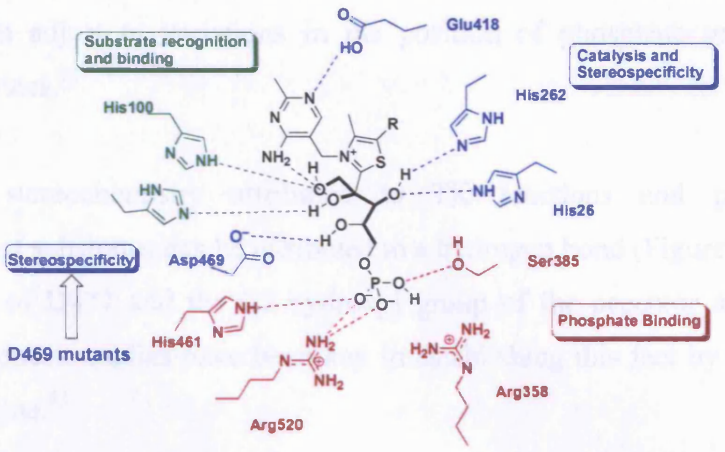


Figure 1.3.3: Proposed model of the covalent donor substrate-TPP adduct in the active site of TK. The function of the conserved residues in the active site channel is indicated[41]

This model (Figure 1.3.3) determined how other donors interacted with the enzyme's active site. In particular, β-HPA (**1**), a pyruvate donor accepted by TK, and the recognition of the C1 hydroxyl group of this donor could be attributed to the two histidine residues (H69 and H103). This provided some explanation as to why pyruvate, which does not possess a C1 hydroxyl group, was not accepted by TK. Substitution of these two conserved histidine residues with more hydrophobic residues (not detailed by the author), meant that TK mutants were able to accept pyruvate, though at lower rates of conversion.[53]

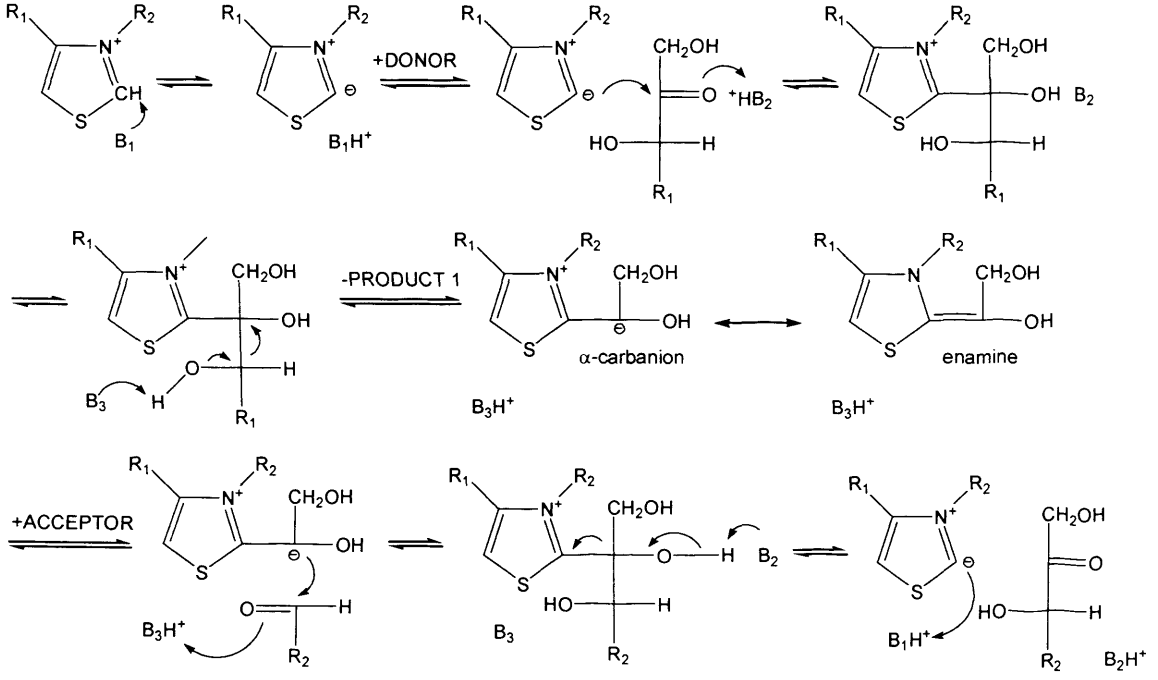
Residues R359, R528 and H469 were highly conserved in TK sequences and were associated with the phosphate binding of phosphorylated substrates. Replacement of any of these three residues with alanine yielded mutant enzymes with considerable residual activities, but large K_m values for phosphorylated substrates. This provided a molecular explanation as to why non-phosphorylated sugars were poor substrates for TK.[53]

With regards to acceptor chain length, TK had a broad substrate range of between a 3 and 7-carbon skeleton. This was associated with the architecture of the substrate channel and the phosphate binding area (Figure 1.3.3). The 'business' end of the substrate was always anchored in the right place by the phosphate-binding site, allowing the α-carbon of the acceptor to always come into contact with the C-2 carbon atom of the thiazolium ring or the α-carbon atom of the dihydroxy-ethyl TPP intermediate **25**, respectively. Phosphate binding was *via* two arginine residues, which through flexible

side chains can adjust to variations in the position of phosphate groups on chain extended substrates.[53]

The D-*threo* stereochemistry attributed to TK reactions and preferred C2-D configurations of substrates can be attributed to a hydrogen bond (Figure 1.3.3) between the side chain of D477 and the C2-hydroxyl group of the acceptor aldehyde. Site-directed mutagenesis studies have been key in establishing this fact by replacement of D477 with alanine.[53]

Finally, the histidine residues located in the active site in addition to H69 and H103 are believed to play a role in transition state stabilisation and various proton transfers. In Scheme 1.3.7, a reaction mechanism refers to three histidine bases (B_1-B_3).



Scheme 1.3.7: Reaction mechanism of TK. The identity of bases B_1-B_3 were discussed in the text

During the first half of the catalytic cycle, two proton transfer steps occur; the C-2 carbon atom of the TPP **20** is deprotonated and upon cleavage of the TPP-donor product, the C-3 hydroxyl group is deprotonated to yield the aldose product. Also the negative charge developing at C-2 of the carbonyl oxygen was stabilised electrostatically. It was thought that 4′ imino group could act as a sufficiently strong enough base at B_1 to abstract the C-2 atom of the thiazolium ring, as previously discussed.

The C-2 carbanion of TPP nucleophilically attacked the carbonyl carbon of the donor substrate. For covalent bond formation to occur, stabilisation of the developing negative charge at the carbonyl oxygen was required. B_2 had two candidates (from the model shown in Figure 1.3.2), His481 and the charged 4′-imino group of TPP. Through various site directed mutagenesis studies, it was concluded that the charged 4′-imino group of TPP might act as both B_1 and B_2 in the reaction mechanism (Scheme 1.3.7). The groups involved in steps 1 and 2 cannot catalyse proton abstraction at the C-3 atom of the acceptor substrate. Two conserved residues; H30 and H263 were both within hydrogen bonding distance to the carbonyl oxygen of the acceptor substrate and the C-3 hydroxyl group of the reaction intermediate (Scheme 1.3.7). Both residues were candidates for the acid/base catalyst in this reaction, referred to as B_3.

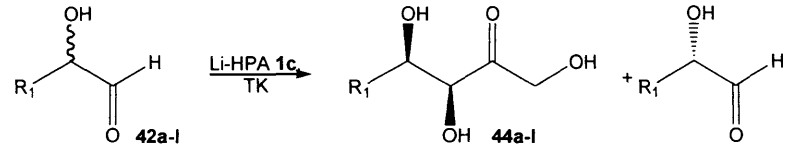
In an attempt to assess the viability of TK as a preparative biocatalyst for the synthesis of carbohydrates, Whitesides *et al.*, produced ketoses in yields comparable to ketoses formed typically from fructose-1,6-bisphosphate aldolase (FB aldolase) biotransformations.[51] TK, however, had several advantages over FB aldolase, in its ability to resolve racemic aldehyde substrates without requiring the phosphorylated substrates, unlike FB-aldolase. This enabled more straightforward monitoring of the progress of TK reactions, as well as the ease in isolation of the products.

TK-catalysed carbon-carbon bond formation was both enantiospecific, in that the new chiral centre formed in the product had the (*S*)-configuration (the same configuration as C-3 of the natural donor substances), and stereoselective in that the enzyme has a preference for α-hydroxyaldehydes with an (*R*)-configuration at C-2. A number of reports described the use of non-natural aldehydes substrates with TK from spinach leaves, yeast (*S. cerevisiae*) and *E.Coli*. Generally, TK from yeast and *E.Coli* generated products with an (*S*)-chiral centre, though with spinach TK, some loss of enantiospecificity was observed with certain substrates, notably α-unsubstituted aldehydes.[54] The (*S*)-stereochemistry with racemic aldehyde substrates can be proved by derivatization to a known standard and can be compared by chiral gas chromatography (GC), or from existing optical rotation data.[49]

Fewer reports detailed the use of spinach TK due to the loss of some enantioselectivity. Yet, in understanding heptulose synthesis in higher plant metabolism, Villafranca *et al.*, reported the use of spinach TK[46] with non-phosphorylated pentoses. Despite the ability

of TK to accept unnatural non-phosphorylated ketol donors, the rate of heptulose formation was reported to be 150 times greater with D-ribose-5-phosphate than with D-ribose as a donor.[46]

Changes in the R_1 group of glycolaldehydes **42a-l**, (Scheme 1.3.4) resulted in [3*S*,4*R*] or D-*threo* ketotriols (**44a-l**).[54] Early studies included chemoselectivity investigations and carbohydrate syntheses with yeast TK on 1-to 5-mmol scale. These results are presented below in Table 1.3.2.



			Adduct		Recovered aldehydes	
R_1	Aldehyde	$V_{R1}/V_{R1=H}$	Ketotriol	Yield (%)[1]	Yield (%)[1]	Ee (%)[2]
H	**42a**	100	**44a**	60	n/a	n/a
CH_3	**42b**	20	**44b**	44	21	>95
CH_3CH_2	**42c**	33	**44c**	45	23	95
$CH_3(CH_2)_2$	**42d**	22	**44d**	39	21	77
$MeOCH_2$	**42e**	27	**44e**	38	9	94
$MeSCH_2$	**42f**	33	**44f**	-	-	-
$H_2C=CH$	**42g**	56	**44g**	30	-	-
$H_2C=CHCH_2$	**42h**	28	**44h**	45	20	95
(*S*)-$H_2C=CHCHOH$[3]	**42i**	36	**44i**	60	n/a	n/a
(*R*)-$H_2C=CHCHOH$[4]	**42j**	32	**44j**	63	n/a	n/a
$(CH_3)_3C$	**42k**	11	**44k**	-	-	-
$HO(CH_2)_2$	**42l**	<10	**44l**	7	-	-

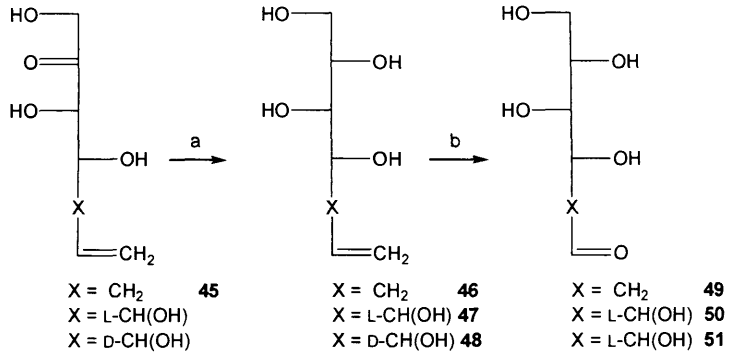
[1]Isolated yield based on total quantity of starting aldehydes. [2]Enantiomeric excesses were determined on the corresponding diol (obtained by reduction of the aldehydes with sodium borohydride). [3]Optically active aldehyde derived from D-xylose. [4]Optically active aldehydes derived from D-ribose.

Table 1.3.2: Substrates for yeast TK[51]

A number of conclusions were drawn from the results in Table 1.3.2. Yeast TK was shown to be chemoselective to a broad range of aldehydes provided they had an (*R*)-configuration at C-2. Size effects exemplified by branching at C-3 (aldehyde **42c**, R = Et to aldehyde **42k**, R = *t*-butyl) were indicated by moderate yields of the corresponding ketotriols **44c-k** and low yields of the corresponding aldehydes recovered. Hindered aldehydes also decreased the relative rates of reaction. The stereochemistry at the β-carbons of aldehyde entries (*S*)-**42i** and (*R*)-**42j** showed no particular effect on their rates of reaction.

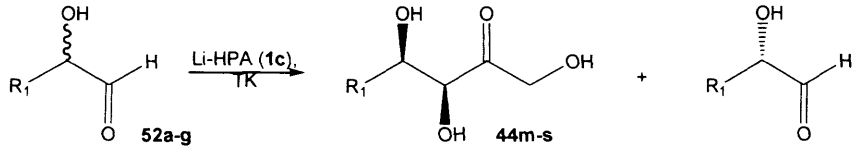
TK-catalysed syntheses of carbohydrates were compared with the comparable FB-aldolase reaction. Adduct **44g** can only be synthesised readily from TK, using aldehyde **42g**. FB-Aldolase does not accept acrolein as a substrate, but other alkene-containing

aldehydes (**42g-j**) may act as substrates for FB-aldolase. These would result in diastereomeric adducts (**44g-j**), whereas the TK adducts would result in single enantiomers (**44g-j**). Enzymatic reduction of these adducts (**44g-j**) by iditol dehydrogenase (E.C. 1.1.1.14), coupled with formate dehydrogenase (E.C. 1.1.1.27) and sodium formate (Scheme 1.3.8) would generate NADH furnished alkene-polyols (**45-48**). Oxidative cleavage of these alkenes with ozone, followed by reductive workup with sodium sulfite afforded 2-deoxy-L-gulose (**49**), L-gulose (**50**) and L-idose (**51**), respectively.



Scheme 1.3.8: Aldose synthesis: a) iditol dehydrogenase (EC 1.1.1.14), FDH (EC 1.1.1.14), NADH, $NaH\text{-}CO_2$; b) 1. O_3, 2. Na_2SO_3

Yeast TK can additionally be used for the kinetic resolution of R_1-substituted L-2-glyceraldehydes from racemic R_1-substituted glycoaldehydes (**52a-g**),[55] which allowed access to ketotriols (**44m-s**) in good chemical yields and in excellent optical purity (Table 1.3.3).



R_1	Aldehydes	Ketotriol	Adduct Yield (%)	Aldehyde Yield (%)	Aldehyde *Ee*[1]
$PhCH_2O$	**52a**	**44m**	79	75	98
CH_3O	**52b**	**44n**	72	72	99
SH	**52c**	**44o**	80	-	-
EtS	**52d**	**44p**	74	52	99
F	**52e**	**44q**	79	71	96
CH_3	**52f**	**44r**	80	50	99
CN	**52g**	**44s**	82	78	97

[1]Ee's were determined by conversion of the L-aldehydes into the corresponding diethyl acetals and were measured using chiral gas chromatography.

Table 1.3.3: Yeast TK-catalysed reaction of racemic R-substituted 2-glycolaldehydes with equimolar amounts of lithium HPA **1c**

In a further study, 3-*O*-benzylglyceraldehyde (**52a**) was used as a substrate for an *E. coli* transketolase, which was later used in a chemoenzymatic synthesis of a novel

glycosidase inhibitor, *N*-hydroxypyrrolidine.[56] A range of other R-substituted glycolaldehydes (Table 1.3.4) were assessed in unbuffered media by the controlled addition of 1M HCl through the use of an autotitrator, and also in 0.1M-glycylglycine buffer (pH 7.0). The authors supplied no *ee* values, but it is assumed that chiral derivatization was used to assign the D-*threo* or (*S*)-stereochemistry observed.[49]

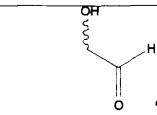
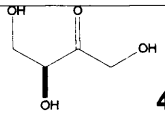
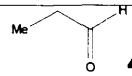
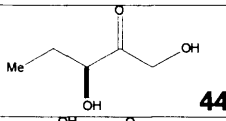
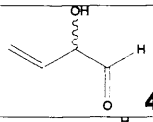
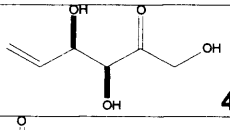
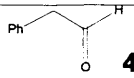
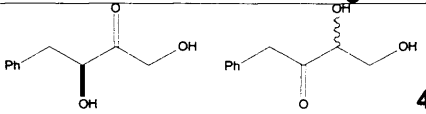
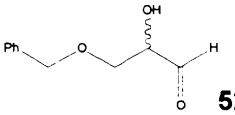
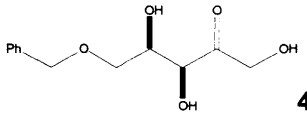
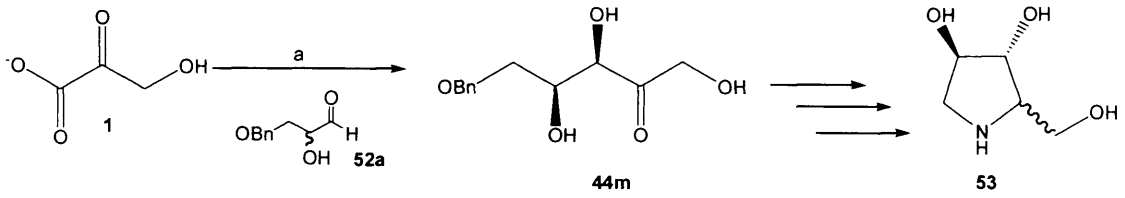
Entry	Substrate	Products	Yield (%)
1	 **42a**	 **44a**	74 (43)
2	 **42c**	 **44c**	29 (23)
3	 **42g**	 **44g**	23 (12)[1]
4	 **42m**	 **44t**	33:18 (26)
5	 **52a**	 **44m**	76

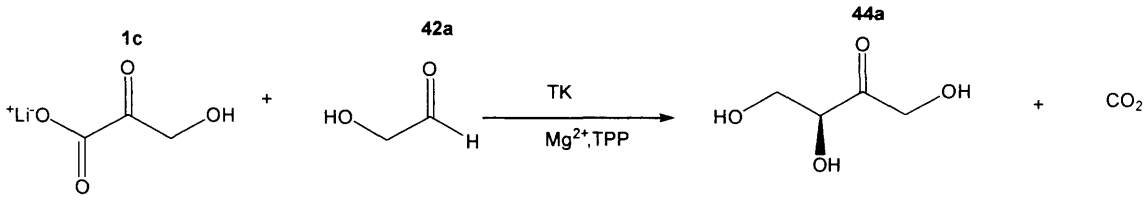
Table 1.3.4: Results of *E. coli* TK catalysed couplings in unbuffered medium ([1]yields in brackets are for corresponding reactions in 0.1M glycylglycine buffer, pH 7.0)

β-HPA (**1**) and (±)-3-*O*-benzylgylceraldehyde (**52a**) are subjected to *E. coli* TK (entry **5**, Table 1.3.4) to give 5-*O*-benzyl-D-xylulose (**44m**) in 76% yield on a 2-3g scale (Scheme 1.3.9). This is used as a convenient starting material in the chemoenzymatic preparation a novel glycosidase inhibitor, *N*-hydroxypyrrolidine (**53**).[56]



Scheme 1.3.9: Synthesis of *N*-hydroxypyrrolidine **53** using a) transketolase, TPP, Mg^{2+}, pH 7.0

The synthetic and commercial usefulness of TK biotransformations are best demonstrated by the potential scale up of Li-hydroxypyruvate (HPA) **1c** as the pyruvate donor (Scheme 1.3.10), with achiral glycolaldehyde **42a** as the aldehyde acceptor. This reaction with wild-type *E. coli* transketolase is known to have the fastest reaction rate[57] when compared with other aldehyde acceptors[58] and the resulting product, L-erythulose **44a** is formed with the irreversible concomitant release of carbon dioxide.



Scheme 1.3.10: Synthesis of L-erythulose

The limitation of scaling these reactions, at present, is the high cost of hydroxypyruvic acid **1a**, or its salt **1c**, due to the difficulty in its synthesis and isolation.[59,60,61] Progress has been made in improving the efficacy of TK bioconversions by immobilizing *E. coli* TK onto two commercial supports Eupergit-C and Amberlite XAD-7. *E. coli* TK biotransformed Li-HPA **1c** and glycolaldehyde **42a**, which produced ketotriol, L-erythulose (**44a**). This was with an 80 to 100-fold improvement in the half-life of the immobilized TK preparations.[62] Such improvements may avoid the need for reactant feeding strategies in subsequent reactor designs.

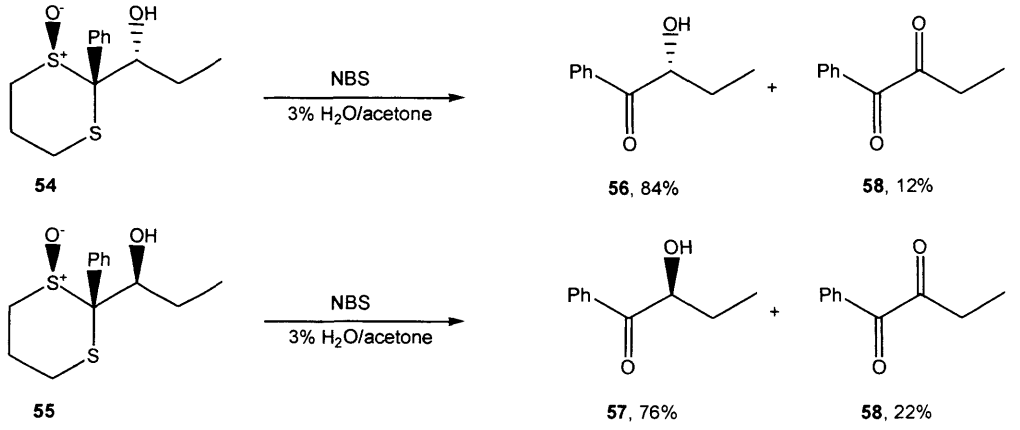
The synthesis of the chiral α-hydroxyketone functionality in ketotriols by TK was usually successful and performed in a single step biotransformation. The chemical synthesis of asymmetric α-hydroxyketones was also possible and is shown.

1.4 Chemical Synthesis of α-hydroxyketones: TK alternatives

α-Hydroxyketones, whether in ketodiols or ketotriols, as discussed in the previous section are an important structural feature of many biologically active molecules (See section 1.3).[63] If it is possible to establish the chiral centre of an α-hydroxyketones as *R* or *S*, before subjecting it to chemical reductive amination or a TA bioconversion, the dual enzymatic or chemoenzymatic process heading towards chiral aminodiols **4** can be realised. Syntheses of α-hydroxyketones *via* other chemical routes are usually step-intensive and challenging. The use of organometallic reagents[64,65] to produce racemic α-hydroxyketones is, however, known.

Bulman-Page *et al.*, reported an asymmetric route to α-hydroxyketones using DiThiane OXide (DiTOX) methodology, which did not require symmetrical α-diketones as a precursor.[66] A predecessor to this methodology used a chiral mono-dithioacetal sulfoxide as the enantiocontrol element in a diastereoselective reduction, but could only

be used with symmetrical α-diketones as a precursor.[67] The DiTOX methodology, shown in Scheme 1.4.1, relied upon hydrolysis of the dithiane 1-oxide moieties, produced in 3 steps, of phenyl derivatives (**54**) and (**55**). Hydrolysis of these derivatives was achieved using *N*-bromosuccinimide (NBS)/acetone/water mixtures and gave α-hydroxyketones (**56**) and (**57**) with high enantioselectivities. Unfortunately the diketone side product (**58**) also resulted due to oxidation under these conditions.[66]



Scheme 1.4.1: Preparation of chiral α-hydroxyketones **56** and **57**

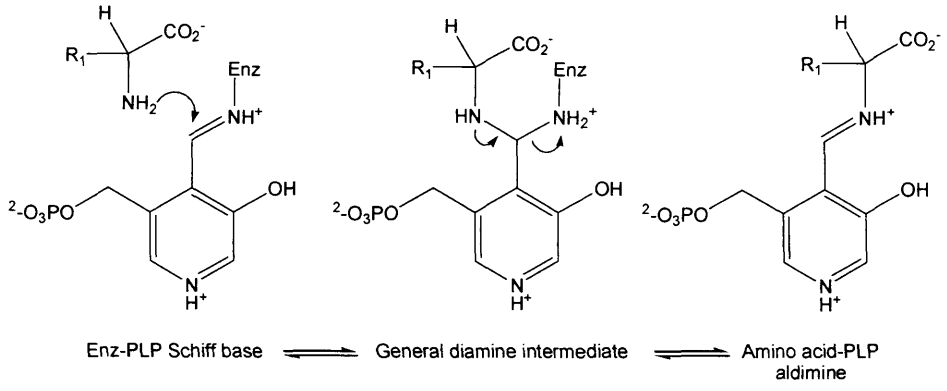
Having seen a limited synthetic method for the preparation of chiral α-hydroxyketones in a relatively short number of steps, the use of TK to produce such molecules in one step outweigh a potentially lengthy asymmetric chemical synthesis. The formation of chiral α-hydroxyketones can be implemented using TK or asymmetric synthesis as seen, but the next section focuses on the asymmetric introduction of nitrogen by transamination at the carbonyl centre of α-hydroxyketones.

1.5 Transaminase (TA)

The asymmetric synthesis of chiral amines *via* prochiral precursors as a strategy is usually favoured over kinetic resolution of enantiomers, as the theoretical yield is 100%.[68] The majority of the enzymatic syntheses of chiral amines utilize hydrolases in kinetic resolutions.[69] Amino acid dehydrogenases[70], amine dehydrogenases[71] or aminotransferases[72] may act as biocatalysts for the synthesis of chiral aminoalcohols from keto alcohols. All of these enzymes are known to convert a carbonyl group to an amino group, but would require genetic engineering to accept substrates other than keto acids or ketones. Recently, Ito *et al.* described an aminoalcohol dehydrogenase[73] from genera *Streptomyces*, *Pseudomononas*, *Burkholderia*, and *Arthobacter*. In particular, a purified aminoalcohol dehydrogenase from *Streptomyces virginiae* IFO 12827 showed

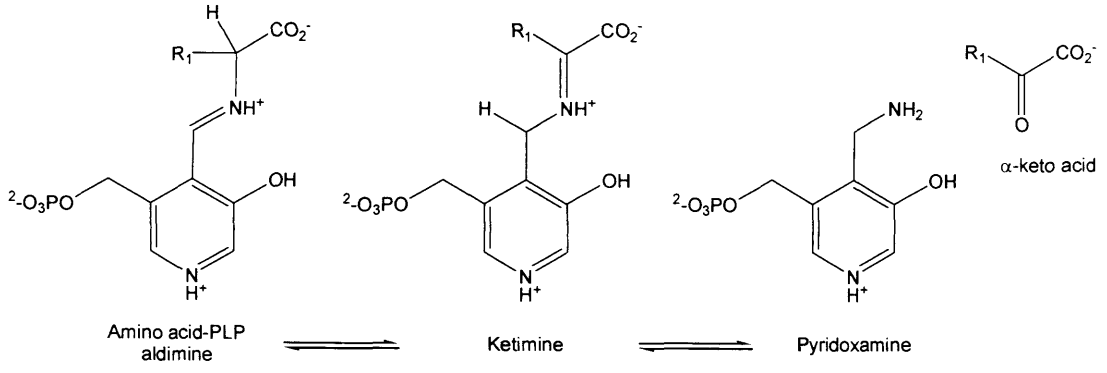
promise as a potential biocatalyst, by converting a wide range of ketols and amines, but no information was available on the stereoselectivity of the reaction. Furthermore, this biocatalyst required cofactor recycling, a disadvantage in an industrial processes due to the additional cost.

Transaminases (TAs) catalyse the enzymatic amino transfer by a ping-pong bi-bi mechanism.[72] In the first half of the reaction (Scheme 1.5.1), the amine donor is bound to the enzyme and the pyridoxal 5′-phosphate (PLP) coenzyme is aminated to pyridoxamine 5′ phosphate (PMP).



Scheme 1.5.1: Transaminase mechanism I

In the second half of the reaction (Scheme 1.5.2), the transamination cycle is complete by transferring the amino group from the enzyme-bound PMP to the acceptor.

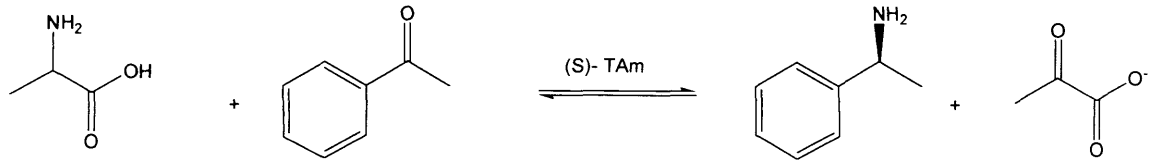


Scheme 1.5.2: Transaminase mechanism II

TAs such as the (*S*)-specific ω-transaminase (ω-TA) from *Vibrio fluvialis* JS17 is known to catalyse the asymmetric synthesis of chiral amines from prochiral ketones.[4] Whilst the use of an ω-TA possesses advantages over a kinetic resolution, the ω-TA reaction has an unfavourable thermodynamic equilibrium and could suffer from severe product inhibition. If these problems are overcome by *in situ* product removal[4] or by enzyme membrane reactors,[74] TA promises to be a useful enzyme in the production of

chiral amines and/or amino acids, as it has a broad chemoselectivity and no external cofactor regeneration requirements.[2] This is an important issue in evaluating the cost of industrial processes.[5]

A report by Shin *et al.*, shows that ω-TA, using L-alanine (**59**, 300 mM) as an amine donor with acetophenone (**60**, 30 mM), gives (*S*)-α-methylbenzylamine (*S*)-α-(MBA) **11a** and pyruvate **22** (Scheme 1.5.3). Yet, the concentration of (*S*)-α-MBA **11a** formed is only 2.7 mM (9% of the theoretical concentration).[74]

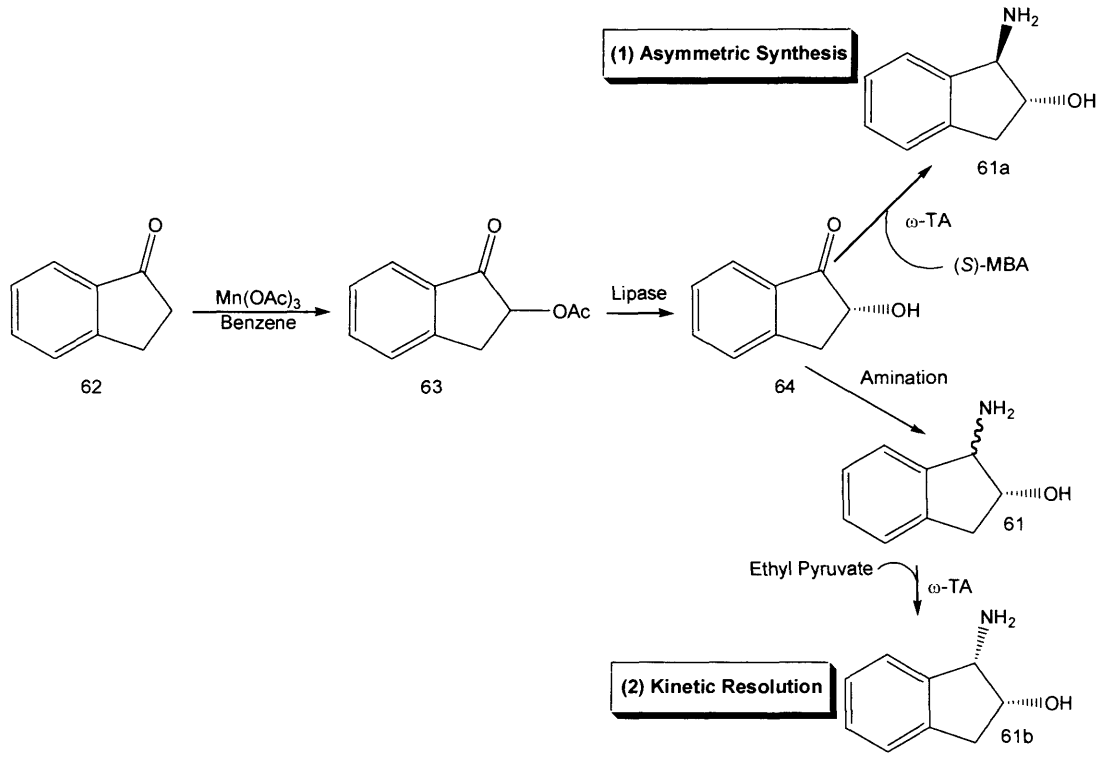


59 **60** **11a** **22**

Scheme 1.5.3: Synthesis of (*S*)-α-methylbenzylamine **11a**

Shin *et al.*, addresses this problem by the removal of pyruvate **22** using lactate dehydrogenase (LDH) in a cell free extract and by using whole cells for the biotransformation. The whole cell reaction results in 90% and 92% reaction yields for formation of (*S*)-α-MBA **11a**, with enantiomeric excesses >99%.[4] Since this report, the value of *V. fluvialis* TA for biotechnological applications has been recognised, and several studies published on single step biotransformations[4,74,75] and multi-step biotransformations involving ω-TAs.[2,76,77]

The synthesis of enantiomerically pure *trans*-(1*R*,2*R*)- and *cis*-(1*S*,2*R*)-1-amino-2-indanol (AI) (**61a** and **61b**) were afforded using lipase and ω-TA as part of a chemoenzymatic strategy (Scheme 1.5.4.)[77]



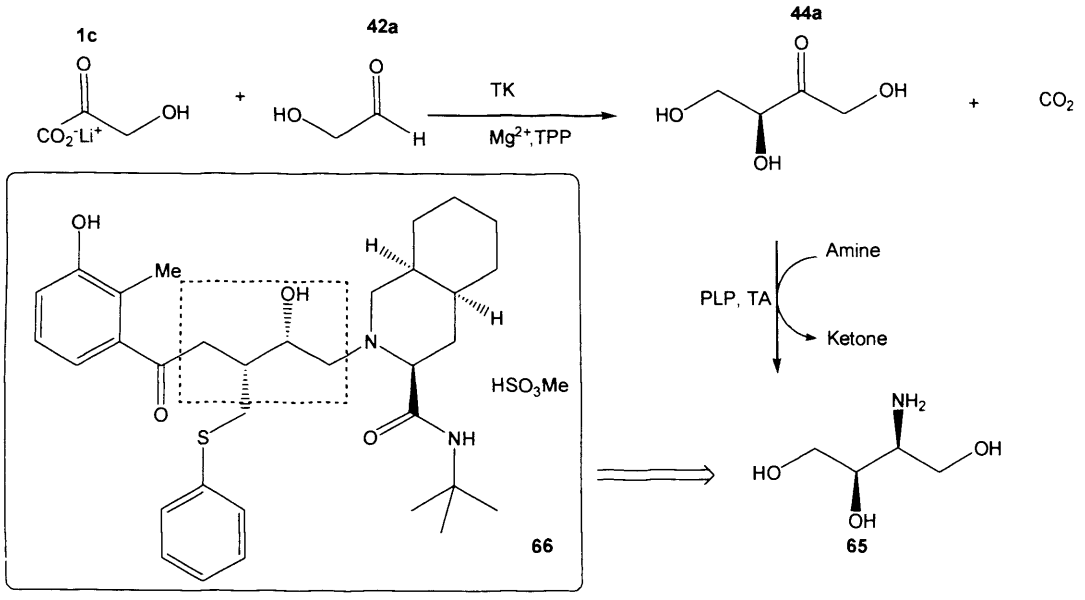
Scheme 1.5.4: Synthesis of *trans*-(1*R*,2*R*) and *cis*-(1*S*,2*R*)-1-amino-2-indanol by lipase and ω-TA

Treatment of 1-indanone (**62**) and manganese(III) acetate gives 2-acetoxy indanone (**63**) in high yield. The enantioselective hydrolysis of racemic **63** is performed using Amano *Pseudomonas* lipase, yielding ketoalcohol (**64**). This is then subjected to an asymmetric transamination using *V. fluvialis* TA in organic solvents with ethyl pyruvate as the amino acceptor and (*S*)-α-MBA **11a** as the amino donor. Studies of the amino donors[77] are extended to include (*S*)-1-aminoindan, (*S*)-1-aminotetralin, (*S*)-1-methyl-3-phenylpropylamine and alanine **59**. It was found that in non-aqueous media (*S*)-1-aminoindan has the fastest rate of reaction and proceeds with 25% conversion, yielding *trans*-**61a**, in the first example of a transamination reaction in non-aqueous media.

Reductive amination of **64** using ammonium acetate and sodium cyanoborohydride yields a 1:1 diastereomeric mixture of *cis*- and *trans*-AI **62**. *V. fluvialis* TA is shown to have some selectivity towards *trans*-**61a**, but the diastereoselectivity is low. This is expected as there is a precedent for poor selectivity where substrates bear other functional groups on their β-carbons relative to the amino group, such as phenylglycinol.[78] Additions of γ-cyclodextrin improves the enantioselectivity from 4 to 21% *ee*, (though the mechanism for this is not clearly understood), yielding *cis*-**61b**.[77]

The possible development of multienzyme reactions is one of the aims of our work, so work by Kim *et al.*, identifies that V. *fluvialis* TAs appears to be good candidates for the preparation of chiral aminoalcohols. Unfortunately, due to V. *fluvialis* being a highly toxic bacterium, TA homologues in the genomes of fully sequenced bacteria, have to be used instead. These allow access to a range of V. *fluvialis*-like TAs through a published protein sequence.[79] Other TA homologues have also been identified.

A β-alanine: pyruvate TA homologue from *Pseudomonas aerouginosa* PAO1 is shown to aminate the aliphatic ketotriol L-erythulose **44a** in a *de novo* TK and β-Alanine: Pyruvate-TA pathway[80] by the cloning and expression of the β-alanine: pyruvate TA homologue from *Pseudomonas aerouginosa* PAO1 into *E. coli*. This means that *E. coli* would express both TK and TA at the same time. This total enzymatic synthesis (Scheme 1.5.5) is performed by the established biotransformation of Li-HPA (**1c**) with glycolaldehyde (**42a**) in the presence of TK, to give L-erythulose (**44a**). This then underwent transamination with TA in the same reaction pot and affords 2-amino-1,3,4-butanetriol (ABT) (**65**), a key component in protease inhibitor drug Nelfinavir™ (**66**).

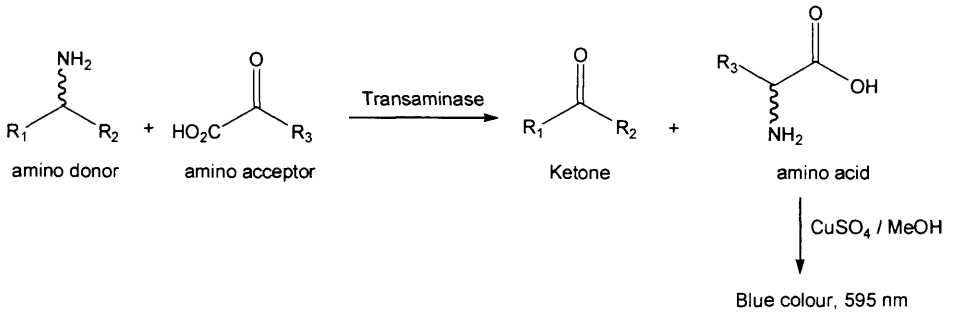


Scheme 1.5.5: Synthetic scheme towards 2-amino-1,3,4-butanetriol (**65**) as a component of Nelfinavir™ (**66**)

The TK/TA dual plasmid *E. coli* strain is able to process Li-HPA (**1c**) and achiral substrate, glycolaldehyde **42a** and generates L-erythulose **44a**. The synthesis of compound **44a** was possible in a one-pot process using either a lysate of the dual plasmid strain or unlysed cells. Unfortunately, the TA efficiency is quite low and the best amine donor iss (*S*)-α-MBA **11a**. The low efficiency is attributed to long reaction

times and the reactivity of Li-HPA **1c** with both TK and TA.[80] Mutagenesis of TA and screening using L-erythulose **44a** could be key for developing high throughput protocols.

Prior to this development, Hwang *et al.*, had studied high-throughput screening methods for the identification of active and enantioselective ω-TAs.[3] A number of high throughput screens utilise immunoassays, conventional chromatography (HPLC, GC), mass spectrometry, NMR spectrometry and UV-Vis spectrometry. Using UV-Vis spectrometry, the conversion of amino donors into ketones can be monitored. Addition of MeOH/$CuSO_4$ to the reaction mixture leads to formation of a blue complex and easy quantification, using a UV-Vis spectrophotometer, is possible (Scheme 1.5.6).



Scheme 1.5.6: TA-catalysed reaction and spectrophotometric assay

This method is particularly effective with aromatic amino donors. Unfortunately, cupric sulphate solution can generate coloured complexes with any amino acid and phosphate buffers make it less useful in a one-pot assay method.

The potential of using a TK/TA enzymatic pathway, along with genetic engineering and potential high throughput protocols, provides the opportunity for access to a number of chiral aminodiol synthons. These are found in many bioactive molecules and are presently discussed.

Chapter 2

2.1 Synthetic strategies to aminoalcohol functionalities found in bioactive molecules

Chiral aminoalcohols and derivatized chiral aminoalcohol motifs are present in many biologically active molecules including alkaloids[81], amino sugars[82], enzyme inhibitors[83] and antibiotics.[84] Chiral aminoalcohols are easily derivatized into a number of useful compounds, *N*-protected aminoalcohols (**67**), oxazolidinones (**68**), *bis*-(oxazolines) (**69**) and phosphinooxazolines (**70**), some of which may be directed towards the preparation of bioactive molecules or used as chiral ligands in asymmetric catalysis (Figure 2.1.1).[85]

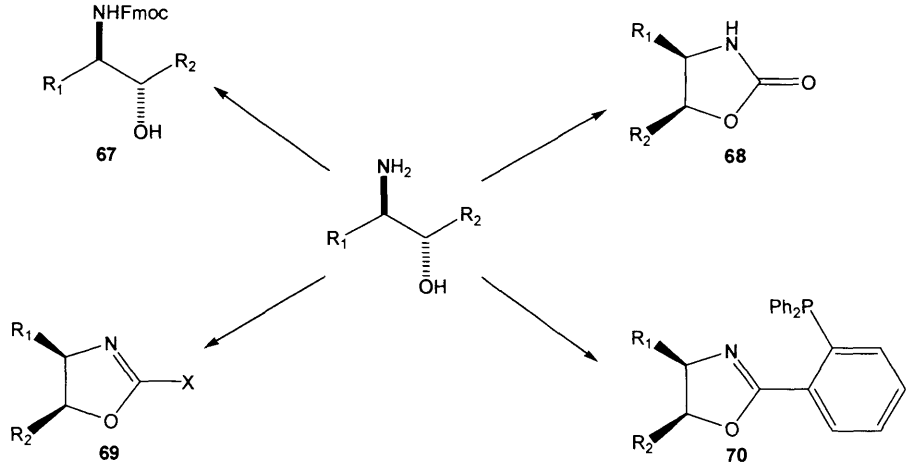
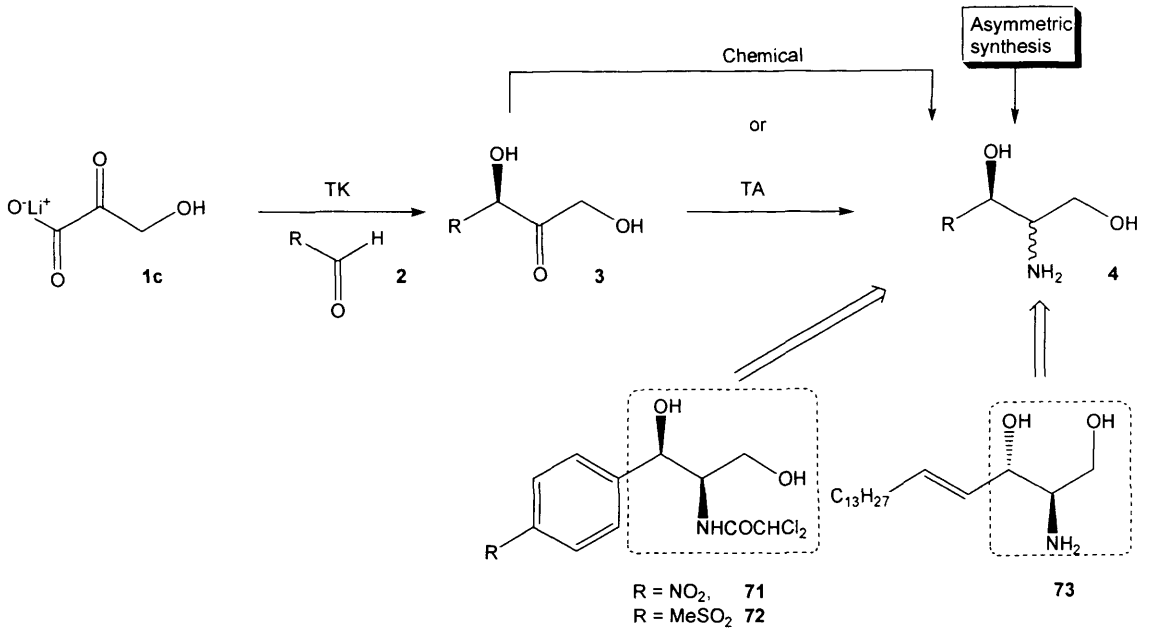


Figure 2.1.1: Applications of chiral aminoalcohols

Chiral aminodiols **4** are also key functional motifs and are found in a range of natural products (Scheme 2.1.1), including chloramphenicol (**71**) and thiamphenicol (**72**) antibiotics, and sphingosines (**73**), which are important cell membrane components. As outlined in the aims of this project, we are interested in synthesising chiral aminodiols **4** *via* chemical, enzymatic or chemoenzymatic strategies.



Scheme 2.1.1: Possible strategies to aminodiols as bioactive molecules

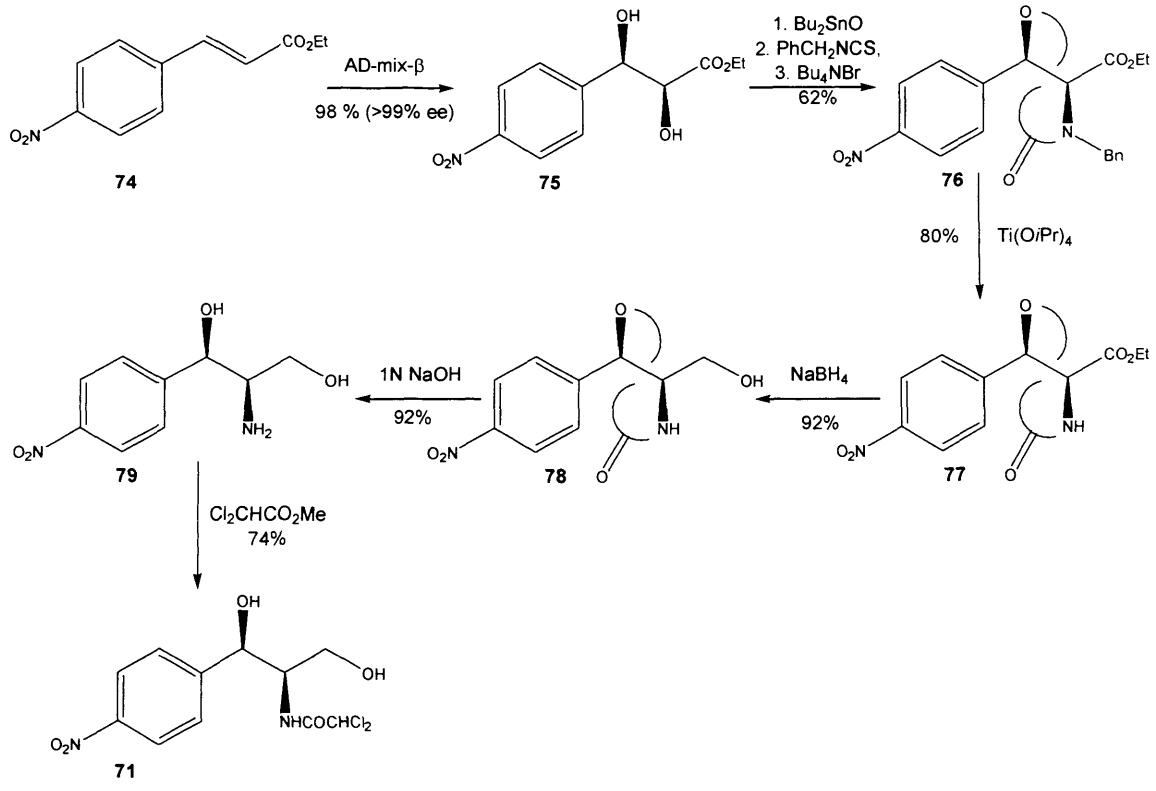
The synthetic routes to selected chiral aminodiol-containing bioactive molecules **71**, **72** and **73** are discussed below as key examples of the arduous chemical syntheses often required for such bioactive molecules.

2.1.1 Chloramphenicol and thiamphenicol syntheses

(2*R*,3*S*)-Chloramphenicol **71** was isolated from *Streptomyces venezuelae* in 1954 and is one of the first antibiotics produced in an optically active form by chemical synthesis, rather than fermentation techniques.[86] Thiamphenicol **72**, a synthetic analogue of chloramphenicol **71** shows antimicrobial activity against both Gram-positive and Gram-negative bacteria. Currently 100 tons per year of thiamphenicol **72** are produced by kinetic resolution.[87]

Chloramphenicol **71** and thiamphenicol **72** have been produced by a number of synthetic protocols. A cyclic iminocarbonate rearrangement is utilised by Park *et al.* to afford **71** in six steps (Scheme 2.1.2).[88] One of the main problems identified by Park *et al.*, is that whilst asymmetric epoxidation (AE) and aminohydroxylation (AA) (covered in Section 2.2) could potentially provide access to **71**, poor regio- and diastereocontrol hindered its access. The asymmetric dihydroxylation (AD) reaction, however, is usually more useful in the synthesis of *anti*-aminoalcohol diastereoisomers, as an extra stereoinversion step is required for the synthesis of *syn*–aminoalcohol diastereoisomers. Park *et al.*, report a rearrangement protocol, allowing divergent regiocontrol with

cinnamate substrates, in a synthesis towards *syn*-aminodiols from *syn*-diols in one step.[89]

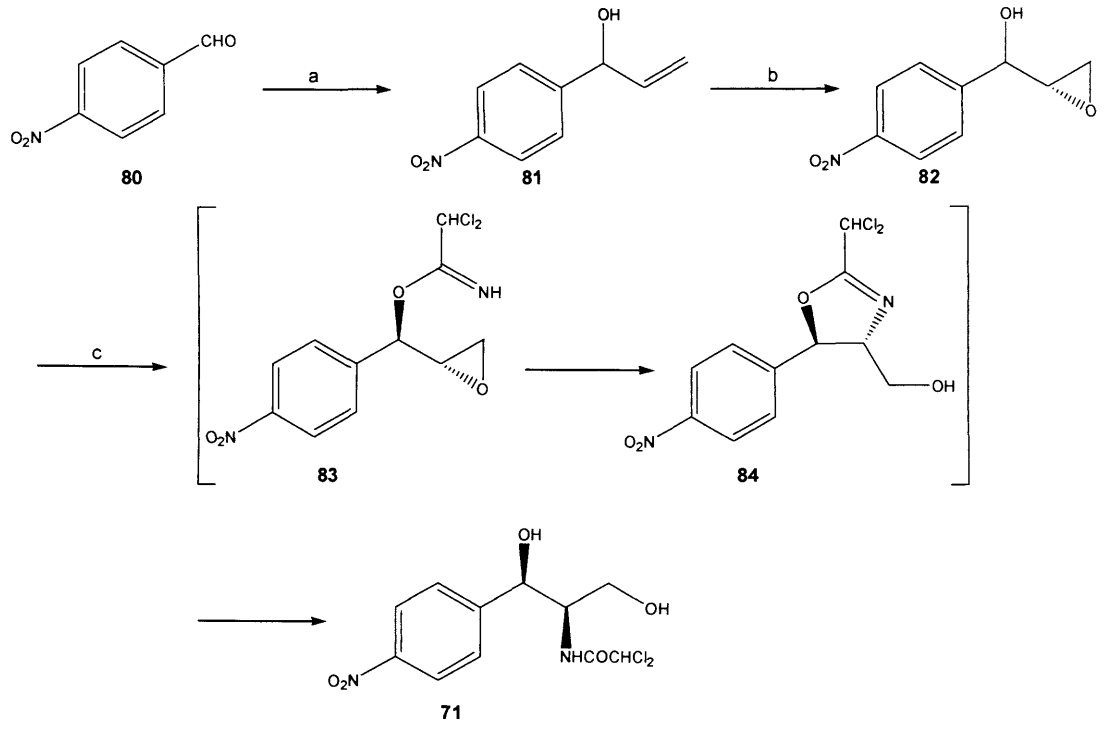


Scheme 2.1.2: Synthesis of Chloramphenicol **71**

Ethyl *p*-nitrocinnamate ester (**74**) is successfully converted into (2*S*,3*R*)-diol (**75**) in 98% yield and >99% *ee* using the AD reaction. Through a short sequence using dibutyl tin oxide (reflux, 24 h with Dean-Stark removal of water), benzoyl isothicyanate (reflux, 6 h) and finally reaction with tetrabutylammonium bromide, (2*S*,3*R*)-diol **75**, *via* a cyclic iminocarbonate rearrangement, gives the *syn* oxazolidinone (**76**). The remaining functional group transformations are straightforward: debenzoylation of **76** under transesterification conditions gives oxazolidinone ester (**77**). Reduction of oxazolidinone ester **77**, using sodium borohydride then gives aminoalcohol (**78**), which by hydrolysis with sodium hydroxide, gives the (*S*,*R*)-aminodiol (**79**). This is amidated with dichloroacetonitrile and gives chloramphenicol **71** in 30% overall yield and in >99% *ee*.[88]

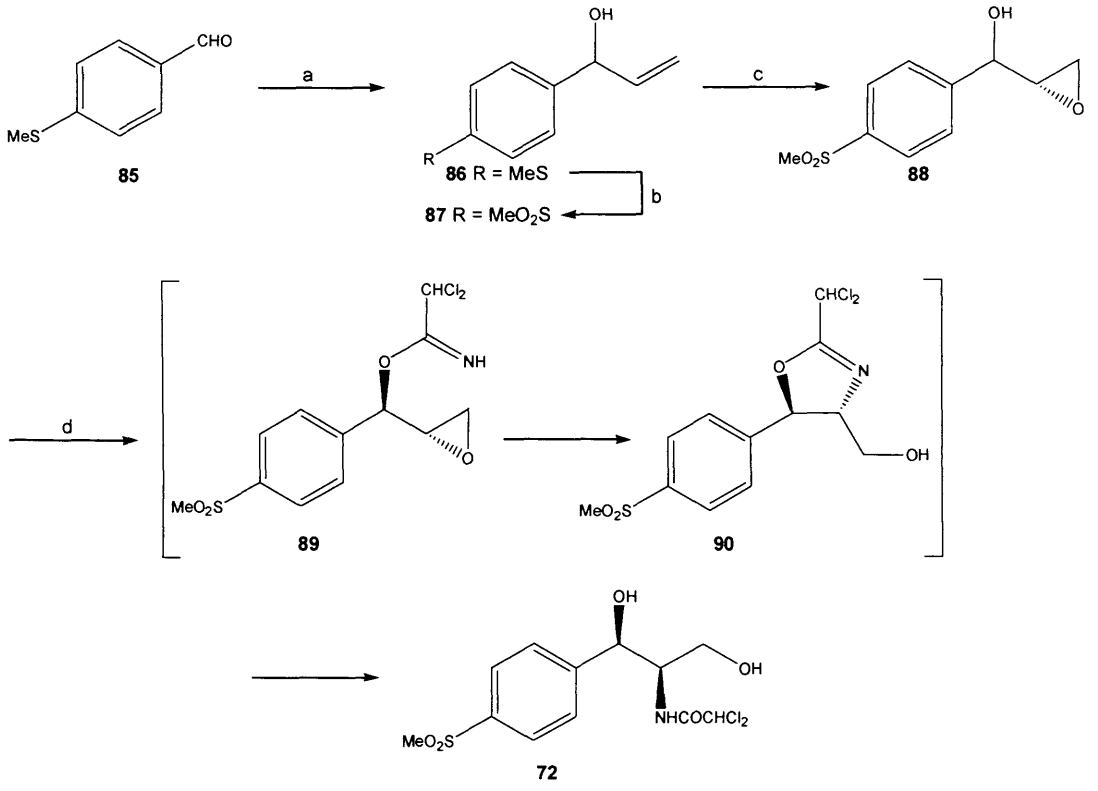
Since this reaction, synthetic sequences have been shortened and methods changed. Bhaskar *et al.*, utilise an asymmetric epoxidation (AE) reaction, but despite the problems previously mentioned (poor regio- and diastereocontrol), this method affords

chloramphenicol **71** (Scheme 2.1.3) and thiamphenicol **72** (Scheme 2.1.4) in three and four steps, respectively.



Scheme 2.1.3: Reagents and conditions: (a) Divinyl zinc, THF, Et_2O, -78 °C to rt, 10 h, 72%; (b) (-)-DIPT, $Ti(OiPr)_4$, TBHP, CH_2Cl_2, -20 °C, 14 h, 45%; (c) NaH, dichloroacetonitrile, CH_2Cl_2, 0 °C to rt, 1 h, then BF_3OEt_2, -78 °C to rt. 3 h, 71%

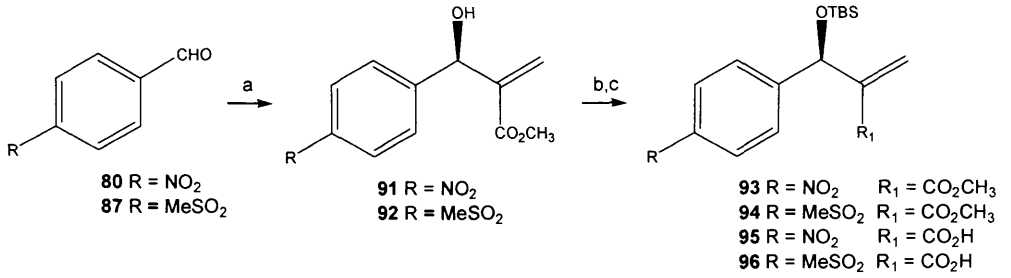
4-Nitrobenzaldehyde (**80**) is reacted with divinylzinc to give 1-(4-nitrophenyl)allyl alcohol (**81**). Allyl alcohol **81** is subjected to AE conditions (kinetic resolution) using (-)-diisopropyl tartarate ((-)DIPT) to yield the chiral epoxy alcohol (**82**) in 45% yield and in 95% *ee*. Then epoxy alcohol **82** is directly converted to chloramphenicol **71** in a one-pot reaction. This is achieved by treatment of the chiral epoxy alcohol **82** with dichloroacetonitrile in the presence of sodium hydride, followed by an *in situ* epoxide ring-opening reaction of the resulting epoxy dichloroimidate (**83**) using boron trifluoride etherate. Excess boron trifluoride etherate complexes with oxazoline (**84**), which when hydrolysed, yields chlroamphenicol **71** in 71% yield. In an analogous synthesis (Scheme 2.1.4), thiamphenicol **72** is prepared in an additional step.[86]



Scheme 2.1.4: Reagents and conditions: (a) Vinyl magnesium bromide, THF, 0 °C to rt, 2 h, 88%; (b) Oxone, MeOH-H_2O-THF (1:1:2), 0 °C to rt, 6 h, 87%; (c) (-)-DIPT, $Ti(OiPr)_4$, TBHP, CH_2Cl_2, -20 °C, 24 h, 42 %; (d) NaH, dichloroacetonitrile, CH_2Cl_2, 0 °C to rt, 4 h, then BF_3OEt_2, -78 °C to rt, 4 h, 64%

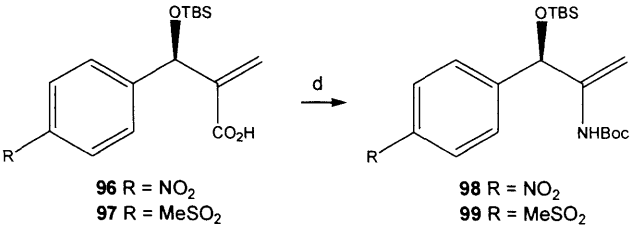
Commercially available 4-(methylthio)benzaldehyde (**85**) is converted to allyl alcohol (**86**) using vinyl magnesium bromide. Oxone is used to oxidise **86** to the sulphone (**87**). The allylic alcohol is subjected to AE conditions using (-)-DIPT to give the chiral epoxyalcohol (**88**) in 42% yield (95% *ee*). Using the same protocol as in Scheme 2.1.3, thiamphenicol **72** is obtained in 64% yield by an *in situ* rearrangement of compound (**89**) and its hydrolysis to oxazoline (**90**).[86]

In a different synthethic strategy, utilised by Mateus and Coelho, chloramphenicol **71** and thiamphenicol **72**, are accessible *via* ene-carbamates (**98**) and (**99**).[90] Reaction (Scheme 2.1.5) of methyl acrylate with 4-nitrobenzaldehyde **80** and 4-methyl-sulfonylbenzaldehyde (**95**), in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) and ultrasound, gives the adducts **91** and **92**, in 97% and 95% yields, respectively. To facilitate the Curtius rearrangement, adducts **91** and **92** are treated with *tert*-butyldimethylsilyl triflate in dichloromethane and triethylamine to yield the silylated ethers (**93**) and (**94**) in almost quantitative yield. Subsequent hydrolysis of silylated methyl esters **93** and **94** using lithium hydroxide in a mixture of acetonitrile and water (1:1) at 40-50°C gives the acids (**96**) and (**97**) in 96% and 90% yields, respectively.[90]



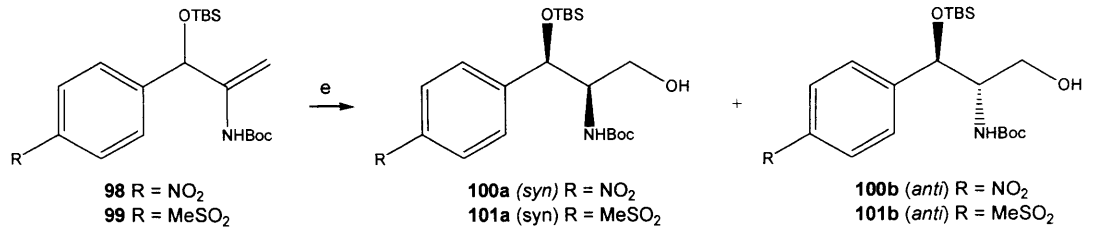
Scheme 2.1.5: Preparation of Baylis-Hillman adducts and hydrolysis of the ester. Reagents and conditions: (a) DABCO, methyl acrylate; (b)TBSOTf, CH_2Cl_2, Et_3N, 0 °C to rt, 4 h, 99% (for **93** and **94**); (c) LiOH, CH_3CN:H_2O (1:1), 40-50 °C, 2-4 h, 96% (for **95**) and 90% (for **96**)

To insert nitrogen atoms into the acids **95** and **96**, ethyl chloroformate and triethylamine is added for 15 min at 0 °C (Scheme 2.1.6). The corresponding anhydrides are not isolated and are then directly treated with sodium azide in water at 0 °C for 50 min. The resulting mixtures are then heated at reflux in toluene to give vinyl isocyanates. These are heated at reflux in *tert*-butanol and form the corresponding *tert*-butyloxycarbonyl ene-carbamates (**98**) and (**100**).[90]



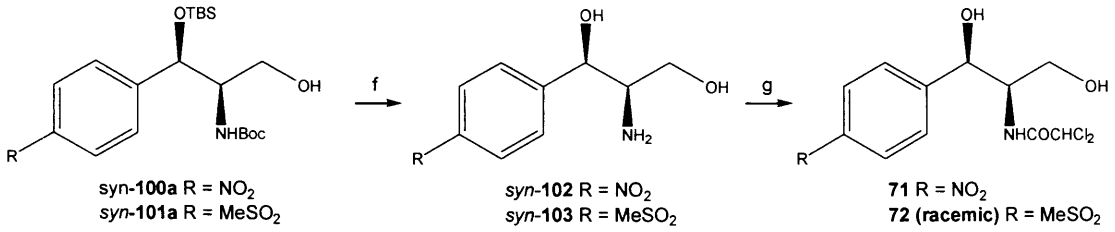
Scheme 2.1.6: Reagents and conditions: (d) i) ethyl chloroformate, acetone, Et_3N, 0°C, 15 min.; ii). NaN_3, H_2O, 0°C, 50 min, then toluene, reflux, 1h; iii) *t*-BuOH, reflux, 18 h, 52% overall yield

The hydroboration of ene-carbamates **98** and **99** is effected by treatment with two boranes (borane dimethylsulfide and 9-borobicyclo[3.3.1]nonane (9-BBN)). This is in the anticipation that the size of the borane has good effects on the diastereoselectivity of the reaction. Ene-carbamates **98** and **99** are independently treated with borane dimethylsulfide and 9-BBN (Scheme 2.1.7) in THF at 0 °C for 18 h. Then sodium hydroxide and hydrogen peroxide are used to yield the *N*-protected aminoalcohols *syn*-(**100a**) and *syn*-(**101a**). The highest *syn* diastereoselectivities obtained from the carbamates **98** and **99** are approximately 2:1 with borane dimethylsulfide. *Anti* isomers are shown as (**100b**) and (**101b**), respectively.[90]



Scheme 2.1.7: Reagents and conditions: (e) i) $BH_3.S(CH_3)_2$, THF, 0 °C to rt, ii) 3N NaOH, H_2O_2, 0 °C-rt

Syn-**100a** and *syn*-**101a** are then treated with HCl in ethyl acetate (Scheme 2.1.8) at room temperature for 3 h. Purification by ion exchange chromatography yields the corresponding *syn*-aminoalcohols (**102**) in 98% yield and (**103**) in 90% yield, respectively. Subsequent treatment of *syn*-**102** and *syn*-**103** with methyl dichloroacetate yields chloramphenicol **71** in 79% yield and thiamphenicol **72** as a racemate in 65% yield, respectively.



Scheme 2.1.8: Reagents and conditions: (f) ethyl acetate: HCl (6N) (1:1), rt, 3 h, 90%; (g) methyl dichloroacetate, reflux, 1 h, 65%

For relatively simple bioactive aminodiols **71** and **72**, the number of synthetic strategies and the number of synthetic steps within each strategy highlight the benefits of developing shorter and more versatile reaction sequences. Furthermore, when dealing with more than one bioactive enantiomer, facile stereodivergent routes are sought after. Considering the synthetic routes towards sphingosines illustrate this point further.

2.1.2 Sphingosines

D-*erythro*-Sphingosines **73** (Figure 2.1.2) are the backbone of D-*erythro*-sphingolipids **104**. Saturated sphingolipid derivatives are known and are called, D-*erythro*-sphinganines **105**.[91,92] The D-*erythro* conformation is that found in natural sphingosines. All sphingosine enantiomers, however, including L-*threo*-**73** and resultant sphingolipids, L-*threo*-**104** and/or L-*threo*-sphinganine-**105** are reported as potent inhibitors of protein kinase, as well as stimulators of DNA synthesis and cell proliferation.

Sphingolipids **104** may vary at the head group R_1, and in the structure of the *N*-acyl group, R_2. It is also possible to produce sphingolipids **104** with variations at R_3. D-*erythro*-sphingolipids **104** are important membrane components and play crucial roles in cell recognition events, such as growth, differentiation, the immune response and as receptors for HIV cells lacking the CD4 receptor.[93]

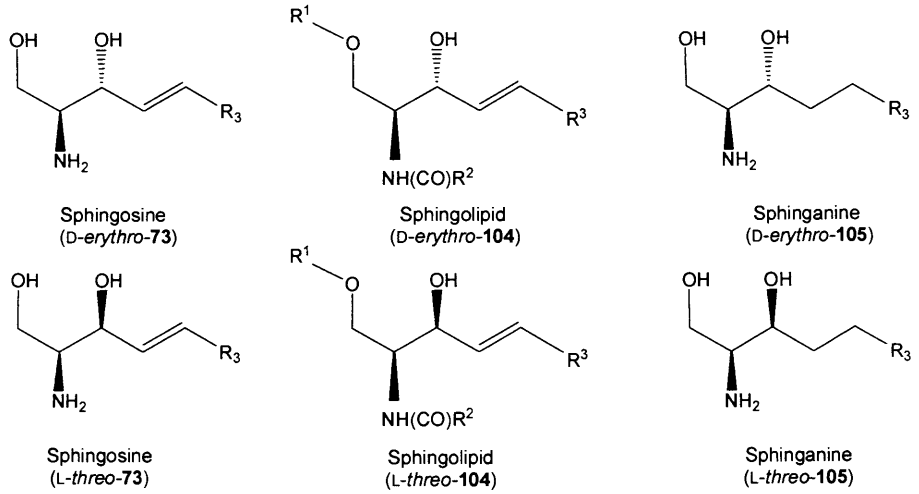


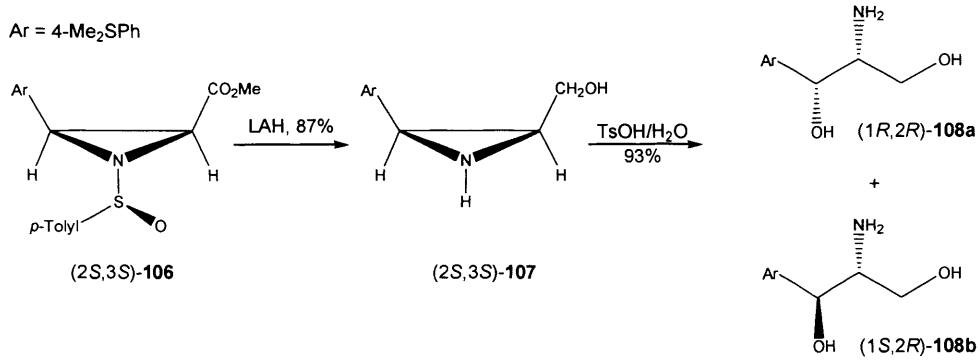
Figure 2.1.2: Sphingosines, sphingolipids and sphinganines[92]

Sphingosines **73** have been the targets of synthetic interest for several decades, but most strategies have relied on the chiral pool for their production. There are few suitable asymmetric reactions that have been used and even fewer stereodivergent synthetic routes.[94] The common theme in the synthesis of these molecules has been to establish stereochemistry early on in the synthesis and then attach or manipulate at R_3. It is crucial that the stereochemistry at C-2 and C-3 was carefully controlled, as there are four possible stereoisomers, each with different bioactivities.[95] Furthermore, the attachment of the tail group must be considered carefully as the *trans* stereochemistry is generally required.

Sphingolipids **104** may be directly isolated from cell membranes, but they are heterogeneous materials where R_1, R_2 and R_3 vary vastly. Epimerisation of the allylic alcohol during isolation or manipulation occurs readily, so synthetic chemical routes are highly sought after for these compounds.[92]

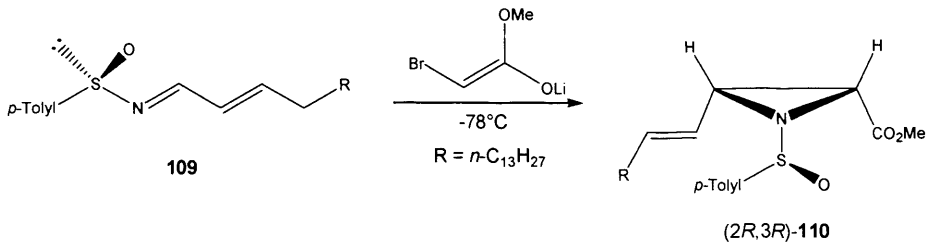
Davis and Reddy utilise an enantiopure aziridine, *cis*-*N*-(*p*-toluenesulfinyl)-2-carbomethoxyaziridine (**106**) in a thiamphenicol **72** synthesis (Scheme 2.1.8), which is first transformed by a lithium aluminium hydride reduction to the corresponding aziridine-2-methanol (**107**). Subsequent regio- and stereospecific ring opening of **107**

with *p*-toluenesulfonic acid and water allow access to (1*R*,2*R*)-(-)-2-amino-1-[(4-methylthio)phenyl]-1,3-propanediol (**108a**) in a 93% chemical yield (Scheme 2.1.9); already identified as a key intermediate in the synthesis of thiamphenicol **72**. Interestingly, no (1*S*,2*R*)-(**108b**) is observed (Scheme 2.1.9).[93]



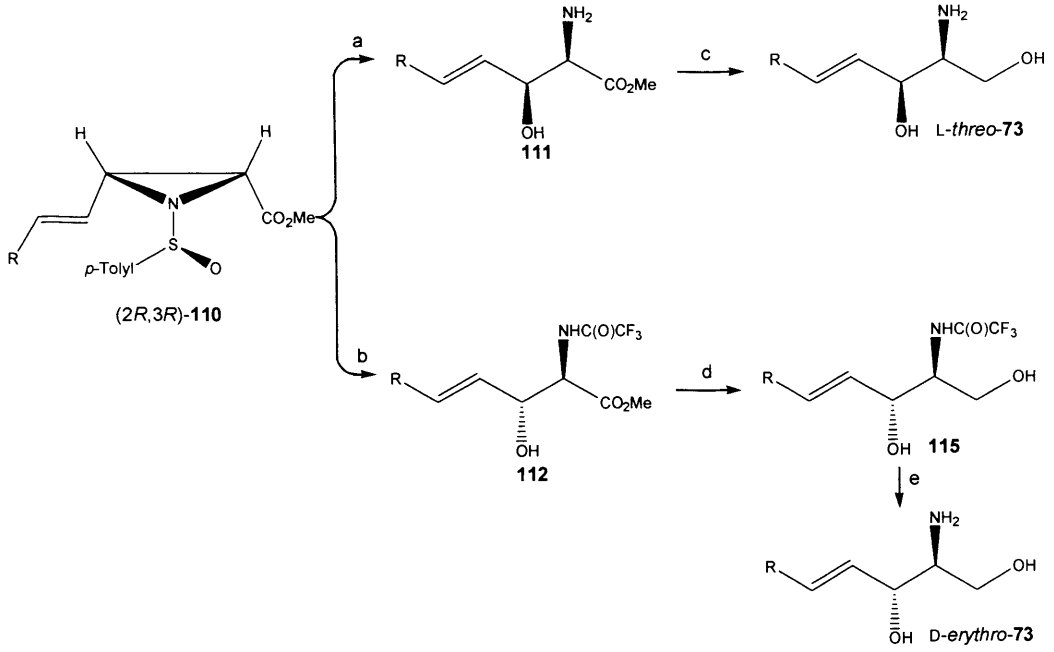
Scheme 2.1.9: Synthetic route to (1*R*,2*R*)-**108a** and (1*S*,2*R*)-**108b**

A similar approach is used to access the sphingosine D-*erythro*-sphingosine **73** (where $R_3 = C_{13}H_{27}$) (Figure 2.1.2). Sulfinimine **109** is treated with the lithium enolate of methyl bromoacetate (Scheme 2.1.10) at –78 °C and gives (2*R*,3*R*)-(-)-aziridine **110**, which acts as a common intermediate, allowing access to both D-*erythro*-(**73**) and L-*threo*-(**73**).[93,96]



Scheme 2.1.10: Synthesis of aziridine **110**

Treatment of **110** (Scheme 2.1.11) with acetone/TFA/water gives the β-hydroxy-α-amino acid ester (**111**) as a single isomer in 72% yield. Reduction of **111** with lithium borohydride yields L-*threo*-sphingosine-(**73**).



Scheme 2.1.11: Reagents and conditions: (a) 50% aq. TFA/acetone, 0 °C, 24 h, 72%; (b) TFAA/CH_2Cl_2, 35 °C, 15 min., radial chromatography, 59%; (c) $LiBH_4$/MeOH, rt., 30 min, 80%; (d) $LiBH_4$/MeOH, rt., 1 h, 71%; (e) K_2CO_3/EtOH, 50 °C, 5 h, 75%

Treatment of **110** with trifluoroacetic anhydride (Scheme 2.1.11) results in the unexpected formation of aminoalcohol (**112**) with 88% *erythro* diastereoselectivity. Usually, formation of *erythro*-**112** requires nucleophilic attack from the same side as the departing amino group. Rationalisation of this step is that an activated sulfoxide complex (**113**) is formed (Figure 2.1.3) and undergoes a stereospecific [3,3]-sigmatropic rearrangement of the trifluoromethyl acetoxy group to a developing carbocation of the ion pair (**114**). The aziridine *N*-activating group that promotes the ring opening is identified as the amino-sulfonium salt.[93]

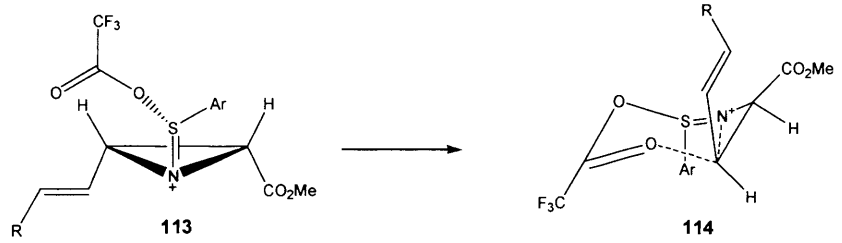


Figure 2.1.3: Stereospecific [3,3]-sigmatropic rearrangement of the trifluoromethyl acetoxy group in **113** to a developing carbocation of the ion pair **114**

Reduction of **112** is achieved using lithium borohydride, as before (Scheme 2.1.11), yielding **115**. Hydrolysis of *N*-trifluoroacetyl group from aminoalcohol (**115**) is achieved using potassium carbonate in ethanol at 50 °C and gives D-*erythro*-**73**. So far, this covers only two sphingosine enantiomers, D-*erythro*-**73** and L-*threo*-sphingosine-**73**.

Olofsson and Somfai have developed a divergent strategy to produce all eight isomers of sphingosines, **73** and their regioisomers *ent*-(**73**) (Figure 2.1.4).[94]

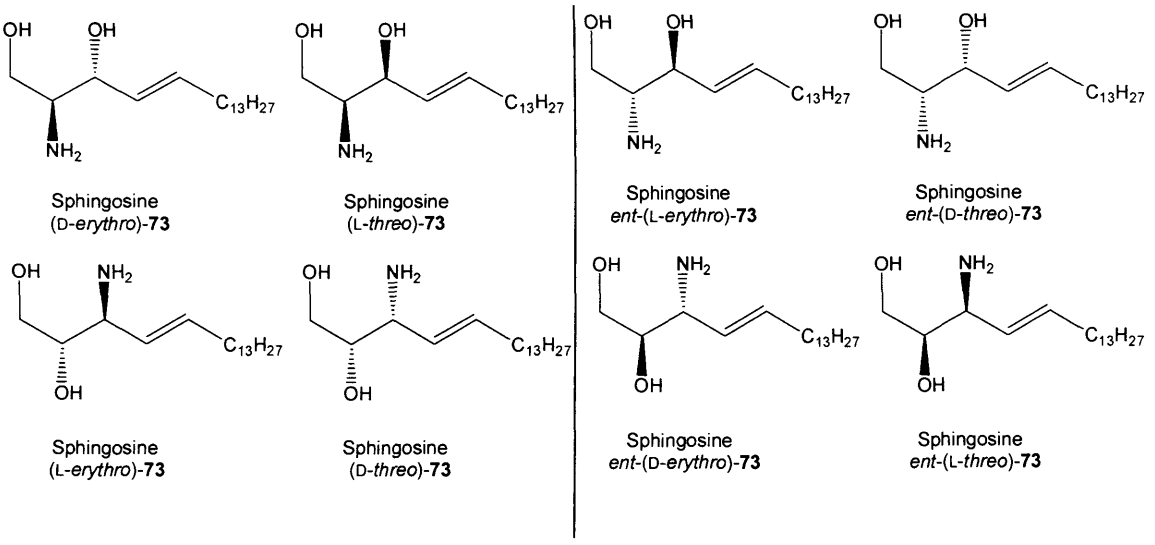
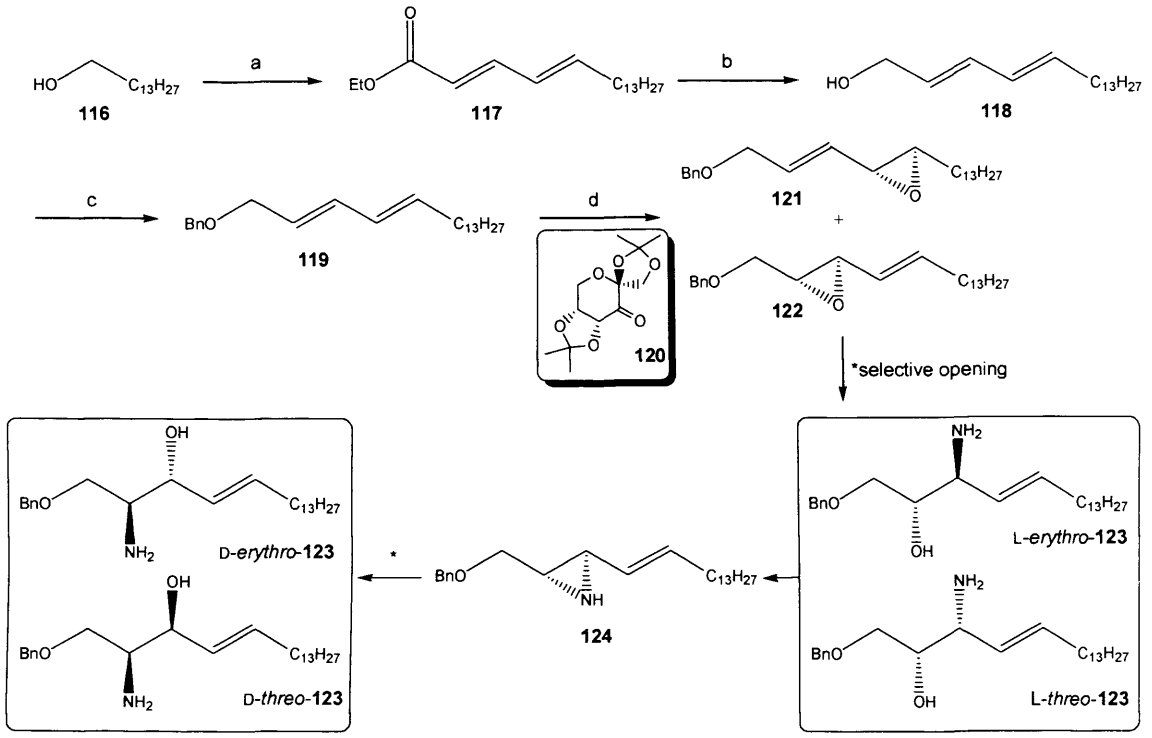


Figure 2.1.4: All possible regio- and stereoisomers of sphingosines

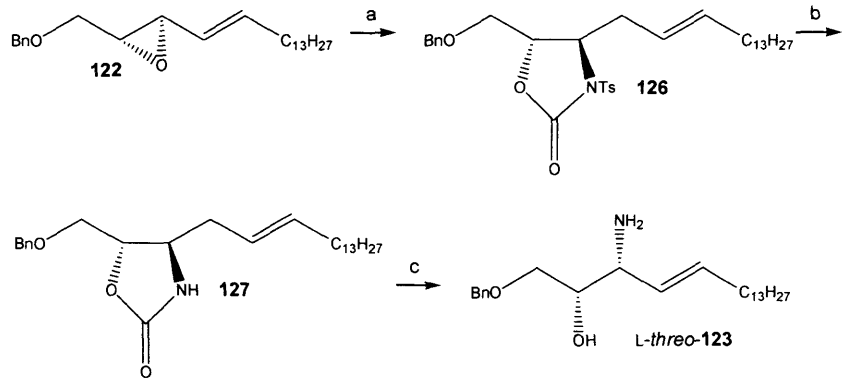
The synthetic strategy employed by Olofsson and Somfai starts from the commercially available tetradecanol (**116**) (Scheme 2.1.12). A 2-iodoxybenzoic acid (IBX) promoted oxidation of **116**, followed by a modified Horner-Emmons reaction with triethyl phosphonocrotonate leads to diene ester (**117**). Reduction of this ester with DIBAL gives the dienol (**118**). This is then benzylated using sodium hydride, benzyl bromide and phase transfer reagent, tetrabutylammonium iodide and gives diene (**119**). Diene **119** undergoes a Shi epoxidation using catalyst (**120**) to give common precursors, vinyl epoxides (**121**) and (**122**) in a 1:1 ratio. Selective ring-opening of **122** yields L-*erythro*-(**123**) and D-*threo*-(**123**), benzyl protected versions of L-*erythro*-**73** and D-*threo*-**73**, respectively. Ring closure of L-*erythro*-**123** and D-*threo*-**123** to aziridine (**124**), act as a common precursor for the selective ring-opening to D-*erythro*-(**123**) and L-*threo*-(**123**), benzyl protected versions of D-*erythro*-**73** and L-*threo*-**73**, respectively.[94]



Scheme 2.1.12: Reagents and conditions: (a) i) IBX/DMSO, 90 min rt; ii) $LiOH.H_2O$, triethyl phosphonocrotonate, THF, 2.5 h; (b) $DIBAL/CH_2Cl_2$, -40 °C, 59%; (c) NaH, Bu_4NI, BnBr, THF, reflux, 80 min, 95%; (d) DMM, MeCN, $Na_2B_4O_7.10H_2O$, Bu_4NHSO_4, cat. **120**, Oxone in EDTA, K_2CO_3, H_2O, 90 min, 90%

Vinyl epoxide **122** is heated in ammonium hydroxide at 125 °C giving a diastereospecific (>20:1) and regiospecific opening at the allylic position in 98 % yield. Even though vinyl epoxides **121** and **122** are formed in a 1:1 ratio, separating the two diminishes the yield of L-*erythro*-aminoalcohol **123** and D-*threo*-aminoalcohol **123**. Fortunately, the crude vinyl epoxides **121** and **122** can be reacted together, as only **122** undergoes ring-opening. L-*erythro*-aminoalcohol **123** is achieved using an aminolysis protocol (Scheme 2.1.12).[94]

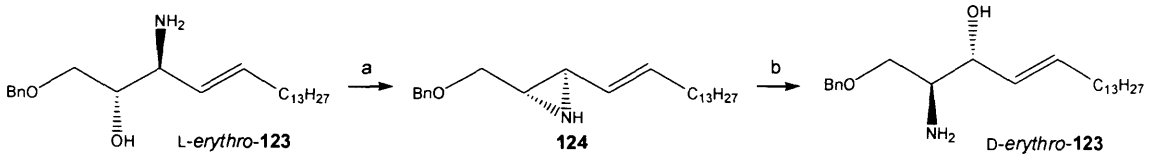
A Pd(0)-catalysed ring-opening (Scheme 2.1.13) of vinyl epoxide **122** in the presence of tosyl isocyanate, yields oxazolidinone **126**.



Scheme 2.1.13: Reagents and conditions: (a) Pd (0), TsNCO; (b) Na, naphthalene; (c) KOH, Δ

Detosylation of **126** is achieved by titration at –78 °C of sodium naphthalide to yield oxazolidinone (**127**) in 88% yield. Finally, basic hydrolysis with potassium hydroxide yields L-*threo*-**123**, in 98 % yield. Even though **121** and **122** are vinyl epoxides formed in a 1:1 ratio, separating the two diminishes the yield. Fortunately, the crude vinyl epoxide **121** and **122** can be reacted together, as only **122** epoxidises forming the oxazolidinone **126**, which allows ease of separation in the purification stage of this reaction.[94]

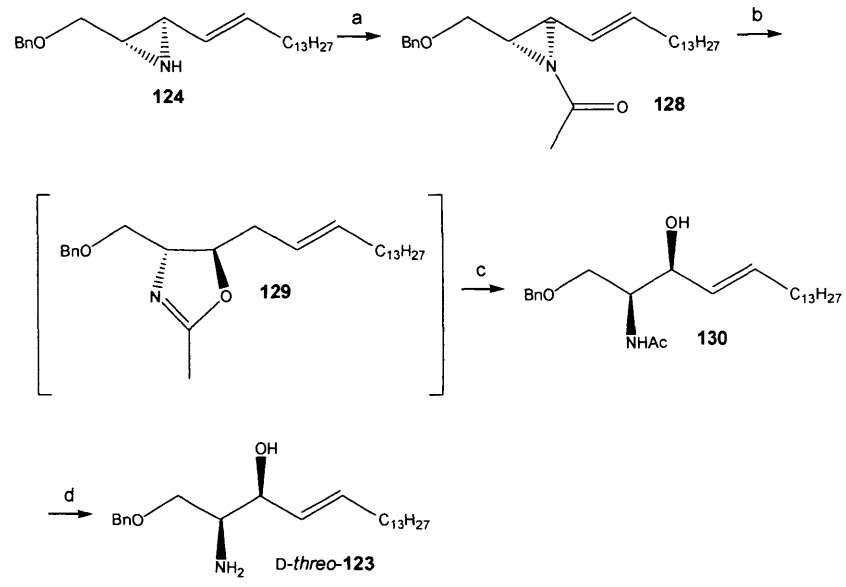
Benzyl-protected D-*erythro*-sphingosine **125** and benzyl-protected L-*threo*-sphingosine **125** (Scheme 2.1.12) are regioisomers of the aminoalcohols L-*erythro*-**123** and L-*threo*-**123**, respectively. These are accessible *via* vinyl aziridine **124**. The L-*erythro*-aminoalcohol **123** is ring closed under Mitsunobu conditions to yield vinyl aziridine **124** (Scheme 2.1.14).[94]



Scheme 2.1.14: Reagents and conditions: (a) PPh_3, DIAD; (b) TFA

Treatment of aziridine **124** with trifluoroacetic acid allow access to D-*erythro*-**123** in high diastereo- and regioselectivity (>20:1) in 62% yield.

Synthesis of benzyl-protected L-*threo*-sphingosine (**123**) is shown in Scheme 2.1.15.

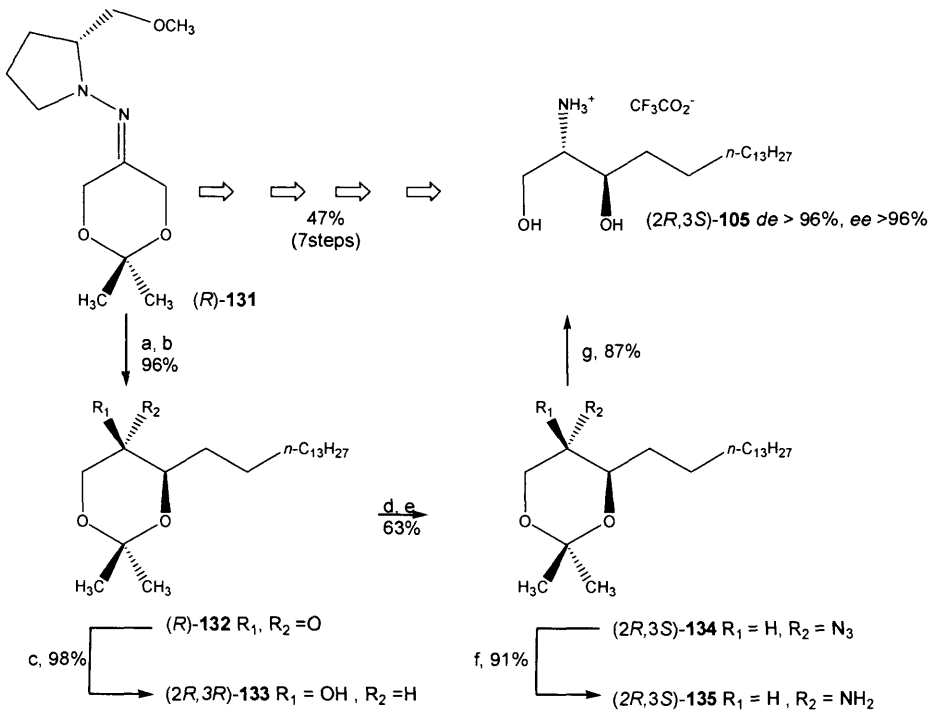


Scheme 2.1.15: Reagents and conditions: (a) reduced DIAD, Et_3N, DMAP, -78 °C, Ac_2O; (b) $BF_3.OEt_2$; (c) H_2O; (d) H_2SO_4

Acetylation of aziridine **124** occurs in almost quantitative yield to give acetylated amino aziridine (**128**). This is then rearranged to oxazoline (**129**) using boron trifluoride etherate, followed by an *in situ* hydrolysis of **129** to hydroxyamide (**130**). A high diastereoselectivity (*de* >20:1) and regioselectivity (>20:1) is achieved. Hydroxyamide **130** is hydrolysed with 5% aqueous sulphuric acid and gives the D-*threo*-aminoalcohol **123** in good yield.[94]

All of the benzylated aminoalcohols of **123** are converted into their corresponding sphingosines **73** using sodium in liquid ammonia in excellent yields. The corresponding regioisomers (*ent*-**73**) could be obtained by starting with *ent*-**122** (Scheme 2.1.12).[94]

Enders *et al.*, describeS the asymmetric synthesis of 2-amino-1,3-diols and D-*erythro*-sphinganine **105** (Scheme 2.1.16) using RAMP-hydrazone methodology.[97,98]



Scheme 2.1.16: Reagents and conditions: (a) *t*BuLi, THF, -78 °C, then pentadecyl bromide, -100 °C; (b) aq. Oxalic acid; (c) L-selectride®, THF, -78 °C; (d) MsCl, CH_2Cl_2, Et_3N, -0 °C → rt.;(e) NaN_3, 18-crown-6, DMF, 100 °C; (f) LAH, THF, rt.; (g) TFA, THF/H_2O, rt.

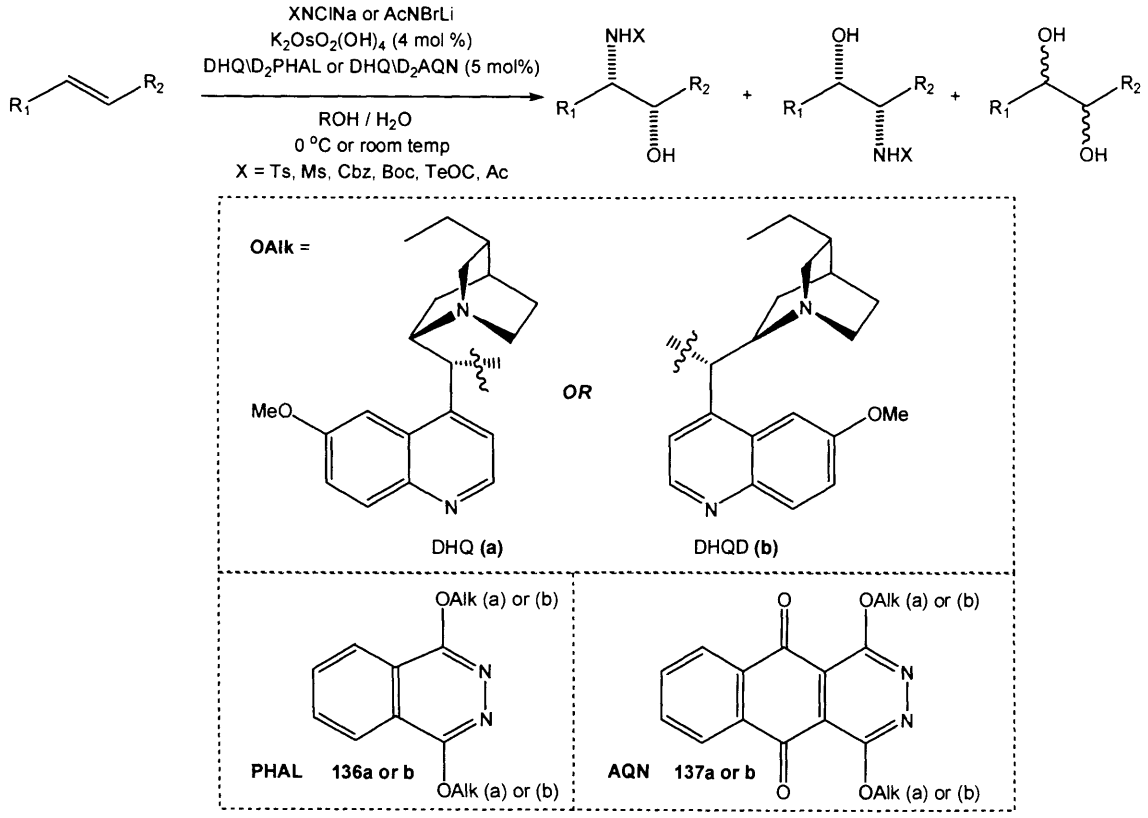
In the first step, hydrazone (*R*)-(**131**) is alkylated with pentadecyl bromide (Scheme 2.1.16). Subsequently, the hydrazone is cleaved with a saturated solution of oxalic acid. From this, ketone (*R*)-(**132**) is obtained in an excellent 96% yield and *ee* (>96%). Subsequent reduction with L-selectride® gives alcohol (2*R*,3*R*)-(**133**) in a practically quantitative 98% yield and very high diastereoselectivity (*de* >96%). Treatment of **133** with methanesulfonyl chloride yields the corresponding mesylate, which is then converted to the azide (2*R*,3*S*)-(**134**) by nucleophilic substitution. Reduction of **134** with LAH, yields amine (**135**), which followed by hydrolytic cleavage of the acetonide group with TFA in THF/water affords the ammonium salt (2*R*,3*S*)-**105** in an overall 47% yield , a *de* of >96% and an *ee* of >96%.

There is a possibility of simplifying the asymmetric synthesis of aminodiols seen above by introducing two chiral centres simultaneously, but this requires a separate discussion, as follows.

2.2 Sharpless Asymmetric Aminohydroxylation (AA)

Synthetic routes to aminoalcohols can require arduous protecting group strategies and require numerous synthetic steps, as previously discussed *vide supra*. The Sharpless asymmetric aminohydroxylation (AA) has significant potential in synthesis due to its ability to set up two chiral centres in a single step.

The aminohydroxylation of olefins dates back to the 1970s,[99] but the Sharpless asymmetric aminohydroxylation (AA) was not discovered until 1996[100], when the same research group used osmium tetroxide, a chinchona alkaloid derived ligand, typically 1,4-*bis*(dihydroquinyl)phthalazine $(DHQ)_2PHAL$ **136a**, or 1,4-bis(dihydroquinidinyl)phthalazine $(DHQD)_2PHAL$ **136b** and 1,4-*bis*(dihydroquinyl)anthraquinone $(DHQ)_2AQN$ **137a** or 1,4-*bis*(dihydroquinidinyl)anthraquinone $(DHQD)_2AQN$ **137b** with an oxidant and a nitrogen source. These components would then convert *trans*-alkenes to *N*-protected aminoalcohols with good regio- and enantioselectivities. The typical reaction protocol used in the AA transformation is shown in Scheme 2.2.1.[101]



Scheme 2.2.1: Overview of the Sharpless asymmetric amino hydroxylation (AA).[101]

It is by an appropriate choice of alkaloid ligands **136** or **137**, that the regio- and enantioselectivity of this reaction could be controlled to a certain degree.[101] The main problems with this reaction are identified as:

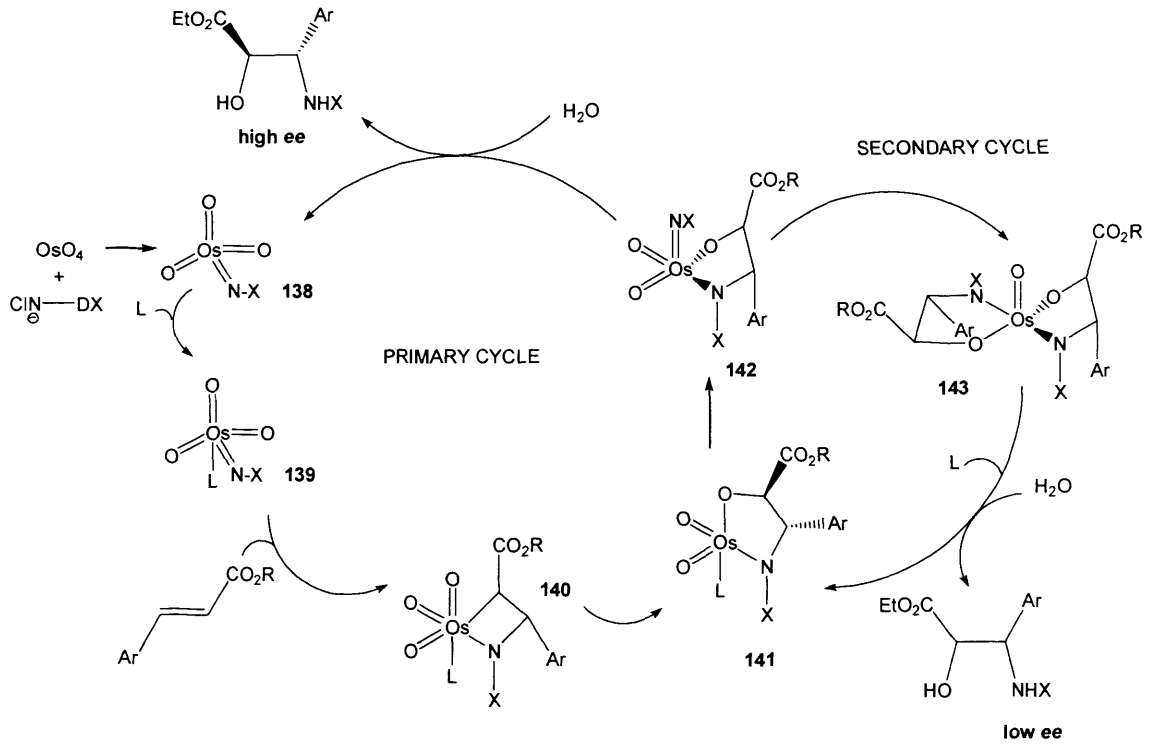
- Selectivities (chemo-, regio, and enantioselectivities);
- Substrate scope;
- Formation of an unwanted diol product and
- Activity of the catalyst.[102]

Despite these problems, the AA transformation plays a key role in the synthesis of many important biological molecules, including amino cyclitols, the Taxol™ side chain, β-amino-α-hydroxyphosphonic acid derivatives and α-amino acids.[101,103]

The following sections highlight how altering various components of this reaction have an effect on the regio- and enantioselectivities, and how the problems encountered have been overcome; but first a consideration of the mechanism is important in defining how the two chiral centres are established.

2.2.1 Mechanism

The predecessor to the AA transformation is the asymmetric dihydroxylation reaction (AD), which is similar to the AA mechanism, shown below in Scheme 2.2.2.[101]



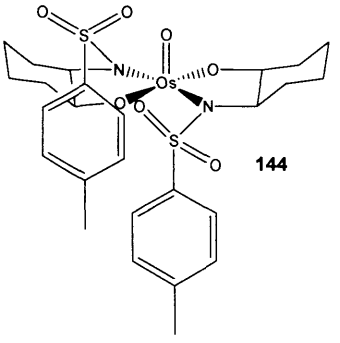
Scheme 2.2.2: AA catalytic cycles.

The formation of an imidotrioxoosmium(VIII) (**138**) species results from the reaction of osmium tetroxide with an appropriate nitrogen source.[101] The chiral ligand L, is coordinated and forms the active complex (**139**). An osmaazetidine (**140**) is then afforded. This subsequently undergoes a 1,2-migration of the carbon-osmium bond and gives the azaglycolate adduct (**141**). Displacement of the chiral ligand L by another nitrogen reagent results in species (**142**). It is at this point that hydrolysis by solvents containing 50% water (v/v) can release the aminoalcohol with high enantioselectivities (primary cycle) and regenerate **138**, or could result in the unwanted diol formation (Scheme 2.2.1). Often alcohol-water mixtures are used to reduce the hydrolysis and hence the diol product. Otherwise, the secondary catalytic cycle is initiated by reaction with another alkene, forming complex (**143**) and releasing the aminoalcohol with low enantioselectivity.[101]

The point of controversy is whether **142** is achieved through a [2+2] cycloaddition, or is directly achieved through a [3+2] cycloaddition (Scheme 2.2.2). Whilst weight is lended to the [3+2] cycloaddition, through kinetic isotope effects[104] and theoretical calculations hypotheses[104], the dual cycles represented (Scheme 2.2.2) give an overall account.[105]

Effective AA reactions result from the suppression of the secondary catalytic cycle, which relies on the effective hydrolysis of **142**. The diol formation is problematic as although less water in the reaction decreases the formation of diol by-products, water is also required to speed the hydrolysis of **142** to aminoalcohols in high enantiomeric excesses.

The second catalytic cycle is, however, considered important by Wu *et al.*, to give aminohydroxylation products with good to moderate enantioselectivities, *without* the need of chiral cinchona ligands for a range of substrates. By considering the osmium azaglycolate species (**144**) (Figure 2.2.1), through X-ray crystallography, the second catalytic cycle is exploited. This means no diol is formed, appreciably quicker turnovers occur, higher yields occur and lower osmium catalyst loadings are required.[106]

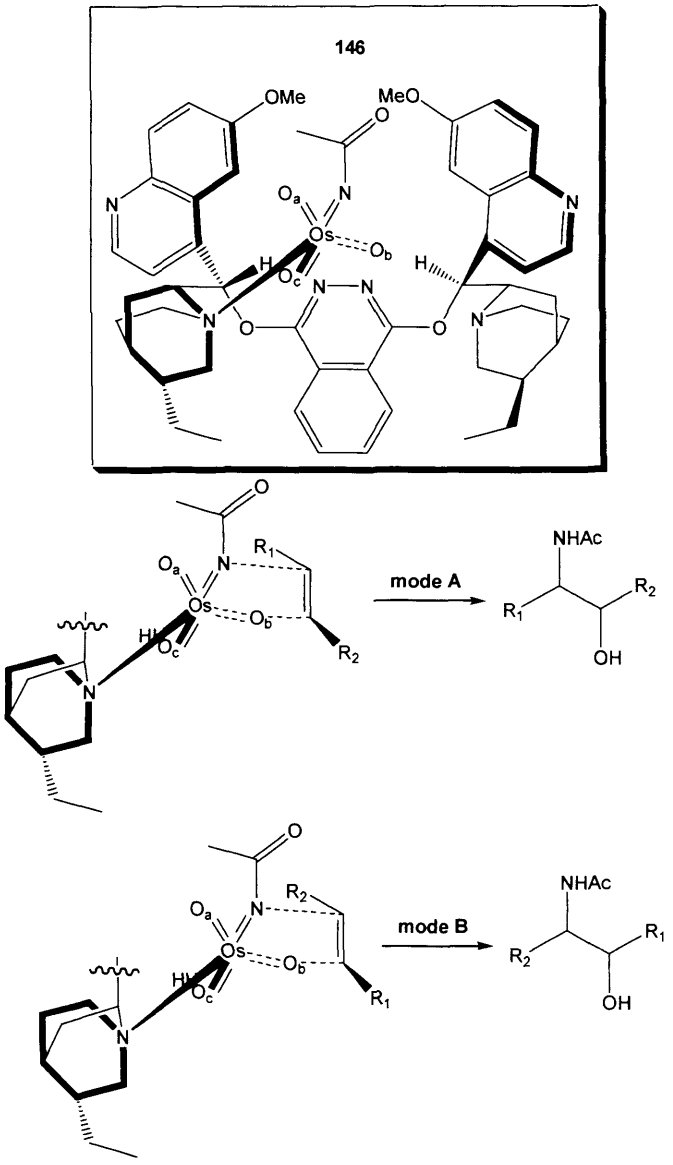


144

Figure 2.2.1: Osmium *bis*(azaglycolate) complex **144**[105]

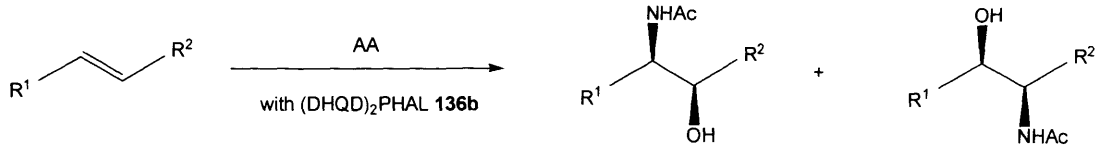
Complex **144** has a greater steric hindrance along the path by which water is needed to approach the only open coordination site of osmium. This is to initiate the hydrolysis step. The hydrophobic pockets created by the two-tosyl groups may also slow the approach of water. The tosyl groups are pointing "down" and around the vacant coordination site. These features generally explain the observation that catalytic aminohydroxylation (AA) is slower than its asymmetric dihydroxylation (AD) counterpart.

Janda *et al.*, study the active catalytic species of the AA reaction *with* cinchona derived ligands and confirm the possibility that a substrate-based method can be used to control the regioselectivity imposed on unsymmetrical alkenes. This is achieveable by having an understanding of the active species (**145**) (Scheme 2.2.3). For example, when considering the $(DHDQ)_2PHAL$ **136b** (Scheme 2.2.1), Janda *et al.*, shows that the regioselectivity of the reaction could be inferred from the catalyst-substrate complex, and is termed either mode **A** or **B** (Scheme 2.2.3) depending on the substrate used.[107]



Scheme 2.2.3: Proposed structure of the Ac-N=OsO_3-$(DHQD)_2PHAL$ catalyst.

Sharma *et al.*, investigates the steric, electronic and substrate-catalyst shape with a range of alkenes **146a-g** to support the concept of "pre-programming" the regioselectivity of the reaction (Table 2.2.1).[107]



146a-g 147a-g 148a-g

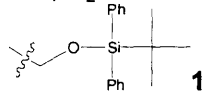
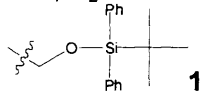
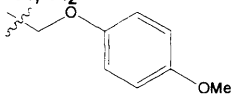
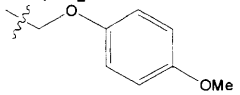
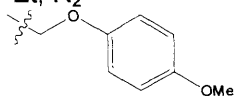
Entry	Alkene	Regioselectivity (147a-g: 148a-g)
1	R_1 = H, R_2 =  **146a**	>20.0:1
2	R_1 = Et, R_2 =  **146b**	2.0:1
3	R_1 = H, R_2 = CO_2Et **146c**	15.2:1
4	R^1 = Me, R^2 = CO_2Et **146d**	1.4:1
5	R_1 = H, R_2 =  **146e**	1.2:1
6	R_1 = Me, R_2 =  **146f**	1:3.2
7	R_1 = Et, R_2 =  **146g**	1:3.5

Table 2.2.1: AA reaction with selected olefins

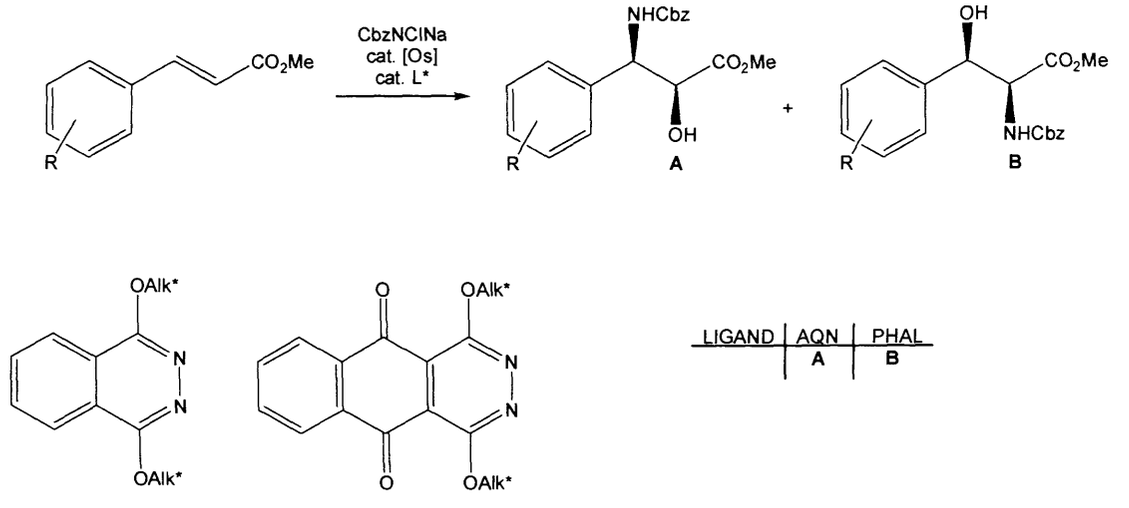
The sterically demanding olefins **146a** and **146b** are shown not to fit the relatively narrow U-shaped binding pocket of the catalyst and the preferred mode **A**. Whereas R_2 groups from (**147a**) and (**147b**) are directed towards the solvent (Scheme 2.2.3).[107]

The electronic influence on the regiochemistry is exhibited by the alkenes **146c** and **146d**, where the nucleophilic nitrogen ligand of the osmium complex prefers to add to the more electrophilic β-carbon of the α,β-unsaturated ester and to the oxygen to the α-carbon atom. It is thought that the polar ester group would be more stable in the hydrophilic environment and lead to mode **A** (Scheme 2.2.3) products (**147c-d**).[107]

Finally, it is noted by Janda *et al.*, that the subtle interplay between substrate-catalyst bindings results in the regioselectivities seen when alkenes **146e-146g** are reacted to give products (**147e-g**). Yet, if steric effects are the only issue, mode **A** would be preferred due to the large *p*-methoxyphenoxy moieties, but the regioselectivity favours

mode **B** (**148f-g**), as aryl-aryl interactions between the substrate and catalyst-binding cleft may prevail over such steric interactions. Other examples are reported, but the problem of regioselective control is non-trivial and can sometimes be difficult to rationalise.[107]

Sharpless *et al.*, performs an investigation using an anthraquinone core (AQN) **137** (Scheme 2.2.1) and finds a reversal in the regioselectivities with various methyl cinnamate precursors. This is linked to the electronic effects displayed by varying the R groups on methyl *p*-cinnamate ester. The typical reaction protocol involves the carbamate modification (Scheme 2.2.4).[8]



Scheme 2.2.4: AA of cinnamates [L* = dihydroquinyl (DHQ) or -dihydroquinidinyl (DHQD)][8]

$(DHQ)_2PHAL$ **136a** core gives the corresponding 3-phenylisoserine regiosisomers **A**. It is found that by using *N*-chloro-*N*-sodio benzylcarbamates as the nitrogen source, 3-phenylserine regioisomer **B** (Scheme 2.2.4) is favoured when the $(DHQ/D)_2AQN$ **137a/b** core are used (Table 2.2.2).[8,108]

Entry	R	B:A	$(DHQ)_2AQN$ 137a *Ee* (yield)/% of (2*R*,3*S*)-B	$(DHQD)_2AQN$ 137b *Ee* (yield)/% of (2*S*,3*R*)-B
1	H	79: 21	95 (58)	92 (62)
2	4-F	82: 18	91 (67)	92 (68)
3	4-Cl	77: 23	91 (58)	92 (54)
4	4-Br	80: 20	89 (51)	89 (52)
5	4-Me	78: 22	93 (n. d.)	96 (52)
6	4-MeO	78: 22	94 (67)	93 (65)
7	$2,6\text{-}(MeO)_2$	75: 25	91 (50)	91 (51)
8	4-BnO	66: 34	87 (40)	87 (40)

Table 2.2.2: AA of R-substituted methyl *p*-cinnamate esters using CbzNClNa.[8]

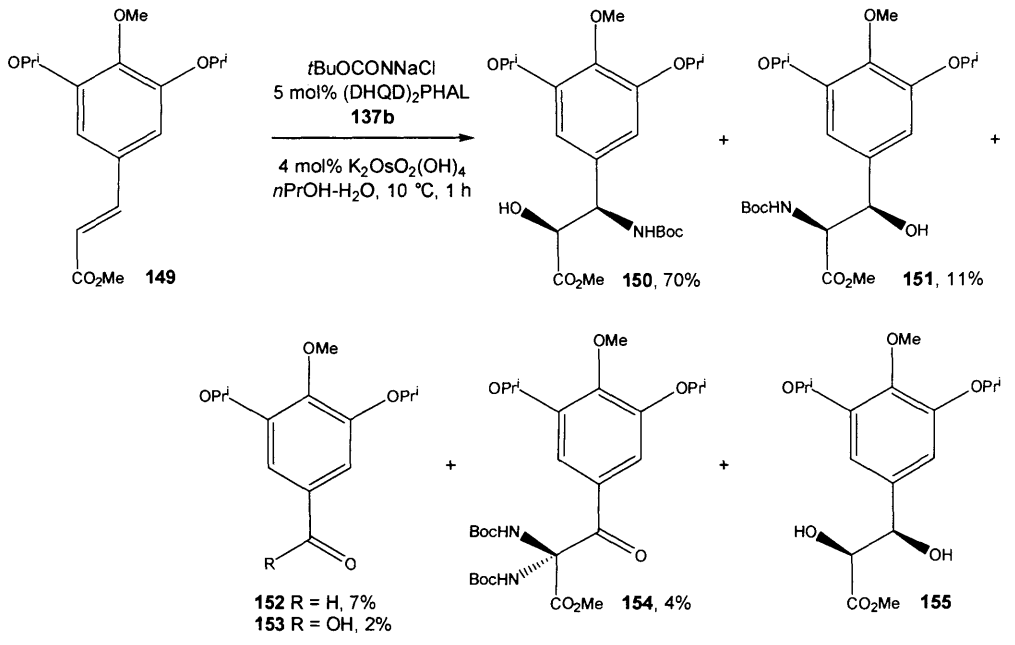
Sharpless *et al.*, find from these results that cinnamates act as a good model for SAA reactions, with resulting high asymmetric inductions and a good reversal in

regioselectivity from 3-phenylisoserine enantiomers **A** to 3-phenylserine enantiomers **B** (Table 2.2.2). The electronic ring effect, due to the nature of either electron donating or electron withdrawing substituents at the *p*-position of cinnamate methyl ester, results in a variety of 3-phenylserine enantiomers. The regioisomeric ratio is as high as 82:18 (entry **2**).

The reversal in regioselectivity is not fully explained, but it is theorized that the substrate orientation within the binding pockets of these ligands is altered in such a way that, whilst the regioselectivity was reversed, enantioselectivities of the AA transformation remain high.[8]

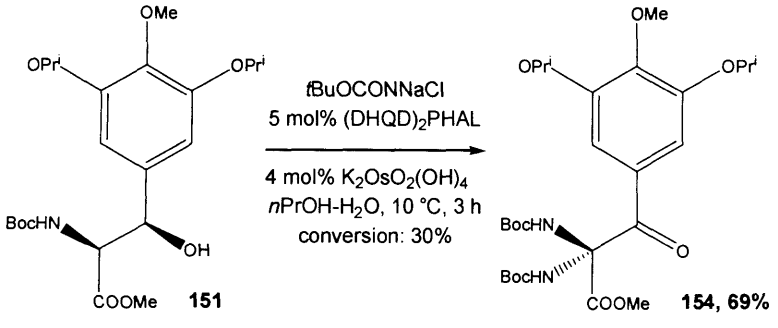
A complete study on the electronic effects on the regio- and enantioselectivity of the AA of *O*-substituted 4-hydroxyl-2-butenoates is also known, in an attempt to understand the aromatic–aromatic interactions.[107] These are crucial in achieving high regio- and enantioselectivities with this class of substrates and investigates a range of electron donating/withdrawing groups.[109]

Finally an analysis of the by-products may aid a further insight into understanding the reaction mechanism. Recently, Liu *et al.*, describe the by-products from the reaction of cinnamates with *tert*-butoxy carbamate as the nitrogen source and with $(DHQD)_2PHAL$ **136b**.[110] Cinnamate precursor (**149**) undergioes AA and gives the expected enantiomers (**150**) and (**151**) (Scheme 2.2.5). Careful analysis of this reaction mixture, however, lead to the identification of aldehyde (**152**), acid (**153**), and novel α,α-di-*tert*-butyloxycarbamoyl-β-ketone ester (**154**). The ratio of these compounds is found to be time dependent. Prolonged reaction times lead to increased formation of side products **152-154**.[110]



Scheme 2.2.5: By-products analysis

The origin of the by-product **154** is elucidated by resubmitting aminoalcohols **150** and **151** and the diol (**155**) back into the reaction. Transesterification of the methyl ester to a propyl ester by the solvent results in the formation of 4 methoxy-3,5-diisopropyloxy benzoic acid **153** in all of these reactions; but 4-methoxy-3,5-diisopropyloxybenzaldehyde **152** only results from the diol **155**. Ketone ester **154** is only formed from β-hydroxy-α-amino ester **151** (Scheme 2.2.6).[110]

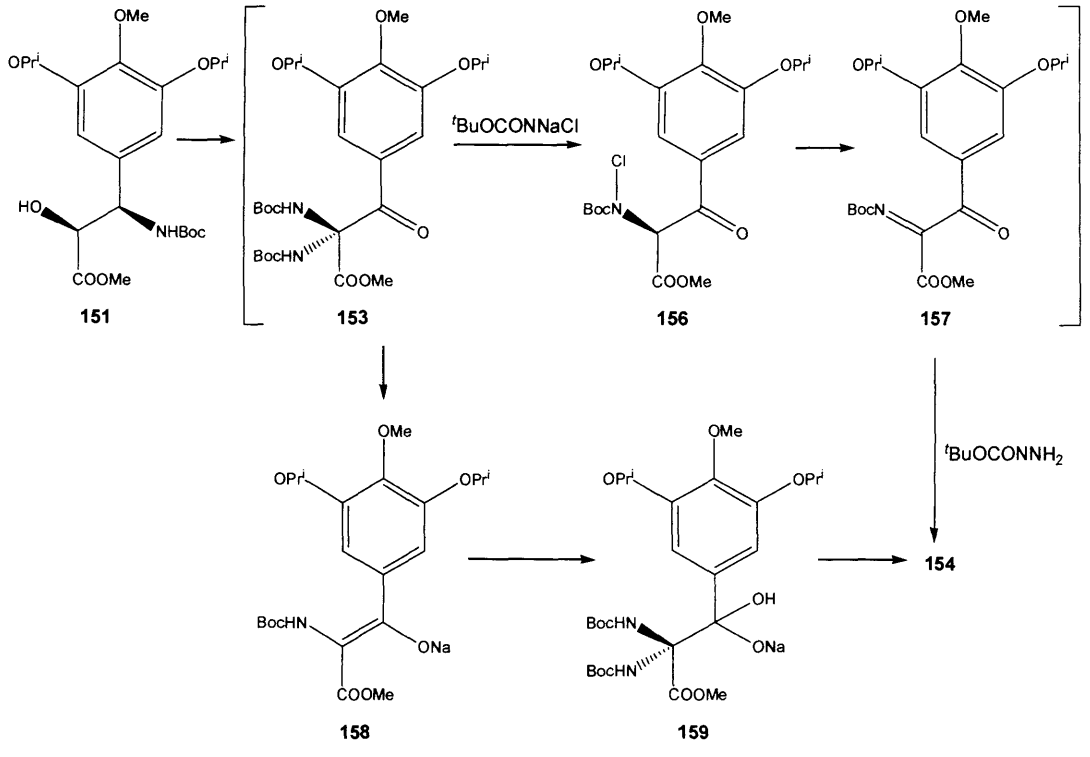


Scheme 2.2.6: Formation of ketone ester **154**

Mechanistic implications arising from these observations (Scheme 2.2.7) are that an oxidation of benzylic alcohol **151** followed by *N*-chlorination of **154** gives intermediate (**156**). Deprotonation and elimination of chloride from compound **156** lead to the formation of imine (**157**). Nucleophilic addition of *tert*-butyl carbamidate anion onto the resulting imine gives compound **153**. Two possible accounts for this are that *N*-chloro, *N*-sodio carbamate acts as a chloronium ion, as a base and then as a nucleophile. Alternatively, the enolization of β-keto ester **154** followed by another AA reaction on

the resultant enolate (**158**), leads to the intermediate (**159**), which on hydrolysis forms **154**.[110]

The overall process (Scheme 2.2.7), however, is attributed to oxidation of the benzylic alcohol to the ketone, which is initiated by the complex formed between osmium tetroxide and *N*-chloro-*N*-sodio *tert*-butyl carbamate. Though studies are complete with $(DHQD)_2PHAL$ **136b** ligand, implications for the mechanism with ligands [$(DHQ)_2AQN$ **137a** & $(DHQD)_2AQN$) **137b**] and the 3-phenylserine enantiomers concur with the literature results, generally showing a lower yield.[7,8] The partial decomposition of the enantiomers following the pathway shown in Scheme 2.2.7 may account for this reduced yield.



Scheme 2.2.7: Mechanistic implications of resubmitting **151** to Sharpless AA conditions

The nature of the nitrogen source may have mechanistic implications itself, by its size and electronic effects, but none of these are mentioned in the above discussion. What follows now is a brief overview of the many nitrogen sources that have been used in the AA reaction.

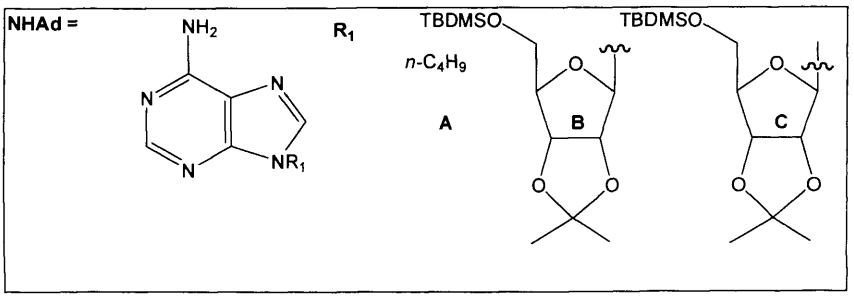
2.2.2 Nitrogen sources

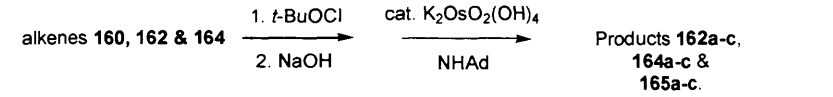
Sharpless AA reactions are successfully performed with a number of nitrogen sources and substrates. Such nitrogen sources include, aminoheterocycles[111], carbamates[112], primary amides[113] and sulfonamides[114]. Each of the following examples provides a way

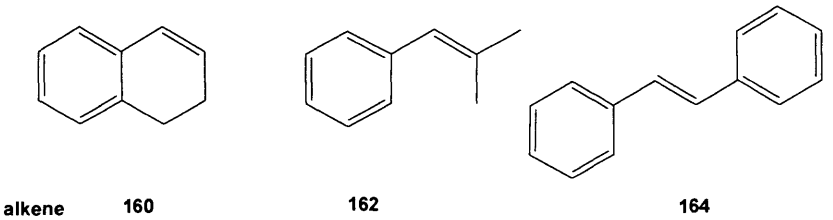
of controlling enantio- and regioselectivities in the final product by their size or electronic effects.

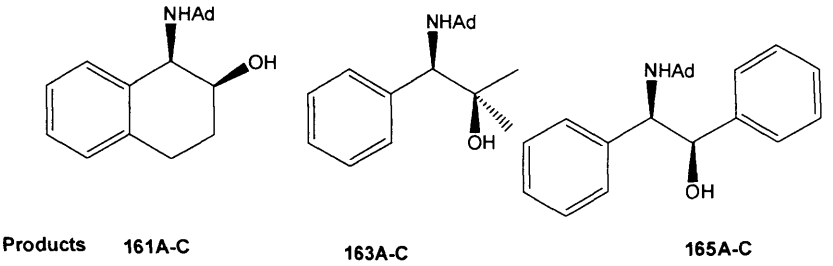
2.2.2.1 Aminoheterocycles

N-chloro-*N*-sodio salts of adenine derivatives are found to be suitable nitrogen sources for the Sharpless AA transformation, achieving suprafacial, vicinal addition of adenine and a hydroxyl group, but unfortunately not in a regio- or enantioselective fashion.[111] Dress *et al.*, report that the poor reactivity and side reactions, such as ring chlorination have prevented the use of heterocyclic compounds in the Sharpless AA, but good reactivity is found between three derivatized adenosine molecules. These are labelled R_1 = **A**, **B** and **C** (below). Three alkenes, 1,2-dihydronapthalene (**160**), 2-methyl-1-phenyl-1-propene (**162**) and 1,2-diphenyl ethene (**164**) are reacted with **A**, **B** and **C**, as summarized in Table 2.2.3.









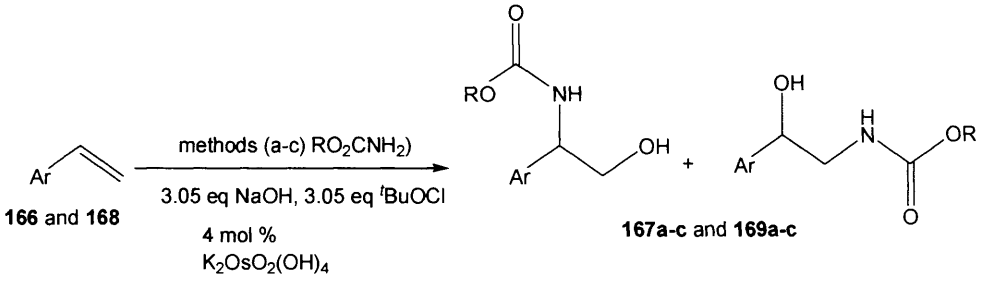
$AdNH_2$	Alkene	product	% yield
A	160	161A	79
B	160	161B	68
C	160	161C	71
A	162	163A	75
B	162	163B	64
C	162	163C	58
A	164	165A	83
B	164	165B	76
C	164	165C	65

Table 2.2.3: Aminohydroxylations with adenine derivatives.[111]

Dress *et al.*, notes that the only regioisomer produced is where the N^6-adenine nitrogen is in the benzylic position (**161**, **163** & **165**). The R_1 substituent on the adenine derivatives has no effects on the regio- or diastereoselectivities of the reaction. The addition of $(DHQ)_2PHAL$ **136a** does not result in any particular asymmetric induction in the products.[111] Whilst this is a good nitrogen source for producing high regioselectivities, though these are not reported, the enantioselectivities are poor, and therefore other *N*-chloro-*N*-sodio salts are investigated, as follows.

2.2.2.2 *N*-chloro-*N*-sodio-carbamates

The use of carbamates in the Sharpless AA transformations of styrene (**166**) and 2-chlorostyrene (**168**) show that *tert*-butyl carbamate formation results in fair yields, but with high enantioselectivities for the resulting phenylglycinols (**167a-c**) and (**169a-c**).[115] *t*-Butyl carbamate is compared to ethyl carbamate and benzyl carbamate. The main difficulty seen with this reaction protocol is that careful column chromatography is needed to separate the regioisomers from the excess carbamate used in the reaction. The resultant *N*-Boc protected aminoalcohols **167a-c** and **169a-c** have the advantage that *N*-deprotection is usually straightforward (Table 2.2.4).[115]



Method (a) : 3:1 eq BnO_2CNH_2, 5 mol% ligand, 1:1 ^{n}PrOH-water, 0 °C, 1.5 - 4.5 h
Method (b) 3:1 eq EtO_2CNH_2, 5 mol% ligand, 1:1 ^{n}PrOH-water, 0 °C, 0.5 - 3.5 h
Method (c) 3:1 eq ^{t}BuO$_2$CNH$_2$, 5 mol% ligand, 1:1 ^{n}PrOH-water, 0 °C, 1 h

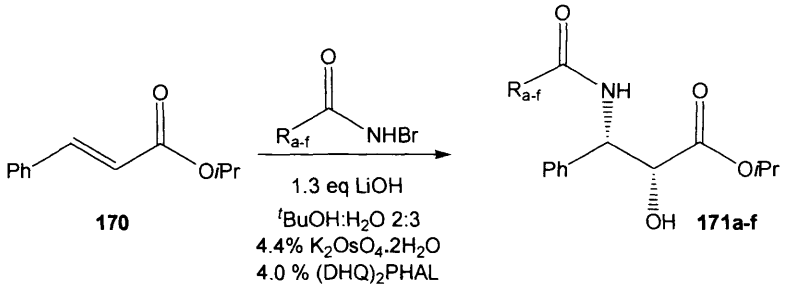
Ar	Alkene	Method	Ligand 136a or 136b	R	Product	Yield (%)	*Ee* (%)
Ph	**166**	a	$(DHQ)_2PHAL$ **136a**	Bn	(*S*)-**167a**	8	-
Ph	**166**	b	$(DHQD)_2PHAL$ **136b**	Et	(*R*)-**167b**	44	88
Ph	**166**	c	**136a**	^{t}Bu	(*S*)-**167c**	58	94
2-ClC_6H_4	**168**	a	**136b**	Bn	(*R*)-**169a**	0	-
2-ClC_6H_4	**168**	b	**136b**	Et	(*R*)-**169b**	44	84
2-ClC_6H_4	**168**	c	**136a**	^{t}Bu	(*S*)-**169c**	41	92

Table 2.2.4: AA of styrenes using Benzyl, Ethyl and *t*-Butyl Carbamates.[115]

O' Brien *et al.*, conclude from these results (Table 2.2.4) that the regioselectivity of the reaction is ligand dependent, with better regioselectivities and higher yields being obtained by using $(DHQ)_2PHAL$ **136a** rather than $(DHQD)_2PHAL$ **136b**. Yet, enantioselectivities are not ligand dependent for this nitrogen source with styrene precursors **166** and **168**. *N*-chloro-*N*-sodio salts can induce high level of asymmetric induction, where the size of the R group does not appear to have much effect. The next section shows carboxamide nitrogen sources, which usually form *N*-bromolithium salts and can act as both the oxidant and the nitrogen source in the Sharpless AA. This does not occur with *N*-halocarbamate salts, which require an external oxidant.[115]

2.2.2.3 Carboxamide nitrogen sources

Alternative nitrogen sources include the primary amides. In particular, *N*-bromoacetamide is the only commercially available nitrogen source, but Sharpless *et al.*, report the facile monobromination of a variety of primary amides and their subsequent use in the Sharpless AA transformation. This moves away from the use of *tert*-butyl hypochlorite, an oxidant used with *N*-halocarbamate nitrogen sources. The reason for this move is because *tert*-butyl hypocholorite is difficult to synthesize, difficult to store on a large scale and is photosensitive.[113] Instead, Gottardi reports the use of dibromoisocyanuric acid (DBI),[116] which proves to be an efficient brominating agent (90-99% conversion), allowing the synthesis of a multitude of amides. These amides subsequently act as nitrogen sources for the AA reaction with isopropyl cinnamate (**170**) and the results are summarized below in Table 2.2.5.



Entry	Bromoacetamide R_{a-f}	T (°C)	Conversion (%)	Yield 171a-f (%)	*Ee* (%)	Regio-selectivity
1	a) $CH_3CH_2CH_2$	4	96	94	95	21: 1
2	b) $ClCH_2$	10	86	75	95	23: 1
3	c) Bn	4	81	76	93	12: 1
4	d) C_6H_{12}	4	84	71	80	20: 1
5	e) Ph	4	50	38	77	2.0: 1
6	f) 4-(MeO)Ph	0	70	42	43	2.5: 1

Table 2.2.5: AA of isopropyl cinnamate **170** using various amides as the nitrogen source.[113]

Sharpless *et al.*, report that aliphatic amides (entries **1-2**) are good nitrogen sources with isopropyl cinnamate, resulting in >95% conversions to the corresponding β-hydroxyamides (**171a-b**). Steric hindrance by large amide nitrogen sources (entries **3** and **4**) has little effect on yields or enantioselectivities. Yet aromatic amides (entries **5** and **6** give low yields, regioselectivities and enantioselectivities.

In an investigation of the stereoelectronic effects of the AA reaction, Sharpless *et al.*, show that electron-deficient carboxamides react more slowly, but do not undergo Hoffman rearrangements. This means that higher temperatures could be used in the reaction. Unfortunately, the diol by-product contamination is considered a problem, as

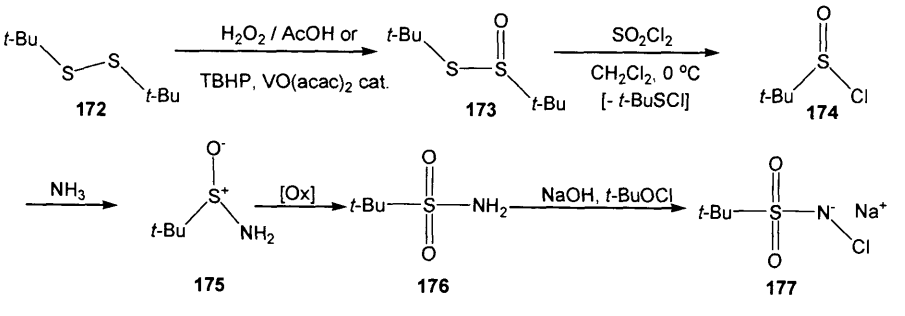
it is difficult to remove by column chromatography and becomes a major product when altering the chloro-group of the acetamide to more electron-deficient halogens. Electron-rich amides react faster, but decompose more readily and so lower temperatures are required to circumvent this problem. When isopropyl cinnamate **170** is used, products are obtained in high regioselectivities, probably due to favourable aromatic interactions in the binding pocket. Styrene and styrene derivatives, however, react well, giving high enantioselectivities, but poor to moderate regioselectivities. This highlights again that the AA reaction is partly dependent on the substrate type.[113]

Despite some of the problems encountered with this nitrogen source, its ease of removal, using thiourea, was considered an attractive option for accessing amino alcohols.[117]

The above studies of primary amides include aliphatic, cyclic and aromatic groups, whilst the next section shows the use of an aliphatic sulfonamide. This has different stereoelectronic properties to the above carboxamide and warrantes a separate discussion, as its synthesis is non-trivial.

2.2.2.4 Sulfonamides

The synthesis of *tert*-butylsulfonamide (Scheme 2.2.8), as an efficient nitrogen source and terminal oxidant for the catalytic AA of alkenes is now described. Usually, alkyl or aryl sulfonyl groups require harsh deprotection conditions,[118] but *tert*-butyl sulfonamide, which is difficult to synthesize (as the intermediates are explosive) is removed under fairly mild acidic conditions. This makes its use as an attractive reagent towards synthesising aminoalcohols.

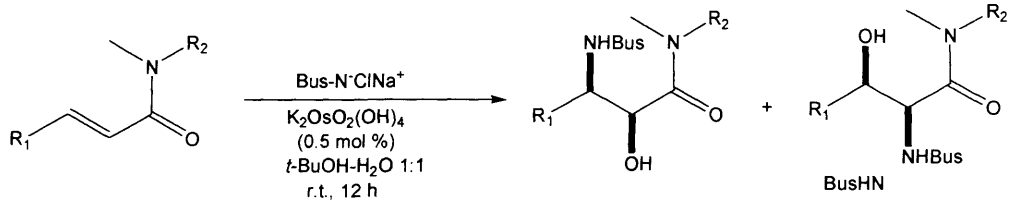


Scheme 2.2.8: Synthesis of sulfonamide **177**

Bis-tert-butyl disulfide (**172**) is reacted with hydrogen peroxide and is subsequently oxidized, forming compound (**173**). This is reacted with sulfonyl chloride and gives

tert-butylsulfinyl chloride (**174**), which when reacted with ammonia forms *tert*-butylsulfinylamine (**175**). Subsequent oxidation with a range of oxidants[114] lead directly to *tert*-butylsulfonamide (**176**), which is treated with *tert*-butyl hypochlorite and sodium hydroxide yielding the corresponding chloramine salt, (**177**).

Sharpless *et al.*, then uses nitrogen source **177** in an AA reaction involving α,β-unsaturated amides. It is noted that this class of compound has the highest turnover rates, chemoselectivities, and yields than any other alkene used with the Sharpless AA. The results of this study are summarized in Table 2.2.6.



A B

Entry	Starting Alkene, R_1 & R_2	Yield (%)	Regioisomer ratio A:B
1	R_1 = Ph, R_2 = OMe	93	3.3: 1
2	R_1 = H, R_2 = Me	84	A only
3	R_1 = Pr, R_2 = Me	91	2.5: 1
4	R_1 = *m*-Xyl, R_2 = Me	82	4.5: 1
5	R_1 = NO_2Ph, R_2 = Me	94	1.5: 1.

Table 2.2.6: AA of α,β-Unsaturated Amides[114]

The results show (Table 2.2.6) that all of the yields (82-94%), whether having electron-donating groups (entry **1-4**) or electron-withdrawing groups (entry **5**) are high. The regioselectivities of the reaction are moderate, except for where electron-withdrawing groups are used, (entry **5**) which is low.

All of the above reactions seen in this section are under homogeneous catalytic conditions. To improve the efficiency of this reaction, solid support catalysis is now considered in terms of chemical yield, regioselectivities and enantioselectivities. A variety of nitrogen sources are seen here, but the most attractive nitrogen source appears to be the carboxamides and is presently discussed.

2.2.3 Solid Support Catalysis

Heterogenization of AA transformations allows for the separation of chiral products, whilst using only small quantities of catalyst and thus presents an obvious industrial advantage.[119] At present, homogeneous reactions using osmium tetroxide on a large

scale are unfeasible due to its volatility and toxicity. Moreover, the use of osmium and chiral alkaloid ligands are uneconomical in large scale processes.[120,121] In an attempt to overcome these problems, the use of solid-supported catalysts for Sharpless AA has been considered by a number of research groups. The first example envisages the use of a silica gel-supported (SGS) *bis*-chinchona alkaloid (Figure 2.2.2), [SGS-$(QN)_2$PHAL (**178**)] to convert *trans*-cinnamate derivatives.[122]

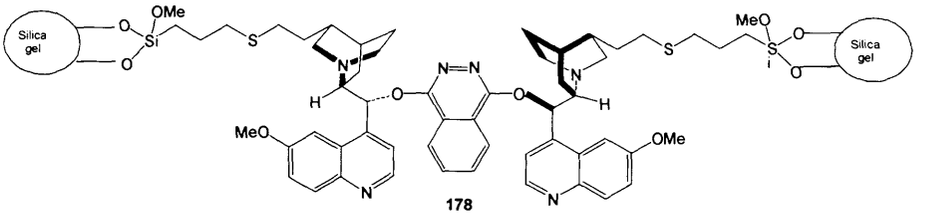
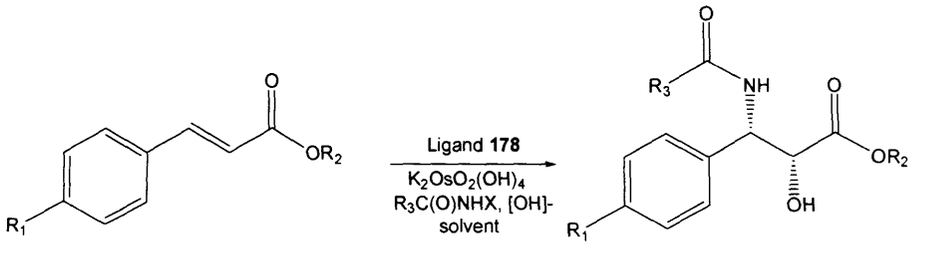


Figure 2.2.2: SGS *bis*-chinchona alkaloid **178**

When SGS-$(QN)_2$PHAL, **178** is used with osmium tetroxide, excellent enantioselectivities are observed in the asymmetric dihydroxylation (AD) of *trans*-cinnamate derivatives. When applied to the asymmetric aminohydroxylation (AA) transformation with the same cinnamate derivatives, using ethyl carbamate or acetamide and under homogeneous reaction conditions;[123,124] all with SGS-$(QN)_2$PHAL, **178**, excellent enantioselectivities and yields are observed. The results of these AA transformations are summarized in Table 2.2.7 for a variety of cinnamates.



170 R_1 = H, R_2 = *i*-Pr
180 R_1 = NO_2, R_2 = *i*-Pr
182 R_1 = H, R_2 = Me
184 R_1 = OMe, R_2 = Me
186 R_1 = NO_2, R_2 = Me

179 R_1 = H, R_2 = *i*-Pr, R_3 = Me
181 R_1 = NO_2, R_2 = *i*-Pr, R_3 = Me
183 R_1 = H, R_2 = Me, R_3 = OEt
185 R_1 = OMe, R_2 = Me, R_3 = OEt
187 R_1 = NO_2, R_2 = Me, R_3 = OEt

Table 2.2.7: Heterogeneous catalytic AA using SGS-$(QN)_2$PHAL **178**

Entry	Substrate	Oxidant	Solvent	t (h)	Product Yield (%)	*Ee* (%) (2*R*, 3*S*)
1	**170**	AcNBrLi	*t*-BuOH-H_2O	7	**179** (71)	>99
2	**180**	AcNBrLi	*t*-BuOH-H_2O	7	**181** (30)	>99
3	**182**	EtOCONClNa	*i*-PrOH-H_2O	12	**183** (40)	88
4	**184**	EtOCONClNa	*i*-PrOH-H_2O	12	**185** (43)	92

5	**186**	EtOCONClNa	CH_3CN-H_2O	12	**187** (52)	92

Song *et al.* note that the use of *N*-bromoacetamide as the nitrogen source (entries **1** and **2**) with cinnamate **170** gives amidoalcohol (**179**) in high yields and enantioselectivity. Electron withdrawing cinnamate (**180**) gives amidoalcohol (**181**) in low yield, but in high enantioselectivity. On changing to ethyl carbamate (entries **3-5**), cinnamates with different electronic properties (**182**), where R = H, gives *N*-protected aminoalcohol (**183**) in reasonable yield and high enantioselectivity. When electron-donating cinnamate (**184**) gives *N*-protected aminoalcohol (**185**), it is in a lower yield than **184**, which is suprising. Interestingly, methyl *p*-nitrocinnamate (**186**) gives *N*-protected aminoalcohol (**187**) in a higher yield than both **185** and **186**. Despite decreases in yields, all of the enantioselectivities are high.

The main advantage of this method is that the osmium complex **178** is recovered by filtration after each reaction. Unfortunately, when the catalyst was studied using XPS (X-Ray Photoelectron Spectroscopy), up to 50% of the catalyst has been lost to the reaction mixture. The repeated use of the catalyst has seen reduced yields, but similarly high enantioselectivities, making the low yields a limitation to its use in industry.[122]

A different approach to solid support catalysis involving osmium tetroxide (OsO_4) in an ion-exchange technique using (layered double hydroxide) LDH-supported OsO_4 is reported. LDH, a class of layered material consists of cationic $M(II)_{1-x}M(III)_x(OH)_2^{X+}$ and anionic $A^{n-}.zH_2O$ layers. $Mg_{1-x}Al_x(OH)_2(Cl)_x.zH_2O$ is synthesised and has been used in the Sharpless AA reaction using various substrates, resulting in moderate yields and enantioselectivities. These results are summarized below in Table 2.2.8.[119]

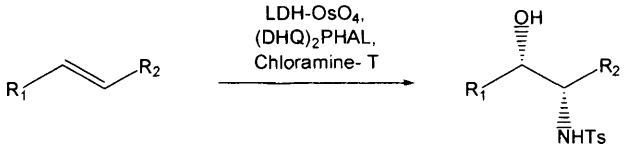
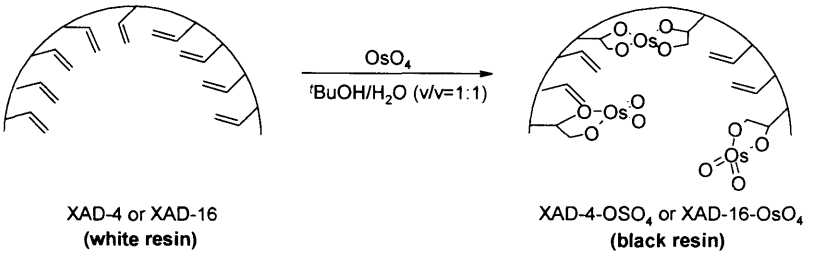


Table 2.2.8: AA of alkenes with LDH-OsO_4

Entry	Substrate	Isolated yield (%)	*Ee* (%)
1	R^1 = Ph, R^2 = CO_2Me	55	78
2	R^1 = CH_3, R^2 = CO_2Me	50	72
3	R^1 = Ph, R^2 = Ph	45	60
4	Cyclohexene	50	40

Unfortunately this method is not particularly successful (Table 2.2.8), mainly due to the diol-by-product being formed in significant quantities, thus lowering the isolated yield of the products (entries **1-4**). Furthermore, osmium leaching occurs. Choudary *et al.*, find that adding the oxidant slowly did not improve the ability to recycle this catalyst.[119]

Finally, Song *et al.*, show promising results using osmylated macroporous resins which they had previous used in the asymmetric dihydroxylation of alkenes. These are more effective than any of the solid-supports outlined above.[125] By immobilizing osmium tetroxide onto resins bearing vinyl groups such as Amberlite™ XAD-4, XAD-7 and XAD-16 (Scheme 2.2.9), osmium exists as both an Os(VI) monoglycolate and Os(IV)*bis*glycolate. This makes the osmium non-volatile, air stable and easier to handle compared with solid osmium tetroxide.[120]



Scheme 2.2.9: Osmylated macroporous resin

The main problem, that has hindered the development of all the solid-supports, is osmium leaching. This example, unfortunately, is no exception. Nevertheless, the catalyst is recovered easily by filtration, but when the osmylated resins are recovered, osmium leaching results in decreased turnover rates after three successive uses. The results are summarized in Table 2.2.9 where 1,2-diphenyl ethene **164**, styrene **166** and isopropyl cinnamate **170** are used as substrates.[120]

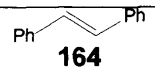
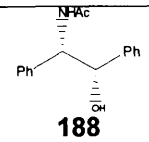
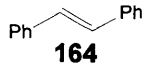
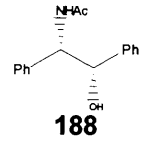
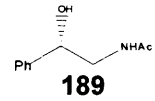
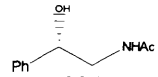
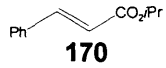
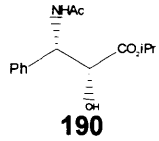
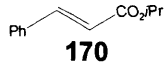
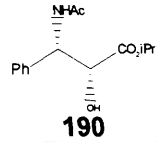
Entry	Resin-Os	Substrates	Products	Time (h)	Regio-selectivity	*Ee* (%)	Yield (%)
1	XAD-4	 **164**	 **188**	24	-	88	48
2	XAD-16	 **164**	 **188**	24	-	90	41
3	XAD-4	 **166**	 **189**	4	5.6:1	84	39
4	XAD-16	 **166**	 **189**	4	5.2:1	84	40
5	XAD-4	 **170**	 **190**	5	>20:1	>99	93
6	XAD-16	 **170**	 **190**	5	>20:1	>99	90

Table 2.2.9: Acetamide-based AA of various alkenes using XAD-4 or XAD-16-Os complex[a]

The results (Table 2.2.9), using various osmylated resins, works well with all of the substrates. 1,2-Diphenyl ethene **164** (entries **1** & **2**) is converted to amidoalcohol (**188**) in moderate yields, moderate regioselectivites and high enantioselectivities. Styrene **166** (entries **3** & **4**) give amidoalcohol (**189**) in moderate yields, but with high enantioselectivities. Isopropyl cinnamate **170** (entries **5** and **6**), however, performs the best and gives amidoalcohol (**190**) in excellent yields, regio- and enantioselectivities

Song *et al.*, hypothesise that the osmium anchored to the resins is highly exposed to the oxidant and as a result, it reacts more readily with the substrate and $(DHQ)_2PHAL$ **136a** in solution. Once the alkenes are consumed, the residual osmium is then trapped back on to the less accessible resin-bound vinyl groups. Unfortunately, osmium leaching is a problem in this reaction, but Song *et al.*, state that future optimisation of these reaction conditions should overcome this caveat, in what is otherwise, the most successful form of solid-support catalysis for the Sharpless AA transformation seen thus far.

[a] All reactions were performed on a 1 mmol scale using 4 mol% of Amberlte XAD-4.OsO_4 or Amberlite XAD-16.OsO_4, 5 mol% of $(DHQ)_2PHAL$, **136a** AcNHBr (1.1 mmol) and LiOH (1.02 mmol) in *t*-BuOH-H_2O (v/v = 1 : 1).

Results and Discussion

Chapter 3

3.1 Transketolase Donor Synthesis

This chapter describes the synthesis of the transketolase donor, shown in (Figure 3.1.1), hydroxypyruvate (**1**) and the methods used heading towards the synthesis of a series of novel nitrogen-containing pyruvate donors, 3-azidopyruvate **5a**, 3-aminopyruvate **5b**, 3-cyanopyruvate **5c** and 3-phthalimido (Phth-) pyruvate **5d**.

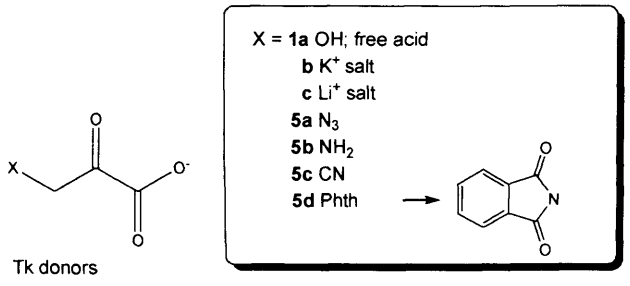


Figure 3.1.1: TK donors

3.1.1 Hydroxypyruvate (HPA)

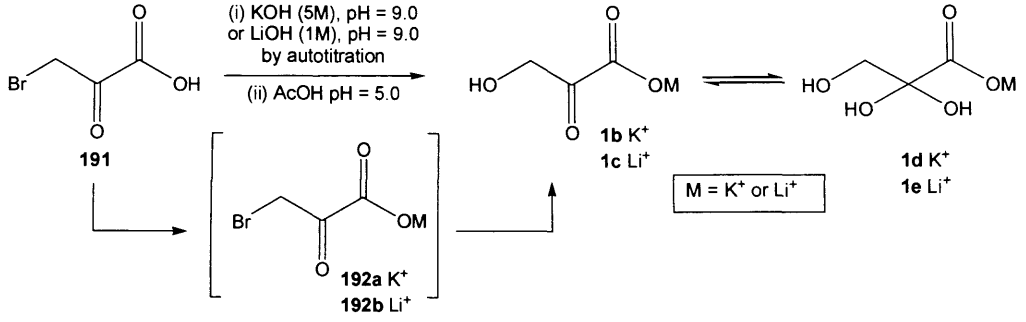
Hydroxypyruvate (HPA) **1** has been used in the past as a TK donor, but more recently, other donors including 3-halopyruvates have been tested for chemoselectivity with TK, as part of the collaboration between chemistry and biochemical engineering. Whilst TK accepts a range of substituted alkyl aldehydes, particularly α-hydroxylated aldehyde acceptors, the donor substrates are still limited to HPA-**1**. Previously, HPA (**1a-c**) has been employed in three forms in the TK biotransformations as:

1. Hydroxypyruvic acid HPA **1a**; This was obtained *via* an ion exchange column from the lithium salt **1c**.[57]
2. Potassium (K)-HPA (**1b**); This was obtained from the reaction of potassium hydroxide with bromopyruvic acid.[49]
3. Lithium (Li)-HPA **1c**; This was obtained from the reaction of lithium hydroxide with bromopyruvic acid.[54]

Whilst hydroxypyruvic acid HPA **1a** and Li-HPA **1c** are commercially available, the cost is high for large-scale investigaions (£36.80 per gram for hydroxypyruvic acid **1a** and £30.20 per gram for Li-HPA **1c**). Moreover, the commercial supply is not always reliable.[b] The K-HPA **1b** and Li-HPA **1c** salts are therefore prepared, as they could be readily converted to the free acid **1a** by ion exchange, if required.

We initially direct our attempts towards the synthesis of K-HPA **1b**, as better yields (36%; >95% purity) and solubilities (39g/L of reaction volume for K-HPA **1b** *cf* 18 g/L for lithium Li-HPA **1c**) are reported for K-HPA **1b**.[49]

Potassium hydroxide is added to bromopyruvic acid (**191**) and then reacts (Scheme 3.1.1) until a steady pH of 9 is obtained using an autotitrator. Subsequent addition of acetic acid to adjust the pH to 5.0 and removal of the aqueous layer at 50 °C by rotary evaporation should yield pure K-HPA **1b**. Yet, ^{1}H NMR spectroscopic and HPLC analyses show this protocol to be ineffective and low yielding. A large-scale (20-30 g) preparation of K-HPA **1b** leads to the formation of potassium bromopyruvate (**192a**), as identified by HPLC[c] (ret. time 9.1 min)[d] when compared with an authentic sample of K-HPA **1b** (ret. time (8.3 min).[e] ^{1}H NMR spectroscopic analysis also shows the tendancy of K-HPA **1b** to exist in equilibrium with the potassium *gem*-diol (**1d**). The methylene protons of **1b** resonate at δ 3.71 ppm and those of the potassium *gem*-diol at δ 4.61 ppm.



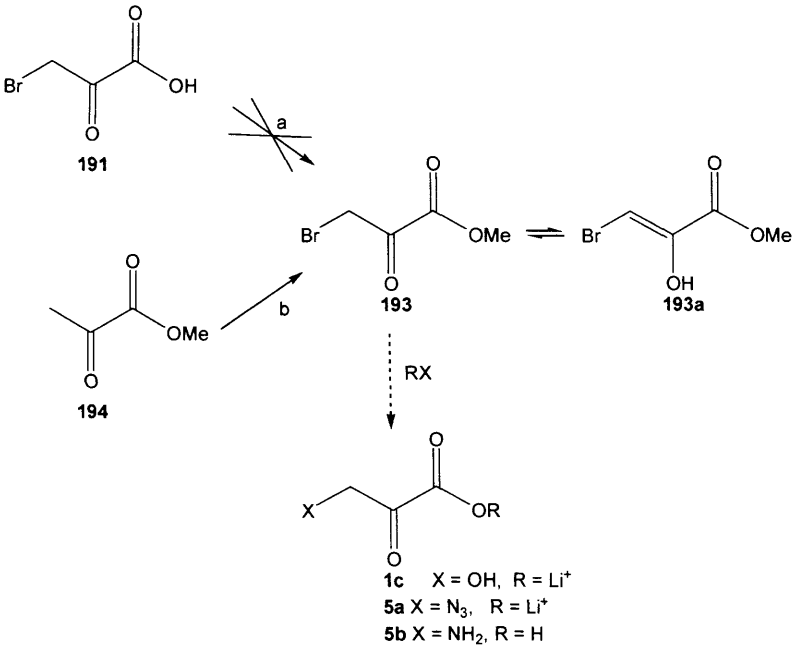
Scheme 3.1.1: Synthesis of hydroxypyruvate salt **1b** and **1c**

Despite the significant precedent of the use of Li-HPA **1c** in TK biotransformations, a protocol[f] for its synthesis is developed due to the lack of experimental details available.[49,55,56,57,62] This is attempted in our lab and the reaction is followed by HPLC.[a] After each hour, an aliquot of reaction mixture is removed and frozen. It is found that

[b] Fluka chemicals made this on a supply and demand basis only
[c] HPLC analysis courtesy of Sean Costello, Biochemical engineering
[d] Aminex 87H (300 x 7.8mm), 0.1%TFA (0.6mL min^{-1})
[e] Sample obtained from Fluka chemicals
[f] Dr M.E.B Smith (Postdoctorate within our collaboration)

after 3 h, there is no evidence of bromopyruvic acid **191** present, even though the autotitrator has not reached a steady pH of 9.0. Beyond 4 h, significant levels of lithium bromopyruvate (**192b**) are formed and so the optimal time is between 3-3.5 h maximum. Heat is considered as possibly aiding the formation of **192b** and accelerating decarboxylation. Hence, the aqueous solution is concentrated *in vacuo* using a high-vacuum Buchi® until crystals are seeded. The concentrated reaction mixture is left to crystallise overnight and crystals are collected by vacuum filtration, yielding lithium HPA **1c** in 30% yield and 95% purity. The lithium *gem*-diol version (**1e**) is also identified by ^{1}H NMR spectroscopic analysis. Further attempts to obtain crystals from the mother liquor are unsuccessful.

In an attempt to improve upon this protocol, it is envisaged that protection of the carboxyl group of bromopyruvic acid **191** by esterification could prevent decarboxylation and may also serve as a common precursor for the synthesis of other nitrogen-containing pyruvate donors, namely azidopyruvate (**5a**) and aminopyruvate (**5b**). This could be achieved by nucleophilic substitution (Scheme 3.1.2) and subsequent ester hydrolysis. Fischer esterification conditions are reported by Noguchi for acid catalysed esterification of **191**, but these fail to yield the methyl ester (**193**).[126] This is likely to be due to the increased rate of decarboxylation versus the formation of *gem*-diol **1d**.



Scheme 3.1.2: Reagents and conditions (a): $MeOH/H_2SO_4$, rt and reflux; (b) Br_2, N_2

Another pathway includes the reaction of methyl pyruvate (**194**) with molecular bromine at room temperature and yield **193** (and **193a**) in 77% yield and a 1:1 ratio, as shown by ^{1}H NMR spectroscopic analysis. Subsequent nucleophilic substitution with lithium hydroxide fails to yield Li-HPA **1c**. It is likely that the base sensitivity of this compound encourages polymerisation as a side reaction.

3.1.2 Azidopyruvate

Two main methods were explored for the synthesis of azidopyruvate **5a** (Scheme 3.1.2). These included nucleophilic substitution and oxidation of a secondary alcohol precursor. Azidopyruvate **5a** was a synthetic precursor to aminopyruvate **5b**, but could potentially be an interesting donor for TK and/or its mutants. Azide compounds are known to have rich reaction chemistry (Figure 3.1.2) and can give clean high yielding reactions usually with few side products.[127]

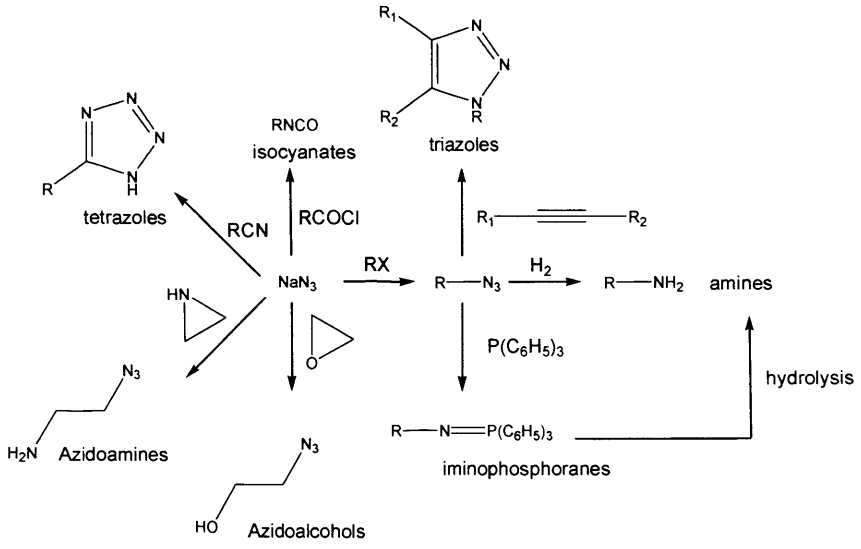
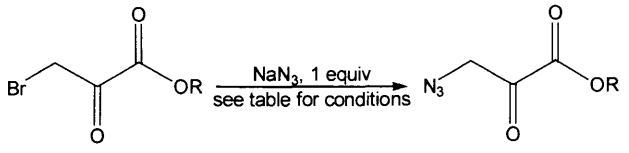


Figure 3.1.2: Azide reactions

3.1.2.1 Substitution methodology

The reactions between bromopyruvic acid **191** and its methyl ester **193** with sodium azide are initially explored in a variety of solvents and at different temperatures (Table 3.1.1).



191 R = H
193 R = Me

5a R = H
195 R = Me

Entry	Temperature (°C)	Solvent
1	-78	$EtOH/H_2O$
2	0	$EtOH/H_2O$
3	+4	$EtOH/H_2O$
4	Room temperature[1]	$EtOH/H_2O$
5	+4	DMF
6	Room temperature[1]	DMF
7	+4	DMF/CH_2Cl_2[2]
8	Room temperature[1]	DMF/CH_2Cl_2[2]
9	+ 4	MeOH
10	Room temperature[1]	MeOH

[1]Room temperature (20 °C), [2]CH_2Cl_2/DMF (1:1) v/v; [3]1 mmol scale

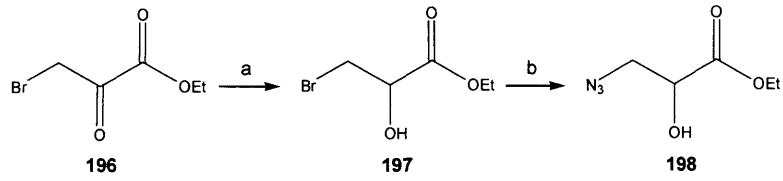
Table 3.1.1: Reaction conditions for attempted synthesis of azidopyruvate **5a**

Bromopyruvic acid **191** is subjected to sodium azide at a range of temperatures (-78 °C to room temperature, entries **1-4**). At these reaction temperatures and in ethanol/water (a common mixed solvent used for azide substitution,[128,129,130,131,132] no reaction is observed by TLC analysis. Mass spectrometry and ^1H NMR spectroscopic analyses are also used on both occasions, but no molecular ions or discernible product signals are observed from the crude reaction mixture. Using the ethanol/water mixture as a reaction solvent may cause formation of hydrazoic acid (HN_3). In all reactions (entries **1-4**), a polymerisation may have occurred due to the high molecular ion peaks [M^+ = 456] being observed. Ester **193**, is reacted under the same reaction conditions (entries **1-4**), but did not yield methyl azidopyruvate ester (**195**).

Attempts to investigate solvent effects on the reaction with bromopyruvic acid **191** or its methyl ester **193** are then conducted. *N,N'*-dimethylformamide (DMF) (entries **5** & **6**) and a mixture of DMF/CH_2Cl_2 solvents are used (entries **7** & **8**), as these solvents have also been used in azide substitution reactions.[133,134] Finally, methanol is used[135] (entries **9** & **10**), but unfortunately the change of solvent systems has no effect on the outcome of the reaction and no azidopyruvate **5a** or its methyl ester **195** is achieved under these reaction conditions.

Nucleophilic substitution is successful when the carbonyl centre of eythl bromopyruvate (**196**) (commercially available) is reduced to the bromohydroxy ethyl ester (**197**) in 65%

yield (Scheme 3.1.3). This is achieved using sodium cyanoborohydride in a methanol-hydrochloric acid mixture.[136]

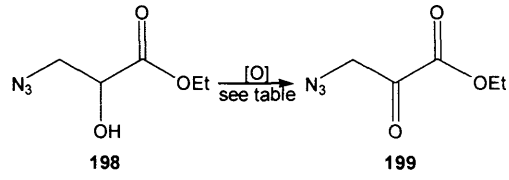


Scheme 3.1.3: Reagents and conditions (a) $NaCNBH_3$, MeOH-HCl, pH 4, bromocresol green, 65%; (b) NaN_3, H_2O/EtOH (1:1, v/v), reflux, 44%

A reduction is also attempted using sodium borohydride with **196**, but interestingly reduction of both carbonyl groups occurs. Bromohydroxy **197** is then treated with sodium azide to give the hydroxyazido ethyl ester (**198**) in 44% yield. This serves as a secondary alcohol precursor for the oxidation to an α-keto ester.

3.1.2.2 Oxidation of secondary alcohol precursors

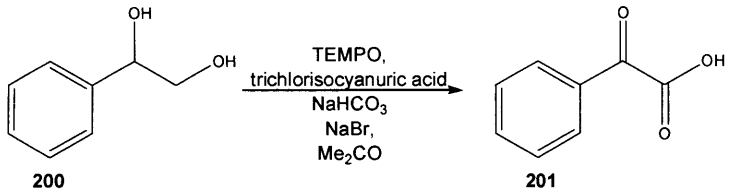
Hydroxyazido ethyl ester **198** is subjected to a number of suitable oxidation methods (Table 3.1.2) in the presence of the azide functionality, in an attempt to synthesise the azido α-keto ethyl ester (**199**).



Entry	Oxidants and conditions	Reaction outcome
1	TEMPO, trichloroisocyanuric acid, Me_2CO, $NaHCO_3$	NO REACTION
2	PCC, EtOAc, (rt and reflux)	NO REACTION
3	Swern: DMSO, Et_3N, Oxalyl chloride, CH_2Cl_2	NO REACTION
4	CrO_3, H_2SO_4, Me_2CO, 60 °C	NO REACTION

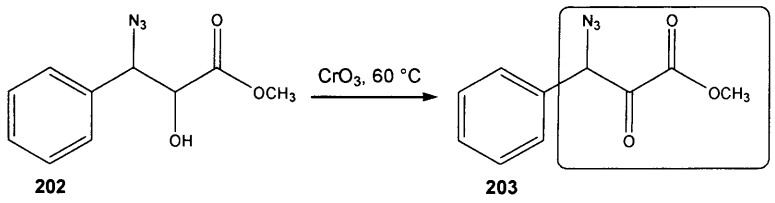
Table 3.1.2: Oxidations attempted with α-hydroxyester **198**

The first attempt (entry **1**) at this oxidation is with trichlorocyanuric acid/TEMPO under mild reaction conditions. This method was previously reported for the conversion (Scheme 3.1.4) of diol (**200**) into the α-keto acid (**201**).[137] No reaction is observed by TLC analysis and by ^1H NMR spectroscopic analysis.



Scheme 3.1.4: TEMPO Oxidation

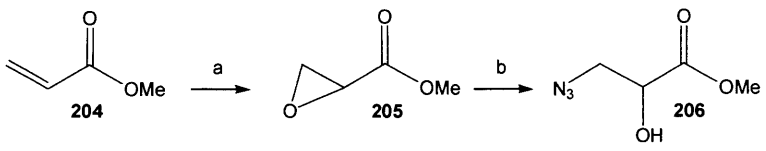
The use of a pyridinium chlorochromate (PCC) oxidation (entry **2**), known to convert α-hydroxyesters to α-ketoesters[138] is attempted, but no reaction is observed. Similarly, no reaction is observed when Swern[139] conditions (entry **3**) are used. Finally, Kayser *et al.*, report using chromic acid at 60 °C (Scheme 3.1.5) for converting α-hydroxyester (**202**) to α-ketoester (**203**), which has a similar carbon framework (highlighted) to yield the desired azidopyruvate ethyl ester **199**.



Scheme 3.1.5: Chromic acid oxidation

A reaction is observed by TLC analysis and a change of colour associated with Cr(VI) to Cr(III) reduction (orange to green) is also observed when we react hydroxyazido **199** under these conditions. ^{1}H NMR spectroscopic analysis also shows a singlet signal at δ 3.93 ppm, which is consistent with the methylene protons of **199**. Disappointingly, only one carbonyl group for the ethyl ester is observed in the ^{13}C NMR spectrum at δ 172 ppm. In order to simplify the number of signals in the ^{1}H NMR spectroscopic analysis, a change of the hydroxyazido ethyl ester **198** to the methyl ester is sought after, so that some of these oxidation protocols could be repeated.

As previously seen (Scheme 3.1.3), it is possible to obtain the ethyl ester **198** in 44% yield by a substitution method. Yet, a number of oxidation protocols followed are previously reported for reactions involving α-hydroxy methylesters to α-keto methylesters. Epoxidation of methyl acrylate (**204**)[140,141] and ring-opening reactions involves epoxide methyl ester (**205**) and are used to access hydroxyazido methyl ester (**206**) (Scheme 3.1.6).[142]



Scheme 3.1.6: Reagents and conditions (a) NaOCl (7%), $NaHCO_3$ (aq), CO_2(s), 13%; (b) NaN_3, MeOH, NH_4Cl, reflux, 56%

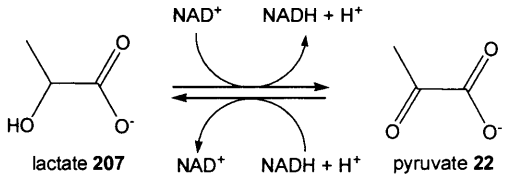
Methyl acrylate **204** is successfully converted to epoxide **205** using sodium hypochlorite and sodium bicarbonate, albeit in a low 13% yield. Methyl acrylate **204**, however, is cheap and the reaction is carried out on a large scale (20-25 g). Nucleophilic oxidants are normally required for the epoxidation of electron-deficient olefins and hydrogen peroxide with bases such as sodium hydroxide, sodium carbonate and potassium hydroxide are often used.[143] This protocol is favoured over a Jacobsen-type epoxidation,[144] which we also utilise (though on a smaller scale *ca* 1 g), mainly due to cost of the transition metal catalyst (manganese trimethyl 1,4,7-triazacyclononane). Despite being a high yielding reaction (85% yield), scale up reactions would be too costly and yields may not be as reliable.

The ring–opening of epoxide **205** (Scheme 3.1.6) is attempted using ceric ammonium nitrate (CAN) in *t*-butyl alcohol with sodium azide at 50 °C, as reported in the literature.[142] The yield of this reaction, however, is only 15% after purification. Therefore, a different reaction protocol is explored. Epoxide **205** is treated with sodium azide and ammonium chloride in methanol. The reaction mixture is heated at reflux. After purification by column chromatography, hydroxyazido methyl ester **206** is obtained in 56% yield.

Azido alcohol methyl ester **206** is subjected to Jones' reagent in sulphuric acid at 60 °C in acetone, but no reaction is observed by TLC analysis and no colour change occurs. It is likely that in the previous case, ethyl ester **199** may have contained a minor unknown impurity, which results in the signal being observed from the ^1H NMR spectroscopic analysis. Finally, a Dess-Martin periodinane reaction is attempted with **206** to yield the azidopyruvate methylester **195** (Table 3.1.1), but no reaction is observed by TLC analysis.[145] At this point an enzymatic oxidation method is explored to access **195**.

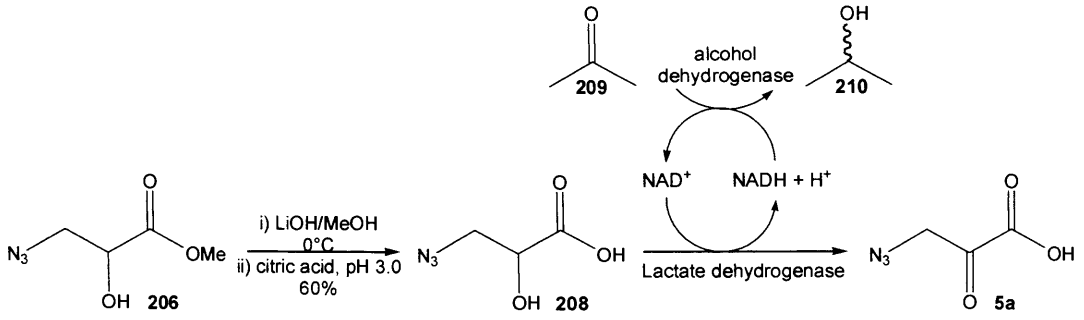
3.1.2.3 Biotransformation using a reversed equilibrium lactate dehydrogenase (LDH) with alcohol dehydrogenase

Lactate dehydrogenase (LDH) is a key enzyme used in energy metabolism which catalyses the reversible oxidation of lactate (**207**) to pyruvate **22** (Scheme 3.1.7), with the concomitant reduction of NAD^+. In muscles (when oxygen levels are low) pyruvate **22** from glycolysis is converted by LDH to lactate **207**. This is then secreted into the blood and transported to the liver where LDH converts lactate **207** back to pyruvate **22**. Pyruvate **22** is mainly converted back to glucose *via* gluconeogenesis. This cycle allows the muscle to keep producing energy (*via* glycolysis) under high load.



Scheme 3.1.7: Reversible oxidation of pyruvate to lactate

In an attempt to favour the oxidation equilibrium, a dual enzyme system is envisaged using lactate dehydrogenase and alcohol dehydrogenase in a one-pot biotransformation. Alcohol dehydrogenases are commonly used in biotransformations to reduce ketones to chiral alcohols and to regenerate NAD^+.[52] We attempt to set up a cofactor recycling system (Scheme 3.1.8), whereby NAD^+ could be regenerated. Ester hydrolysis of methyl ester **206** with lithium hydroxide affords azido α-hydroxyacid (**208**) in 60% yield. Oxidation of α-hydroxyacid **208** with lactate dehydrogenase LDH in a synthesis towards azidopyruvate **5a** is attempted.



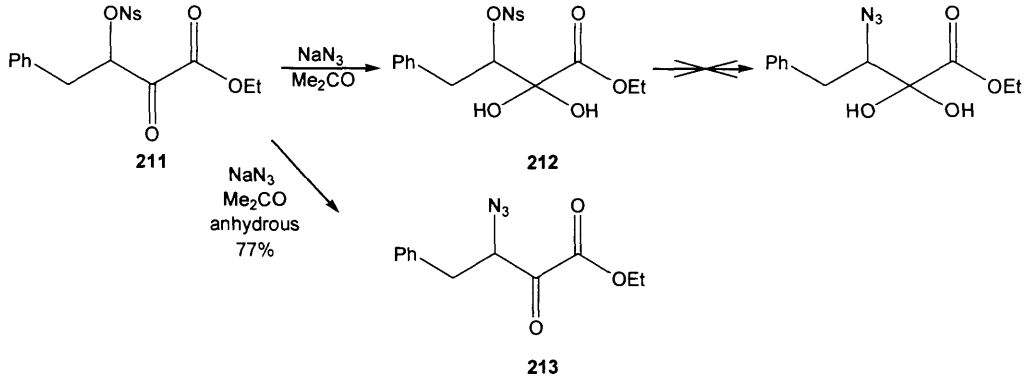
Scheme 3.1.8: Enzyme cofactor regeneration

3-Azido-2-hydroxy-propionic acid **208** in water/acetone at pH 7 (adjusted using sodium hydroxide) is added to a dehydrogenase mixture consisting of lactate dehydrogenase (LDH) (2.18 mg, 2000U), alcohol dehydrogenase (4.43 mg, 2000U) and NADH (20 mg) in tris(hydroxymethyl)aminomethane (TRIS) (50 mM, pH 7.0, 2 mL). The dehydrogenase mix is added to the stirred solution and the reaction mixture is

maintained at pH 7.0 using a pH stat autotitrator. Alcohol dehydrogenase is added to convert acetone (**209**) to isopropyl alcohol (**210**), whilst regenerating a source of NAD^+. After 3 h, a small aliquot was taken (5 mL) into ethyl acetate, dried and evaporated *in vacuo*. Unfortunately, ^1H NMR spectroscopic analysis shows the quantitative recovery of starting material **208**.

In order to explain these observations, an oxidation reaction is sought after from the literature with a similar carbon framework in mind. Hoffman *et al.*, describe the substitution of nosylate ester (**211**) with sodium azide, which results in the formation of the *gem*-diol (**212**), but no azide substitution occurs (Scheme 3.1.9) to give α-ketoester (**213**).[146]

Ns = *p*-nitrobenzenesulfonyl

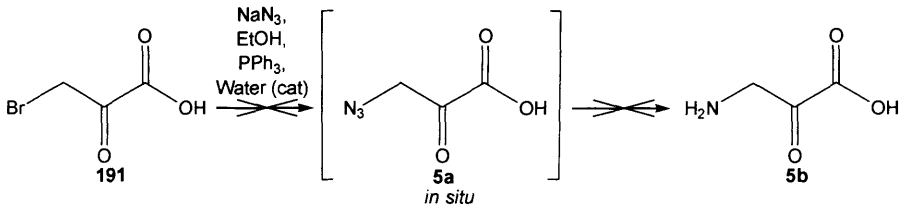


Scheme 3.1.9: Formation of *gem*-diol instead of azide substitution

Trace water present in the acetone solvent is believed to react faster at the carbonyl center than the sodium azide is able displace the nosylate group. When the reaction is repeated in dry acetone, Hofmann *et al.*, access the desired α-azido ketoester **213** in 77% yield. The inherent instability of **213** is noted and reaction times are critical. After 7 h, the extent of conversion of the starting material **211** to the product **213** versus decomposition pathways is optimal. Whereas, after 4 h, only half of **211** is converted to **213**, over 11 h complete consumption of the starting material **211** is observed. Evidence of the decomposition products (not described) after 11 h superseded the formation of **213**.[146] Hoffman's *et al.*, observations support the idea that reactions toward azidopyruvate **5a** and/or its esters **195** and **199** result in unknown polymerisation reactions.

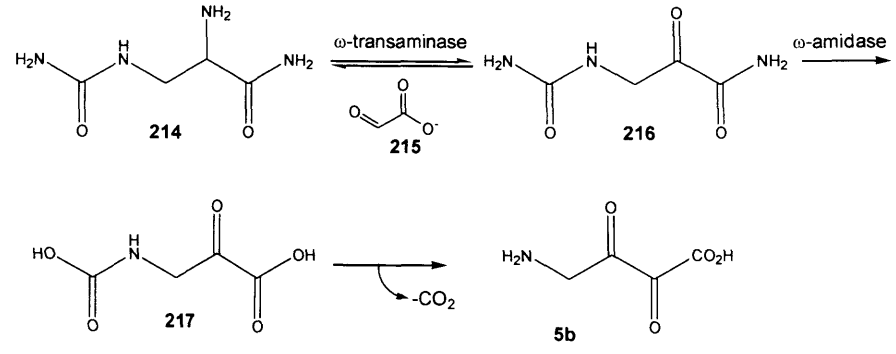
3.1.3 Aminopyruvate

Bromopyruvic acid **191** is treated with sodium azide in ethanol and then triphenylphosphine is added for an attempted *in situ* Staudinger reduction of 3-azidopyruvate **5a**. Although the reaction temperature increases, aminopyruvate **5b** is not observed (Scheme 3.1.10). It is likely that the rate of nucleophilic substitution and its polymerisation pathways occur more rapidly than the reduction of azidopyruvate **5a** to aminopyruvate **5b**.



Scheme 3.1.10: Attempted *in situ* Staudinger reduction of azidopyruvate **5a**

Different methods are explored to synthesise aminopyruvate **5b**. Cooper and Meister previously report its formation in an investigation of ω-transaminases.[147] A reaction (Scheme 3.1.11) between L-albizziin (L-α-amino-β-ureidopropionic acid) (**214**), a glutamine analogue and sodium glyoxylate (**215**) in the presence of ω-transaminases, amide (**216**) is formed. This is then treated with ω-amidase, but does not yield the expected product, β-*N*-carboxy derivative (**217**). Instead, a spontaneous decarboxylation reaction occurs resulting in the formation of aminopyruvate **5b**.[147]



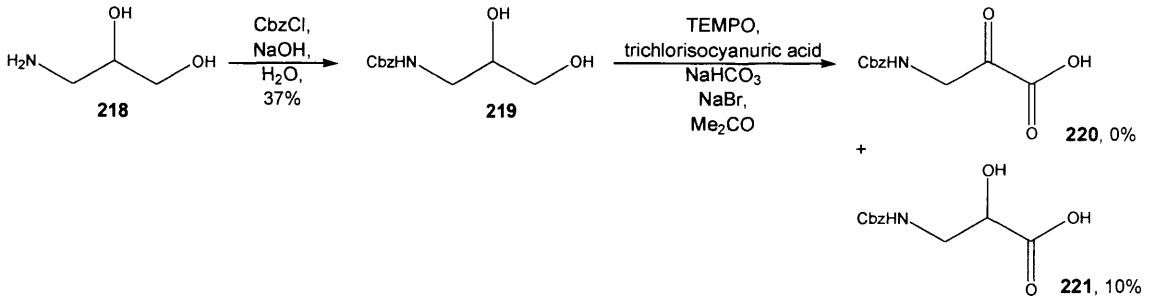
Scheme 3.1.11: Enzymatic route to aminopyruvate **5b**

Although no characterisation data is provided for **5b**, the following inference supports its formation. Cooper and Meister describe a number of control experiments, which show no reaction when selectively omitting any component. Treatment of albizziin **214** in the presence of pyruvate **22** with glutamine transaminase and ω-amidase form **5b** directly. This is supported by the fact that after incubation, the reaction mixture is

adjusted to pH 4.0 and is then treated with hydrogen peroxide (1M). An aliquot is then subjected to paper chromatography which shows the formation of glycine (the expected product between aminopyruvate **5b** and hydrogen peroxide), but no glycine is formed in control studies with reaction mixtures lacking the ω-amidase.[147]

3.1.3.1 Oxidation of secondary alcohols

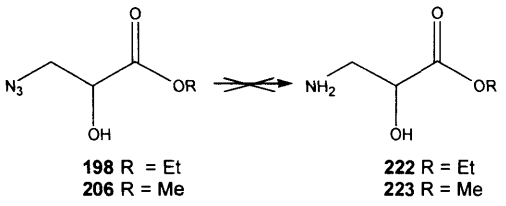
3-Amino-1,2-propane diol (**218**) is reacted (Scheme 3.1.12) with benzyl carbamate to give *N*-Cbz-protected (**219**) in 37% yield. An attempt to utilise TEMPO oxidation, known to convert a similar 1,2 propane diol directly to the α-keto ester, is utilised (Scheme 3.1.4) in attempt to obtain α-ketoester (**220**), but only provides carboxylic acid (**221**) in 10% yield.



Scheme 3.1.12: TEMPO Oxidation

This route is abandoned and the oxidation of α-hydroxy acid **221** is not attempted due to the low 10 % yield obtained.

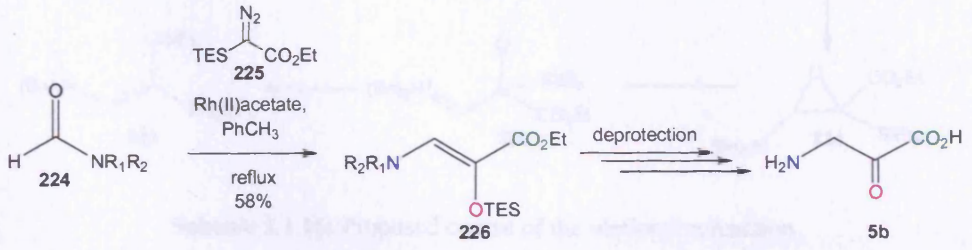
In an attempt to utilise hydroxyazido ethyl ester **198** and methyl ester **206** further, a Staudinger reduction (Scheme 3.1.13) followed by hydrogenation over a palladium/carbon catalyst is attempted. Unfortunately, the Staudinger reduction is unsuccessful and aminohydroxy ethyl ester (**222**) and methyl ester (**223**), for oxidation to aminopyruvate **5a** remain inaccessible. This means a different method is sought, one capable of protecting the highly reactive functional groups in aminopyruvate **5b**, but also allowing ease of their removal.



Scheme 3.1.13: Staudinger reduction of azide using triphenylphosphine

3.1.3.2 Carbene method towards aminopyruvate

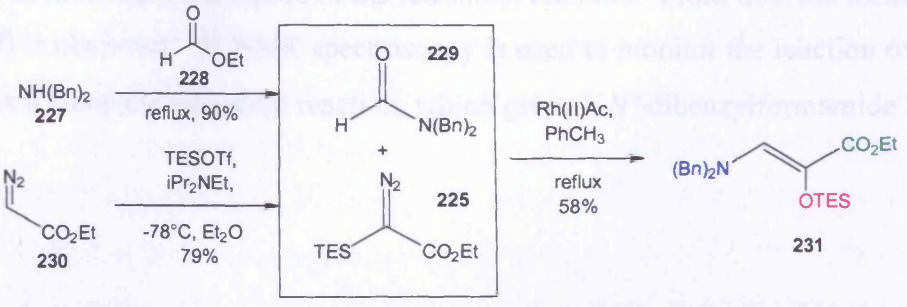
A method for protecting all the reactive functional groups is provided by Nandra *et al.*, after their reactions (Scheme 3.1.14) using rhodium(II)-catalysed olefination of tertiary formamides (**224**) with a silylated diazoester (**225**). These provide vinylogous carbamates (**226**) instead of anticipated ring expanded products.[148]



Scheme 3.1.14: Conversion of formamides to vinylogous carbamates

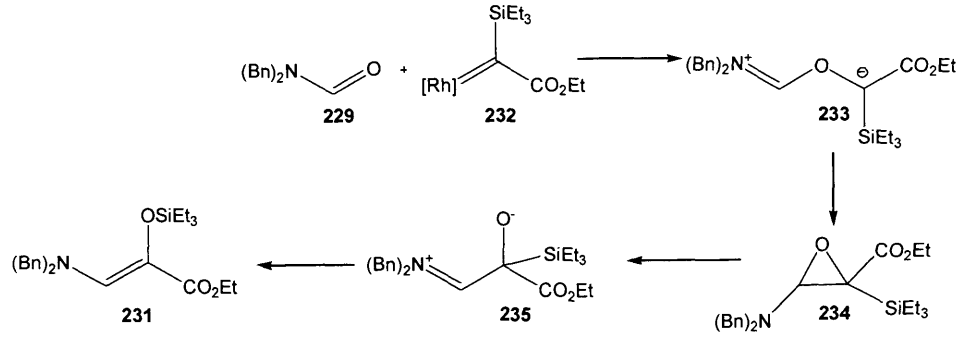
As seen (Scheme 3.1.14), the vinylogous carbamate **226** is essentially a "protected" 3-aminopyruvate **5b**. By selective deprotection of the TES group, favourable ketol-enol tautomerisation equilibrium should favour the α-keto functionality, seen in **5b**. The deprotection (R_1R_2) on the nitrogen atom of **226**, should then allow access to the amino functionality in **5b**. Finally, hydrolysis of the ester **226** should provide the carboxylic acid functionality seen in **5b**.

Using this approach (Scheme 3.1.15), dibenzylamine (**227**) is reacted with ethyl formate (**228**) and heated at reflux to yield *N,N'*-dibenzyl formamide (**229**) in an excellent 90% yield. Diazoester (**230**) is treated with triethylsilyl triflate and *N,N'*-diisopropylethylamine at –78 °C to give the silylated diazoester **225** in 79% yield. Silylated diazoester **225** is reacted with *N,N'*-dibenzyl formamide **229** in the presence of rhodium(II) acetate, heating at reflux. This gives the vinylogous carbamate (**231**) in 58% yield.[148]



Scheme 3.1.15: Synthesis of vinylogous carbamate **231**

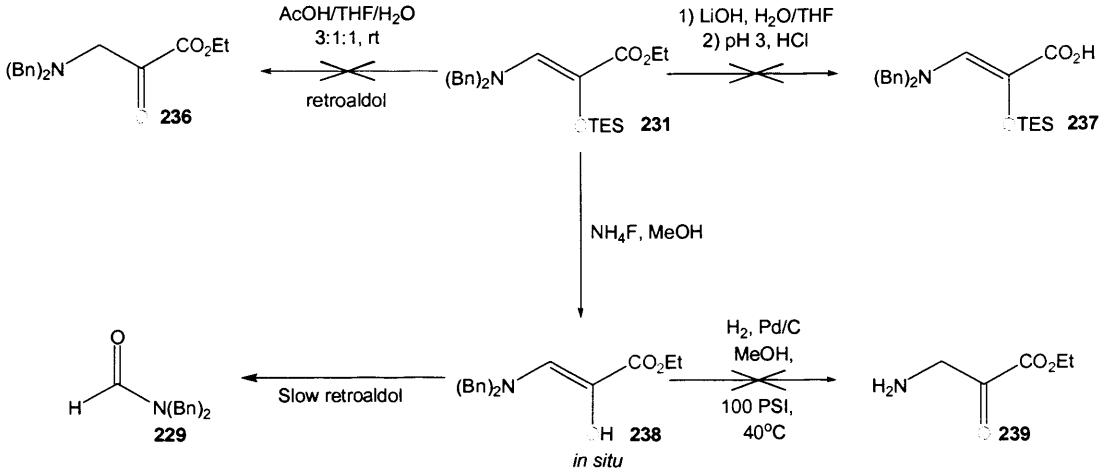
The mechanism for the formation of vinylogous carbamate **231** is postulated by Nandra *et al.*, and is seen below (Scheme 3.1.16).



Scheme 3.1.16: Proposed course of the olefination reaction

Reaction of diazo species **225** with the metal catalyst formed a rhodium carbene species (**232**), which is transferred the carbene moiety to the carbonyl group of **229**. This gives the carbonyl ylid (**233**). An intramolecular reaction forms epoxide (**234**), which is ring-opened to give zwitteriton (**235**). Finally the silyl group migrates from carbon to oxygen to give the vinylogous carbamate **231**.[148]

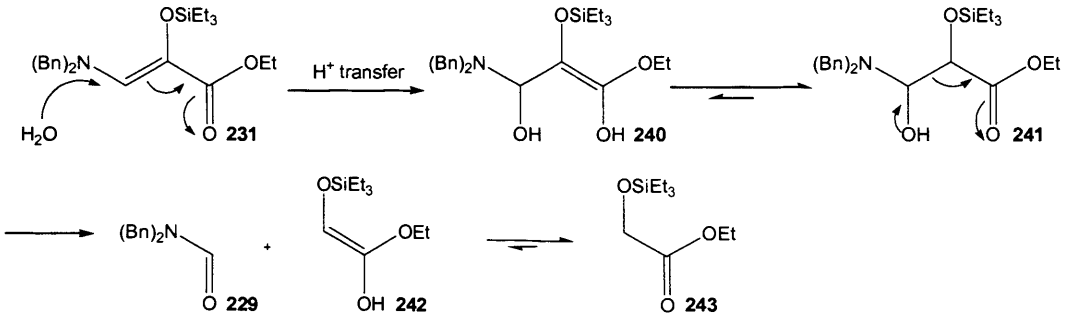
In our reactions (Scheme 3.1.17), we attempt the removal of the triethylsilyl group from **231** using acetic acid in a water-THF mixture to obtain the α-ketoester (**236**). Instead of forming **236**, however, a retroaldol reaction forms *N,N'*-dibenzylformamide **229**. This is inferred from the ^{1}H NMR spectroscopic analysis, which shows a singlet signal at δ 8.42 ppm. Similarly, an attempted ethyl ester hydrolysis (lithium hydroxide in a THF/water mixture)[149] does not give the enolic acid (**237**), but also results in the retroaldol reaction and gives *N,N'*-dibenzylformamide **229**. This is also confirmed by ^{1}H NMR spectroscopic analysis. In a method, which involves neither acid nor base, ^{1}H NMR spectroscopic experiments are conducted using tetrabutylammonium fluoride in methanol to investigate the nature of the retroaldol reaction. From this, the formation of enol (**238**) is observed. ^{1}H NMR spectroscopy is used to monitor the reaction over 24 h and shows a complete retroaldol reaction, which gives *N,N'*-dibenzylformamide **229**.



Scheme 3.1.17: Deprotection strategies towards aminopyruvate **5b**

In an attempt to suppress this retroaldol reaction, by changing the stereoelectronic properties of **238**, removal of the dibenzyl groups by hydrogenation (Scheme 3.1.17), under atmospheric pressure and using 100-psi pressure at 40 °C is attempted. This would have given aminopyruvate ethyl ester (**239**), but no reaction results with either palladium/carbon catalyst or platinum oxide.[150,151]

A mechanism for the retroaldol reaction in aqueous media is shown (Scheme 3.1.18).



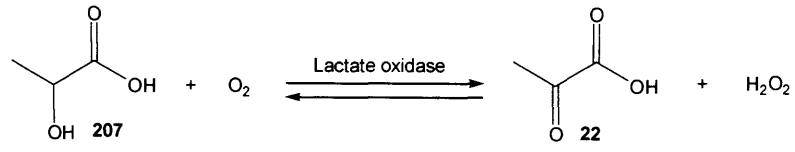
Scheme 3.1.18: Mechanism of retroaldol reaction

The retroaldol reaction occurs in both acid and alkali conditions and seems plausible that the nucleophilic addition of water on the vinylogous carbamate **231** allows the formation of species (**240**). It is possible that only catalytic amounts of water are required to initiate the retroaldol, which would also explain when tetrabutylammonium fluoride (TBAF) was used in methanol, the same retroaldol occurring more slowly. Subsequent keto-enol tautomerisation favour formation of species (**241**). The retroaldol reaction yields formamide **229** and keto-enol tautomerisation of enol (**242**) gives ester (**243**).

3.1.3.3 Biotransformations with lactate oxidase (LAO) and pyruvate oxidase (PAO)

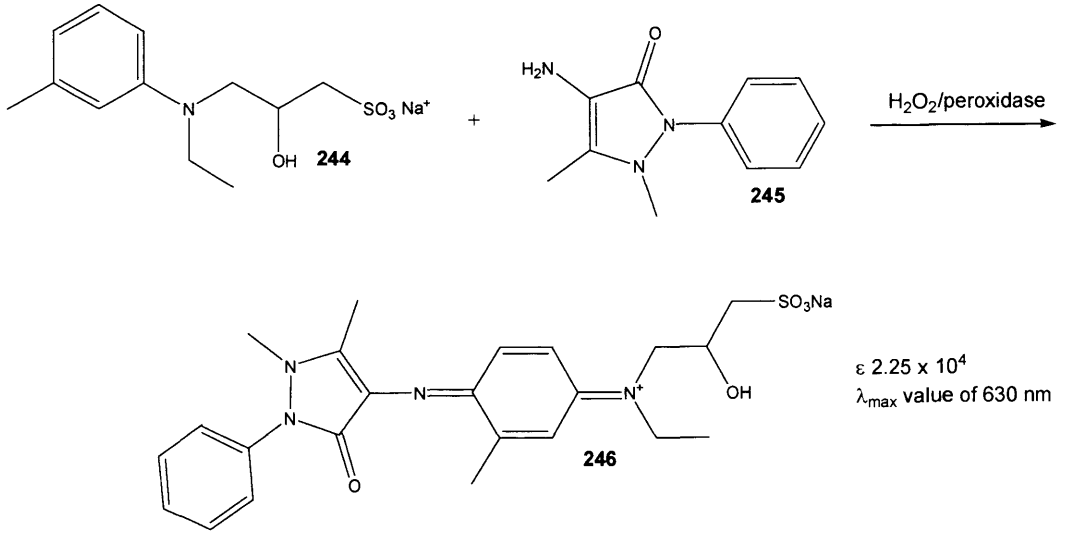
A previous attempt to utilise lactate dehydrogenase and alcohol dehydrogenase (section 3.1.2.3) to regenerate NAD^+ to access azidopyruvate **5a** is unsuccessful. It is considered that such enzyme systems are too complicated, as two substrates, two products, and the coenzyme (NAD^+, which is expensive) are required for the recycling reaction.[152]

The use of lactate oxidase is deemed more useful on a preparative scale as it may directly convert α-hydroxy acid lactate **207** to α-ketoacid pyruvate **22** in one step. Furthermore, a by-product of the lactate oxidase (from *Lactococcus* and *Aeriococcus*) biotransformation of L-lactate **207** (a much cheaper precursor) to pyruvate **22** is hydrogen peroxide (Scheme 3.1.19).[153] Studies on *Streptococcus pyogenes* show the involvement of lactate oxidase with lactate and coupling with aerobic utilisation, the generation of copious amounts of hydrogen peroxide, useful for a colorimetric screen.



Scheme 3.1.19: Reversible lactate oxidase equilibrium

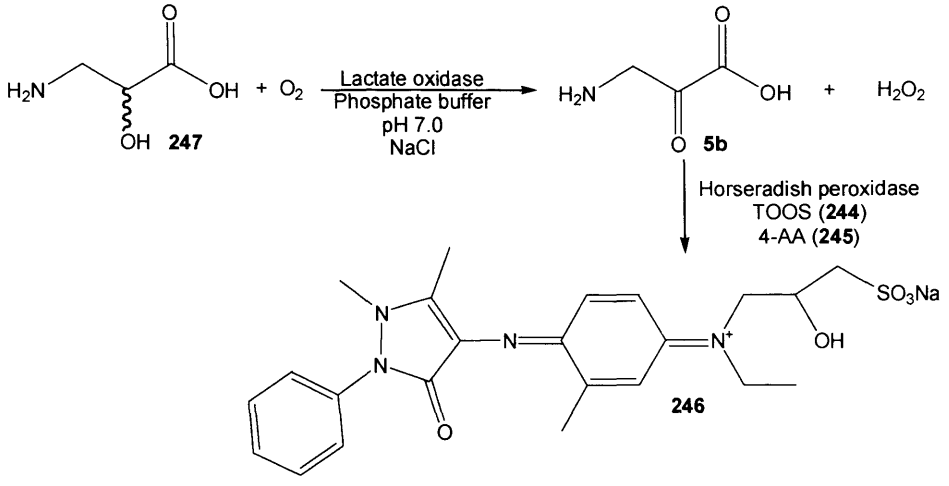
Hydrogen peroxide is deemed deleterious to the formation of pyruvate **22** (Scheme 3.1.19), as it results in breakdown products, acetate, carbon dioxide and water. Despite this, generation of an assay method using hydrogen peroxide as a colorimetric marker for the formation of pyruvate **22** is known, using a Trinder reagent, *N*-Ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline sodium salt dihydrate (TOOS) (**244**) with horseradish peroxidase and 4-aminoantipyrine (4-AA) (**245**). The oxidised condensation product (**246**) results from this oxidative coupling (Scheme 3.1.20).[154]



Scheme 3.1.20: Oxidative coupling of **244** and **245**

The amount of hydrogen peroxide formed corresponds to the substrate concentration and hence, the amount of substrate converted can be determined by the colour development of this oxidative coupling reaction.

We utilise this protocol in an attempt to bioconvert DL-isoserine (**247**) with lactate oxidase, initially and then with pyruvate oxidase using the colorimetric assay *vide supra* to give aminopyruvate **5b** (Scheme 3.1.21).



Scheme 3.1.21: Colorimetric assaying method for potential aminopyruvate formation

A 56-microwell-plate assay format was developed and tested with both lactate oxidase and pyruvate oxidase with DL-isoserine **247**. The results of these biotransformations are shown below (Figure 3.1.3).

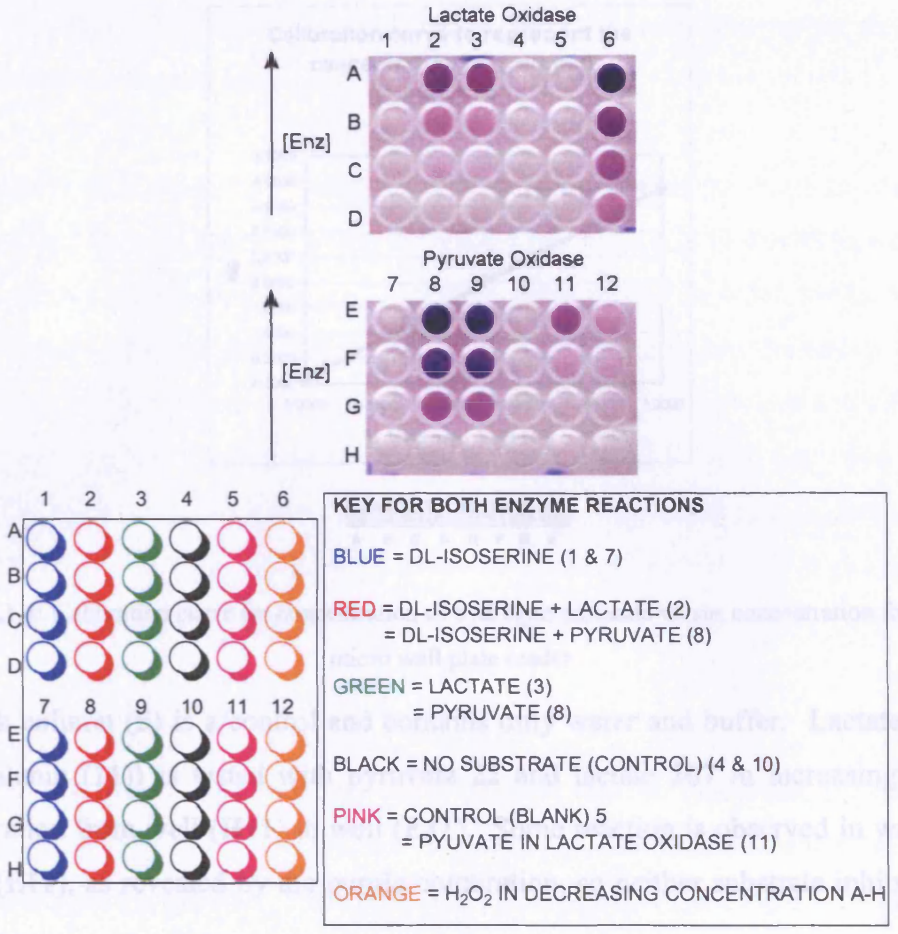


Figure 3.1.3: 56 microwell plate format with DL-isoserine **247**, lactate **207** and pyruvate **22** substrates with lactate oxidase and pyruvate oxidase, respectively

Each microwell contains horseradish peroxidase 8 U/ml, with TOOS **245** (0.4 mg/ml) and 4-AA **245** (0.27 mg/mL), which is responsible for not only providing a binary response as to whether any of the substrates are bioconverted, but could give a quantitative measure of substrate concentration using a microwell plate reader. Cofactors considered important to lactate and pyruvate oxidase experiments are added to the wells, where appropriate. These include TPP (0.6 mM), flavin adenine dinucleotide (FADH) (0.02 mM) and magnesium chloride (5 mM).

Reading from right to left (Figure 3.1.3), the orange columns **6** and **12** show the increase of hydrogen peroxide from 0 to 1 mM and provide a calibration curve (Figure 3.1.4). The sensitivity of the colorimetric assay to the concentration of hydrogen peroxide (H_2O_2) is as low as 0.03 mM.

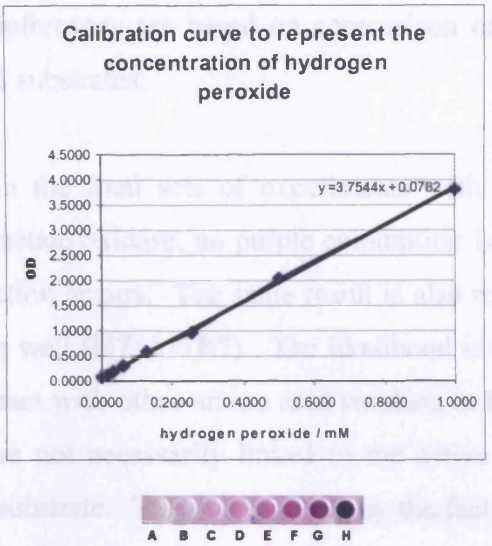


Figure 3.1.4: Calibration curve for concentration of hydrogen peroxide versus concentration found from micro well plate reader

The pink column (**5**) is a control and contains only water and buffer. Lactate oxidase (pink column (**11**)) is tested with pyruvate **22** and lactate **207** in increasing enzyme concentration from well (**H11**) to well (**E11**). Some reaction is observed in well (**F11**) to well (**E11**), as revealed by the purple colouration, so neither substrate inhibit lactate oxidase.

Negative controls, for lactate oxidase (black column (**4**)) and pyruvate oxidase (black column (**10**)) have no substrates added, and as expected no colours develop.

Lactate oxidase in decreasing concentration (green column (**3**)) (2 to 0 U mL^{-1}) with lactate **207** in well (**A3**) to well (**D3**) and pyruvate oxidase with pyruvate **22** in decreasing concentration (2 to 0 U ml^{-1}) in wells (**E3** to **F3**) are tested. Lactate oxidase with lactate **207** is successfully bioconverted to pyruvatem, as shown by the increasing purple colouration produced by the colorimetric screen, seen in well (**C3**) to well (**A3**), with the increasing concentration of lactate oxidase. Pyruvate **22** is also successfully converted to acetate with increasing concentrations of pyruvate oxidase (0 U/mL) to (2U/ mL) as seen by the increasing purple colouration in well (**H9**) to well (**E9**).

As DL-isoserine **247** (the desired test substrate for conversion to aminopyruvate **5a**) has no inhibitory effects on the natural substrates, lactate **207** with lactate oxidase and pyruvate **22** with pyruvate oxidase, DL-isoserine **247** is added. Red columns **2** and **8**

indicate this and these inferences are based on comparison of the purple colouration observed with the natural substrates.

Unfortunately though, in the final sets of experiments with DL-isoserine **247** (blue columns **1** and **7**) with lactate oxidase, no purple colouration is observed in wells (**D1**) to (**A1**). Hence, no reaction occurs. The same result is also recorded for DL-isoserine with pyruvate oxidase in well (**H7**) to (**E7**). The likelihood is that the amino group of DL-isoserine **247** may react with other amino acid residues in both lactate oxidase and pyruvate oxidase that are not necessarily linked to the active site, rendering the DL-isoserine as an inactive substrate. This is supported by the fact that it displays no clear inhibitory behaviour with the natural substrates of these enzymes. If it did act as an inhibitor, it would have to be in the active site to compete with the natural substrates.

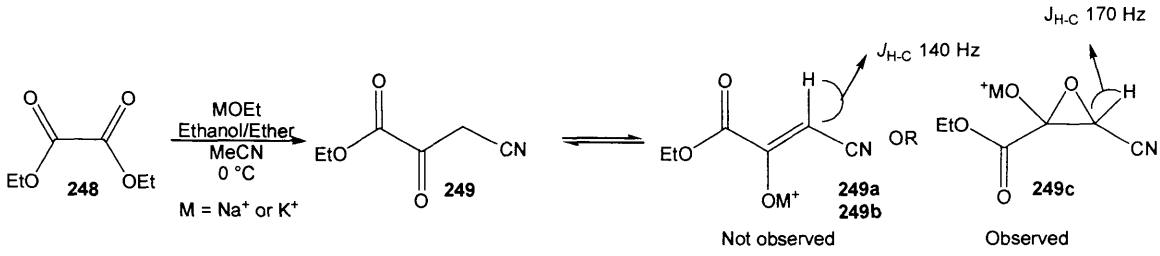
3.1.4 Cyanopyruvate

A literature procedure[155] in which an ethanolic/ether mixture of sodium is treated with diethyl oxalate (**248**) at 0 °C in the presence of acetonitrile is adopted and the precipitated solid is collected. This is thought to be cyanopyruvate ethyl ester (**249**), but ^{1}H NMR spectroscopic analysis shows an enolic signal at δ 4.89 ppm, which might be attributed to the sodium enolate (**249a**) (Scheme 3.1.22). Deuterium exchange shows how labile the enolic proton is, as after 3 h, the signal is no longer visible.

Under slightly different reaction conditions (Scheme 3.1.22) using potassium ethoxide, instead of potassium or sodium metals in ethanol, an almost quantitative yield results for what is thought to be cyanopyruvate ethyl ester **249**. ^{1}H NMR spectroscopic analysis in d^6-DMSO (to prevent deuterium exchange), however, indicates presence of the potassium enolate (**249b**) by a signal at δ 4.19 ppm with 1H integration. The main problem is that the coupling constant $J_{H\text{-}C}$ expected is 140 Hz. Instead, the observed value for $J_{H\text{-}C}$ is 170 Hz, which is more consistent with potassium epoxide structure (**249c**).

An ethanolic contaminant is present in the ^{1}H NMR spectrum of **249** and by diffusion ^{1}H NMR spectroscopic analysis is found to be a lower molecular weight than **249**. The chief concern is that enol **249b** or potassium epoxide **249c** (Scheme 3.1.11) did not correlate with the ^{13}C NMR analysis. This shows all of the key signals, including the cyano group (*C*N) at δ 125 ppm, methyl signals at δ 18 ppm (*C*H$_3$) and ethyl signals at δ

56 ppm (OCH_2), but shows the 1,2-dicarbonyl signals at δ 166 and δ 170 ppm. These are more akin to the desired cyanopyruvate ethyl ester **249**. No enolic carbon signals are observed for structures **249a-b** and no epoxide carbon signals are observed for structure **249c**.



Scheme 3.1.22: ^{1}H NMR analysis of **249**

Whilst uncertainty about the keto-enol form remains, some attempts are made to trap the enol by alkylation with methyl iodide. Unfortunately, these experiments are unsuccessful, thus lending little support to the formation of the enolic structure **249b** (Scheme 3.1.22).

After this, hydrolysis of the ethyl ester **249** is attempted, first with lithium hydroxide, which by ^{1}H NMR and ^{13}C NMR spectroscopic analyses shows the complete degradation of **249**. Then, a milder form of hydrolysis with an esterase is considered. Two experiments are performed; one as a control, only containing phosphate buffer and the substrate **249**. The other contains the phosphate buffer, esterase and the substrate **249**. Interestingly, hydrolysis of the ethyl ester occurs in both and the ethanolic contaminant is still present. From this observation and previous diffusion ^{1}H NMR experiments, it is realised that some potassium ethoxide is present in the sample of ethyl ester **249**.

In order to overcome this oversight, reverse-phase column chromatography is employed to purify **249**. After this, *ab initio* modelling[g] of the carbon positions are compared with the carbon-proton coupled ^{1}H NMR and ^{13}C NMR spectra and show that the cyanopyruvate ethyl ester exists as a carbanionic potassium salt (**249e**) at the methylene position (Figure 3.1.5), as is described in the literature.[156]

[g] Courtesy of Dr. Abil Aliev.

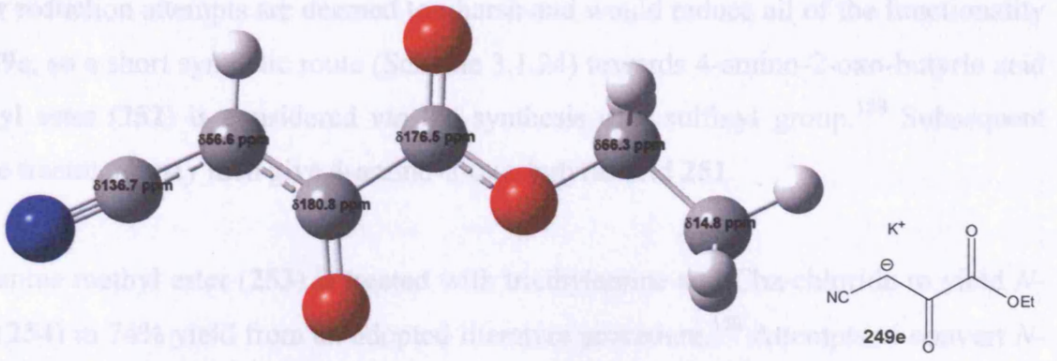
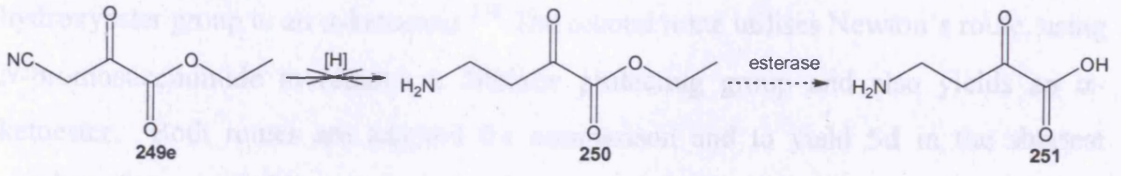


Figure 3.1.5: Cyanopyruvate ethyl ester carbanionic salt **249e** and its ^{13}C NMR predicted peaks

Attempts to hydrolyse ester **249e** are attempted and appear successful, as the ethyl groups by ^{1}H NMR and ^{13}CNMR spectroscopic analyses, are no longer visible. New proton signals for cyanopyruvic acid **5c** at δ 3.20 ppm suggest possible inequivalence of the methylene group in **5c**, which is exemplified by a roofing effect of two doublets (*J* 7.0 Hz). The reason for this inequivalence remains unclear. Attempts to remove trace phosphate buffers in **5c** by reverse phase chromatography fail and so yields for the formation of 3-cyanopyruvic acid **5c** cannot be determined accurately. The presence of trace phosphate in **5c** is identified using ^{31}P NMR spectroscopic analysis and mass spectrometry further aid our analyses of this compound.

To make further use of the cyano functional group in **249e** (Scheme 3.1.23), the possibility of its reduction to an amino group is realised. If this is successful, then esterase hydrolysis could be used on the resultant 4-amino-2-oxo-butyric acid ethyl ester (**250**) and would give 4-amino-2-oxo-butyric acid (**251**), already known from the literature.[157] 4-Amino-2-oxo-butyric acid **251** would have an additional methylene group compared to aminopyruvate **5b** and may act as a potential TK donor itself.

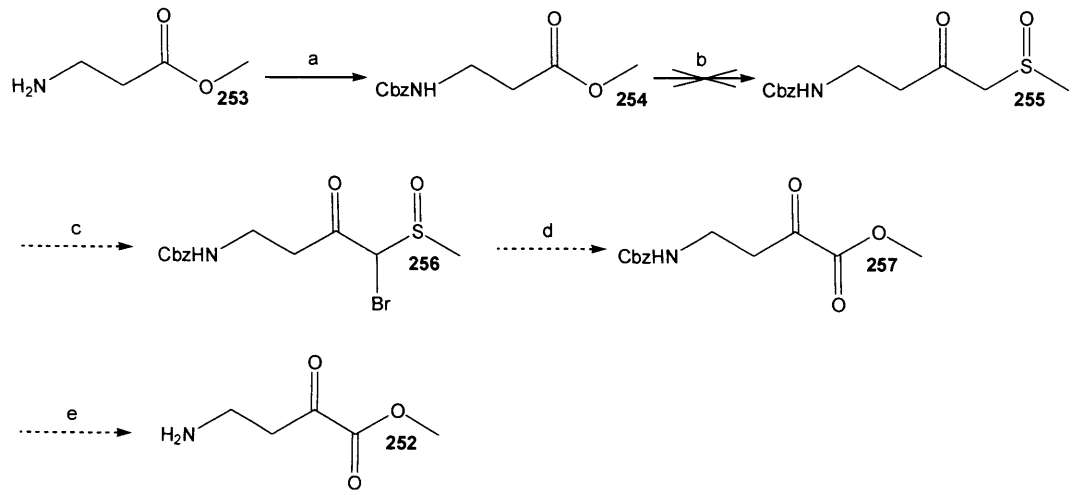
Our initial attempt to give **251** involves treatment of the cyanopyruvate ethyl ester carbanionic salt **249e** with hydrogen over a Raney nickel catalyst at atmospheric pressure and room temperature, though no reaction is observed. The same reaction is repeated at 40 °C and 100 psi to no avail.



Scheme 3.1.23: Attempted reductions of cyano group

Other reduction attempts are deemed too harsh and would reduce all of the functionality in **249e**, so a short synthetic route (Scheme 3.1.24) towards 4-amino-2-oxo-butyric acid methyl ester (**252**) is considered *via* the synthesis of a sulfinyl group.[158] Subsequent acidic treatment may then give 4-amino-2-oxo-butyric acid **251**.

β-Alanine methyl ester (**253**) is treated with triethylamine and Cbz-chloride to yield *N*-Cbz (**254**) in 74% yield from an adopted literature procedure.[158] Attempts to convert *N*-Cbz **254** to *N*-Cbz-4-amino-1-methylsulfinyl-2-butanone (**255**) as described by Iriuchijima are tried, but are unsuccessful.[158] The basis for the rest of this synthesis would have involved the conversion of the sulfinyl group **255** to an α-bromo sulfinyl group (**256**), using *N*-bromosuccinimide and acetone.[159] This then could have been converted by acidic hydrolysis to *N*-Cbz-4-amino-2-oxo-butyric acid methyl ester (**257**). Removal of the *N*-Cbz group by hydrogenation would have yielded 4-amino-2-oxo-butyric acid methyl ester **252**, and in no more than 6 steps.

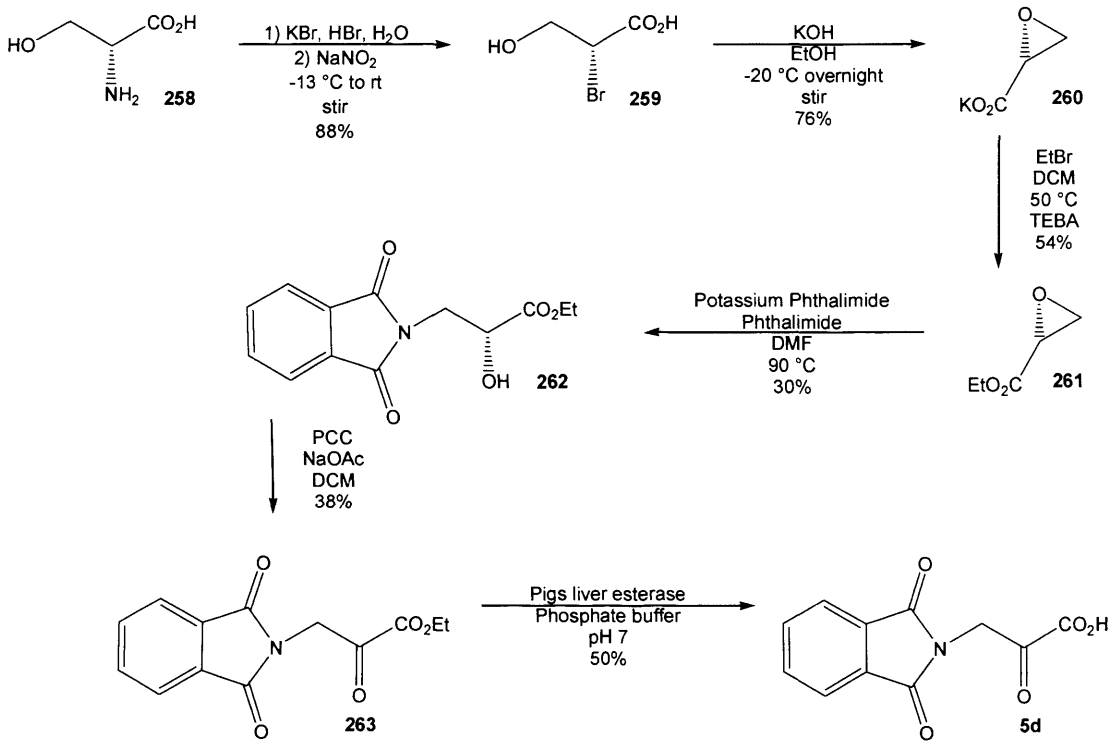


Scheme 3.1.24: Reagents and conditions: (a) Et_3N, CbzCl, $CHCl_3$, 74%; (b) NaH, DMSO, THF 70-75 °C, Ar, 0%; (c) NBS, acetone, 5 min, HCl, MeOH; (d) aq. HCl (e) hydrogenolysis

3.1.5 Phthalimidopyruvate

Synthesis of pthalimidopyruvate **5d** are reported by Williams *et al.*,[138] and Newton *et al.*[160] We utilise the Williams *et al.*, route first as they have successfully oxidised an α-hydroxyester group to an α-ketoester.[138] The second route utilises Newton's route, using *N*-bromosuccinimide to remove a dithiane protecting group and also yields an α-ketoester. Both routes are adapted for comparison and to yield **5d** in the shortest number of steps possible.

We adapt the Williams *et al.*, route, which involves the synthesis of a useful precursor, now described (Scheme 3.1.25). A protocol by Petit *et al.*, is used to achieve this precursor, as it is absent from the experimental details of Williams *et al.* The precursor synthesis starts with L-serine (**258**) and is reacted in the presence of sodium nitrite and hydrobromic acid to give bromoamino acid (**259**), in 88% yield. This is then reacted with potassium hydroxide and results in the ring closure to potassium glycidate (**260**), formed in 76% yield. This is subsequently converted to ethyl glycidate (**261**) using ethyl bromide in the presence of triethylbutylammonium chloride (a phase transfer regent). The reaction temperature of this reaction is critical, as degradation of epoxide **261** occurs above this temperature. Isolation by distillation affords epoxide **261** in 54% yield.[161]



Scheme 3.1.25: Adapted route to phthalimido pyruvate **5d**

Using the synthetic route (Scheme 3.1.25) given by Williams *et al.*, we achieve ring opening of epoxide **261** by using catalytic potassium phthalimide and phthalimide in DMF heated at reflux. This gives α-hydroxyester (**262**) in 30% yield. Pyridinium chlorochromate (PCC) oxidation of **262** affords α-ketoester (**263**) in 38% yield, but only on a 1 mmol scale reaction, as detailed by Williams *et al.*[138] An individual 5 mmol scale reaction is attempted and confirms Williams *et al.* finding, that 1 mmol is the optimal scale for this oxidation. Successful esterase hydrolysis of α-ketoester **257** gives 3-phthalimidopyruvate **5d** in 50% yield. We find that the hydrolysis of the ethyl ester **263**

using acid, reported by Williams *et al.* does not yield 3-phthalimido pyruvate **5d**, but forms a degraded product that we are unable to identify.

The main disadvantage of this route is the oxidation step, as it could not be successfully scaled above 1-mmol. Whilst Williams *et al.*, reports a number of methods to oxidise α-hydroxyester **262** to α-ketoester **263**, including an oxidation with potassium permanganate and copper sulfate in benzene; this is capricious, as yields range from 0 to 80% and so is avoided. Williams *et al.*, subject a number of secondary *N*-protected ethyl esters of isoserine (Figure 3.1.6) to a range of oxidation conditions without any success, including the successful PCC oxidation and highlights the difficulty of this transformation.

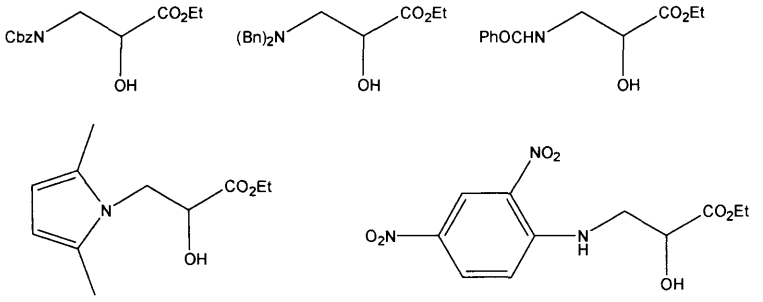
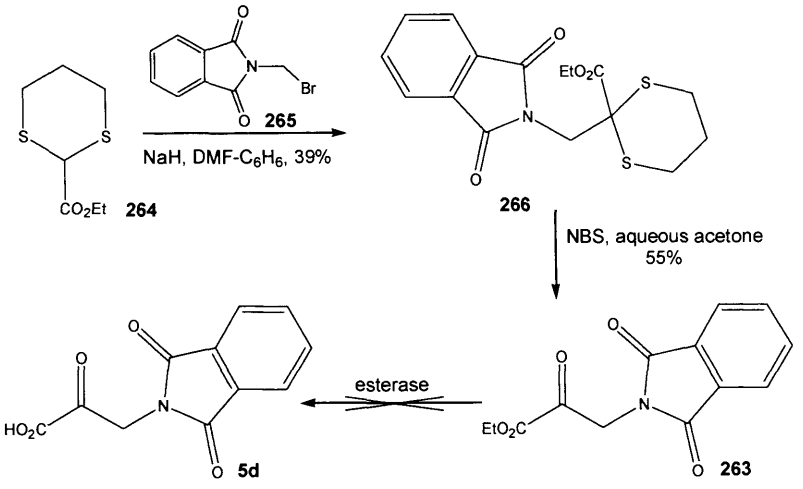


Figure 3.1.6: α-hydroxyesters subjected to various oxidation attempts

In order to scale up the reaction towards synthesis of α-ketoester **263**, a different method (Scheme 3.1.26) was adopted from Newton *et al.*, which shortens the number of steps significantly and is readily scalable.[160]

Alkylation of ethyl 1,3-dithiane-2-carboxylate (**264**) with bromomethyl phthalimide (**265**) gives dithiane protected α-keto ester (**266**) in 39% yield. Subsequent treatment of **266** with *N*-bromosuccinimide in aqueous acetone yields the desired α-ketoester **263** in 55% yield. Unfortunately, attempted repeats of esterase hydrolysis on **263** from this route to afford 3-phthalimido pyruvate **5d** are unsuccessful on a larger scale (1-2 g). Despite numerous recrystallisation attempts of **263** and subsequent repeat of the esterase hydrolysis reaction on a 1-mmol scale; as before, these reactions are unsuccessful. Nonetheless, from the sample of **263** obtained by the oxidation method, enough is available for a biomimetic reaction. It is hypothesised that a contaminant from the latter route involving the dithiane, however minor, is affecting the efficacy of the esterase.

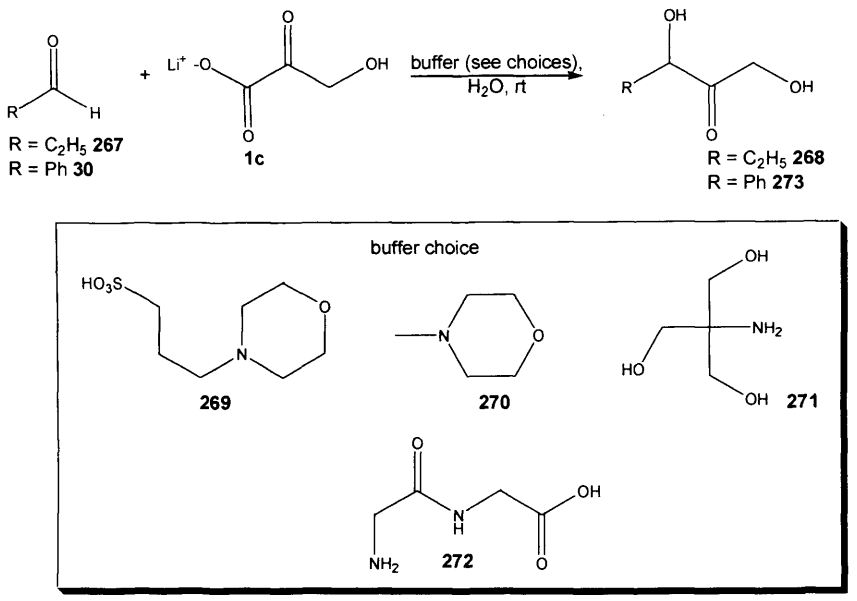


Scheme 3.1.26: Alternative synthesis of **263**

3.1.6 Biomimetic reaction of phthalimidopyruvate with propionaldehyde

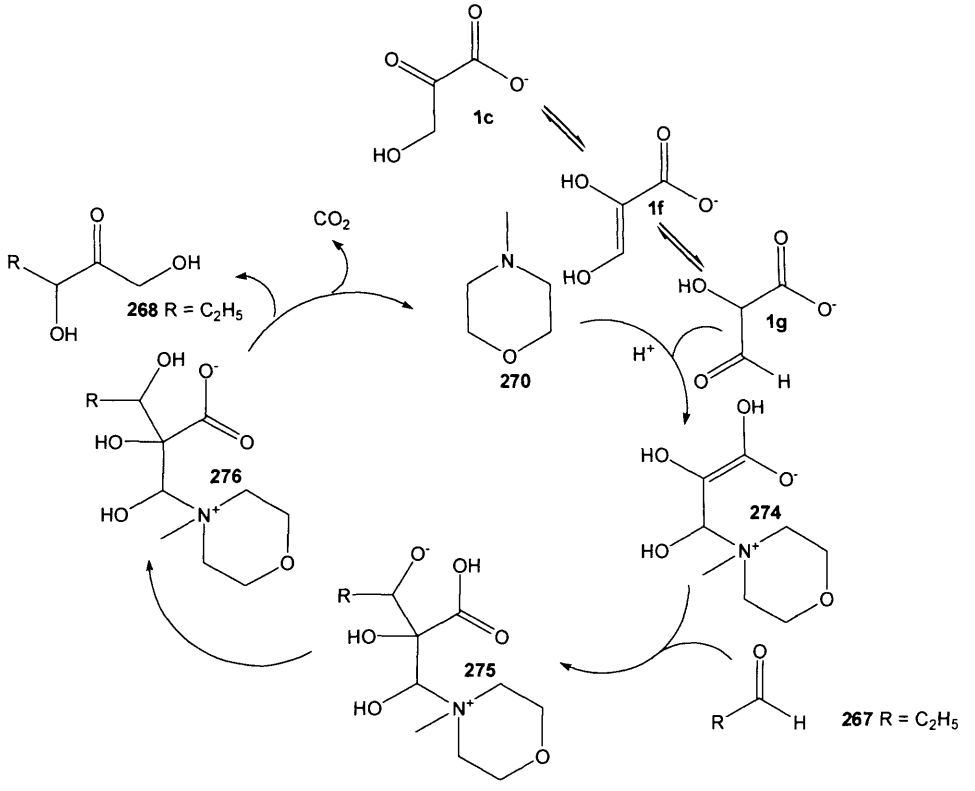
Reactions between Li- HPA **1c** and propionaldehyde (**267**) with TK mutants are carried out and a range of buffers are evaluated for maintaining the pH at 7.0. Interestingly, Smith *et al.*, observe that some control experiments (omitting the TK enzyme) form a ketodiol product, 1,3-dihydroxypentan-2-one (**268**), from reactions containing buffers 3-(-morpholino)propanesulfonic acid (MOPS) (**269**) and *N*-methylmorpholine NMM (**270**).

The mechanistic involvement of TPP **20** and the formation of a thiazolium ylid **21** are ruled out, as other control reactions, utilising *tris*(hydroxymethyl)aminomethane (TRIS) (**271**) or glycylglyine (gly-gly) (**272**) do not yield any ketodiol **268**. Tests with benzaldehyde **30**, however, are also conducted, yielding ketodiol (**273**), but in a low 25 % yield. (Scheme 3.1.27).[6]



Scheme 3.1.27: Biomimetic reaction with various buffers **269-272**

A reaction mechanism is postulated, which involve an enolic intermediate and is presented in Scheme 3.1.28.

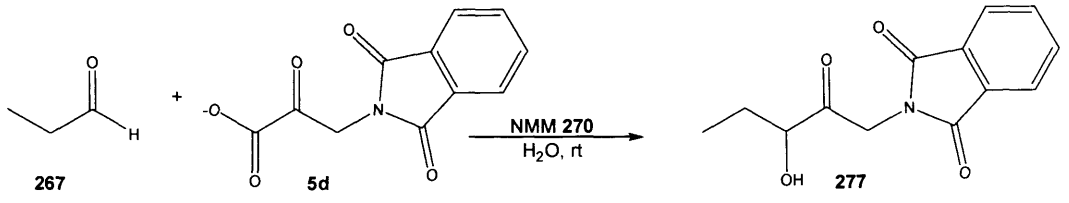


Scheme 3.1.28: Proposed mechanism of the formation of **268** using NMM **270**

It is proposed that in alkaline media, **1c** is known to undergo enolization to enol (**1f**) and tautomerisation to tartonate semialdehyde (**1g**), which could then undergo subsequent addition to NMM **270**, generating enolate (**274**). Addition of propionaldehyde **267** to

enolate **274**, followed by proton transfer gives species (**275**). Finally, decarboxylation of species (**276**), yields keto diol **268** and regenerates NMM **270** in the catalytic cycle.

Following the successful synthesis of phthalimidopyruvate **5d**, no enolization is observed by solution ^{1}H NMR spectroscopic analysis, but it is still possible for it to occur in aqueous media. A small-scale reaction (Scheme 3.1.29) is conducted between phthalimidopyruvate **5d** and propionaldehyde **267** in the presence of NMM **270** and a product spot is observed by TLC analysis.



Scheme 3.1.29: Biomimetic reaction toward ketoalcohol **277**

Preparative TLC is used to purify what appear to be two bands. Though ^{1}HNMR spectroscopic analysis appear promising, not enough ketoalcohol **277** is available for ^{13}C NMR analysis (*ca* 5-6 mg) and contains propionaldehyde contaminants, despite columning. Fortunately, high-resolution mass spectrometry does provide a molecular ion peak (MH^+) 248.09187, which is 1.97 ppm different to the theoretical mass (MH^+) 248.09228 (well within the accepted error limit of for both bands). Both bands, on this basis, are identified as the same product. Unfortunately, it is not possible to conduct tests with phthalimidopyruvate **5d** with TK or its mutants, as there is not enough of this substrate.

Attempts to make more of phthalimido pyruvate **5d** have been futile, despite repeating a number of ester hydrolyses on ethyl ester **263** (Scheme 3.1.26). Methods on a larger scale include: basic hydrolysis with lithium hydroxide, acidic hydrolysis with 1M and 6M HCl and numerous repeats of the esterase hydrolysis.

3.2 Conclusion

In conclusion, despite numerous attempts to obtain novel nitrogen containing pyruvate donors, a number of important factors are highlighted by this research. Firstly, the preference of enol forms over α-ketoester highlights the difficulty in synthesising these small and highly reactive molecules. This is prevalent, even in Li- HPA **1c**, which has a rigorous synthetic protocol; albeit only 30-40% yields are obtainable (Scheme 3.1.1).

Having a number of reactive functional groups in a small molecule makes synthetic manipulation and functional group conversion a challenging task. This has clearly been highlighted in terms of substitution of bromopyruvic acid **191** or its ester **193** with azide, which undergoes unidentifiable polymerisation pathways (Table 3.1.1).

Attempts to obtain the α-ketoesters from α-hydroxyesters by oxidation (Table 3.1.2) are only successful with phthalimidopyruvate **5d**, synthesised using a PCC oxidation route (Scheme 3.1.25). In addition to oxidation protocols utilised by Williams *et al.*, we apply a rigorous set of oxidation conditions aimed toward the synthesis of azidopyruvate **5a** and aminopyruvate **5b**, but these are unsuccessful. Moreover, two enzymatic oxidation protocols, one involving lactate dehydrogenase and the other, lactate and pyruvate oxidases are used in a reversed equilibrium and forward reaction, respectively (Scheme 3.1.7, Scheme 3.1.8 & Scheme 3.1.19).

Nandra *et al.*, provide a unique carbene methodology, which allow the protection of the highly reactive functionalities in aminopyruvate **5b** (Scheme 3.1.15). From this, we discover the preference of enol formation over the desired α-ketoester (Scheme 3.1.17) and the ease at which pyruvate donors can undergo retroaldol reactions. This is deemed important for biomimetic reactions because they utilise basic amine buffers to mimic TK reactions.

Further attempts to lengthen the methylene chain length of a pyruvate donor and thus decrease the proximity of reactive functional groups is attempted by the synthesis of cyanopyruvate **5c** and 4-amino-2-oxo-butyric acid (**251**). Though this is unsuccessful, we demonstrate a wide-range of chemical strategies that could be employed for this synthesis (Scheme 3.1.22 & Scheme 3.1.23).

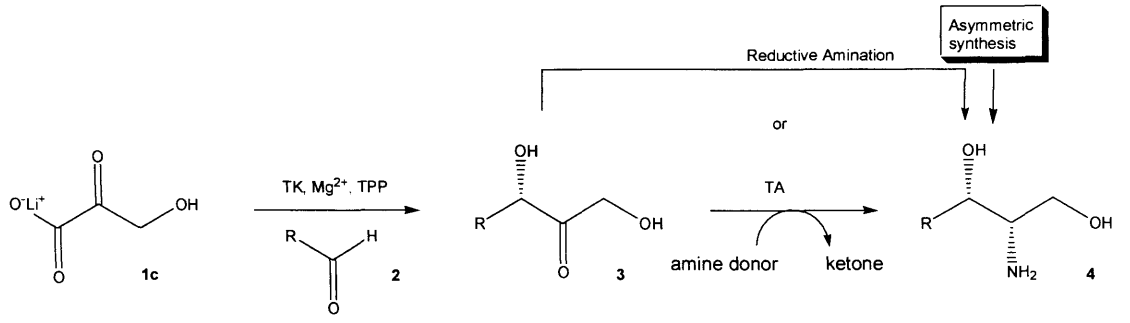
Phthalimidopyruvate **5d** is obtained in a six-step synthesis (Scheme 3.1.26). Unfortunately, synthesis of more of this material is unobtainable despite numerous attempts. Therefore, instead of subjecting **5d** directly to TK and its mutants, we decide to utilise the mimetic TK reaction (Scheme 3.1.28). It is anticipated that the racemic ketoalcohol **277** from this reaction could be used as a standard for determining any enantioselectivity that might arise from TK or its mutant's bioconversion. Despite low conversion of 3-phthalimido pyruvate **5d** into ketoalcohol **277**, phthalimido pyruvate **5b**

has been identified as a nitrogen containing pyruvate donor with potential uses in TK reactions.

Chapter 4

4.1 Syntheses towards syn-aromatic aminodiol and syn-aromatic diol HPLC standards

Recently, Hailes *et al.*, show that Li-HPA **1c** with aldehydic substrates **2** reacted in the presence of TK generate an (*S*)-ketodiol **3**[h]. Furthermore, it is known that TA can be used to accept (*S*)-ketodiols **3** and that some mutants may eventually accept (*R*)-ketodiols, ultimately favouring a *syn* stereochemistry in the final aminodiol product **4**. Attempts have been made to extend this method to include aromatic aldehydes **2**, R = Ar and this meant that chiral HPLC standards were required to aid elucidation of the stereoselectivities of **4** from the overall reaction scheme (Scheme 4.1.1).



Scheme 4.1.1: Asymmetric synthesis of *syn*-aminodiols **4**, where R = Ph

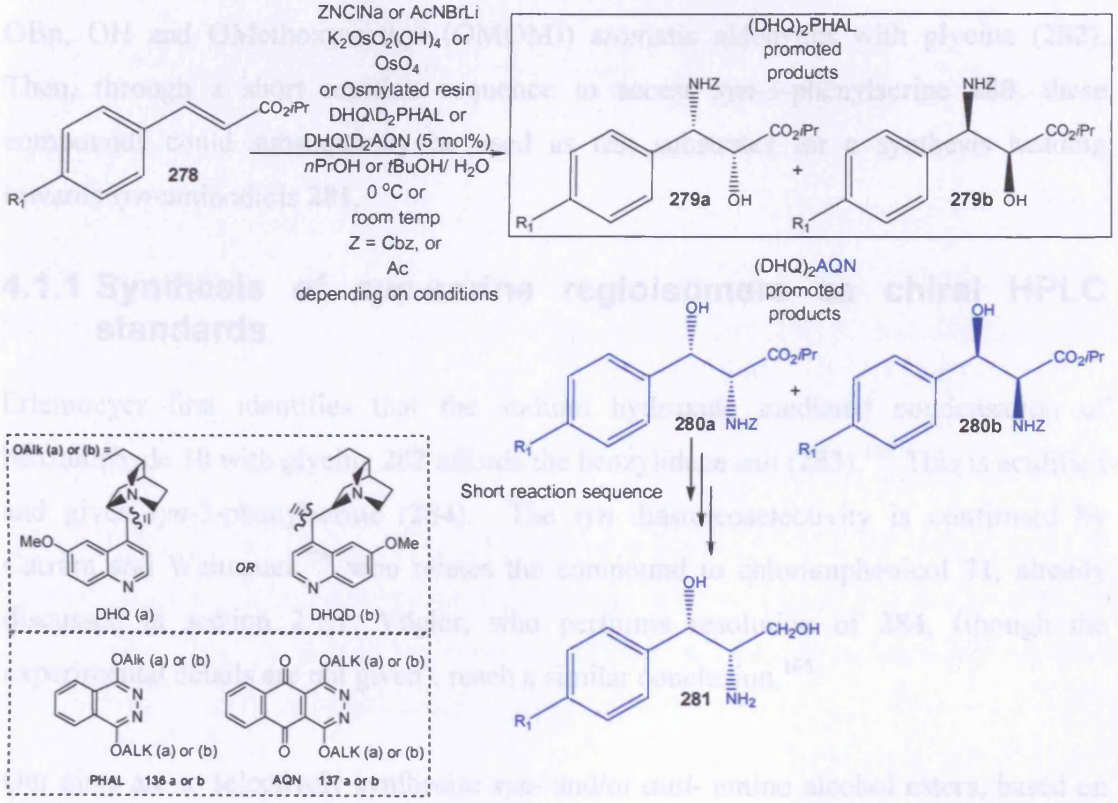
Carrying out the Sharpless asymmetric aminohydroxylation (AA) reaction (Scheme 4.1.2) with isopropyl cinnamate ester substrates (**278**) will give rise to four possible *syn*-stereoisomers, depending on the choice of chiral ligand, either as $(DHQ)_2PHAL$ **136a** or $(DHQ)_2AQN$ **137a** (Scheme 4.1.2).

If an osmium(VIII) species with $(DHQ)_2PHAL$ **136a** is used, two possible *syn*-stereoisomers result. These are the (2*R*,3*S*)-*N*-protected 3-phenylisoserine enantiomer (**279a**), obtained as the major enantiomer and (2*S*,3*R*)-*N*-protected 3-phenylisoserine enantiomer (**279b**), obtained as the minor enantiomer.[124,162] The enantioselectivities are reversed when $(DHQD)_2PHAL$ **136b** is used.

[h] Hailes, H.C., Smith, M.E.B., Smithies, K. *Unpublished results*

When $(DHQ)_2AQN$ **137a** is used, the major enantiomers produced are (2*R*,3*S*)-*N*-protected 3-phenylserine (**280a**), whilst (2*S*,3*R*)-*N*-protected 3-phenylserine (**280b**), results from the use of $(DHQD)_2AQN$ **137b**.[7,8]

This means that the regioselectivity and enantioselectivity can be controlled by the choice of chiral ligands **136** and **137**, and thus presents an attractive one-pot method for establishing a *syn* relationship. This is considered as the lead synthetic route toward aromatic *syn*-aminodiols (**281**) *via* this short reaction sequence (Scheme 4.1.2).



Scheme 4.1.2: Sharpless AA as a route to aminodiol **281**

We focus on the use of $(DHQ)_2PHAL$ **136a** and $(DHQ)_2AQN$ **137a** chiral ligands, to give (2*R*,3*S*) *syn* stereoselectivity, as at the time of the work by Hailes *et al.*, the stereochemistry of aminodiols **4**, are unknown (Scheme 4.1.1).

The regioselectivity of the reaction can be reversed from (2*R*,3*S*)-*N*-protected 3-phenylisoserine **279a** to (2*R*,3*S*)-*N*-protected 3-phenylserine **280a** using either **136** or **137**; but through the use of **137**, the regioselective ratios of **279a**:**280a** can range anywhere between 1:4 to 1:2 for the desired (2*R*,3*S*)-*N*-protected 3-phenylserine **280a**.[8] This means that a significant amount of unwanted (2*R*,3*S*)-*N*-protected 3-phenylisoserine **279a** is present from the synthesis of (2*R*,3*S*)-*N*-protected 3-

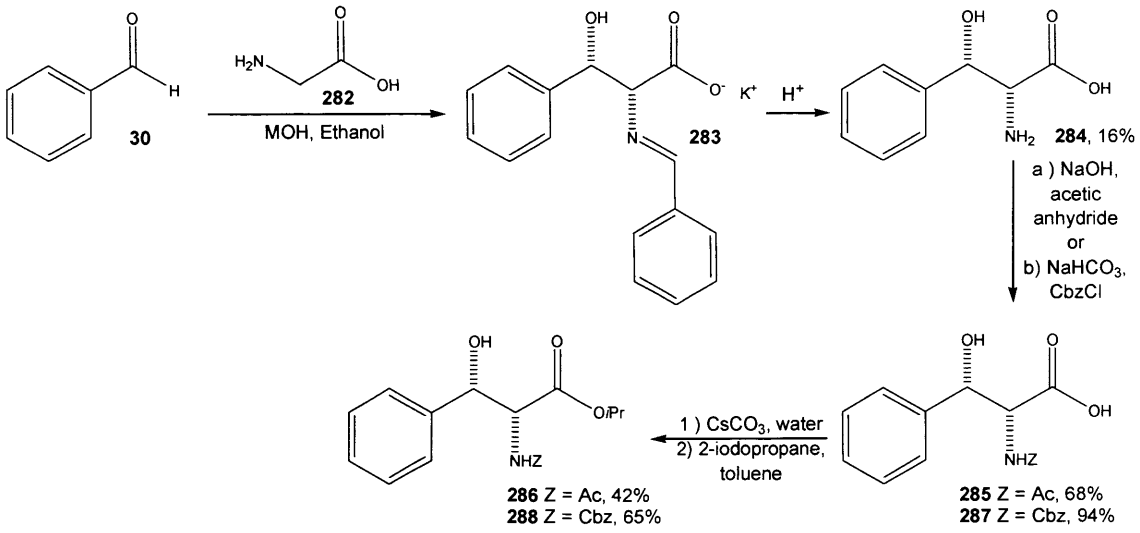
phenylserine **279a**; which, when heading towards *syn* aromatic aminodiol **281** needs removal by careful column chromatography. Furthermore, should it not be possible to remove unwanted regioisomers from the Sharpless AA reactions, they will complicate the chiral HPLC interpretation of the final mixture.

A synthetic route that gave exclusively *syn-N*-protected 3-phenylserine regioisomers **280** as chiral HPLC standards is developed. The attempted syntheses take advantage of known condensation reactions between non *para*-substituted and *para*-substituted (R = OBn, OH and OMethoxymethyl (OMOM)) aromatic aldehydes with glycine (**282**). Then, through a short reaction sequence to access *syn*-3-phenylserine **280**, these compounds could subsequently be used as test substrates for a synthesis heading towards *syn*-aminodiols **281**.

4.1.1 Synthesis of *syn*-serine regioisomers as chiral HPLC standards

Erlenmeyer first identifies that the sodium hydroxide mediated condensation of benzaldehyde **30** with glycine **282** affords the benzylidene salt (**283**).[163] This is acidified and gives *syn*-3-phenylserine (**284**). The *syn* diastereoselectivity is confirmed by Carrara and Weitnauer,[164] who relates the compound to chloramphenicol **71**, already discussed in section 2.1.1. Vögler, who performs resolution of **284**, (though the experimental details are not given), reach a similar conclusion.[165]

Our aims are to selectively synthesise *syn*- and/or *anti*- amino alcohol esters, based on the general structure **281** (Scheme 4.1.2). These will assist chiral HPLC analysis of the Sharpless AA reaction (discussed in Chapter 5), and provide *syn*- and/or *anti*-aminodiols for applications in other screening activities of the BiCE project. Erlenmeyer's method is repeated in our lab, by the reaction of benzaldehyde **30** with glycine **282** in the presence of potassium hydroxide (Scheme 4.1.3). *Syn*-**284** is obtained in 16% yield and is confirmed by ^{1}H NMR spectroscopic analysis against a commercially available sample.

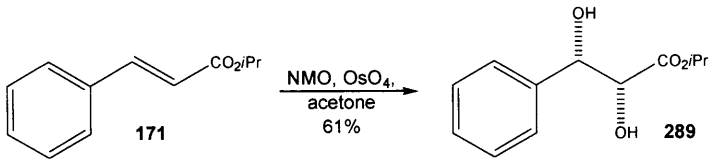


Scheme 4.1.3: Synthetic route towards *syn*-diastereoselective HPLC standards **286** and **288**

In order to test the amide and carbamate nitrogen sources in the Sharpless AA (Scheme 4.1.2), both *N*-acetyl and *N*-Cbz analogues are formed using standard Schotten-Baumann reaction conditions. Reacting acetic anhydride or benzyl chloroformate with **283** under basic conditions yields *syn*-*N*-acetyl aminoacid (**285**) in 68% yield, and *syn*-*N*-Cbz aminoacid (**287**) in 94% yield, respectively. As we have elected to use the isopropyl cinnamate ester **170** in the Sharpless AA reactions (Scheme 4.1.2), cinnamic *N*-Cbz-protected aminoacids **285** and **287** are converted to the corresponding isopropyl cinnamate esters, with *N*-acetyl (Ac)-(**286**) obtained in 42% yield and *N*-Cbz-(**288**) in 65% yield. This is achieved by formation of their corresponding caesium salts and alkylation of these with 2-iodopropane (Scheme 4.1.3).

S*yn*-**286** and *syn*-**288** are then analysed by chiral HPLC and a 1:1 ratio of peaks show both *syn* enantiomers are present. It is anticipated that co-injecting these *syn* standards **286** and **288** against the corresponding Sharpless AA adducts, the identity of each chiral HPLC peak produced could be confirmed as the peak retention times for unsubstituted cinnamate substrates with Sharpless AA had been reported for the (2*R*,3*S*) isomer when using chiral ligand $(DHQ)_2AQN$ **137a**.[8,63,124,162,166]

One of the by-products of the Sharpless AA reaction is a diol (section 2.2, Scheme 2.2.1).[166] So to ensure as many expected products are identified before doing any Sharpless AA work, we synthesise a *syn*-diol for chiral HPLC elucidation by reaction of isopropyl cinnamate ester **170** with *N*-morpholine oxide in the presence of osmium tetroxide. This affords *syn*-diol **289** in a yield of 61% (Scheme 4.1.4).



Scheme 4.1.4 Synthesis of diol product **289**

Fortunately, the retention times of the expected Sharpless adducts *N*-Ac **286** and *N*-Cbz **288** is different enough to allow identification of each product enantiomers. They are also different in retention times to the peaks generated by the diol by-product **289**. The deconvolution is shown in Table 4.1.1.

Enty	Retention time/ min[a]	Z if applicable	Compound
1	14.55	Ac	(2*R*,3*S*)-**286a**
2	15.36	Ac	(2*S*,3*R*)-**286b**
3	16.74	Cbz	(2*R*,3*S*)-**288a**
4	18.00	Cbz	(2*S*,3*R*)-**288b**
5	20.22	N/A	*Syn*-**289**[b]
6	21.98	N/A	*Syn*-**289**[b]

[a]Chirapak AD : (0.6 ml min^{-1}), 210 nm, 85:15 (hexane:*i*PrOH); [b]Absolute configuration not measured

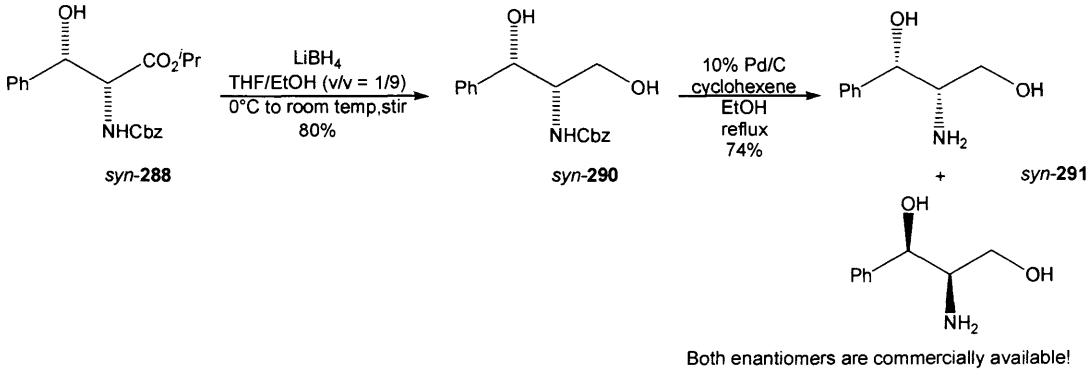
Table 4.1.1: Retention times of chiral HPLC standards

We assume, based on earlier literature reports, that the *N*-Ac-(2*R*,3*S*) **286a** enantiomer (entry **1**) elutes first from our chiral HPLC trace when compared with the (2*S*,3*R*)-enantiomer **286b** (entry **2**). The *N*-Cbz-(2*S*,3*R*)-**288a** and (2*R*,3*S*)-**288b** enantiomers are reported to elute at slightly later retention times (entries **3** and **4**).[166] Both enantiomers of the *syn*-diol **289** (entries **5** and **6**) are identified as much later peaks on the chiral HPLC trace, at retention times 20.22 and 21.98 min, respectively. This is however, only one Sharpless AA reaction and so we decide to explore the synthesis of *p*-substituted 3-phenylserine *syn*-HPLC standards for any other system under investiagtion. This is because we want to confirm the absolute stereochemistry of other *p*-substituted *N*-Cbz–3-phenylserine enantiomers as (2*R*,3*S*)-**288a** when $(DHQ)_2AQN$ **137a** is used in later Sharpless AA reactions. This would be achieved, hopefully by comparison to a known standard.

From the literature, we are aware of how some (2*R*,3*S*)-3-phenylisoserine enantiomers **280a** and (2*S*,3*R*)-3-phenylisoserine enantiomers **280b** will elute by chiral HPLC. This aids the deconvolution process for a few of the *N*-Ac and *N*-Cbz products.[124,162] Having identfied a good chiral HPLC system, we turn our attention to the synthesis of the *syn*-aminodiol (**291**), seen in the next section.

4.1.2 Synthesis of a *syn*-aminodiol

Using the *syn*-*N*-Cbz 3-phenylserine regioisomer **288** (prepared in section 4.1.1) as a test substrate, a short synthesis is developed towards the corresponding *syn*-aminodiol **291** (Scheme 4.1.5). This could then be used to optimise the chiral HPLC purification (a labour intensive process) of Sharpless AA generated enantiomers.



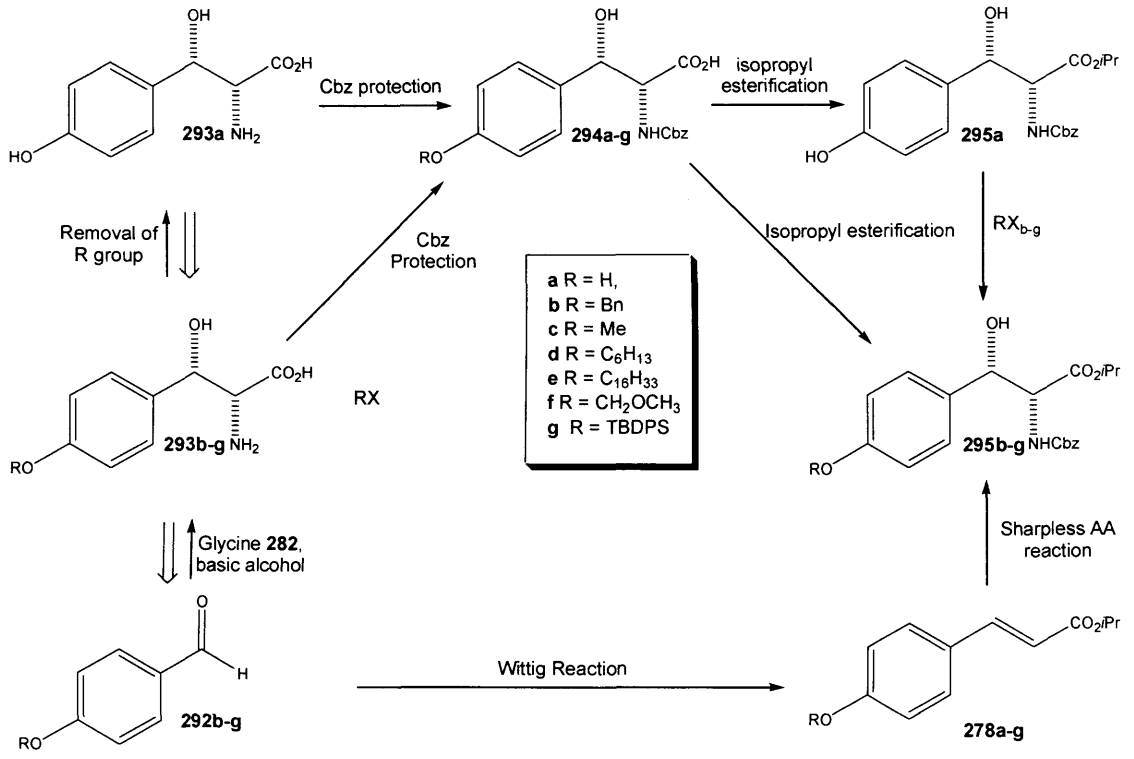
Scheme 4.1.5: Short step reaction sequence to *syn*-aminodiol **291**

Initially sodium borohydride was used to reduce *syn*-ester **288** as Merlo and Fernandes use it to reduce a similar phenylalanine ester, but this is problematic.[167] Increasing the number of equivalents of sodium borohydride is ineffective for the reduction of *syn*-**288**, as determined by TLC and ^{1}H NMR spectroscopic analyses. Instead, lithium borohydride in a mixed solvent system (THF/ethanol) is used, as Gershon and Rodin shows its effectiveness on a similar benzoyl tyrosine ester.[168] The *syn*-ester **288** is reduced to the *syn*-*N*-Cbz aminodiol-**290** in 80% yield. This then undergoes an attempted hydrogenation using a palladium catalyst at room temperature in ethanol to remove the *N*-Cbz protecting group. This is ineffective, so we use a catalytic transfer hydrogenation method. A palladium catalyst in ethanol with cyclohexene is heated at reflux with *N*-Cbz aminodiol **290** and results in the hydrogenolysis of the *N*-Cbz group, giving the *syn*-aminodiol **291** in 74% yield. It is anticipated that this method could be extended towards the synthesis of a number of *p*-substituted aromatic aminodiols, similar to compound **281** (Scheme 4.1.2).

4.1.3 Synthesis of *syn p*-substituted phenylserine chiral HPLC standards

A range of isopropyl *p*-substituted cinnamate substrates **278a-g** (Scheme 4.1.6) is investigated with the Sharpless AA reaction, as seen in the next chapter. These cinnamates are chosen to investigate how varying the chain lengths or having other bulky aromatic groups at the *p*-position will affect regio- and enantioselectivities of

Sharpless AA reactions, under both homogeneous and heterogeneous reaction conditions.



Scheme 4.1.6: Diastereoselective syntheses towards *syn-p*-substituted 3-phenylserine HPLC standards **295a-g**

Reacting glycine **282** with *p*-substituted aldehydes (**293b-g**) is thought to lead to the successful synthesis (Scheme 4.1.6) of *syn*-(*p*-substituted-3-phenyl)serines) (**293b-g**). We are aware that hydroxybenzaldehyde (**292a**) would not be suitable for this type of condensation, but may be required in the synthesis of other aldehydes **292b-g**. *Syn*-(*p*-hydroxy-3-phenyl)serine (**293a**) is of particular interest and is believed to be readily accessible by deprotection of the R groups of (**293b-g**) should there be any problem in the latter stages of the synthesis.

If no problems result, *syn*-(*p*-substituted-3-phenyl)serines **293b-g** could be *N*-Cbz protected to give *syn*-*N*-Cbz-(*p*-substituted-3-phenyl)serines-(**294b-g**). Subsequent isopropyl esterification of *syn*-**294b-g** should give *syn*-*N*-Cbz-(*p*-substituted-3-phenyl)serine isopropyl esters (**295b-g**). *Syn*-*N*-Cbz-(*p*-hydroxy-3-phenyl)serine (**294a**), however, is potentially useful as alkylation of the phenolic group should give access to any of the *syn*-HPLC standards **295b-g**. No chiral HPLC data are reported in the literature for (2*R*,3*S*)-**295b-g** resulting from the use of $(DHQ)_2AQN$ **137a** in the Sharpless AA transformations. Having access to all of the *syn* HPLC standards **295a-g**

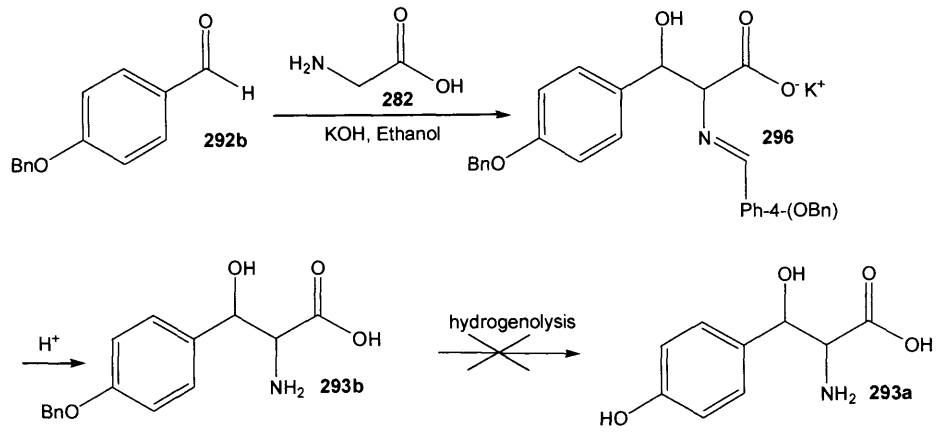
could be useful for identifying the *syn*-(2*R*,3*S*) enantiomers of the subsequent Sharpless AA reactions.

Literature data are only available for synthetic routes to *syn*-(*p*-substituted-3-phenyl)serines **293a**, **293b** and **293f** (Scheme 4.1.6) and is a good place to start the subsequent investigations.

Bolhofer report that the condensation reaction (Scheme 4.1.7) of glycine **282** with *p*-benzyloxybenzaldehyde (**292b**) in alcoholic potassium hydroxide solution gives exclusively the *syn*-(*p*-benzyloxy-3-phenyl)serine (**293b**) in 77% yield. This is achieved *via* acidic hydrolysis of the isolated *p*-benzyloxy benzylidene salt (**296**) in 95% yield.[169] Bolhofer also report a method to remove the benzyl ether from *syn*-**293b** to give *syn*-**293a**, thus providing a good lead synthesis to the two desired *syn*-amino acids, **293a** and **293b**.

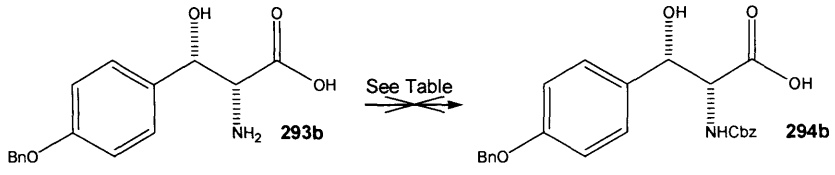
We repeat the condensation reaction (Scheme 4.1.7) and *syn*-**293b** is obtained in 77% yield, *via* the successful isolation and acidic hydrolysis of **296**, obtained in 63% yield. ^{1}H NMR spectroscopic analysis show that *syn*-**293b** is formed in a 60:40 *de*. Cable *et al.*, note the same diastereoselectivities by ^{1}H NMR spectroscopic analysis even after a number of recrystallised samples are obtained from the mother liquor.[170] Bolhofer has relied on the absence of a specific IR band (11.90 μ), to show that the *anti* compound is absent from the condensation reaction, which is not the case.[169,170] A series of repeats using Cable's method of collecting crops of crystals are carried out, but the *de* remained constant at 60:40 (*syn*:*anti*), as determined by ^{1}H NMR spectroscopic analyses.

Attempts to remove the benzyl ether by hydrogenation as described by both Bolhofer and Cable *et al.*,[169,170] (sodium hydroxide & Pd/C or hydrochloric acid/methanol & Pd/C) are unsuccessful, as determined by TLC and ^{1}H NMR spectroscopic analyses.



Scheme 4.1.7: Synthetic protocol towards *syn*-(*p*-hydroxy-3-phenyl)serine **293a**

Even though we have a 60:40 *de* (*syn*:*anti*), we attempt to convert **293b** to *N*-Cbz-(**294b**) using the methods shown in Table 4.1.2. This is because the *anti* isomer present may still prove useful in the chiral HPLC analysis of products formed using dual enzymatic pathways (Scheme 4.1.1). This could be achieved by derivatisation of the corresponding aromatic aminodiols **4**, in other screening activities of the BiCE project.

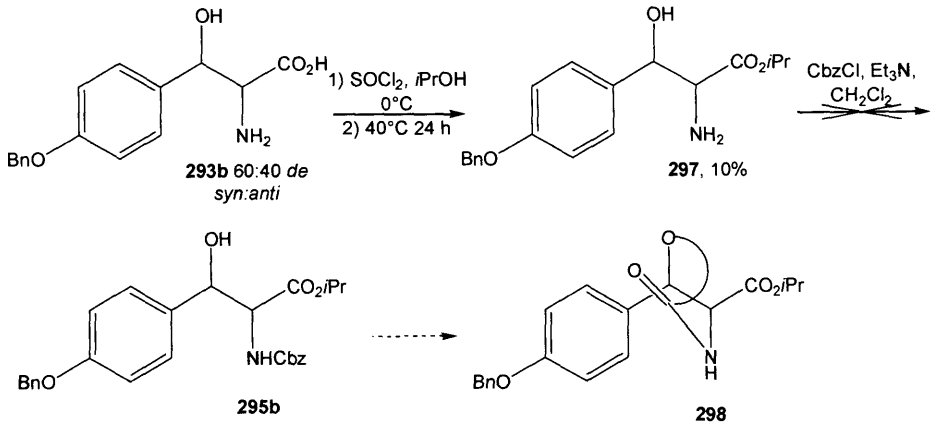


Entry	Reaction conditions
1	$NaHCO_3$ (aq)/0°C/CbzCl
2	NaOH/0°C /pH 9,/CbzCl
3	MgO/EtOAc/reflux/CbzCl

Table 4.1.2: Reaction conditions for the conversion of **293b** to *N*-Cbz-**294b**

We initially try *N*-Cbz protection of *syn*-(*p*-benzyloxy-3-phenyl)serine **293b** under basic conditions (entry **1**), as this method has previously been used on the analogous *syn*-3-phenylserine **284**.[171] *Syn*-(*p*-benzyloxy-3-phenyl)serine **293b** is not fully soluble in the aqueous solution. The reaction is repeated with a small amount of THF added to ensure homogeneity, but still no reaction results. Next we use a method in which the pH is maintained at pH 9 by dropwise addition of sodium hydroxide (entry **2**). The substrate is fully soluble in this mixture, but once again, no reaction results. We hypothesise that using an organic solvent might drive the reaction to completion. Therefore, magnesium oxide and benzyl chloroformate are reacted with **293b** in ethyl acetate and are heated at reflux for 24 h (entry **3**), a method adapted from the literature.[172] This reaction also does not proceed and it is concluded that the amphiphilic and zwitterionic nature of **293b** is responsible for its inability to undergo this transformation. We therefore modify the synthesis so that the (*p*-benzyloxy-3-phenyl)serine isopropyl ester (**297**) is synthesised.

This functional group transformation should increase the solubility of **293b** in organic solvents by reducing the amphiphilic nature of the molecule, allowing the subsequent carbonylation reaction (Scheme 4.1.8).

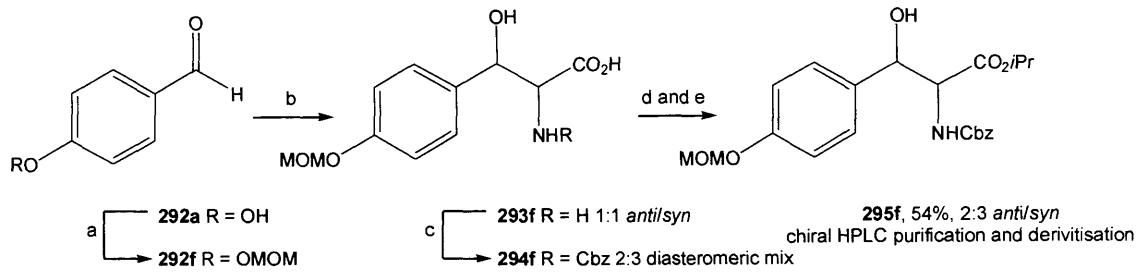


Scheme 4.1.8: Synthesis of isopropyl ester **297** and subsequent *N*-Cbz protection to **295b**

A number of routine esterification protocols on **293b** are attempted, including dicyclohexylcarbodiimide/4-dimethylaminopyridine (DCC/DMAP), *N*-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and Fischer esterification, but without success. It is, however, seen from the ^{1}H NMR spectroscopic analysis of **293b**, that trace water is present in the sample and so, as a hydrate, the carboxylate reactivity may be reduced. Thionyl chloride mediated esterification does work, albeit in low yield (10%) to give **297**, but ^{1}H NMR spectroscopic analysis shows the presence of only one diastereoisomer.

Since the starting material has a 60:40 *de*, we are only isolating either *anti*-(**297**) or *syn*-(**297**) only. To complete the analysis, we anticipate that conversion of either *anti*-**297** or *syn*-**297** to **295b** by *N*-Cbz protection and subsequent ring closure to oxazolidinone **298** should allow for the positive identification of *syn* or *anti*-**297**, by *J* couplings, using ^{1}H NMR spectroscopic analysis. The protection of **297** with a benzyloxycarbonyl group to give **295b** (Scheme 4.1.8) using benzyl chloroformate, however, is unsuccessful.

Shigematsu *et al* report that the reaction of *p*-hydroxybenzaldehyde (**292a**) with methoxymethylchloride yields *p*-methoxymethylbenzaldehyde (**292f**). This aldehyde reacts with glycine **282** in the presence of ethanolic potassium hydroxide and results in the formation of a 1:1 diastereomeric mixture of *syn*- and *anti*- (*p*-methoxymethyl-3-phenyl)serine (**293f**) in 20% yield (Scheme 4.1.9). Subsequent *N*-Cbz protection is possible.[173]



a) Dimethoxymethane, *p*-toluenesulphonic acid, CH_2Cl_2, soxhlet containing molecular sieves (3Å), reflux, 23%; b) glycine **282**, KOH, ethanol, 11%, c) $NaHCO_3$ (aq), CbzCl, 71%; d) K_2CO_3, BTEAC, 2-iodopropane, DMA, 55°C, .e) chiral HPLC purification

Scheme 4.1.9: Synthetic route towards chiral HPLC standard **295f**

By modification of the protocol developed by Shigematsu *et al*, *p*-hydroxybenzaldehyde **292a** is reacted (Scheme 4.1.9) with *p*-toluenesulphonic acid in dimethoxymethane. The mixture is heated to reflux using a soxhlet adaptor containing molecular sieves. *p*-Methoxymethylbenzaldehyde (**292f**) is obtained in 23% yield, which when compared to the literature yield of 60% appears poor. Despite numerous attempts, the literature yield is not achievable.[174] This method is, however, better than using methoxymethyl chloride itself in the synthesis, as methoxymethyl chloride is difficult to obtain commercially and this modification reduces the number of steps.

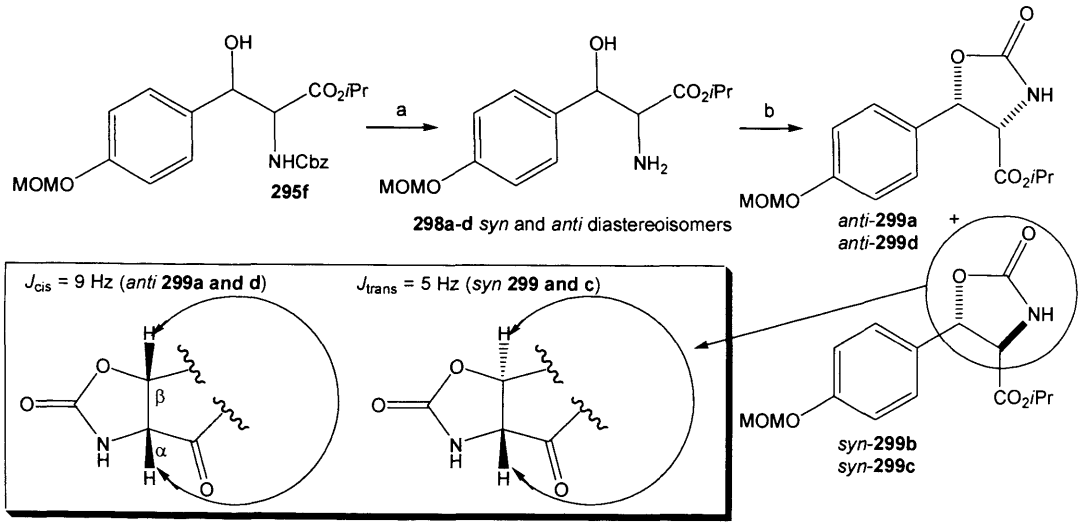
As with the synthesis (Scheme 4.1.9) developed by Shigematsu *et al.*, glycine **282** is reacts with the *p*-methoxymethylbenzaldehyde **292f** in the presence of alcoholic potassium hydroxide (Scheme 4.1.9). This yields a 1:1 diastereomeric mixture of *syn*- and *anti*- (*p*-methoxymethyl-3-phenyl)serine **293f** in 11% yield.[173] Attempts are made to improve upon this yield, but are not successful. *N*-Cbz protection of **293f** in the presence of aqueous sodium hydrogencarbonate with benzyl chloroformate afford a 1:1 diastereomeric mixture of *syn*:*anti*-**294f**. This shows that steric hindrance may be preventing the *N*-Cbz protection of **293b** (Table 4.1.2) or **297** (Scheme 4.1.8).

A number of standard routes to the isopropyl ester **295f** (Scheme 4.1.9) are explored, but are unsuccessful (caesium carbonate/2-iodopropane/r.t. and potassium carbonate/2-iodopropane/DMF/r.t.). The reaction of **294f** with benzyltriethylammonium chloride (BTEAC), potassium carbonate (26 equiv.) and 2-iodopropane (48 equiv.) gives **295f** in 54% yield. This is adapted from a literature precedent.[175]

Isopropyl ester **295f** is then reacted with potassium carbonate (26 equiv.) in dimethylacetamide, to see if the diastereoisomeric ratio changes from 1:1 (*syn*:*anti*), to

favour more of the *syn*-product by epimerisation. *p*-Methoxymethyl (MOM)-benzaldehyde **292f** results from this reaction and is identified by ^{1}H NMR spectroscopic analysis. This suggests that **295f**, instead of epimerisation, undergoes a retroaldol reaction. It is postulated that the high excess of 2-iodopropane (48 equiv.) is required to achieve ester formation, due to its volatility or possible elimination reactions.

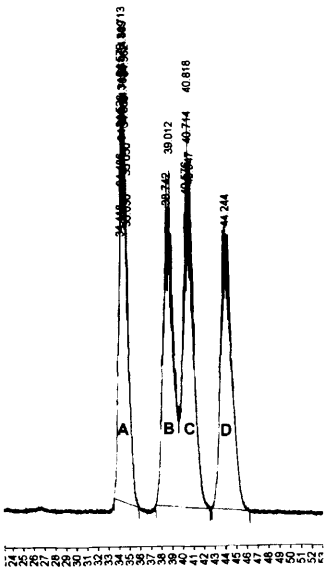
A set of trial reactions (Scheme 4.1.10) are then carried out with the 1:1 diastereoisomeric mixture of **295f** to ensure that derivatization to a ring closed product allows the relative stereochemistry to be deduced. This is done in preparation for **295f**'s purification into 4 diasteroisomers using preparative chiral HPLC. Successful hydrogenolysis on the 1:1 diastereisomeric mixture of **295f** using hydrogen over a Pd/C catalyst ensures the synthesis of *p*-(MOM-3-phenyl)serine isopropyl ester (**298**) in 81% yield. Subsequent ring closure of the diastereomeric mixture **298** using triphosgene with triethylamine ensures the synthesis of *syn* and *anti*-oxazolidinones (**299**) in 94% yield (Scheme 4.1.10), which are readily separable by column chromatography.



Scheme 4.1.10: Determination of the relative *syn* and *anti* stereocenters of **295f** by derivatization to oxazolidinones **299a-d** for ^{1}H NMR spectroscopic analyses. Reagents and conditions: a) Pd/C (5%), H_2, room temperature, methanol (46% for **298a-d**); b) triphosgene, Et_3N, Ar, THF, -10 °C to room temp, (42% yield for **299a-d**)

Having ensured the synthesis of oxazolidinones **299**, preparative chiral HPLC is used to separate all 4 diastereoisomers of **295f** and these syntheses are repeated on each diastereoisomer obtained (Scheme 4.1.10) to access *syn*- and *anti*-(**298a-d**). This leads to *syn*- and *anti*- oxazolidinones **299a-d**.

The trace chromatogram (Figure 4.1.1) shows that each isolated enantiomer of **294f** corresponds to a separate peak **A-D** (Table 4.1.3). ^{1}HNMR spectroscopic *J* coupling analysis of chirally pure oxazolidinones **299a-d** (Scheme 4.1.10) allows correlation of the HPLC peaks (**A-D**) to the identities of **295f** diastereoisomers (Table 4.1.3).



Compound (Peak entry)	Retention time (min)	*Oxazolidinone*	*JsynlJanti (Hz)*
295f (A)	34.7	**299a**	8.8 Hz (*anti*)
295f (B)	38.7	**299b**	5.4 Hz (*syn*)
295f (C)	40.8	**299c**	5.4 Hz (*syn*)
295f (D)	44.2	**299d**	9.0 Hz (*anti*)

Table 4.1.3: Identification of *syn/anti* products by chiral HPLC

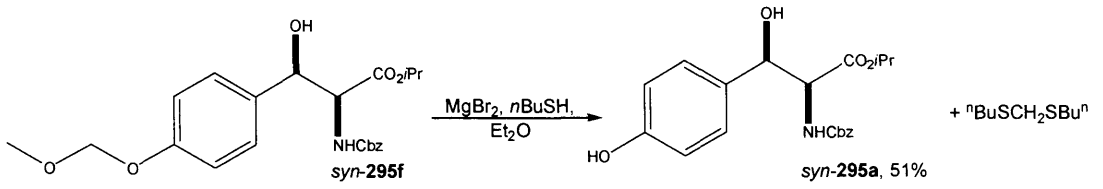
Figure 4.1.1: HPLC trace for **295f**

Anti-**295f** (peaks **A** and **D**) are separable, but *syn*-**295f** (peak **B**) and *syn*-**295f** (peak **C**) are more difficult to separate. On increasing the column loading from 2.5 mg ml^{-1} to 5 mg ml^{-1} to obtain more of the *syn* enantiomers **295f** (peak **B**) and **295f** (peak **C**), the separation becomes even worse.

So that the desired *syn*-(*p*-hydroxy-3-phenyl)serine isopropyl ester **295a** is obtained *via* *syn*-**295f** (Scheme 4.1.6), preparative chiral HPLC is used to isolate peaks **B** and **C** simultaneously. Alkylation of the resultant *syn*-**295a** should provide access to any *syn*-*p*-substituted 3-phenylserine standard **295b-g** for comparison with enantiomers produced by subsequent Sharpless AA reaction. For this protocol to be effective, the *p*-MOM group from the diastereomeric mixture of **295f** has to be removed without removal of other protecting groups (*i.e.* the isopropyl ester or the *N*-Cbz group).

Compound **295f** is reacted (Scheme 4.1.11) with 1-butanethiol and magnesium bromide and the *p*-MOM group is successfully removed in 51% yield, to give **295a**.[176] As this method could be extended by simple alkylation procedures, we next turn our attention

to isolating one *syn*-enantiomer (peak **B**) using preparative chiral HPLC (Figure 4.1.1) for comparison with the corresponding Sharpless adduct.

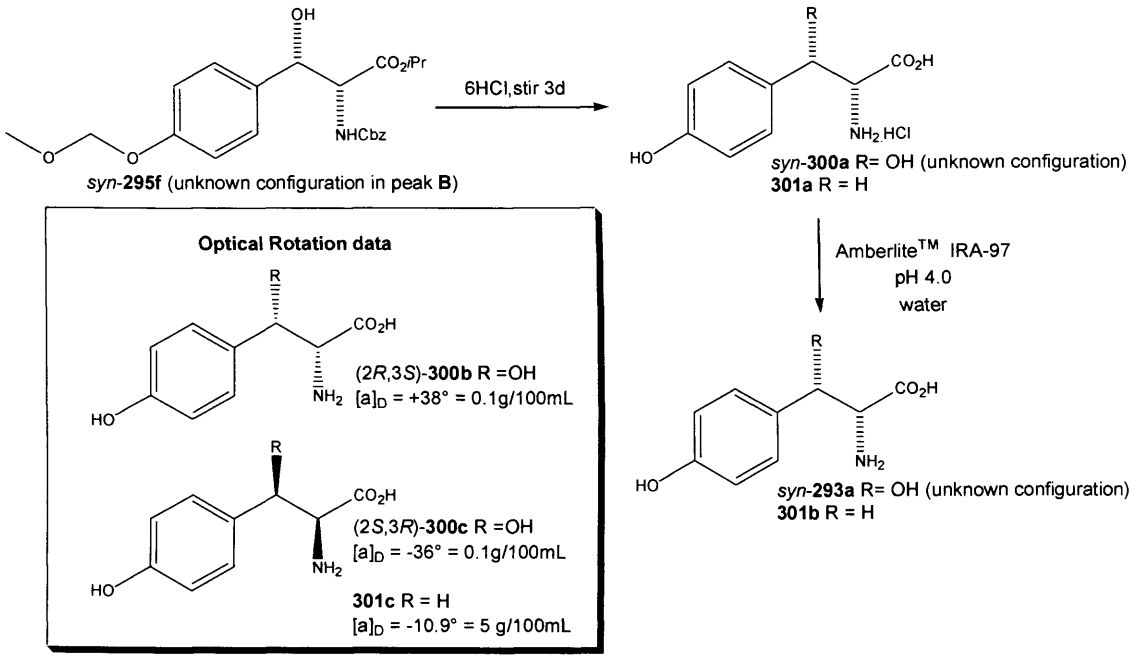


Scheme 4.1.11: Selective cleavage of *p*-methoxymethyl group using a thioacetal protocol

4.1.4 Determination of absolute stereochemistry

It is thought that the absolute stereochemistry of *syn* **295f** could be determined by using optical rotation data for a known derivative. The known derivative could be formed in one step (Scheme 4.1.12) using 6M HCl to remove the *p*-MOM group, *N*-Cbz group and hydrolyse the isopropyl ester from the enantiomer *syn*-**295f** (peak **B**), which would be obtained by chiral HPLC purification (Figure 4.1.1). This should give pure *syn*-3-(4-hydroxyphenyl)serine enantiomer **300a** as a hydrochloric acid (Scheme 4.1.12). An Amberlite IRA-97 resin could then be used (Scheme 4.1.12) to remove the hydrochloric acid from **300a** and give *syn*-3-(4-hydroxyphenyl)serine enantiomer as the amino acid **293a** with unknown configuration. This could then be compared with the optical rotation data supplied by Herbert *et al* for known *syn*-3-(4-hydroxyphenyl)serine (2*R*,3*S*)-(**300c**) and (2*S*,3*R*)-(**300d**) enantiomers (Scheme 4.1.12). From this data, the absolute configuration, deduced by optical measurements of **293a**, should identify the stereochemistry of the original *syn*-enantiomer **295f**, isolated as peak **B** (Figure 4.1.1).[177]

The synthesis is tested (Scheme 4.1.12) by conversion of L-tyrosine (**301a**) to L-tyrosine hydrochloric acid salt (**301b**) under acidic conditions. Lyophilisation of the aqueous mixture, maintaining the pH at 4.0 using a pH meter and the bead-wise addition of Amberlite IRA-97 resin provide **301a** for comparison to known melting points. Isolation is simple, as the resin is removed by filtration and the aqueous mixture is dried by lyophilisation. The optical rotation is measured for **301a** ($[\alpha]_D$ =-11.8°) and found to be within acceptable error limits for the observed optical rotation of $[\alpha]_D$ =-10.9°, seen in the literature for **301c**.[178] It iss found however, that the reliability of this result is dependent on the concentration being greater than 0.1 g/100 mL for this measurement.

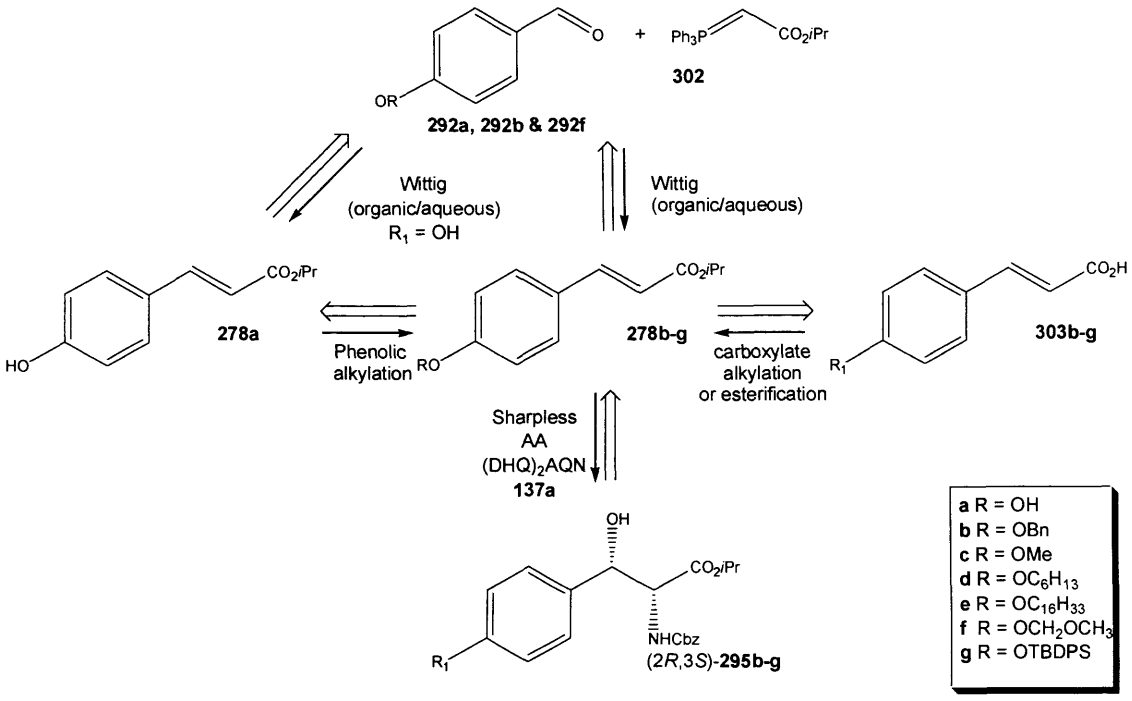


Scheme 4.1.12: Derivatization to known standards **300c** and **300d** for determining the absolute stereochemistry of **293f**

As only milligrams of *syn*-**295f** enantiomer (peak **B**) are obtained by chiral HPLC, a reliable optical rotation is too difficult to obtain. Also purification issues may arise if acidic hydrolysis does not cleave all the groups. Instead, a safer option is considered, using a Mosher's ester route. The small amount of *syn*-enantiomer **295f** (obtained by chiral HPLC (peak **B**)) is kept for comparison with the corresponding Sharpless adduct as synthesised in chapter 5.

4.2 Synthesis of Cinnamate precursors for Sharpless Asymmetric aminohydroxylation

The selected isopropyl cinnamate ester substrates **278a-g** (Scheme 4.2.1) for preparation of Sharpless AA *N*-Cbz 3-(*p*-substituted phenyl)serines **295a-g** are identified in Scheme 4.1.6. The required isopropyl cinnamate ester substrates **278a-g** can be obtained using a number of routes. This section describes the synthesis of **278a-g** using traditional Wittig reactions in organic, an organic/aqueous mixture and aqueous solvents. The Wittig methods are applied to the substrates (**278a**, **278b** and **278f**), which are not readily prepared by phenolic alkylation or carboxylate addition/esterification routes.

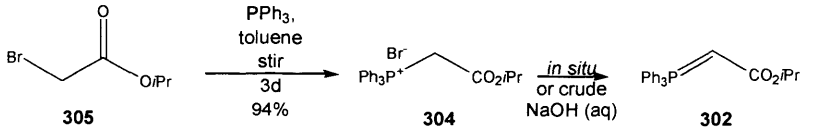


Scheme 4.2.1: Retrosynthetic scheme for the synthesis of cinnamate substrates **278a-g**

4.2.1 Wittig Methodology

The Wittig reaction is used to access cinnamate substrates **278a**, **278b** and **278f**, as high *E*:*Z* ratios can be obtained using this approach. High *E*:*Z* ratios are required for Sharpless AA reactions to occur due to the nature of osmium binding. Aldehydes **292a** (R = OH) and **292b** (R =OBn) are commercially available, whilst the synthesis of **292f** (R =OMOM) has previously been described (Scheme 4.1.9).

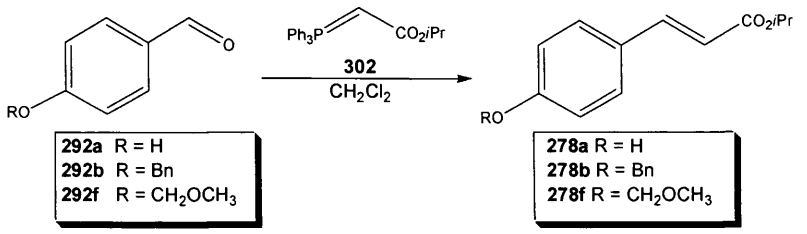
The phosphorous ylid **302** is generated from the phosphonium salt **304** and is used in its crude form in organic reactions, or is made *in situ* in aqueous reactions (Scheme 4.2.2). This is due to the inherent instability of **302**.[179] The salt **304** is obtained in 95% yield from the reaction between isopropyl bromoacetate **305** with triphenylphosphine.



Scheme 4.2.2: Synthesis of ylid **302**

Phosphonium salt **304** is dissolved in dichloromethane and vigorously stirred with a sodium hydroxide solution. Separation of the aqueous layer yields the crude phosphorous ylid **302**. Addition of aldehydes **292a** (R = OH), **29b** (R = OBn) and **292f** (R = OMOM) in dichloromethane to the crude ylid **304** lead to the selected isopropyl

cinnamate esters **278a** (R = OH), **278b** (R = OBn) and **278f** (R = OMOM) as shown in Table 4.2.1 in good yields and with high *E*:*Z* selectivities.



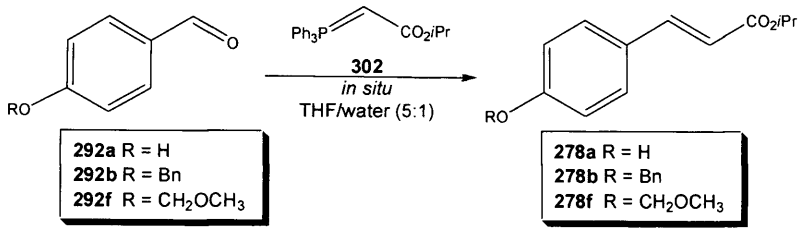
Entry	Aldehyde	Product (% yield)	*E*:*Z* ratio[a]
1	**292a**	**278a** (90)	26:1
2	**292b**	**278b** (96)	25:1
3	**292f**	**278f** (75)	6:1

[a]As determined by ^{1}H NMR spectroscopic analysis

Table 4.2.1: Wittig reactions in dichloromethane

One disadvantage of using chlorinated organic solvents is that it costs four times as much to dispose of chlorinated waste than it does for non-chlorinated waste. Therefore, performing these reactions an aqueous environment may make the process more environmentally benign. In addition, the development of a one-pot reaction for forming Sharpless AA adducts directly from the *in situ* formation of cinnamates **278a**, **278b**, **278d** (R = C_6H_{13}), **278e** (R = $C_{16}H_{33}$) and **278f** may be possible.

Test reactions are initially carried out to see what effects a water-THF mixture (5:1 v/v) has on yields and *E*:*Z* selectivities with the aldehydic substrates **292a** (R = OH), **292b** (R = OBn) and **292f** (R = OMOM). These results are shown in Table 4.2.2.



Entry	Aldehyde	Product (% yield)	E:Z ratio[a]
1	**292a**	**278a** (83)	26:1
2	**292b**	**278b** (90)	56:1
3	**292f**	**278f** (93)	14:1

[a]As determined by ^{1}H NMR spectroscopic analysis

Table 4.2.2: Wittig reactions in THF/water

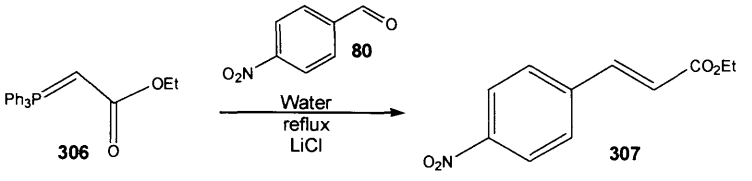
Wittig reactions in water/THF (5:1 v/v) using substrates **292a**, **292b** and **292f** with phosphorus ylid **302** give improved *E*:*Z* ratios for cinnamates **278a**, **278b** and **278f** compared to the reactions in dichloromethane (Table 4.2.1). The yields, however,

decrease slightly for cinnamates **278a** and **278b** (entries **1** and **2**), but increase from 75% to 93% for cinnamate **278f** (R = OCH_2OCH_3; entry **3**).

With the substantial improvement in *E:Z* selectivities on going from dichloromethane to aqueous THF, it is decided to test a wider range of substrates in water. Wu *et al.*, report reactions of triethyl phosphonoacetate and diethyl 2-oxoalkanephosphonates with aldehydes in a Horner-Wadsworth-Emmons reaction in an aqueous environment, but decomposition of the phosphonium salts and phosphorus ylids caused by aqueous bases are observed.[180] They also report that nitrobenzaldehyde **80** with (carbethoxymethylene)triphenylphosphorane (**306**), in the presence of aqueous lithium chloride, heated to reflux (Scheme 4.2.3) affords cinnamate (**307**) in 97% yield and with a 91:9, *E:Z* selectivity. Wu *et al.*, investigates a wide range of aldehydes but they have to have a boiling point higher than that of water, due to volatility issues. The order of reactivity of the *p*-substituted aromatic aldehydes follows the expected pattern:

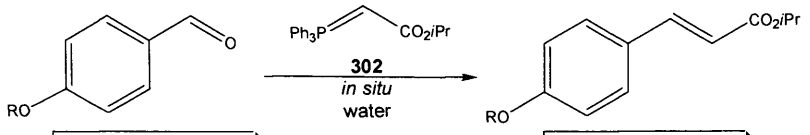
Electron withdrawing groups>> unsubstituted > electron donating groups.

All of the yields are high (72-98%) and good *E:Z* selectivities (85:15 to 95:5) are observed.



Scheme 4.2.3: Synthesis of cinnamate **307** under aqueous conditions

Based on our previous observations, we decide to investigate a range of aldehydes under aqueous conditions, without heating or with the addition of additives. These results are shown in Table 4.2.3. Triphenylphosphine oxide, a by-product of the Wittig reaction should not be soluble in water and would be removed by filtration at the end of the reaction. Unfortunately, this is not the case and a chlorinated solvent has to be used for the workup as we are concerned that ether may not dissolve all of the cinnamate products; whilst ethyl acetate has the potential to react unreacted triphenylphosphine forming ethyl crotonate and thus makes chromatographic separation of the desired product difficult to perform.



292a R = H
292b R = Bn
292d $R_1 = C_6H_{13}$
292e $R_1 = C_{16}H_{33}$
292f R = CH_2OCH_3

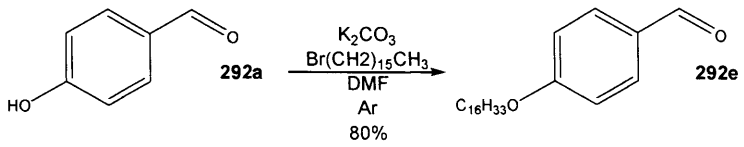
278a R = H
278b R = Bn
278d R = C_6H_{13}
278e R = $C_{16}H_{33}$
278f R = CH_2OCH_3

Entry	Aldehyde	Product (% yield)	*E*:*Z* ratio[a]
1	**292a**	**278a** (47)	26:1
2	**292b**	**278b** (67)	2:1
3	**292d**	**278d** (80)	30:1
4	**292e**	**278e** (80)	30:1
5	**292f**	**278f** (65)	15:1

[a]As determined by ^{1}H NMR spectroscopic analysis

Table 4.2.3: Wittig reactions in water

Aldehyde **292a** (entry **1**) gives the cinnamate **278a**, but the reaction yield is low (47%). The *E*:*Z* ratio is maintained at 26:1 from the previous reaction (Table 4.2.2). Aldehyde **292b** (entry **2**) gives the cinnamate **278b** 67% yield (Table 4.2.3), which is lower than both reactions in dichloromethane (Table 4.2.1) or with aqueous THF (Table 4.2.2). Also the *E*:*Z* ratio is substantially lowered by using aqueous THF from 26:1 to 2:1 when compared with water. Aldehyde **292d** is commercially available, but **292e** requires synthesis (Scheme 4.2.4) and is readily prepared in 80% yield.



Scheme 4.2.4: Synthesis of **292e**

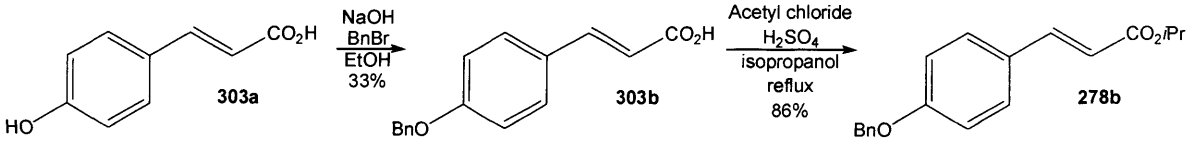
Both aldehydes **292d** and **292e** (entries **3** and **4**) give cinnamates **278d** and **278e** in high 80% yields, with excellent *E*:*Z* selectivities (30:1). This is not expected. We postulate that these aldehydes form reaction pockets in the aqueous media, whereby micelles envelope the ylid **302** and keep water out of the reaction pocket. Moreover, creating localised areas of high concentration causes the reaction to be driven to completion and one face of the aldehyde is more readily available to the ylid. Finally, the use of aldehyde **292f** (entry **5**) gives cinnamate **278f** and is obtained in good yields, in all solvents (65%-93% yields). The *E*:*Z* selectivity improves slightly from 14:1 in dichloromethane (Table 4.2.1) when compared with 15:1 in aqueous THF (Table 4.2.2) and water. The water based Wittig reactions performs better than the Wittig reactions in

dichloromethane. Furthermore, we find that additives (lithium chloride) and the need to reflux at high temperatures are unnecessary for these syntheses.

4.2.2 Other methods towards the synthesis of cinnamates

Standard esterification methods, carboxylate additions and phenolic alkylations are also used to access the desired isopropyl cinnamate esters **278a-g**, all using commercially available compounds. The results of these syntheses are reported in this section.

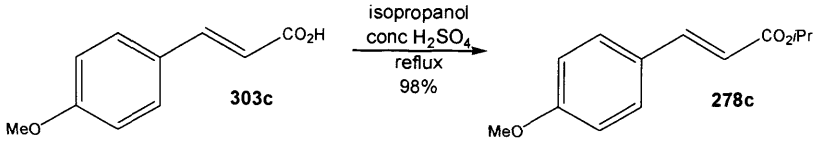
Commercially available 4-hydroxycinnamic acid (**303a**) is alkylated at the phenolic position with benzyl bromide in alcoholic sodium hydroxide solution (Scheme 4.2.5), yielding 4-benzyloxycinnamic acid (**303b**) in 33% yield.



Scheme 4.2.5: Synthesis of **278b**

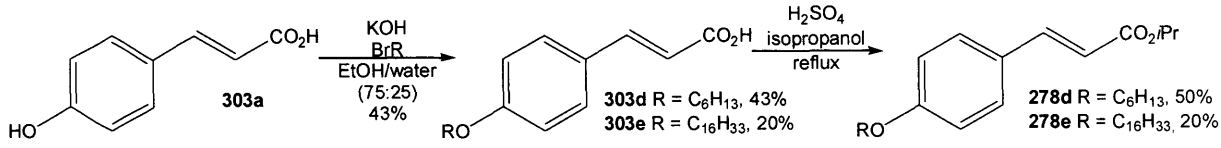
Esterification of **303b** (Scheme 4.2.5) is achieved using acetyl chloride with concentrated sulphuric acid in isopropanol heated at reflux and affords 4-benzyloxy isopropyl cinnamate (**278b**) in 86% yield. The overall yield for this route is 60%, which compared to the Wittig routes (even in water) is generally lower yielding (67%-96%) and requires more steps.

Fischer esterification (Scheme 4.2.6) of commercially available 4-methoxycinnamic acid (**303c**) in isopropanol, heated to reflux with an acid catalyst, leads to the successful synthesis of 4-methoxy isopropyl cinnamate ester (**278c**) in 98% yield, thus providing the most direct route.



Scheme 4.2.6: Fischer esterification of 4-methoxycinnamic acid **303c**

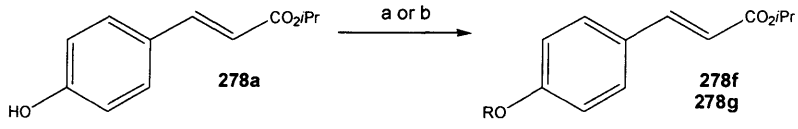
Commercially available 4-hydroxycinnamic acid (**303a**) is alkylated at the phenolic position with 1-hexyl bromide and 1-hexadecyl bromide in an alcohol/water mixture of potassium hydroxide (Scheme 4.2.5), yielding 4-hexyloxycinnamic acid (**303d**) in 43 % yield and 4-hexadecyloxycinnamic acid (**303e**) in 20% yield, respectively.



Scheme 4.2.7: Synthesis of **278d** and **278e**

A typical Fischer esterification protocol of **303d** and **303e** (Scheme 4.2.7) affords cinnamates **278d** in 50% yield and **278e** in 20% yield, respectively. The overall yields for these reactions are 47% and 20% yields, which compared to the aqueous Wittig route are far more inefficient.

Cinnamate **278a** (R = OH) is converted by conventional alkylation methods, as shown (Scheme 4.2.8).



Scheme 4.2.8: Reagents and conditions: (a) MOMCl, DIPEA, CH_2Cl_2 → **278f** (24%); (b) imidazole, TBDPSiCl, DMF → **278g** (65%);

Cinnamate **278a** in both instances are successfully alkylated at the phenolic position to give cinnamates **278f** and **278g**. As expected, the single Wittig reaction provides a better yield of **278g** than using a two-step reaction.

4.3 Conclusion

A simple one-pot reaction (Scheme 4.1.3) of benzaldehyde **30** with glycine **282** under basic conditions yields the *syn*-3-phenylserine **284**. Subsequent acetamide or benzyloxycarbonyl protection yields **285** and **287** (Scheme 4.1.3), respectively. Isopropyl ester formation by alkylation of an *in situ* caesium salt of **285** and **287**, yields desired *syn* chiral HPLC standards **286** and **288** (Scheme 4.1.3). The *syn*-diol by-product **289**, associated with the Sharpless reaction, is also readily synthesised (Scheme 4.1.4). A short reaction sequence (Scheme 4.1.5) involving the reduction of isopropyl ester **288** and removal of the benzyloxycarbonyl group of **290** yields the desired *syn* aminodiol **291** and could be readily applied to the synthesis of other *syn*-3-*N*-Cbz-(*p*-substituted phenyl)serines substrates, **295a-g**.

An investigation into the synthesis of a range of *syn*-3-(*p*-substituted phenyl)serines using glycine **282** is attempted. First benzyloxybenzaldehyde (**292b**) is reacted with

glycine **282** and this leads to the formation of 3-(*p*-benzyloxyphenyl)serine **293b** (Scheme 4.1.7) in a 60:40 *syn*/*anti* ratio. Subsequent hydrogenolysis methods fails to yield a desired 3-(*p*-hydroxyphenyl)serine (**293a**). Attempts to form a chiral HPLC standard by benzyloxycarbonyl protection of **293b** fail (Table 4.1.2). This is due to its amphiphilic nature. Isopropyl ester formation using **293b** (Scheme 4.1.8) in the presence of acetyl chloride with isopropanol is successful and affords **297**, but only in 10% yield. Attempts to synthesise *N*-Cbz-**294b** using **297** are unsuccessful.

Then glycine **282** is reacted under basic conditions with *p*-MOM benzaldehyde (**292f**) and provides a divergent route (Scheme 4.1.9), allowing access to a 1:1 diastereomeric mixture of 3-(*p*-methoxymethylphenyl)serine (**293f**). Subsequent *N*-Cbz protection and isopropyl ester formation, as before, gives chiral HPLC standard **295f**. Chiral HPLC separation of the 4 peaks and derivatization of each peak collected (peaks **A-D**) to give oxazolidinones **299a-d** (Table 4.1.3) allow elucidation of the relative stereochemistry in peaks A-D of **295f**. *Syn* products of **295f** (peaks **B** and **C**) are collected as one product using preparative chiral HPLC and removal of the *p*-MOM group using *n*-butane thiol in the presence of magnesium bromide afford **295a** (Scheme 4.1.11), which could be alkylated to give any other *syn*-*p*-substituted alkoxy Sharpless adduct.

The determination of the absolute stereochemistry of **295f** is defined in the next chapter by comparison against Mosher's ester formation. This is achievable using the Sharpless AA reaction, as not enough purified chiral HPLC **295f** is available for the tentative route outlined in Scheme 4.1.12.

Three main routes are investigated towards the formation of cinnamate substrates used in the Sharpless AA reaction. The Wittig reactions of *p*-hydroxybenzaldehyde (**292a**), *p*-benzyloxybenzaldehyde (**292b**) and *p*-methoxymethylbenzaldehyde (**292f**) with the phosphorous ylid **302** in dichloromethane (Table 4.2.1), aqueous THF mixture (Table 4.2.2) and in water (Table 4.2.3) are investigated. There are no major differences between the three reaction media, with yields ranging from 47%-96%. The lower yields are observed generally in aqueous media, but the *E*:*Z* selectivities improve on going from dichloromethane to aqueous solvents.

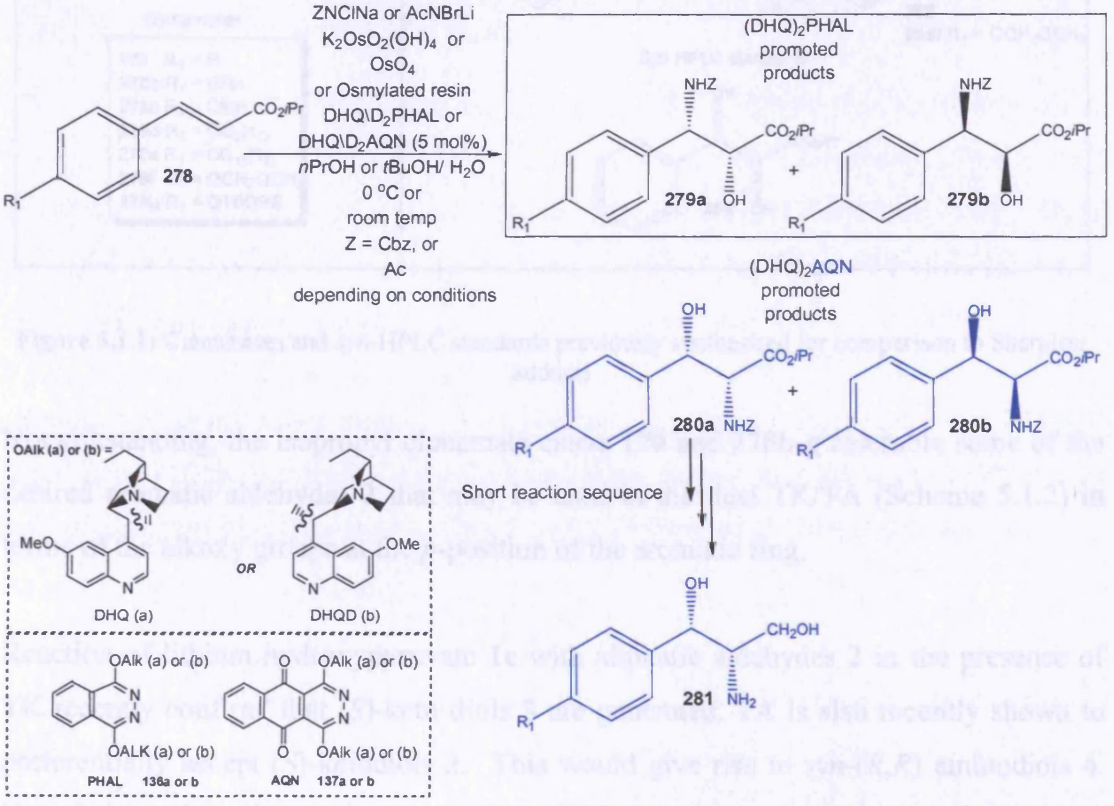
Other methods, including esterification and phenolic alkylation, are used with commercially available cinnamic acid to access a variety of cinnamates (Scheme 4.2.6,

Scheme 4.2.7 and Scheme 4.2.8). Those cinnamic acids that are not commercially available are readily synthesised by phenolic alkylation. Fisher esterification protocols are used to access cinnamates **278b-g**. On the whole, the Wittig route is superior in terms of yields and is performed in one step. This provides all the selected test substrates for the Sharpless AA.

Chapter 5

5.1 Sharpless Asymmetric Aminohydroxylation (AA) using cinnamates

As shown previously (Scheme 5.1.1) the products generated using cinnamates **278** with chiral ligand $(DHQ)_2AQN$ **137a** are 3-phenylserine enantiomers **280a** and **280b** and are now used as part of a synthetic strategy towards the desired chiral aminodiols **281**.



Scheme 5.1.1: *N*-protected aminoalcohol esters generated from cinnamates **278** by Sharpless AA

The isopropyl cinnamate esters **170** (R_1 = H) and **278b-g** (R_1 = Alkoxy group), as seen in Figure 5.1.1, were synthesised and are detailed in section 4.2. The isopropyl cinnamate esters **278b-g** are elected to investigate the size effects of various *p*-substituted alkoxy groups on the yields, regio- and enantioselectivities in the Sharpless AA reaction. A one-pot synthesis provides a route towards *syn* chiral HPLC standards **286** and **288**, whereas **295f** is only accessible in a 1:1 diastereomeric ratio (Figure 5.1.1). These standards are for the purposes of chiral HPLC elucidation and to test the synthetic methods towards *syn*-(*S*,*S*)-aminodiols **281**. The Sharpless AA reaction is

employed to synthesise either (2*R*,3*S*)-*N*-Cbz 3-phenylserine enantiomer **280a** or (2*S*,3*R*)-*N*-Cbz 3-phenylserine enantiomer **280b**. These can be achieved using chiral ligands $(DHQ)_2AQN$ **137a** or $(DHQD)_2AQN$ **137b**, respectively. We elect to use $(DHQ)_2AQN$ **137a** in the Sharpless AA reaction to yield (2*R*,3*S*)-*N*-Cbz 3-phenylserine enantiomer **280a**. (2*S*,3*R*)-*N*-Cbz 3-phenylserine enantiomer **280a** could be isolated using preparative chiral HPLC and then converted to the (*S*,*S*)-aminodiols **281** (as previously detailed in section 4.1.2).

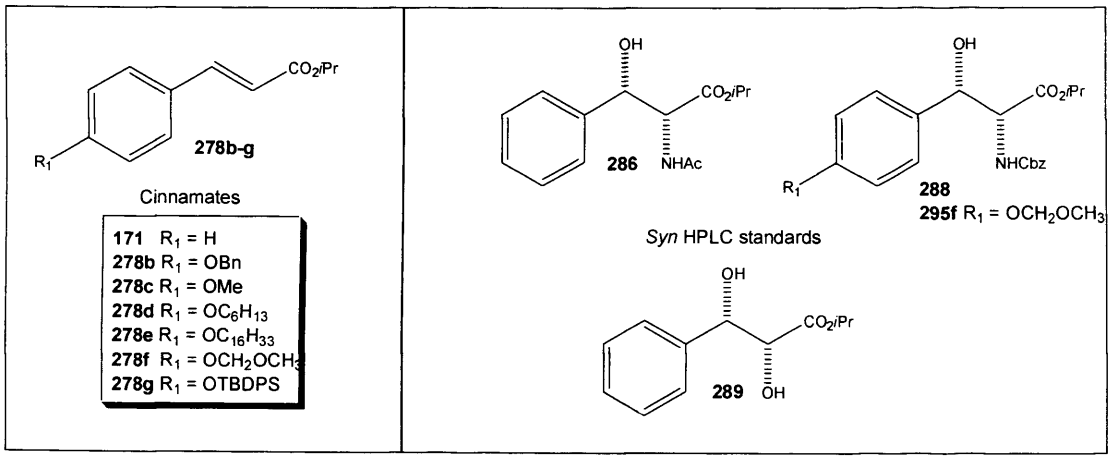
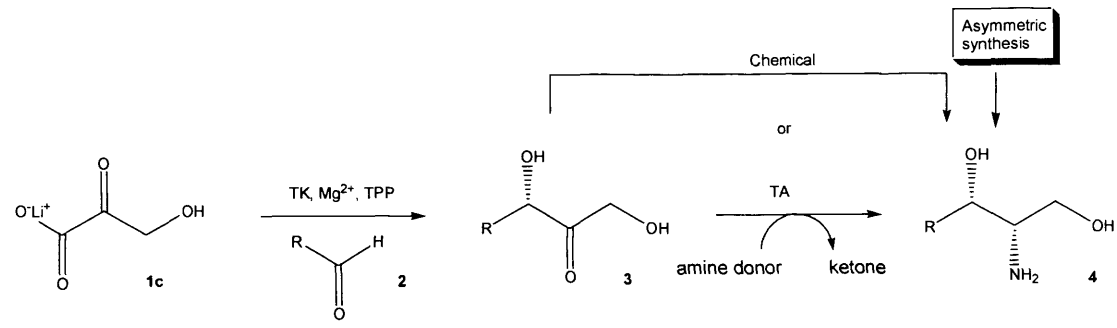


Figure 5.1.1: Cinnamates and *syn*-HPLC standards previously synthesised for comparison to Sharpless adducts

Notwithstanding, the isopropyl cinnamate esters **170** and **278b-g** resemble some of the desired aromatic aldehydes **2** that may be used in the dual TK/TA (Scheme 5.1.2) in terms of the alkoxy groups at the *p*-position of the aromatic ring.

Reaction of lithium hydroxypyruvate **1c** with aliphatic aldehydes **2** in the presence of TK recently confirm[i] that (*S*)-keto diols **3** are generated. TA is also recently shown to preferentially accept (*S*)-ketodiols **3**. This would give rise to *syn*-(*R*,*R*) aminodiols **4**. Nonetheless, through genetic engineering of TA, it may be possible to accept aromatic keto diols with (*R*)-stereochemistry, giving rise to aminodiols with *syn*-(*S*,*S*)-stereochemistry. In terms of asymmetric synthesis, the Sharpless AA route provides the most direct route in establishing the *syn*-(2*R*,3*S*) stereocenters in the *N*-Cbz 3-phenylserine enantiomers.

[i] *Unpublished results*, Hailes, H.C., Smith, M.E.B.



Scheme 5.1.2: Synthesis of aminodiols using TK/TA dual enzyme or chemoenzymatic route

Homogeneous and heterogeneous catalytic Sharpless AA reactions are performed using the $(DHQ)_2AQN$ ligand **137a** and the results are presently discussed.

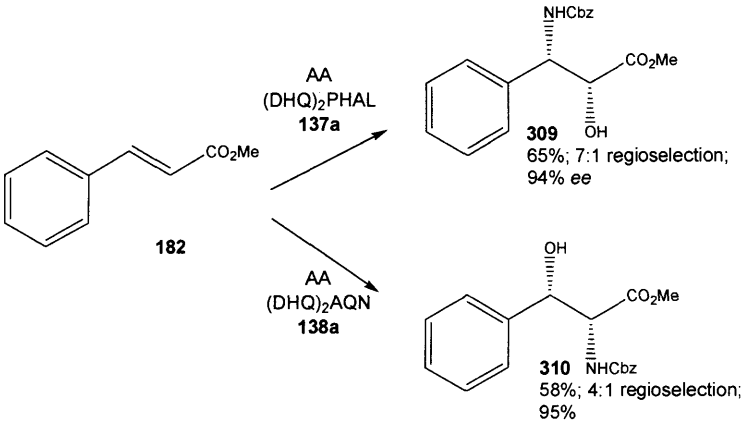
5.1.1 Homogeneous catalysis

There is a large literature precedent for the use of $(DHQ)_2PHAL$ **136a** in Sharpless AA reactions,[101,102,103,105,110,124,162,181,182,183] with various isopropyl cinnamates **170** (R_1 = H) and **278b-g** (R_1 = Alkoxy), ethyl cinnamates **308** and methyl cinnamates **182**. These yield the expected (2*R*,3*S*)-3-phenylisoserine enantiomers **279a**. The corresponding $(DHQD)_2PHAL$ **136b** give rise to (2*S*,3*R*)-3-phenylisoserine enantiomer **279b** (Scheme 5.1.1). Sharpless AA reactions with **170** (R_1 = H) and **278b-g** (R_1 = Alkoxy) in the presence of $(DHQ)_2PHAL$ **136a** under homogeneous catalytic conditions are undertaken for the main purpose of determining regioselectivities and enantioselectivities when compared with the analogous reactions using $(DHQ)_2AQN$ **137a**, giving (2*R*,3*S*)-3-phenylserine enantiomers **280a**.

Chiral HPLC elucidation of the semi-purified product mixtures resulting from Sharpless AA reactions using $(DHQ)_2AQN$ **137a** would still contain the (2*R*,3S)-3-phenylisoserine enantiomer **279a** and so these HPLC peaks need to be identified against the desired (2*R*,3*S*)-3-phenylserine enantiomers **280a** (Scheme 5.1.1). The synthesis of (2*R*,3*S*)-3-phenylserine enantiomer **280a** heading to *syn*-(*S*,*S*)-aminodiols **281** (Scheme 5.1.1),[7,8] warrants an investigation into improving the regio- and enantioselectivities of the Sharpless AA reaction. This is done so that the final yield of *syn*-(*S*,*S*)-**281** would be increased by minimizing the formation of undesired by-products. This, we thought could be achieved by either homogeneous or heterogeneous catalysis.

Morgan *et al.* and Tao *et al.*, previously report that yields and regioselectivities of (2*R*,3*S*)-3-phenylisoserine enantiomers **279a**, formed using $(DHQ)_2PHAL$ **136a** exceed

those from the analogous Sharpless AA reactions using $(DHQ)_2AQN$ **137a**, which would yield (2*R*,3*S*)-3-phenylserine enantiomer **280a** (Scheme 5.1.1).[7,8] For example, O'Brien reports a direct comparison of the Sharpless AA with a Cbz-carbamate nitrogen source with methyl cinnamate **182** in the presence of both **136a** and **137a**, yielding (2*R*,3*S*)-3-phenylisoserine enantiomer (**309**) and (2*R*,3*S*)-3-phenylserine enantiomer **310**, respectively (Scheme 5.1.3).[183]



Scheme 5.1.3: Sharpless AA reaction with methyl cinnamate in the presence of either $(DHQ)_2PHAL$ **136a** and $(DHQ)_2AQN$ **137a**

A number of nitrogen sources are used in the Sharpless AA reaction and are discussed in the introduction. Several of these nitrogen sources (certain carboxamides,[181] aminohetereocycles[111] and sulfonamides[118]) require synthesis. Therefore these nitrogen sources are not explored, so as to achieve the shortest possible synthetic route to (2*R*,3*S*)-**280a**. The most successfully used nitrogen sources giving (2*R*,3*S*)-3-phenylisoserine enantiomers **279a** (Scheme 5.1.1) are *N*-bromoacetamides[124] and *N*-halocarbamates.[162] Adopting this strategy, unsubstituted isopropyl cinnamate ester **170** and *p*-substituted isopropyl cinnamate esters (**278b-g**) are used with *N*-bromoacetamide or benzyl carbamate in the presence of $(DHQ)_2PHAL$ **136a** under homogeneous Sharpless AA conditions.

Many Sharpless AA reactions utilise isopropyl cinnamate ester **170** with an *N*-bromoacetamide nitrogen source and $(DHQ)_2PHAL$ **136a**, but methyl and ethyl cinnamate esters when *N*-halocarbamates are used.[63,124,166] Sharpless AA reactions with *N*-bromoacetamide and *N*-halocarbamates with $(DHQ)_2AQN$ **137a** are performed, as this type of comparison has not been reported with electron rich cinnamates - known to be good substrates for the Sharpless AA reaction.[7,8]

The use of styrenes though, are reported, both using chiral ligands **136a** and **137a**, in the presence of *N*-bromoacetamide and with some *N*-halocarbamates. These reports show varying levels of success. Tao *et al.*, for instance finds the amide-based AA preference is to place the nitrogen on the terminal non-benzylic carbon and is accentuated using $(DHQ)_2AQN$ **137a**. This is to our advantage.[184,115]

Therefore, we rationalise that a similar effect may occur with isopropyl cinnamate esters **170** and **278b-g**, owing to some of the successes reported by O'Brien (Scheme 5.1.3), but we decide to use benzyl carbamate as the nitrogen source in these transformations. This is after considering the following advantages and disadvantages of each nitrogen source and then trialling them with isopropyl cinnamate.[183]

N-Bromoacetamide acts as both the oxidant for osmium and as the nitrogen source, whereas carbamates require an external oxidant.[166] For, *N*-bromoacetamide, it is observed that the stereochemical outcome of Sharpless AA reactions (irrespective of the regiochemical outcome) is analogous to the asymmetric dihydroxylation (AD) reaction. The empirical rule states that dihydroquinyl ligands (DHQ)-**136a** or **137a**, should give rise to oxidation predominantly at the α-side of the olefin and exhibit (2*R*,3*S*) product stereochemistry, exemplified by **279a** (Scheme 5.1.1).[102,106,185,186] The outocme has not been established in detail for carbamate nitrogen sources, but was assumed to be similar.[124] It is also known that higher yields and enantioselectivities could be obtained when a less sterically demanding substituent is placed at the nitrogen atom of the aminoalcohol, so favouring the use of *N*-bromoacetamide. Furthermore, chromatographic separation of the *N*-Ac-3-phenylserines is reported as easier than with *N*-Cbz-3-phenylserines.[162]

Nevertheless, removal of *N*-acetamide groups is not as easy as with *N*-Cbz groups, which are cleaved by hydrogenolysis.[162] Additionally, *N*-bromoacetamide reactions are conducted at 4 °C to minimize the formation of Hoffman rearranged products;[124] but *t*-butyl hypochlorite, an oxidant used with the carbamate nitrogen source in Sharpless AA reactions[162] could be problematic due to its light sensitivity, difficulty of synthesis and short shelf life.[187] Barta *et al.* however, detail the use of two solid oxidants which could be substituted for *t*-butyl hypochlorite and claim that they have no deleterious effects on yields, regio and enantioselectivities.[176] These are 1,3-dichloro-5,5-dimethylhydantoin (**311**) or dichloroisocyanuric acid (**312**), both commercially available (Figure 5.1.2).

Neither oxidant have been used in homogeneous Sharpless AA reactions with cinnamates in the presence of $(DHQ)_2AQN$ **137a**.

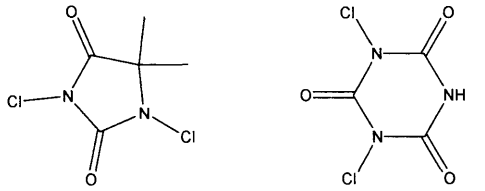
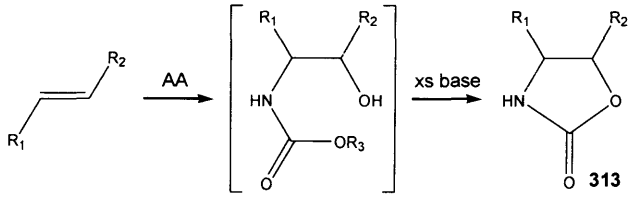


Figure 5.1.2: 1,3-dichloro-5,5-dimethyl hydantoin (**311**) and dichloroisocyanuric acid (**312**) oxidants

Moreover, Barta *et al* also states that by using a small excess of base, chromatographic separation of *N*-carbamate Sharpless AA adducts could be simplified by using an *in situ* conversion to oxazolidinones (**313**) (Scheme 5.1.4).

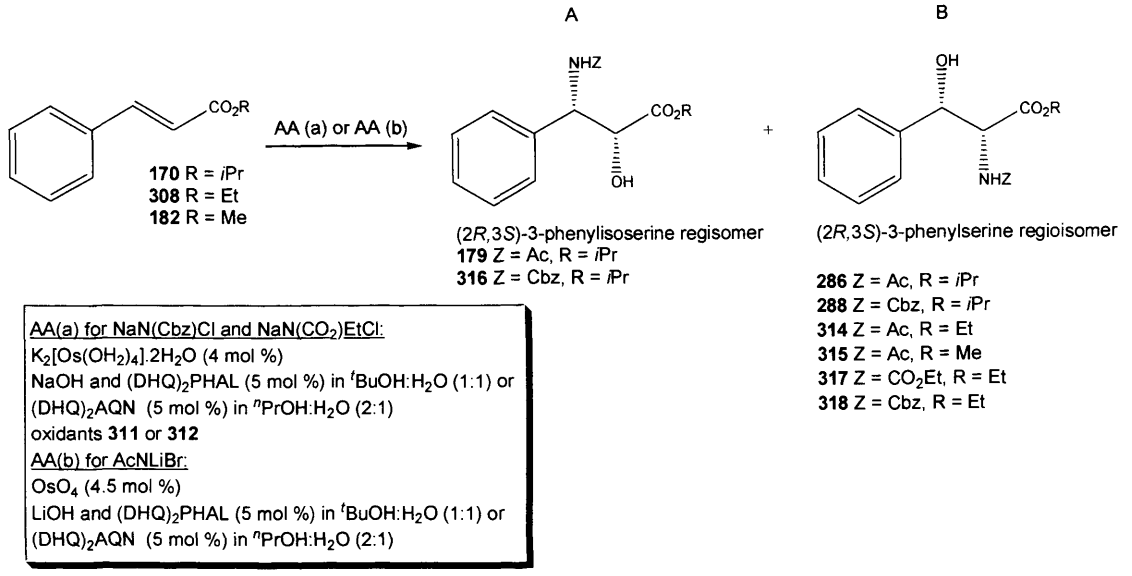


Scheme 5.1.4: Synthesis of oxazolidinone from using a nitrogen carbamate source

Taking into account these facts, we perform a series of reactions with unsubstituted cinnamate esters.

5.1.1.1 Homogeneous Sharpless AA reactions with unsubstituted cinnamate esters and *N*-halocarbamates or *N*-bromoacetamide

The results of Sharpless AA reactions (Scheme 5.1.5) are shown for various unsubstituted cinnamate esters (Table 5.1.1). Regioselective ratios (A:B) are for A, the 3-phenylisoserine enantiomer, the product generally formed using $(DHQ)_2PHAL$ **136a**. Whilst, B represents the desired 3-phenylserine enantiomer formed using $(DHQ)_2AQN$ **137a**.



Scheme 5.1.5: Sharpless AA reactions with unsubstituted cinnamate esters

Entry	Nitrogen Source	Cinnamate	Ligand	(2*R*,3*S*) Product (% yield) (lit. Yield, R = ester group, if avail)	A:B[a] (A:B from literature, if avail)	*Ee* (%) (2*R*,3*S*) (Lit Ee, If avail)
1	AcLiNBr	**170**	$(DHQ)_2AQN$	**286**, 15[d], 11[e]	1:3[c], 2:5[d]	17[c]
2	AcLiNBr	**170**	$(DHQ)_2PHAL$	**179**, 79[d], (72, *i*Pr)[124]	15:1 (>20:1, *i*Pr)[124]	90[c], (99)[124]
3	AcLiNBr	**308**	$(DHQ)_2AQN$	**314**, 20[d]	1:1	12[c]
4	AcLiNBr	**182**	$(DHQ)_2AQN$	**315**, 16[d]	1:1	30[c]
5	NaNCbzCl	**170**	$(DHQ)_2AQN$	**288**, 66[d], (58, Me)[8]	2:3 (1:4, Me)[8]	94[b] (95)[8]
6	NaNCbzCl	**170**	$(DHQ)_2PHAL$	**316**, 80,[d,f] (65, Me)[162]	18:1 (7:1, Me)[162]	94[c], (94)[162]
7	$NaNCO_2EtCl$	**308**	$(DHQ)_2AQN$	**317**, (48)[f,g],	3:2	n/a
8	NaNCbzCl	**182**	$(DHQ)_2AQN$	**318**, 48[d,g], 16[d,h],	1:1 2:3[e]	n/a

[a]As determined by ^1H NMR spectroscopic analysis, [b]Chiralpak AD, 80:20 (hexane:*i*PrOH), 0.6 mL min^{-1}; [c]Chiralpak ODH, 60:40 (hexane:*i*PrOH), 0.5 mL min-1; [d]1 mmol scale; [e]5 mmol scale; [f]6.5 mmol scale; [g]1,3-dichloro-5,5-dimethyl hydantoin (**311**); [h]dichloroisocyanuric acid (**312**);

Table 5.1.1: Results of Sharpless AA reactions with unsubstituted cinnamate esters using differing nitrogen carbamate and amide sources

O'Brien states that the enantioselectivity would not be affected by altering the ligand from **136a** to **137a** with the same nitrogen source, which proves true for *N*-Cbz carbamate sources.[183] Contrary to this observation, however, are the levels of asymmetric induction seen by changing the nitrogen source to *N*-bromoacetamide. *N*-bromoacetamide (Table 5.1.1) with isopropyl cinnamate ester **170** under homogeneous

conditions (entry **1**) gives (2*R*,3*S*)-*N*-Ac-3-phenylserine enantiomer-**286** in 15% yield. This is worse than the analogous (2*R*,3*S*)-*N*-Cbz-3-phenylserine enantiomer-**288**, which is obtained in 66% yield (entry **5**). The regioselectivity of (2*R*,3*S*)-*N*-Ac-3-phenylserine enantiomer **286** is 1:3, whilst the enantioselectivity is poor, 17% *ee*.

Yet, a switch to $(DHQ)_2PHAL$ **137a** (entry **2**) gives 3-phenylisoserine enantiomer (2*R*,3*S*)-**179** (as expected) in high yield 79%, with excellent regioselectivities (15:1) and excellent enantioselectivities, 90% *ee*. Our results are directly comparable to the literature results available for (2*R*,3*S*) -*N*-Ac-3-phenylserine regioisomer **179**, though we observe a small drop in *ee*. It is rationalised that the drop in enantioselectivity to 17% *ee* observed with (2*R*,3*S*)-**286** from 90% *ee*, observed with (2*R*,3*S*)-**179** is a result of changing the chiral ligand from **136a** to **137a**. It is likely that unfavourable stereoelectronic effects and unfavourable binding interactions in the pocket of the chiral ligand were responsible for such a decrease in enantioselectivity.

To ensure this behaviour is not being affected by the size of the ester group alone, the corresponding ethyl ester (2*R*,3*S*)-**314** (entry **3**) and methyl ester (2*R*,3*S*)-**315** (entry **4**) *N*-Ac-3-phenylserine enantiomers are synthesised using $(DHQ)_2AQN$ **137**. These are obtained in 20% and 16% yields, respectively. The regioselectivity is affected, going from 1:3 observed with the isopropyl ester (entry **1**) to 1:1 with both (2*R*,3*S*)-**314** and **315**. The enantioselectivities are poor, 12% *ee* for (2*R*,3*S*)-**314**, but an improvement is noted for **315**, obtained in 30% *ee*.

From these results, it is decided that *N*-halocarbamates (Table 5.1.1) urethane (ethyl carbamate) and Cbz-carbamate are to be used as nitrogen sources in the following Sharpless AA reactions. Isopropyl cinnamate ester **170** (Table 5.1.1) using $(DHQ)_2AQN$ **137a** with Cbz carbamate (entry **5**), provides (2*R*,3*S*)-*N*-Cbz-3-phenylserine enantiomer-**288** in 66% yield and in a 2:3 regioselective ratio. The enantiomeric purity of (2*R*,3*S*)-**288** is 94% *ee* and our result is comparable to the literature result available for the corresponding methyl ester. A change of ligand from $(DHQ)_2AQN$ **137a** to $(DHQ)_2PHAL$ **136a** (entry **6**) gives (2*R*,3*S*)-*N*-Cbz-3-phenylisoserine enantiomer **316** in excellent yields, regio- and enantioselectivities. Again this result is comparable to the available literature result for the corresponding methyl ester. The (2*R*,3*S*) absolute configuration is assigned by analogy to 3-phenylserine enantiomers formed in the literature, where $(DHQ)_2AQN$ **137a** is used

(Scheme 5.1.3).[8] This assumes that oxidation was occurring predominantly at the α-face of the cinnamate, as previously detailed.[124]

Ethyl cinnamate **308** is reacted with urethane (entry **7**), a smaller nitrogen source than Cbz-carbamate (entry **8**). Both reactions give similar yields and regioselectivities. To establish whether oxidants 1,3-dichloro-5,5-dimethylhydantoin (**311**) and dichloroisocyanuric acid (**312**) would improve yields or regioselectivities, assuming that similar enantioselectivities are achieved, ethyl cinnamate **308** is reacted under homogeneous Sharpless AA conditions. As shown in Table 5.1.1 (entry **8**), oxidant **311** gives a yield of 48% for 3-phenylserine enantiomer (2*R*,3*S*)-**317**. This is reduced to 16% when oxidant **312** is used, but a slight improvement in regioselectivity is observed for (2*R*,3*S*)-*N*-Cbz-**318**.

From these results (Table 5.1.1), further investigations are then performed with Cbz carbamate and *p*-substituted isopropyl cinnamates **278b-g** due to the reasonable yields, regio- and enantioselectivities observed (entry **5**).

5.1.1.2 Homogeneous Sharpless AA reactions with *p*-substituted isopropyl cinnamate esters with *N*-Cbz carbamate

Electron poor cinnamates are described as non-ideal substrates with *N*-Cbz carbamates from previous studies.[8] Therefore, electron-rich *p*-substituted isopropyl cinnamate esters **278a-g** (Figure 5.1.3) with $(DHQ)_2PHAL$ **136a** are reacted under homogeneous conditions to give various (2*R*,3*S*)-3-phenylisoserine enatiomers-**279a** (Scheme 5.1.1), aiding HPLC elucidation as before.

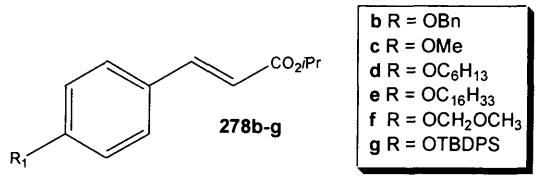
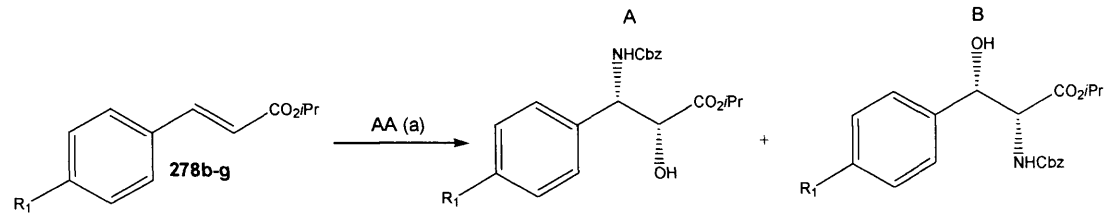


Figure 5.1.3: *p*-substituted cinnamate esters

The results of these homogeneous Sharpless AA reactions are shown first for homogeneous Sharpless AA reactions with $(DHQ)_2PHAL$ **136a** (Table 5.1.2) and then with $(DHQ)_2AQN$ **137a** (Table 5.1.3), which forms part of the synthetic strategy towards *syn*-(*S*,*S*)-aminodiols **281** (Scheme 5.1.1). The regioselective ratio A:B is where A is the (2*R*,3*S*)-3-phenyisoserine enantiomer (**319b-g**) formed using $(DHQ)_2PHAL$

136a. Whilst, B is the desired (2*R*,3*S*)-3-phenylserine (**295b-g**) enantiomer formed using $(DHQ)_2AQN$ **137a**.



AA(a) for NaNCbzCl:
$K_2[Os(OH_2)_4].2H_2O$ (4 mol %)
NaOH and $(DHQ)_2PHAL$ (5 mol %) in $^tBuOH:H_2O$ (1:1) or
$(DHQ)_2AQN$ (5 mol %) in $^nPrOH:H_2O$ (2:1)
oxidant **311**

Entry	R	(2*R*,3*S*) Product (% yield)[a]	A:B[b]	*Ee* (%) (2*R*,3*S*)[c]
1	OBn	**319b**, 80	18:1	94
2	OMe	**319c**, 48	28:1	96
3	OC_6H_{13}	**319d**, 65	5:3	90
4	$OC_{16}H_{33}$	**319e**, 6	4:1	87
5	OMOM	**319f**, 15	19:1	94
6	OTBDPS	**319g** 49	3:1	95

[a]Yields isolated as both regioisomers; [b]As determined by ^1H NMR spectroscopic analysis, [c]Chirapak AD, 80:20 (hexane:*i*PrOH), 1 mL min^{-1}

Table 5.1.2: Results of the homogeneous Sharpless AA reactions with the $(DHQ)_2PHAL$ **136a** ligand

No literature results are available for 3-phenylisoserine enantiomers (2*R*,3*S*)-**319b-g** as these are novel compounds (Table 5.1.2). The additional benzyl group on cinnamate **278b** results in 3-phenylisoserine enantiomer (2*R*,3*S*)-**319b** (entry **1**) and is thought to be responsible for favourable π-π stacking interactions within the binding pocket of either chiral ligand **136a** and **137a**. This is exemplified by having the highest (80% yield) of this reaction series, giving excellent regioselectivities (18:1) and having excellent enantioselectivities (94% *ee*). The smallest substituent at the *p*-alkoxy position, R = OMe of cinnamate **278c** (entry **2**) gives 3-phenylisoserine enantiomer (2*R*,3*S*)-**319c** in a moderate 48% yield, but has the highest regio- and enantioselectivities for this reaction series.

Aliphatic olefins do not show high levels of regioselectivities or high levels of asymmetric induction with either chiral ligand **136a** or $(DHQ)_2AQN$ **137a**.[105] p-Alkoxy groups (R = OC_6H_{13} and R = $OC_{16}H_{33}$) attached to cinnamates **278d** and **278e** (entries **3** and **4**) are reacted to see if the favourable π-π stacking interactions within the binding pocket of $(DHQ)_2PHAL$ **136a** are interrupted by these alkyl chains. 3-Phenylisoserine enantiomer (2*R*,3*S*)-**319d** with a *p*-hexyloxy side chain (entry **3**) is given in 65% yield,

but the regioselectivities is 5:3. This effect is observed also with the longer alkyl substituent ($OC_{16}H_{33}$) and gives 3-phenylisoserine enantiomer (2*R*,3*S*)-**319e**, isolated in 6% yield. The regioselectivity for (2*R*,3*S*)-**319e** was 4:1, but both (2*R*,3*S*)-**319d** and (2*R*,3*S*)-**319e** are formed in lower enantioselectivities, 90% and 87% *ee*'s, respectively when compared with (2*R*,3*S*)-**319b** (entry **1**) or (2*R*,3*S*)-**319c** (entry **2**).

A *p*-methoxymethyl isopropyl cinnamate **278f** gives 3-phenylisoserine enantiomer (2*R*,3*S*)-**319f** in 15% yield (entry **5**), in high regioselectivity (19:1) and enantioselectivity (94% *ee*).

The "bulkiest" group used on cinnamate **278g** (R = OTBDPS) gives 3-phenylisoserine enantiomer (2*R*,3*S*)-(**319g**) and interesting results (entry **6**). The yield achieved is 49%, but the regioselectivities are low (3:1), without having a detrimental effect on the enantiomeric purity, 95% *ee*.

Using the same cinnamates **278b-g**, homogeneous Sharpless AA conditions are repeated, but with chiral ligand $(DHQ)_2AQN$ **137a** (Table 5.1.3).

Entry	R	(2*R*,3*S*) Product/ (% yield)[a]	A:B[b]	*Ee* (%) (2*R*,3*S*)[c,d]
1	OBn	**295b** (76)	3:4	93
2	OMe	**295c** (60)	1:5	95
3	OC_6H_{13}	**295d** (75)	5:7	91
4	$OC1_6H_{33}$	**295e** (34)	1:1	86
5	OMOM	**295f** (76)	1:1	93[e]
6	OTBDPS	**295g** (40)	1:8	95

[a]Yields isolated as both regioisomers; [b]As determined by ^1H NMR spectroscopic analysis, [c]Chirapak AD, 80:20 (hexane:*i*PrOH), 1 mL min^{-1};[c]By analogy to previous literature data, [e]A discussion in the text follows

Table 5.1.3: Results of the homogeneous Sharpless AA reactions with $(DHQ)_2AQN$ **137a**

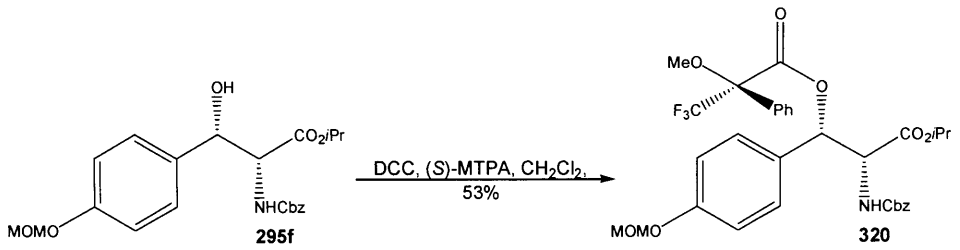
As before, an additional benzyl group on cinnamate **278b** (entry **1**) is thought to improve the favourable π-π stacking interactions within the binding pocket of **137a**, affording 3-phenylserine enantiomer (2*R*,3*S*)-**295b** in 76% yield. This yield is comparable to (2*R*,3*S*)-**319b** (entry **1**, Table 5.1.2), but a large decrease in regioselectivity for 3-phenylserine enantiomer (2*R*,3*S*)-**295b** (nearly 1:1) is observed when compared with 3-phenylisoserine enantiomer (2*R*,3*S*)-**319b** (Table 5.1.2, entry **2**). The enantioselectivities are unaffected, with a 93 % *ee* observed for 3-phenylserine enantiomer (2*R*,3*S*)-**295b**. Cinnamate **278c** (entry **2**) with the smallest substituent at the *p*-alkoxy position gives 3-phenylserine enantiomer (2*R*,3*S*)-**295c** in 60% yield. This is unexpected, as generally yields for Sharpless AA reactions with $(DHQ)_2PHAL$ **136a**

(48%, entry **2**, Table 5.1.3) are higher than those with $(DHQ)_2AQN$ **137a**. The regioselectivities, however, decrease to 1:5 for 3-phenylserine enantiomer (2*R*,3*S*)-**295c** from 3-phenylisoserine regioisomer (2*R*,3*S*)-**319c** (28:1). The enantioselectivities remain unaffected by changing from $(DHQ)_2PHAL$ **136a** to $(DHQ)_2AQN$ **137a** and are high (95% *ee*) for (2*R*,3*S*)-**295c**.

With large alkyl groups at the *p*-alkoxy position of cinnamates **278d** and **278e**, the corresponding 3-phenylserine enantiomers (2*R*,3*S*)-**295d** and (2*R*,3*S*)-**295e** (entries **3** and **4**) are obtained. Yields are comparable with 3-phenylisoserine enantiomers (2*R*,3*S*)-**319d** (75% yield) and (2*R*,3*S*)-**319e** (34% yield), as shown in Table 5.1.2. The trend in regioselectivities and enantioselectivities follow the traits already seen thus far.

p-Methoxymethyl cinnamate **278f** (entry **5**, Table 5.1.3) gives 3-phenylserine enantiomer (2*R*,3*S*)-(**295f**) in a 76% yield. This is compared to the 15% yield obtained for the 3-phenylisoserine enantiomer (2*R*,3*S*)-**319f**, seen in Table 5.1.2. The regioselectivities are affected significantly when $(DHQ)_2PHAL$ **136a** (entry **5**, Table 5.1.2) are compared to the regioselectivities of reactions involving $(DHQ)_2AQN$ **137a** (Table 5.1.3). Little effects on the enantioselectivities are observed when either ligand **136a** or **137a** are used. The only major difference observed is when cinnamate **278g** is used. This has a bulky OTBDPS group. The yield of 3-phenylserine enantiomer (2*R*,3*S*)-(**295g**) (entry **6**, Table 5.1.3) is similar to 3-phenylisoserine enantiomer (2*R*,3*S*)-**319g** (entry **6**, Table 5.1.2) being in the 40-50% region. The regioselectivity, however, improves to 1:8 for the 3-phenylserine enantiomer (2*R*,3*S*)-**295g** from 3:1 for the 3-phenylisoserine enantiomer (2*R*,3*S*)-**319g**. This is also achieved without having any deleterious effects on enantioselectivities of (2*R*,3*S*)-**295g**, which is obtained in 95% *ee*.

Co-injecting an analytical sample of previously obtained **295f** (via a diastereoselective synthesis, see section 4.1.4) by chiral HPLC separation confirms the identity of Sharpless AA product **295f**. Sharpless AA product (2*R*,3*S*)-**295f** is isolated and is converted to a Mosher's (*S*)-MTPA ester **320** (Scheme 5.1.6) for confirmation of the absolute configuration as (2*R*,3*S*).



Scheme 5.1.6: Synthesis of Mosher's ester **320**

A DCC coupling of the secondary alcohol **295f** with (S)-(+)-α-methoxy-α-trifluoromethylphenylacetylacid (*S*)-MTPA gives the Mosher's ester **320** in 53% yield. The use of Mosher's esters to assign chiral centres is well known from the literature[188] and useful conformation correlation models exist, which are used with **320a** (Figure 5.1.4) and aid the confirmation of the absolute configuration of the alcohol as a (3*S*)-stereocenter.

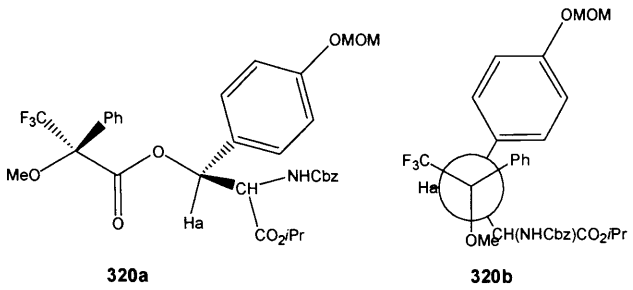


Figure 5.1.4: Conformational correlation model **320a** and **320b** for (*S*)-MTPA **320**

Our model, in accordance with the literature,[188] places the α-trifluoromethyl group and carbonyl oxygen eclipsed.[189] Ha is eclipsed by the carbonyl group. Assuming a *trans*-ester conformation is present, shown as the extended Newman projection **320b**, the 4-(MOM)Ph group would be close to the shielding influence of the magnetically anisotropic aromatic group and is expected to resonate at higher fields (typically $\Delta\delta_H$ = 0.04 ppm). In our result, the OMOM group has $\Delta\delta_H$ = 0.07 ppm, supporting the first part of this model.

In the second part of the literature model, the OMe group of (*S*)-MTPA (Figure 5.1.5) is oriented towards the anisotropic carbonyl group and means $\Delta\delta_H$ should be > 0.04 ppm for Ha. In our case, this is observed for Ha and it has a $\Delta\delta_H$ = 0.1 ppm, indicating that the *S,S* diasteroisomer is present in **320b** (Figure 5.1.4).[188]

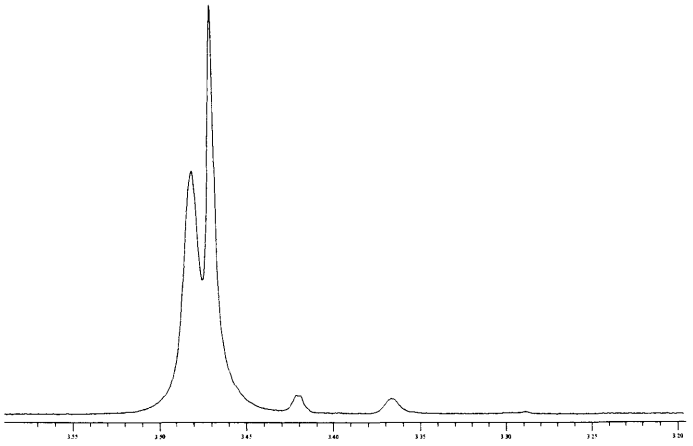
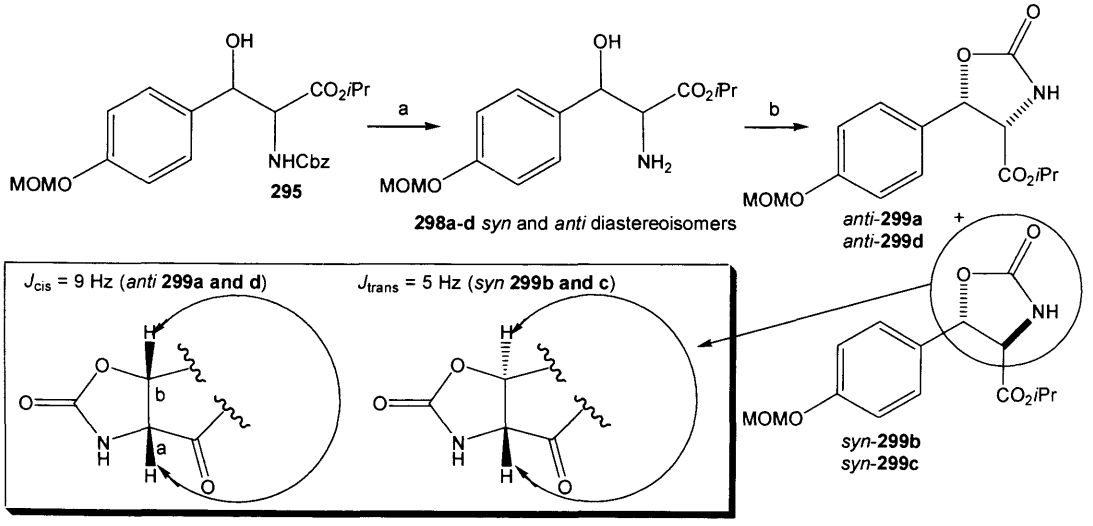


Figure 5.1.5: Extended ^{1}H NMR region of (*S*)-Mosher's esters **320**

Confirmation of the stereocenter as (3*S*) in **320**, gave the second stereocenter as (2*R*), because a *syn* product always resulted from the Sharpless AA reaction. Furthermore, ring closing to the oxazolidinone **299b** (Scheme 5.1.7), as seen from section 4.1.4, determined the relative stereochemistry of this peak as *syn*, by measuring the *J* coupling of the hydrogens in this oxazolidinone. This aided the assignment of the (2*R*,3*S*) absolute configuration, which was fortunately consistent with the literature and the analogous Sharpless asymmetric dihydroxylation rule in assigning absolute configurations using dihydroquinyl chiral ligands $(DHQ)_2PHAL$ **136a** and $(DHQ)_2AQN$ **137a**.



Scheme 5.1.7: Confirmation of *syn* stereochemistry by ring closure to oxazolidinone **299b**

5.1.2 Heterogeneous catalysis

The use of a solid supported catalysis is also investigated to establish whether improved regioselectivities could be obtained and compared to those from homogeneous catalysis. As described in the introduction (section 2.2.3), some precedent for work on

heterogeneous Sharpless AA reactions already exists, but using an *N*-bromoacetamide nitrogen source with $(DHQ)_2PHAL$ **136a** chiral ligand and an osmylated macroporous resin to access 3-phenylisoserine enantiomers (2*R*,3*S*)-**279a** (Scheme 5.1.1).[120] The advantages of investigating this method includ isolating osmium tetroxide onto a resin, making it less volatile and less toxic. Jo *et al.*, determine through X-ray photoelectron spectroscopy (XPS) the oxidation state of osmium in osmylated resins bearing residual vinyl groups, such as Amberlite™ XAD-4, XAD-7 and XAD-16 resins. The oxidation state varies between an Os(VI) monoglycolate and an Os(IV)bisglycolate species.[185]

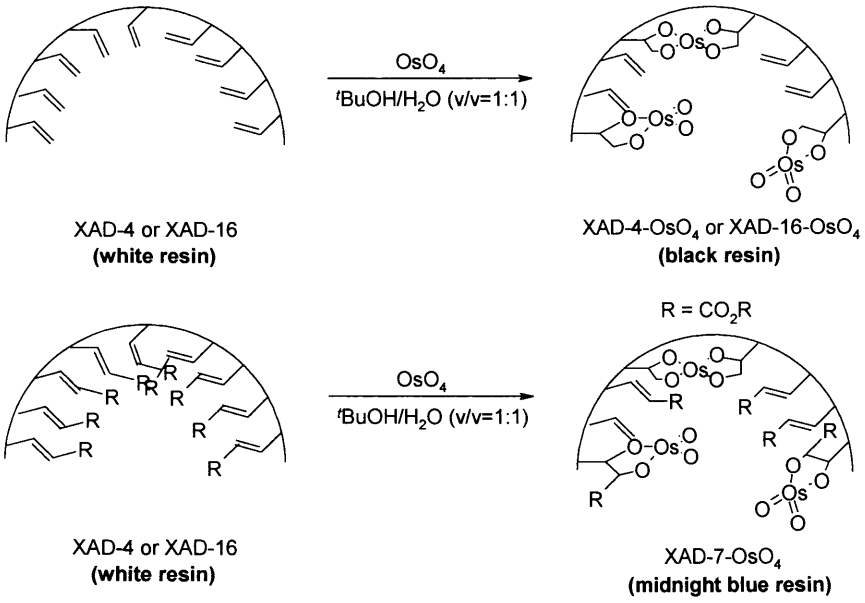
No work has been previously been reported in the literature to access 3-phenylserine enantiomers (2*R*,3*S*)-**279a** (Scheme 5.1.1) with the $(DHQ)_2AQN$ **138a** ligand. We investigate our chosen nitrogen sources (*N*-bromoacetamide and *N*-Cbz carbamate) with isopropyl cinnamate ester **170** and *p*-substituted isopropyl cinnamate esters **278b-g** for comparison with the homogeneous Sharpless AA reactions.

5.1.2.1 A comparative study of osmium anchored Amberlite XAD resins with homogeneous methodology.

Preparation of a number of Amberlite XAD-osmylated resins is undertaken to investigate the effects of resins bearing different vinyl residues. Amberlite XAD-4 is a polystyrene-based resin, whilst XAD-7 is a polyacrylate-based resin. XAD-16 is similar to XAD-4, but has a higher surface area.[j] These are synthetic non-ionic adsorbents and are highly cross-linked macroporous resins bearing residual vinyl groups (from 0.2 to over 0.4 mol of vinyl groups per mole of polymer repeating unit).[185]

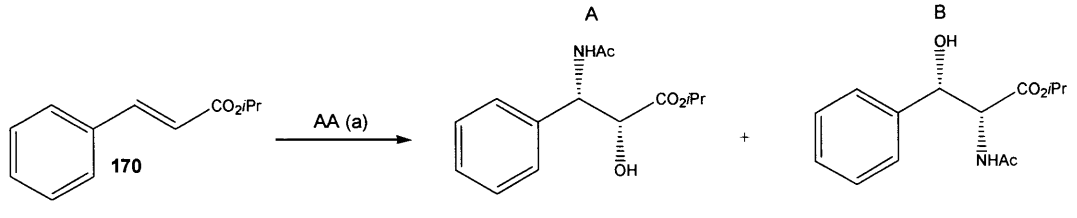
The use of this method for Sharpless AA reactions is interesting because *N*-bromoacetamide acts as both the nitrogen source and *in-situ* oxidant. On reaction, osmium(VI) and osmium(IV) species could be released by the oxidant from the resin and once utilised, are trapped by other vinyl groups on the resin. This immobilises the active osmium species and makes the resin potentially available for reuse.[120] Furthermore, synthesis of the osmylated resins is a facile process (Scheme 5.1.8).

[j] From Sigma-Aldrich Catalogue 2005-2006



Scheme 5.1.8: Synthesis of osmylated macroporous resins

XAD-4, XAD-7 and XAD-16 are reacted with osmium tetroxide in a *tert*-butyl alcohol and water mixture and give the corresponding osmylated resins. Isolation is by filtration and the osmylated resin is believed to contain 3.5-4.0 mol% of the equivalent osmium tetroxide per gram of resin, as described for XAD-4 and XAD-16 osmylated resins.[120,185] Initially, experiments are conducted with XAD-4 and XAD-7 osmylated resins with isopropyl cinnamate **170** to compare the yields, regio- and enantioselectivities of the corresponding (2*R*,3*S*)-phenylisoserine enantiomers **179** and (2*R*,3*S*)-3-phenylserine regiosisomers **286** with those from the homogeneous Sharpless AA reactions. This is achieved using chiral ligands $(DHQ)_2PHAL$ **136a** and $(DHQ)_2AQN$ **137a** with an *N*-bromoacetamide nitrogen source (Table 5.1.4).



AA (a) for NaNAcNLiBr:
XAD4/7- Os Resin (*ca* 4 mol %)
LiOH and $(DHQ)_2PHAL$ (5 mol %) in $^tBuOH:H_2O$ (1:1) or
$(DHQ)_2AQN$ (5 mol %) in $^nPrOH:H_2O$ (2:1)

Entry	XAD-Os resin	Chiral Ligand	(2*R*,3*S*)[a] Product (% yield) (lit. Yield %, R = ester group, where avail)	A:B[b] (A:B from literature, where avail)	*Ee* (%) (2*R*,3*S*)[c,d] (Ee from literature %, where avail)
1	XAD-4-Os	$(DHQ)_2AQN$	**286**, 9	3:7	34
2	XAD-4-Os	$(DHQ)_2PHAL$	**179**, 79 (93)[120]	7:3 (>20:1)[120]	93 (>99)[120]
3	XAD-7-Os	$(DHQ)_2AQN$	**286**, 9	5:9	46
4	XAD-7-Os	$(DHQ)_2PHAL$	**179**, 32	4:5	>99

[a]Yields isolated as both regioisomers; [b]As determined by ^1H NMR spectroscopic analysis, [c]Chirapak AD, 80:20 (hexane:*i*PrOH), 1 mL min^{-1};[d]Ee's based upon analogy with current literature

Table 5.1.4: Heterogeneous Sharpless AA reactions with XAD osmylated resins in the presence of chiral ligands **136a** and **137a** using an isopropyl cinnamate ester substrate **170**

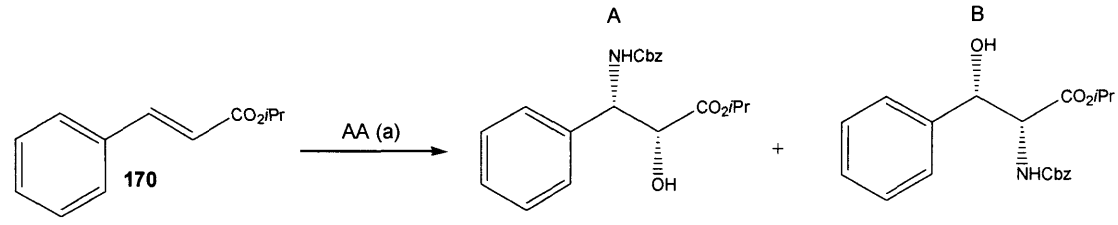
Using $(DHQ)_2AQN$ **137a** with the osmylated resin XAD-4-Os and substrate **170**, the behaviour observed is analogous to the homogeneous reaction (Table 5.1.1), whereby yields and moderate regioselectivitiesare similar (entry **1**, Table 5.1.4). The enantioselectivities, however, are improved by the use of osmylated resin XAD-4 with chiral ligand **137a**. In agreement with the literature (Table 5.1.4), a heterogeneous Sharpless AA reaction using chiral ligand **136** with the isopropyl cinnamate ester **170** affords (2*R*,3*S*)-**179** in 79% yield (entry **2**).[120]

We next compare the use of polystyrene resin XAD4-Os with polyacrylate resin XAD 7-Os. XAD-7-Os resin, which has not been used by Jo *et al.*, in heterogeneous Sharpless AA reactions.[120] XAD-7-Os resin has been used in a Sharpless asymmetric dihydroxylation reaction.[125] The reaction is performed using $(DHQ)_2AQN$ **137a** (entry **3**, Table 5.1.4) with XAD-7-Os and gives (2*R*,3*S*)-**286** in 9% yield, which is comparable to the yield obtained with XAD-4-Os resin (entry **2**, Table 5.1.4). No improvements in regioselectivity or enantioselectivity occur with either osmylated resin. However, the use of $(DHQ)_2PHAL$ **136a** reverses the regioselectivity of (2*R*,3*S*)-**179**, giving a 1:1 ratio of (2*R*,3*S*)-**179**:(2*R*,3*S*)-**286** (A:B), where B is the 3-phenylserine regioisomer.

The yield for this reaction, however, is a poor 32% yield. Although interestingly, the enantioselectivity of (2*R*,3*S*)-**179** is high (90% *ee*).

On the basis of these results, we are able to discount the use of *N*-bromoacetamide as a nitrogen source to access of 3-phenylserine enantiomer (2*R*,3*S*)-**286**. We are still interested in the possibility of using the osmylated resins, but with a carbamate nitrogen source. An oxidant is required to release the active osmium(VIII) species from the resin and *N*-bromoacetamide seems the perfect choice for this, as it acts as an *in situ* oxidant and as the nitrogen source. Yet, the use of *N*-Cbz with 1,3-dichloro-5,5-dimethylhydantoin (**311**) as the oxidant could perform the same role.

Experiments are conducted using isopropyl cinnamate ester **170** (Table 5.1.5) in the first instance; and also include the XAD-16 Os resin. No comparable literature results are available for comparison with these results as these are novel compounds.



(2*R*,3*S*)-3-phenylisoserine **316**

(2*R*,3*S*)-3-phenylserine **288**

AA (a) for NaNCbzCl:
XAD-4, XAD-7 and XAD-16- Os Resin (*ca* 4 mol %)
NaOH and $(DHQ)_2PHAL$ (5 mol %) in $^tBuOH:H_2O$ (1:1) or
$(DHQ)_2AQN$ (5 mol %) in $^nPrOH:H_2O$ (2:1)
Oxidant (**311**)

Entry	XAD-Os resin	Chiral ligand	(2*R*,3*S*)[a] Product (% yield)	A:B[b]	*Ee* (%) (2*R*,3*S*)[c,d]
2	XAD-4-Os	$(DHQ)_2AQN$	**288** (62)	5:7	>99
3	XAD-7-Os	$(DHQ)_2AQN$	**288** (88)	5:9	95
4	XAD-16-Os	$(DHQ)_2AQN$	**288** (50)	2:3	87
1	XAD-7-Os	$(DHQ)_2PHAL$	**316** (43)	9:1	87

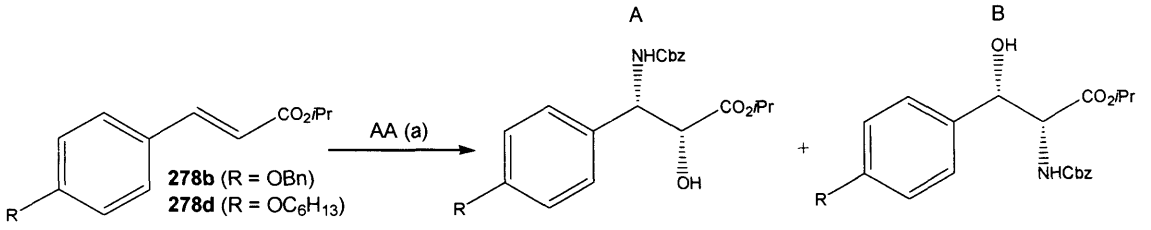
[a]Yields isolated as both regioisomers; [b]As determined by ^1H NMR spectroscopic analysis, [c]Chirapak AD, 80:20 (hexane:*i*PrOH), 1 mL min^{-1};[d]Ee's based upon analogy with current literature

Table 5.1.5: Heterogeneous Sharpless AA reactions with XAD-4, XAD-7 and XAD-16-osmylated resins in the presence of chiral ligands **136a** and **137a** using an isopropyl cinnamate substrate **170**

XAD-4-Os (polystyrene) resin and XAD-7-Os (polyacrylate) resin are reacted with **170** using $(DHQ)_2AQN$ **137a** (entry **1** and **2**, Table 5.1.5) to give 3-phenylserine enantiomer (2*R*,3*S*)-**288**. The XAD-7-Os resin slightly outperforms the XAD-4-Os resin in terms of an 88% yield and a 5:9 regioselectivity. The enantioselectivity is 95% *ee*, but gives more of the desired 3-phenylserine enantiomer. The XAD-16-Os resin (entry **3**) has a

greater surface area than XAD-4, but performs worst out of all three osmylated resins when used with $(DHQ)_2AQN$ **137a**. Overall, the homogeneous reaction conditions are slightly more successful (Table 5.1.1). Due to the unexpected reversal of regioselectivity observed with *N*-bromoacetamide and isopropyl cinnamate **170** (entry **4**, Table 5.1.4) with the $(DHQ)_2PHAL$ **136a** chiral ligand, the same experiment is repeated, this time using the *N*-CBz carbamate nitrogen source with $(DHQ)_2PHAL$ **136a**. Under these reaction conditions (entry **4**, Table 5.1.5), the normal regioselectivity favouring the 3-phenylisoserine enantiomer (2*R*,3*S*)-**316** results in a 9:1 regioisomeric ratio. A moderate 43% yield of 3-phenylisoserine enantiomer (2*R*,3*S*)-**316** is observed with a good enantioselectivity of 87% *ee*.

We then decide to investigate a couple of the cinnamate substrates **278b** (R = OBn) and **278d** (R = OC_6H_{13}) with the osmylated resins to determine whether yields, regio and enantioselectivities could be improved by switching from homogeneous to heterogeneous Sharpless AA reaction conditions with benzyl carbamate, using $(DHQ)_2AQN$ **137a** (Table 5.1.6) to favour the 3-phenylserine regioisomer B in the regioisomeric ratio (A:B).



AA (a) for NaNCbzCl:
XAD-4, XAD-7 and XAD-16- Os Resin (*ca* 4 mol %)
NaOH and $(DHQ)_2AQN$ (5 mol %) in ^{n}PrOH:H_2O (2:1)
Oxidant (**311**)

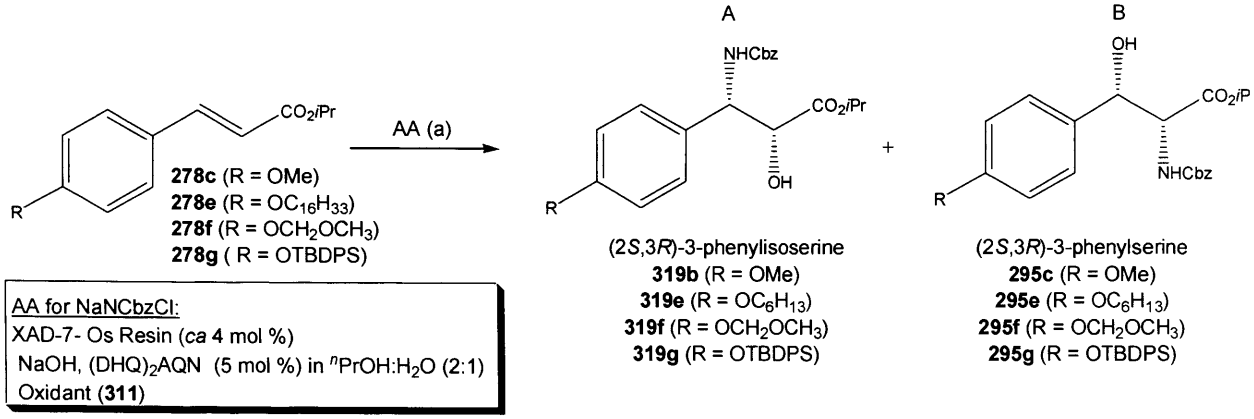
Entry	R	XAD-Os resin	(2*R*,3*S*)[a] Product (% yield)	A:B[b]	*Ee* (%) (2*R*,3*S*)[c,d]
1	OBn	XAD4-Os	**295b** (62)	5:6	72
2	OBn	XAD7-Os	**295b** (88)	2:3	70
3	OBn	XAD16-Os	**295b** (50)	5:6	75
4	OC_6H_{13}	XAD4-Os	**295d** (37)	5:6	75
5	OC_6H_{13}	XAD7-Os	**295d** (33)	2:3	76
6	OC_6H_{13}	XAD16-Os	**295d** (44)	5:6	80

[a]Yields isolated as both regioisomers; [b]As determined by ^{1}H NMR spectroscopic analysis, [c]Chirapak AD, 80:20 (hexane:*i*PrOH), 1 mL min^{-1};[d]Ee's based upon analogy with current literature

Table 5.1.6: Heterogeneous Sharpless AA reactions with XAD-4, XAD-7 and XAD-16-osmylated resins using $(DHQ)_2AQN$ **137a** with isopropyl cinnamate esters **278b** and **278d**

Based on the results (Table 5.1.6) of using isopropyl cinnamate ester **278b** (entry **1-3**) to access the 3-phenylserine enantiomer (2*R*,3*S*)-**295b**, the best results are obtained in entry **2** using XAD 7-Os. Despite having the lowest enantioselectivity (70% *ee*), this is outweighed by having the highest 88% yield and 2:3 regioselectivity for the 3-phenylserine ernantiomer. Similar results are observed using isopropyl cinnamate ester **278d** (entry **4-6**) to access 3-phenylserine enantiomer (2*R*,3*S*)-**295d**.

Having selected the XAD-7-Os resin as having the best overall performance, it is tested under the same heterogeneous Sharpless AA conditions with the remainder of the isopropyl cinnamate esters, **278c** (R = OMe), **278e** (R = $OC_{16}H_{33}$), **278f** (R = OCH_2OCH_3) and **278g** (R = OTBDPS) (Table 5.1.7).



Entry	R	(2*R*,3*S*)[a] Product (% yield)	A:B[b]	*Ee* (%) (2*R*,3*S*)[c,d]
1	OMe	**295c** (67)	1:1	89
2	$OC_{16}H_{33}$	**295e** (35)	1:1	90
3	OCH_2OCH_3	**295f** (62)	1:1	91
4	OTBDPS	**295g** (41)	1:1	89

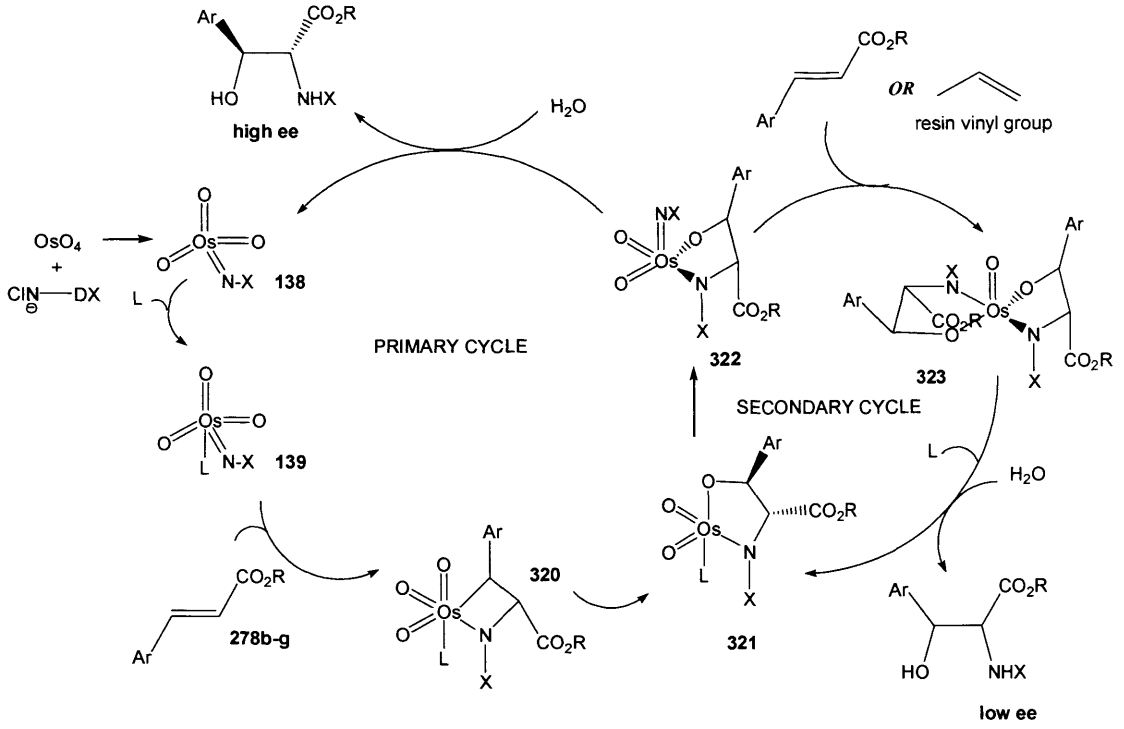
[a]Yields isolated as both regioisomers; [b]As determined by ^{1}H NMR spectroscopic analysis, [c]Chirapak AD, 80:20 (hexane:*i*PrOH), 1 mL min^{-1};[d]Ee's based upon analogy with current literature

Table 5.1.7: Reaction of isopropyl cinnamate cinnamate esters with XAD-7-Os resin

Unfortunately, despite yields (Table 5.1.7, entries **1-4**) being comparable with homogeneous reactions (Table 5.1.3) and to other heterogeneous resin results (Table 5.1.5 and Table 5.1.6), not much of an improvement is observed. This observation is based on regio- and enantioselectivities going from homogeneous to heterogeneous Sharpless AA reactions. Overall, it appears that the homogeneous reaction performs better than the heterogeneous reactions, with higher regio- and generally enantioselectivities observed, when $(DHQ)_2AQN$ **137a** is employed. Nevertheless, heterogeneous catalysis is a safer option and more environmentally benign.

5.1.2.2 Mechanistic Considerations

The mechanism postulated for these results is based upon a known literature mechanism, which typifies the $(DHQ)_2PHAL$ **136a** ligand (Scheme 5.1.9), but is adapted to give (2*R*,3*S*)-3-phenylserine enantiomers.[105]



Scheme 5.1.9: Sharpless AA mechanism with the $(DHQ)_2AQN$ chiral ligand **137**

As discussed in the literature, the formation of an imidotrioxoosmium(VIII) complex **138**, (section 2.2.1) with a nitrogen source occurs. Following this, coordination with a chiral ligand, in this case $(DHQ)_2AQN$ **137a** leads to the formation of an active complex **139**, which is ready to react with cinnamate substrates **278b-g**. On reaction with these alkenes, subsequent rearrangement of **320** to **321** should occur. In the Sharpless AA reaction using the $(DHQ)_2PHAL$ **136a** chiral ligand, the mechanism is based on electronic effects and the nitrogen is introduced at the β-position to an electron-withdrawing group in the alkene, simultaneously coordinating the electrophilic osmium centre at the α-position.

The reversal of regioselectivity observed by use of the $(DHQ)_2AQN$ **137a** chiral ligand from the literature[7,8] and from our own homogeneous and heterogeneous Sharpless AA reactions cast doubts on this explanation. It is clear that the complexes observed **320** and **321** occur not only by stereoelectronic effects, but rather by the substrate orientation within the binding pocket of the $(DHQ)_2AQN$ **137a** chiral ligand.[101] Whilst this appears to be the only explanation available for the homogeneous Sharpless AA, lending credit

to the [3+2] cycloaddition, the behaviour observed from our results also require explanation. The most important complex within this mechanism is **322**. It is at this point that hydrolysis could result in *N*-protected amino alcohols with high ee's. It is also at this point where a second cinnamate **278b-g** can complex with osmium, forming **323**.

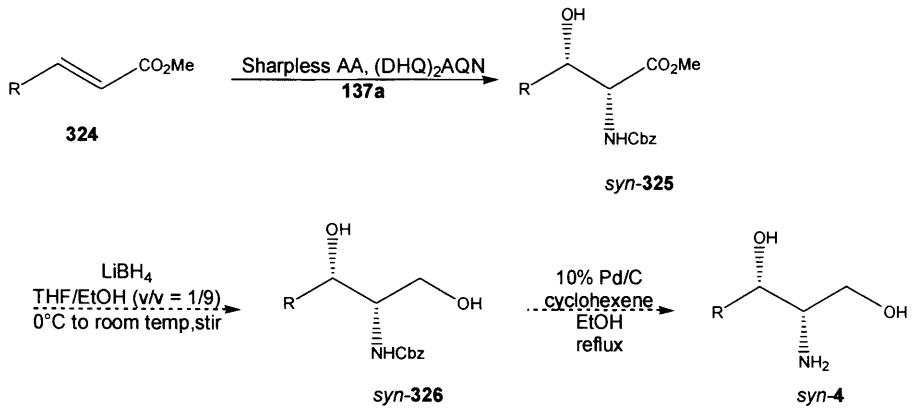
It is possible that the different sizes of resin, particularly XAD 4-Os (polystyrene) and XAD-7-Os (polyacrylate) have no deleterious effects on enantioselectivity (as seen), but there are effects on regioselectivity which affects the substrate–ligand orientation. It is also likely that, should a vinylic group from the osmylated resins be bound on the formation of **323**, the second cinnamate **278b-g** cannot be bound. From this observation, hydrolysis at this point would not have deleterious effects on enantioselectivities, but the regioselectivities observed from our results can be rationalised by subtle interplay between the size of the group attached to cinnamates **278b-g** and the size of the groups on the vinyl resins (*i.e.* polystyrene or polyacrylate groups) and how well they bind to complex **322**.

XAD-7-Os resins, even with $(DHQ)_2PHAL$ **136a**, give 1:1 mixtures of *3*-phenylserine enantiomers (entry **4**, Table 5.1.4). Therefore, it is even more probable that the size of the groups attached at the *p*-alkoxy position and the vinylic groups within the resin are closely related in affecting the regioselective outcome, but not the enantioselectivities.

One notable observation from our studies is the loss of enantioselectivity when using an *N*-bromoacetamide nitrogen source with $(DHQ)_2AQN$ **137a**. From our investigations, either using homogeneous or heterogeneous Sharpless AA conditions, a behavioural trait that is common is the number of factors that could determine the yield, regioselectivity, and enantioselectivity of the reaction. By this, it appears that cinnamates bearing *p*-alkoxy groups that are electron-donating work well with this reaction. These are the stereoelectronic factors. Notable, is having a TBDPS group at *p*-alkoxy position of cinnamate **278g**. Furthermore, π-π stacking interaction also appears favourable, as exemplified by both cinnamates bearing a TBDPS group **278g** or an OBn group **278b**. These are steric factors that affect substrate binding within the chiral ligands "U-shaped binding pocket".

5.2 Sharpless Asymmetric Aminohydroxylation (AA) using methyl 1-trans-2-octenoate

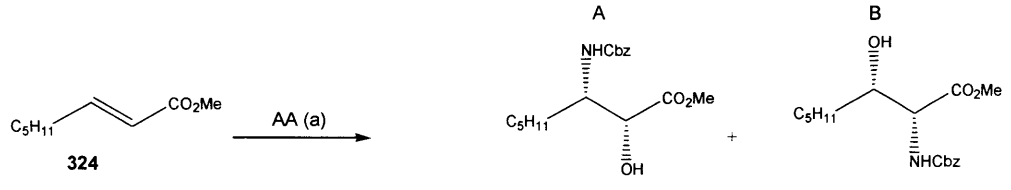
We decide to extend the methodology to the alkyl olefin methyl *trans*-2-octenoate (R = C_5H_{11}) (**324**) to increase the versatility of the Sharpless AA reaction. This serves as a model for the short step synthesis towards aliphatic aminodiols **4** through ester reduction of pentylserine regioisomer (**325**), followed by the catalytic hydrogenolysis of (**326**) (Scheme 5.2.1). There is also some literature precedent for this substrate being used with $(DHQ)_2AQN$ **137a** in a homogeneous reaction, giving the desired pentylserine enantiomer **325** in 3:1 regioselectivity and 93% *ee*, though no yield is cited.[8]



324 *syn*-**325** *syn*-**326** *syn*-**4**

Scheme 5.2.1: Short step synthesis to (*S*,S)-aliphatic aminodiol **4**

We react this substrate **324** under homogeneous conditions with both chiral ligands **136a** and **137a**. The XAD-7-Os resin is used with **137a** for the heterogeneous reaction and the results are shown below (Table 5.2.1).



324 (2*R*,3*S*)-pentylisoserine **327** (2*R*,3*S*)-pentylserine **325**

AA (a) for NaNCbzCl:
$K_2[Os(OH_2)_4].2H_2O$ (4 mol %) (Homogeneous)
XAD-7-Os (Heterogeneous)
NaOH and $(DHQ)_2PHAL$ (5 mol %) in $^tBuOH:H_2O$ (1:1) or
$(DHQ)_2AQN$ (5 mol %) in $^nPrOH:H_2O$ (2:1)
oxidant (**311**)

Entry	Ligand	(2*R*,3*S*) Product/ (% yield)[a]	A:B[b]	*Ee* (%) (2*R*,3*S*)[c]
1	$(DHQ)_2AQN$	**325**, 37	1:1	26
2	$(DHQ)_2PHAL$	**327**, 35	1:1	64
3	(DHQ)AQN[d]	**325**, 30	1:1	45

[a]Yields isolated as both regioisomers; [b]As determined by ^1H NMR spectroscopic analysis, [c]Chiralpak AD, 80:20 (hexane:*i*PrOH), 1 mL min^{-1}; [d]Heterogeneous reaction with XAD-7-Os resin

Table 5.2.1: Results of the homogeneous and heterogeneous Sharpless AA reactions with $(DHQ)_2PHAL$ **136a** and $(DHQ)_2$ AQN **137a**

The results (Table 5.2.1) show that regardless of the ligand choice (**136a** or **137b**) and whether the reaction is performed under homogeneous or heterogeneous Sharpless AA conditions (entries **1-3**), ester **324** is not a suitable substrate for the Sharpless AA reaction. These results contradict those of Tao *et al.*, and our results may need to be optimised.[8] Disappointing yields, regioselectivities and *ees* resulted from this reaction, but also suggested that the substrate was influential in determining these factors, rather than yield optimisations.

5.3 Conclusions

The homogeneous Sharpless AA reactions utilises the $(DHQ)_2PHAL$ **136a** chiral ligand and is useful for the synthesis of (2*R*,3*S*)-3-phenylisoserine enantiomers **279a** (Scheme 5.1.1). These products are used for chiral HPLC elucidation. As expected, the yields, regio and enantioselectivities for unsubstituted **170** and *p*-substituted **278b-g** isopropyl cinnamate esters work well (Table 5.1.1 and Table 5.1.2) with **136a** and conform to the literature results, where available.

Homogeneous Sharpless AA reactions using the chiral ligand $(DHQ)_2AQN$ **137a** with isopropyl cinnamate ester **170** show deleterious effects on enantioselectivities when combined with *N*-bromoacetamide (Table 5.1.3). This is addressed by swapping to an *N*-Cbz carbamate source (Table 5.1.3). Oxidant 1,3-dichloro-5,5-dimethylhydantoin (**311**) works well with isopropyl cinnamate ester **170**, but reaction of **170** with dichloroisocyanuric acid (**312**), proved otherwise (Table 5.1.3). This is despite claims that neither oxidant would have deleterious effects on yields, regio and enantioselectivities.[182]. Size effects are deemed important at the *p*-alkoxy position of the isopropyl cinnamate esters **278b-g**, with bulky benzylic groups (**278b**, R = OBn) and (**278g**, R = OTBDPS) having considerable effects on the regioselectivities (Table 5.1.3).

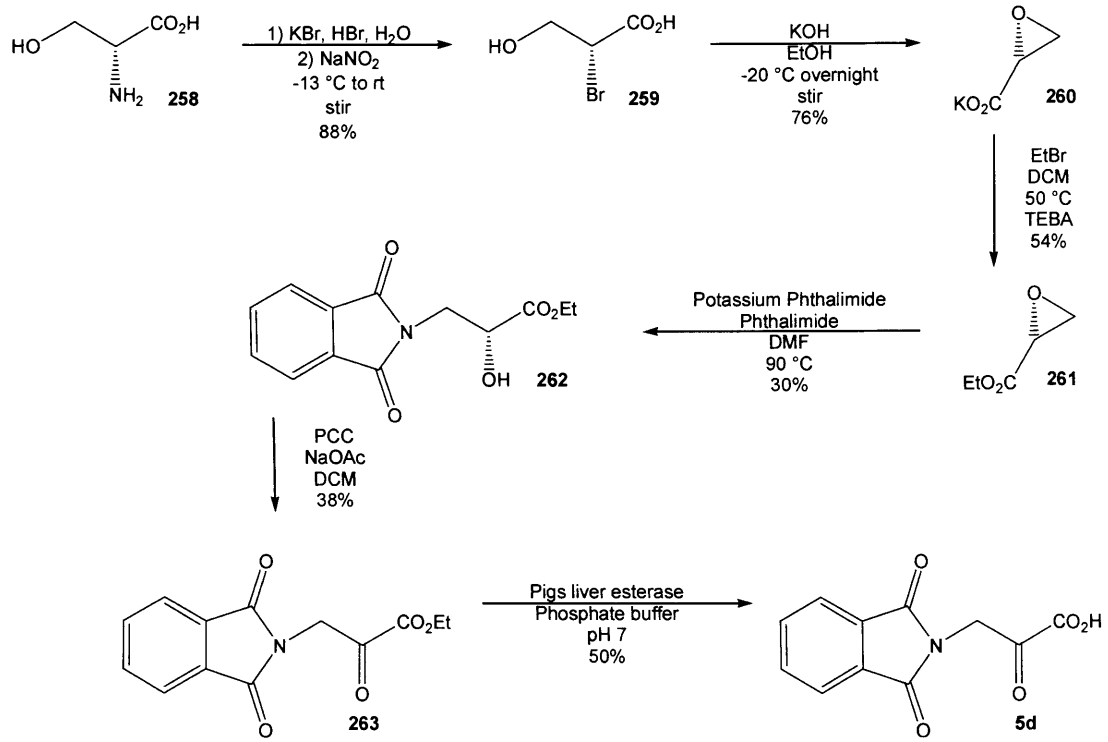
HPLC standard **295f** is compared with the Sharpless AA product **295f** by chiral HPLC and corresponds as the same HPLC peak. This allows us to confirm that the leading enantiomer from chiral HPLC is (2*R*,3*S*). Isolation of the Sharpless AA product **295f** using preparative chiral HPLC and subsequent synthesis of Mosher's ester **320** (Scheme 5.1.6) confirming the absolute configuration as (2*R*,3*S*)-**295f** by ^{1}H NMR spectroscopic analysis and uses a pre-existing conformational model (Figure 5.1.4). This is consistent with the Sharpless asymmetric dihydroxylation prediction of stereochemistry for dihydroquinyl chiral ligands **136a** and **137a**.[8,162]

Heterogeneous Sharpless AA reactions are then investigated using, XAD-4, XAD-16 and XAD–7-osmylated resins. Both *N*-bromoacetamide and *N*-Cbz carbamate nitrogen sources are tested with isopropyl cinnamate **170** and with both chiral ligands $(DHQ)_2PHAL$ **136a** and $(DHQ)_2AQN$ **137a** (Table 5.1.4). Poor levels of asymmetric induction are observed with the *N*-bromoacetamide source with the $(DHQ)_2AQN$ **137a** chiral ligand, similar to the poor enantioselectivities observed in the homogeneous Sharpless AA reactions. Interestingly, XAD-7-Os when used with *N*-Ac and $(DHQ)_2PHAL$ **136a** gives **179** (Table 5.1.4) in a 1:1 regioselectivity, without any effects on the enantioselectivity of the reaction. On moving to *p*-alkoxy substituted cinnamates, with *N*-Cbz carabamate, the same behaviour is not observed. To test which resin has the best overall performance, determined by yields, regioselective ratios and enantioselectivities, a couple of representative isopropyl cinnamate esters (**278b**, R = OBn and **278d**, R = OC_6H_{13}) are reacted with XAD-4, XAD-16 and XAD-7–Os resins. Overall, from these results (Table 5.1.6), the XAD 7-Os resin is chosen for the remaining *p*-substituted isopropyl cinnamate esters (Table 5.1.7). Unfortunately, the yields and regioselectivities are not particularly high, making the homogeneous Sharpless AA an attractive alternative. From these results, it is hypothesised that the mechanism (Scheme 5.1.9) is affected by having other vinyl residues present on an osmylated resin. In terms of providing a useful methodology towards (*S*,*S*)-aromatic aminodiols **281**, the Sharpless AA reaction is a powerful method that is worth further investigation. We attempt to extend the Sharpless AA methodology to include alkyl olefin **324**, under homogeneous and heterogeneous catalytic conditions to access pentylserine regioisomer **325**, but this is not very successful (Table 5.2.1).

Summary & Future Work

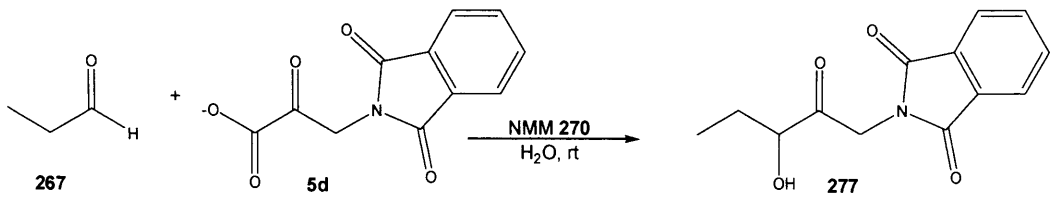
Chapter 6

In Chapter 3 several attempts to synthesise novel nitrogen containing pyruvate donors are investigated and phthalimidopyruvate **5d** is prepared in seven steps (Scheme 6.1.3) from L-serine **258**.



Scheme 6.1.1: Adapted route to phthalimido pyruvate **5d**

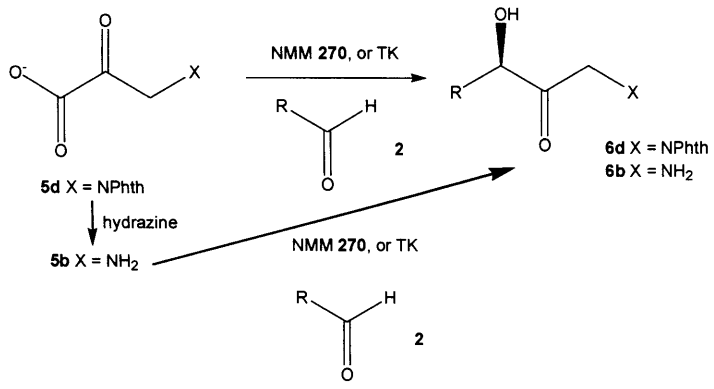
Synthesis and isolation of more of this material is unsuccessful and therefore, instead of subjecting this substrate **5d** directly to TK or its mutants, it is decided to utilise it in the mimetic TK reaction (Scheme 6.1.2).



Scheme 6.1.2: Biomimetic reaction toward ketoalcohol **277**

It is anticipated that the racemic ketoalcohol (**277**) from this reaction could be used as a standard for determining any enantioselectivity that might be achieved from future TK or TK mutant bioconversions.

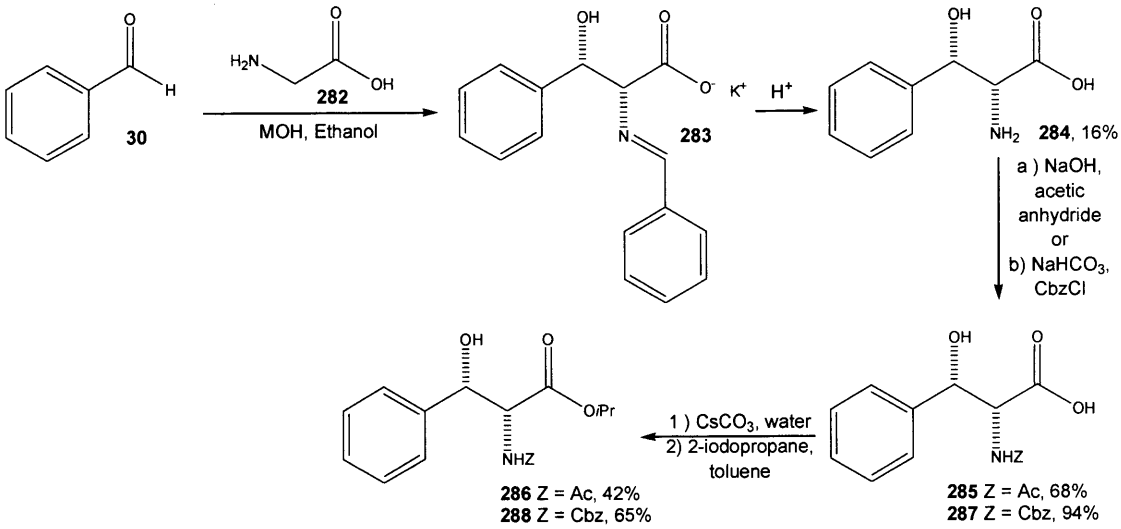
Despite the low conversion of phthalimidopyruvate **5d** into ketoalcohol **277**, phthalimidopyruvate **5d** is an exciting novel nitrogen containing pyruvate donor. Future work would see that the synthesis of this molecule is optimised, allowing future studies and investigating the biomimetic reaction (discussed in section 3.1.6) with this substrate and an array of aldehydic acceptors. Furthermore, the removal of the phthalimido group from **5d** could give access to aminopyruvate **5b**, which may also be used in the TK mimetic screen or for activity with TK and its mutants, providing racemic **5d** or **5b** from the mimetic TK reaction or chiral **6d** or **6b** from a TK bioconversion (Scheme 6.1.3) .



Scheme 6.1.3: Biomimetic reactions with amine catalysts, such as NMM **270**

If chiral ketoalcohols **6d** and **6b** were inaccessible from TK or its mutants, a chiral amine catalyst, such as L-proline may give access to ketoalcohols, as it is known from the literature to catalyse asymmetric carbon-carbon bond forming reactions.[190]

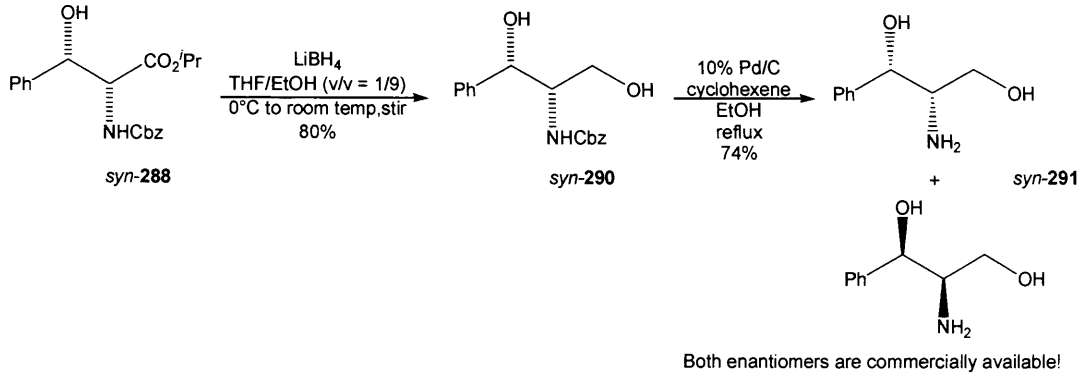
A one-pot reaction of benzaldehyde **30** with glycine **282**, under basic conditions gives access to *syn*-3-phenylserine **284** and is presented in chapter 4. The subsequent acetamide or benzyloxycarbonyl protection yields **285** and **287** (Scheme 6.1.4), respectively.



Scheme 6.1.4: Synthetic route towards *syn*-diastereoselective HPLC standards **286** and **288**

Following this, isopropyl ester formation by alkylation of an *in situ* caesium salt of **285** and **287**, yields *syn* chiral HPLC standards **286** and **288** (Scheme 6.1.4) for use in the chiral HPLC elucidation of Sharpless AA adducts formed in Chapter 5. The *syn*-diol by-product **289** also associated with the Sharpless reaction is readily synthesised.

A short reaction sequence (Scheme 6.1.5) involving the reduction of isopropyl ester **288** and the removal of the benzyloxycarbonyl group in **290** yields the desired *syn* aminodiol **291** and could be readily applied to other *syn*-(*p*-substituted-3-phenyl)serines **281**.



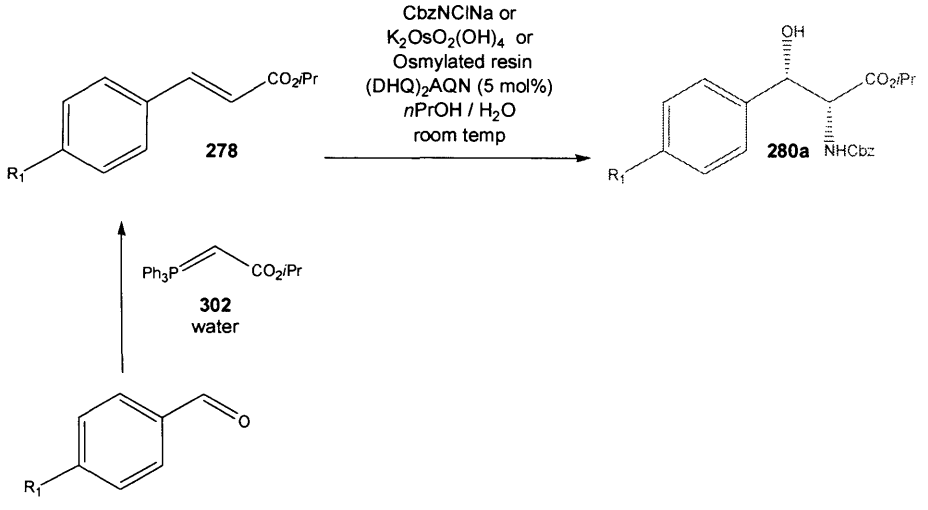
Scheme 6.1.5: Short step reaction sequence to *syn*-aminodiol **291**

Glycine **282** with *p*-methoxymethyl benzaldehyde **292f** provides a divergent route (Scheme 4.1.9), allowing access to a 1:1 diastereomeric mixture of (*p*-methoxymethyl-3-phenyl)serine **293f**. Subsequent *N*-Cbz protection of **293f** and isopropyl ester formation, as before, gives chiral HPLC standard **295f**. Chiral HPLC separation of the 4 peaks and derivatization of each peak collected (peaks **A-D**) to oxazolidinones **299a-d** (Table 4.1.3) allows the elucidation of the relative stereochemistry in peaks A-D of **295f**. *Syn* products of **295f** (peaks **B** and **C**) are collected as one product using

preparative chiral HPLC. Removal of the *p*-methoxymethyl group of crude **295f** using *n*-butane thiol in the presence of magnesium bromide affords **295a** (Scheme 4.1.11), which can be alkylated to give any other *p*-substituted alkoxy Sharpless adduct (**295b-g**). Also described in chapter 4 is the formation of the cinnamate substrates used in the Sharpless AA reaction.

The Wittig reactions of *p*-hydroxybenzaldehyde **292a**, *p*-benzyoxybenzaldehyde **292b** and *p*-methylmethoxybenzaldehyde **292f** with phosphorous ylid **302** in dichloromethane (Table 4.2.1), aqueous THF mixture (Table 4.2.2) and in water (Table 4.2.3) are investigated and allow access to the corresponding cinnamate. Surprisingly water works really well in this reaction, providing an environmentally benign route to cinnamate substrates **278a**, **278b** and **278f**. Other methods are also used to provide cinnamates **278b-g**, but the Wittig reaction is the most successful reaction where comparisons are available.

Aqueous Wittig reactions (discussed in section 4.2.1), through a mixture of solvents, may in future be used to directly access enantiomeric *syn*-3-phenylserine **280a** in a one-pot reaction (Scheme 6.1.6). This is either through homogeneous Sharpless AA reactions or using osmylated macroporous resins as seen in sections 5.1.1and 5.1.2. Water dilutions and filtration of triphenylphosphine oxide may be possible by controlling the solvent concentrations and would also feature as part of any future work.



Scheme 6.1.6: Potential one-pot reaction to generate **280a**

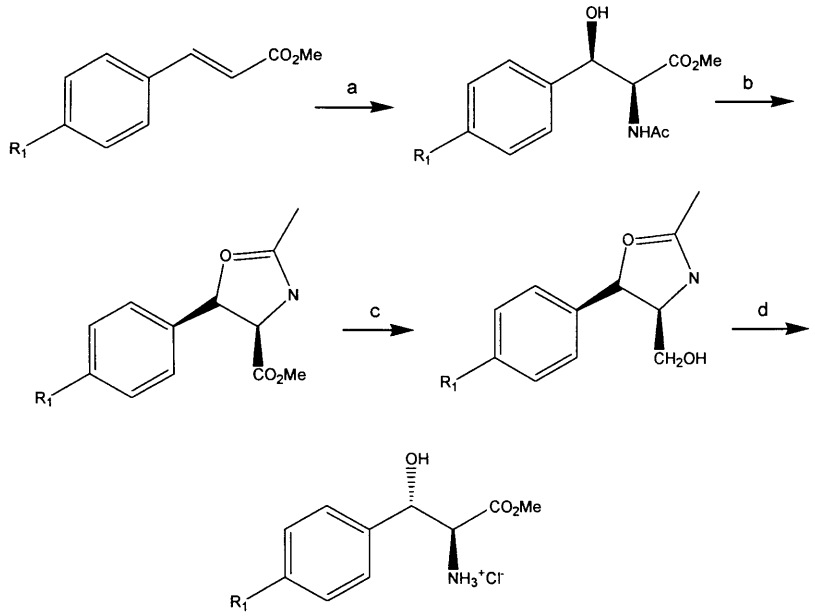
Once all of the cinnamates are synthesised, they are then subjected to a range of Sharpless AA reactions, as described in chapter 5. The regioselectivity of the reaction is explored by swapping the chiral ligand from $(DHQ)_2PHAL$ **136a** to $(DHQ)_2AQN$ **137a**.

Both homogeneous and heterogeneous Sharpless AA reactions with two different nitrogen sources (*N*-bromoacetamide and *N*-Cbz carbamate) are investigated. Homogeneous Sharpless AA reactions utilises the $(DHQ)_2PHAL$ **136a** chiral ligand and is useful for the synthesis of (2*R*,3*S*)-**279a** (Scheme 5.1.1). These compounds are then used for chiral HPLC elucidations.

Homogeneous Sharpless AA reactions using the chiral ligand $(DHQ)_2AQN$ **137a** with isopropyl cinnamate ester shows deleterious effects on the enantioselectivities when combined with *N*-bromoacteamide (Table 5.1.3), which are addressed by swapping to an *N*-Cbz carbamate source (Table 5.1.3). The oxidant 1,3-dichloro-5,5-dimethylhydantoin **311** works well with isopropyl cinnamate ester **170**, but the Sharpless AA reaction of **170** with dichloroisocyanuric acid (**312**) is poor (Table 5.1.3). Size effects were deemed important at the *p*-alkoxy position of the isopropyl cinnamate esters **278b-g**, with bulky benzylic groups (**278b**, R = OBn) and (**278g**, R = OTBDPS) having considerable effects on the regioselectivities (Table 5.1.3). At this point, through homogeneous Sharpless AA conditions, HPLC standard **295f** (from chapter 4) is elucidated by the synthesis of a Mosher's ester **320**.

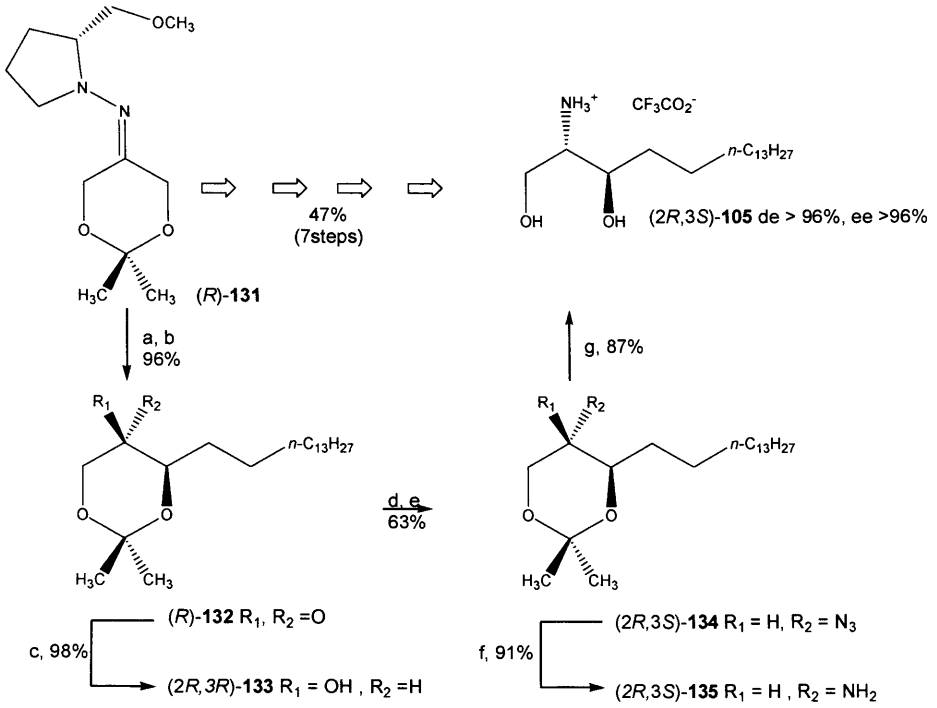
Heterogeneous Sharpless AA reactions are then investigated using, XAD-4, XAD-16 and XAD–7-osmylated resins. Both *N*-bromoacetamide and *N*-Cbz carbamate nitrogen sources are tested with isopropyl cinnamate ester **170** and with both chiral ligands $(DHQ)_2PHAL$ **136a** and $(DHQ)_2AQN$ **137a** (Table 5.1.4). Poor levels of asymmetric induction are observed with the *N*-bromoacetamide source with $(DHQ)_2AQN$ **137a**. To test which resin has the best overall performance, measureble by yields, regioselective ratios and enantioselectivities, a couple of representative isopropyl cinnamate esters (**278b**, R = OBn and **278d**, R = OC_6H_{13}) are reacted with XAD-4, XAD-16 and XAD-7–Os resins. Overall, from these results (Table 5.1.6), the XAD-7-Os resin is selected for use with the remaining *p*-substituted cinnamates (Table 5.1.7). Unfortunately, the yields and regioselectivities are not outstanding, making the homogeneous Sharpless AA an attractive alternative. From these results, it is hypothesised that the mechanism (Scheme 5.1.9) is affected by having other vinyl residues present on an osmylated resin. We attempt to extend the Sharpless AA methodology to include alkyl olefin **321**, under homogeneous and heterogeneous catalytic conditions to access pentylserine enantiomer **325**.

Finally, once a generic one-step reaction is possible towards *syn*-(2*R*,3*S*)-3-phenylserine enantiomer **280a**, optimisation studies on the Sharpless AA reaction or by a different methodology could be used to access the shortest reaction sequence to aminodiols (discussed in section 4.1.2). This would then be implemented to produce any desired chiral HPLC standard for comparison with those generated from the TK/TA screens. The work carried out in this thesis has seen *syn*-(2*R*,3*S*) or (2*S*,3*R*) enantiomers as the focal point of discussion. *Anti*-aminodiols may be accessed if the SAA conditions are optimised. One such method to achieve stereoinversion may include an adapted route used to access (-)-cytoxazone, presented in Scheme 6.1.7.[191]



Scheme 6.1.7: Reagents and conditions: (a) SAA conditions, **137a;** (b) Tf_2O, DMAP, CH_2Cl_2; (c) $LiBH_4$ reduction; (d) 12% HCl, heat

Failing the ability to access *anti*-aminodiols *via* this route, another route, already mentioned in section 2.1.2 may also be used. This involves the RAMP-hydrazone method, which Enders uses to access D-*erythro*-sphinganine **105** (Scheme 6.1.8).[98]



Scheme 6.1.8: Reagents and conditions: (a) *t*BuLi, THF, -78 °C, then pentadecyl bromide, -100 °C; (b) aq. Oxalic acid; (c) L-selectride®, THF, -78 °C; (d) MsCl, CH_2Cl_2, Et_3N, -0 °C → rt.;(e) NaN_3, 18-crown-6, DMF, 100 °C; (f) LAH, THF, rt.; (g) TFA, THF/H_2O, rt.

The only problem with this route is the lengthy synthetic protocol, but it is more versatile in that the lipid chain can be substituted for aryl groups of other alkyl groups and may be adequately scaled.

Experimental

Chapter 7

7.1 General Experimental Procedures

Unless otherwise stated, all reagents were obtained from Sigma-Aldrich Co Ltd., BDH laboratory supplies, Fischer Scientific UK and Lancaster synthesis and are used without any further purification.

Dry solvents, where used were obtained from a Sharpe dry column system (using activated alumina; bead size 1-2 mm) and were stored under nitrogen, or were bought in from commercial suppliers and then used immediately. Ether refers to diethyl ether, ethanol refers to absolute ethanol (>99.7%) and brine refers to a saturated solution of sodium chloride. For all air and moisture sensitive reactions, glassware was dried in a hot oven (120 °C) and a nitrogen atmosphere was used.

The term *in vacuo* refers to the removal of solvents by means of evaporation at a reduced pressure, which was provided by the in-house vacuum or an oil pump, using a Buchi® rotary evaporator.

Thin layer chromatography (TLC) was performed on plastic backed, precoated Merck 60-F_{254} silica plate with 0.25 mm thickness. Visualisation was achieved through absorption of U.V. light or by staining with potassium permanganate solution [$KMnO_4$ (1.25 g), Na_2CO_3 (6.25 g), water (250 mL)] or phosphomolybdic acid (PMA) solution [PMA hydrate (12 g), conc. sulphuric acid (10 mL), ethanol (250 mL). Normal phase flash column chromatography was carried out using silica gel (40-63 μm). Preparative TLC was carried out on Whatman preparative TLC plates (silica gel 60 Å, 1000 μm thickness, 20 x 20 cm).

Normal phase high performance liquid chromatography (HPLC) was carried out using a Varian Prostar machine with hexane/*i*PrOH mixtures. Preparative HPLC was achieved using the same machine with an attached fraction collector.

Melting points (m.p.) were determined using a Gallenkemp instrument and are uncorrected.

I.R. spectra were recorded on Shimadzu FTIR-8700 spectrophotometers for solutions in chloroform (with a KBr cell) unless otherwise stated.

^{1}H NMR and ^{13}C spectra were recorded using Bruker® AMX300, AMX400 and AVANCE500 machines. All samples were dissolved in deuterated chloroform ($CDCl_3$), unless otherwise stated. The chemical shifts (δ) were given in units ppm relative to tetramethylsilane (TMS). Couplings constants (*J*) were measured in hertz (Hz). Multiplicities for ^{1}H were shown as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), dd (doublet of doublets), bt (broad triplets), m (complex multiplet). ^{13}C chemical shifts were listed where characterisation of peaks were carried out from experiments described in parentheses following the "δ_C". The term "*ca*" in front of a ^{13}C chemical shift indicates an approximate value for two or three signals for the same carbon that were indistinguishable (regioisomers and/or diastereoisomers) and ^{13}C chemical shifts expressed as (X + Y) indicated two distinct diastereomeric carbon signals.

Mass spectra were recorded on a VG-7070 instrument, available for low mass range EI methods. Ionisation methods include electron impact (EI), positive and negative and desorptional chemical ionisation (CI). Fast atom bombardment (FAB) was recorded on a UG70FE. In addition, (+ES and -ES) were recorded on a Micromass Quattro LC spectrospray (MassLynx software) on a MAT 900XP spectrometer. The same spectrometer was used for (+HRFAB and +HRES) for accurate mass spectrometry. Major peaks were reported with intensities quoted as percentages of the base peak.

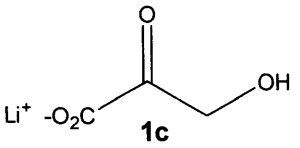
Microanalyses were determined, using a Perkin Elmer 2400 Elemental Analyser (CHN) and Perkin Elmer 240 Elemental Analyser (S, halogen).

Optical rotations were recorded on Optical Activity: polAAR2001 polarimeter at 589 nm in chloroform solutions (unless otherwise indicated) quoted in 10^{-1} deg cm^2 g^{-1}. Concentrations (*c*) were in g/100 mL.

7.2 Transketolase Donor Synthesis

7.2.1 Hydroxypyruvate (HPA)

7.2.1.1 Lithium hydroxy-2-oxo-propionate (1c)



1c

Lithium hydroxide monohydrate (8.39 g, 200 mmol) was dissolved in water (200 mL). Half of this solution was added to bromopyruvic acid (1.67 g, 10.0 mmol) in 100 mL water, by autotitration (radiometer ABU91), with the pH not exceeding 9.5. The reaction was stopped after 3 h and the pH adjusted to 5.0 using dilute acetic acid. The mixture was concentrated *in vacuo* at room temperature until crystals started to appear. At this point, the concentrated solution was cooled to 0 °C for 16 h and the product was precipitated and collected by filtration, to give the title compound as a colourless solid (1.18 g, 32%).

ν_{max} (KBr)/cm^{-1}: 3433br (OH), 1751s, (C=O), 1635s (C=O);

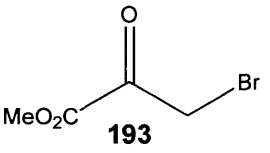
^{1}H NMR (400 MHz, D_2O): δ 3.40 (2H, s, C*H*$_2$OH);

^{13}C NMR (100 MHz, D_2O): δ 66.5 (*C*H$_2$OH), 177.0 (*C*O$_2$H), 203.0 (*C*OCO$_2$H);

***m/z* (+ES):** 104 (M$^+$, 100%);.

HPLC (reverse phase)**:** Column: Aminex 87H (300 x 7.8 mm) (Bio-Rad Laboratories) Mobile Phase: Isocratic, 0.1% TFA in water, 0.6 mL/min, Electrochemical Detector (Dionex), HPA **1c** 8.38 min.

7.2.1.2 Methyl 3-Bromo-2-oxo-propionate (193)



193

The reaction was carried out under anhydrous conditions. To methyl pyruvate (5.00 g, 48.9 mmol) was added bromine (7.28 g, 45.6 mmol) over 1 h. Nitrogen was bubbled through the reaction mixture to remove any hydrogen bromide. The product was purified by distillation under reduced pressure (110 mmHg; 82-84 °C) to yield the title compound as a gold oil (6.93 g, 77%).

ν_{max} (neat)/cm^{-1}: 1736s (C=O), 671s (C-Br);

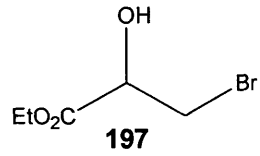
^{1}H NMR (400 MHz): δ 3.87 (3H, s, O*Me*), 4.30 (2H, s, C*H*$_2$Br);

^{13}C NMR (100 MHz): δ 30.73 (OCH_3), 53.96 (CH_2Br), 158.28 ($(CO)CH_2Br$), 177.93 (CO_2Me);

***m/z* (CI):** 180 (M^+, 25%), 165(M^+ -$[CH_3]^+$, 8), 150 ($[BrCH_2COCOH]^+$, 76), 123 ($[BrCH_2COH]^+$, 85).

7.2.2 Azidopyruvate

7.2.2.1 Ethyl 3-Bromo-2-hydroxy-propionate (197)



197

Ethyl 3-bromopyruvate (2.80 g, 14.4 mmol) dissolved in methanol (4 mL) was added to a stirring solution of $NaCNBH_3$ (0.39 g, 6.21 mmol) and a trace of bromocresol green in methanol (4 mL). HCl (1M) was added dropwise to maintain the pH at 4, as indicated by the yellow colour of the solution over 90 min. The solvent was removed *in vacuo* and the residue dissolved in sat. ammonium sulfate (10 mL). The aqueous layer was then extracted with ether (5 x 20 mL). The organic layers were combined, dried ($MgSO_4$) and then evaporated *in vacuo*. The crude was purified by flash column chromatography (100% dichloromethane), which yielded the title compound as an oil (1.82 g, 65%).

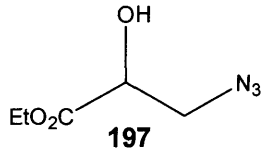
ν_{max} (neat)/cm^{-1}: 3358br (C-OH), 3019s, 2926m, 2854m, 1716 (C=O);

^{1}H NMR (400 MHz): δ 1.33 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 3.22 (1H, d, *J* 6.6 Hz, O*H*), 3.70 (2H, dt, *J* 7.2, 3.5 Hz, CH_2Br), 4.29 (2H, dq, *J* 7.1, 3.2 Hz, (OCH_2CH_3), 4.50 (1H, dt, *J* 6.5, 3.2 Hz, C*H*(OH));

^{13}C NMR (100 MHz): δ 14.1 (OCH_2CH_3), 35.1 (CH_2Br), 62.5 (OCH_2CH_3), 69.7 (*C*H(OH)), 171.4 (CO_2Et);

***m/z* (EI)**: 199 (M + ^{81}Br, 38%), 197 (M + ^{79}Br, 38), 125 ($[^{79}BrCH_2CH_2OH]^+$, 100).

7.2.2.2 Ethyl 3-Azido-2-hydroxypropionate (197)



197

3-Bromo-2-hydroxypropionic acid ethyl ester (1.82 g, 9.10 mmol) was dissolved in water/ethanol (1:1 v/v, 100 mL). Sodium azide (0.86 g, 13.1 mmol) was added and the mixture heated to reflux for 24 h. The solvent was removed *in vacuo* and the residue

taken up in water (30 mL). The aqueous layer was extracted with dichloromethane (5 x 30 mL) and the organic layers were combined, dried (Na_2SO_4) and the solvent was removed *in vacuo*, yielding an oil that was purified by flash column chromatography (3:7, EtOAc: hexane). This gave the title compound as an oil (503 mg, 34%).

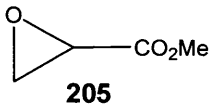
ν_{max} (KBr)/cm^{-1}: 3415br, (C-OH), 2956s, 2108s (N_3), 1743s (C=O);

^{1}H NMR (400 MHz): δ 1.30 (3H, t, *J* 7.0 Hz, OCH_2CH_3), 3.51 (1H, d, *J* 4.4 Hz, $CHHN_3$), 3.64, (1H, d, *J* 4.4 Hz, $CHHN_3$), 4.28 (2H, q, *J* 7.0 Hz, OCH_2CH_3), 4.34 (1H, m, CH(OH));

^{13}C NMR (100 MHz): δ 14.0 (OCH_2CH_3), 53.7 (CH_2N_3), 62.4 (CH(OH)), 69.7 (OCH_2CH_3);

***m/z* (FAB):** 160 (MH^+, 45%).

7.2.2.3 Methyl Oxirane-2-carboxylate (205)



205

Sodium hypochlorite (6.8% wt/v; 176.5 mL) was added to a stirred solution of sodium carbonate (16.00 g in 40 mL water) and methyl acrylate (12.26 mL, 136.1 mmol) at 10 °C. Stirring was continued for 1 h, whilst a stream of CO_2 (8.00 g) was passed through the solution. The mixture was extracted with dichloromethane (4 x 150 mL), dried (Na_2SO_4) and the solvent was removed *in vacuo*. The product was purified by fractional distillation under vacuum (20 mmHg; 45-49 °C) to give the title compound as yellow oil (1.84 g, 13%).

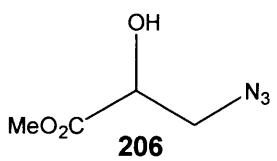
ν_{max} (neat)/cm^{-1}: 2986s (C-H), 1736 (C=O), 1203 (C-O);

^{1}H NMR (500 MHz): δ 2.98 (2H dt, *J* 4.0, 2.5 Hz, CH_2CO), 3.42 (1H, dd, *J* 4.0, 2.5 Hz), 3.77 (3H, s, O*Me*);

^{13}C NMR (125 MHz): δ 46.2 (CH_2O), 47.1 (CHO), 53.0 (OCH_3), 169.6 (CO_2Me);

***m/z* (CI):** 103 (MH^+, 46%).

7.2.2.4 Methyl 3-Azido-2-hydroxy-propionate (206)



206

The reaction was carried out under anhydrous conditions. To a stirred solution of oxirane-2-carboxylic acid methyl ester (1.50 g, 14.7 mmol) in methanol (70 mL) was

added sodium azide (2.35 g, 36.2 mmol) and ammonium chloride (1.41 g, 26.3 mmol). The mixture was stirred and heated at reflux. The reaction was monitored by TLC and stopped after 24 h. The solvent was removed *in vacuo* and the residue was extracted with hot $CHCl_3$ (3 x 150 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent was then removed *in vacuo*. The crude mixture was purified by flash column chromatography (1:1, EtOAc: petroleum ether 40-60 °C), yielding the title compound (1.70 g, 80%).

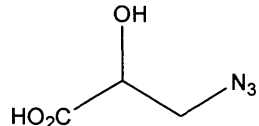
ν_{max} (KBr)/cm^{-1}: 3420br (OH), 2111 (N_3), 1740 (C=O), 1220 (C-O);

^{1}H NMR (400 MHz): δ 3.41 (1H, d, *J* 4.3 Hz, O*H*), 3.50 (1H, d, *J* 4.4 Hz, CH(OH)), 3.60 (1H, d, *J* 4.4 Hz, C*H*HN_3), 3.80 (3H, s, O*Me*), 4.36 (2H, d, *J* 3.5 Hz, CH_2N_3);

^{13}C NMR (100 MHz): δ 53.5 (*C*H_2N_3), 53.9 (O*C*H_3), 70.0 (*C*H(OH)), 172.9 (*C*O_2Me);

***m/z* (EI):** 144 (M^+, 30%), 126 (M – [OH]$^-$, 28).

7.2.2.5 Azido-2-hydroxy-propionic acid (208)



208

3-Azido-2-hydroxy-propionic acid methyl ester (300 mg, 2.07 mmol) was dissolved in methanol, with stirring and cooled to 0 °C. Lithium hydroxide (51 mg, 2.1 mmol) was added. The mixture was stirred for 24 h and warmed to room temperature. The mixture was then diluted with water (10 mL) and extracted with EtOAc (3 x 100 mL) to remove any unreacted starting material. The aqueous layer was acidified to pH 3 with citric acid solution (10% w/v), then extracted with EtOAc (3 x 100 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the title compound as an orange oil. No further purification was required (163 mg, 60%).

ν_{max} (neat)/cm^{-1}: 3358br (C-OH), 2920s, 2112 (N_3), 1702m (C=O), 1265 (C-O);

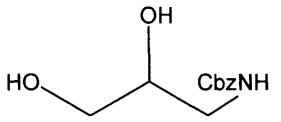
^{1}H NMR (400 MHz): δ 3.43 (1H, d, *J* 4.4 Hz, CH*H*N_3) 3.73 (1H, d, *J* 4.4 Hz, C*H*HN_3), 4.44 (1H, t, *J* 3.5 Hz, C*H*(OH));

^{13}C NMR (100 MHz): δ 53.5 (*C*H_2N_3), 70.1 (*C*H(OH)) 175.4 (*C*O_2H);

***m/z* (CI):** 132 (MH^+, 75%), 118 (M^+ - N, 90).

7.2.3 Aminopyruvate

7.2.3.1 Benzyl (2,3-Dihydroxy-propyl)-carbamate (219)



219

3-Amino-1, 2-propane diol (1.00 g, 11.0 mmol) was dissolved in water (50 mL). The reaction mixture was cooled to 0 °C and sodium hydroxide (1M) added to increase the basicity to pH 10. Benzyl chloroformate (1.4 mL, 10 mmol) was then added over a 10 min period and the reaction was then warmed to room temperature and stirred for 24 h. The product was extracted into dichloromethane (3 x 40 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* and the product was then purified by recrystallisation using dichloromethane. This gave the title compound as colourless crystals (0.83 g, 37%).

m.p. (°C): 62-63, Lit[192] (60.5);

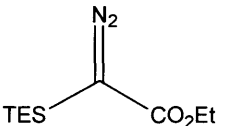
ν_{max} (KBr)/cm^{-1}: 3340br (C-OH), 2931s, 1697s (C=O);

^{1}H NMR (400 MHz): δ 2.29 (2H, m, C*H*$_2$OH), 3.54 (2H, m, C*H*$_2$N), 3.75 (1H, quint, *J* 6.3 Hz, C*H*(OH)), 5.11 (2H, s, NCO$_2$C*H*$_2$Ph-), 5.25 (1H, br, O*H*). 7.31-7.36 (5H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 43.2 (*C*H$_2$NH), 63.7 (*C*H$_2$OH), 67.2 (O*C*H$_2$Ph), 71.2 (*C*H(OH)), 128.1, 128.2, 128.5, 136.0 (Ar-*C*), 157.1 (N*C*O$_2$Bn);

***m/z* (CI):** 226 (MH$^+$, 35%), 118 (M – [$C_6H_5CH_2O$]$^+$, 60), 107 ([$C_6H_5CH_2O$]$^+$,), 91 ([$C_6H_5CH_2$]$^+$,100).

7.2.3.2 Ethyl Diazo-triethylsilanyl-acetate (225)



225

Triethylsilyl trifluoromethanesulfonate (8.6 mL, 38 mmol) was added to a stirring solution at –78 °C of ethyl diazoacetate (4.0 mL, 38 mmol) and *N,N'*-diisopropylethylamine (6.6 mL, 38 mmol) in ether (50 mL). The reaction was stirred at –78 °C for 0.5 h and allowed to warm to room temperature. Stirring was continued overnight. A white precipitate was filtered off and the solvent removed *in vacuo.* The

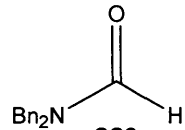
crude product was purified by flash column chromatography (1:99 EtOAc: hexane) to afford the title compound as a yellow oil (6.90 g, 79%).

ν_{max} (neat)/cm^{-1}: 2987s, 2905s, 2877s, 2083s (N_2), 1689s, (C=O), 1211s (C-O);

^{1}H NMR (500 MHz): δ 0.69 (6H, q, *J* 6.9 Hz, Si(CH_2CH$_3$)), 0.93 (9H, t, *J* 7.1 Hz, Si(CH$_2$$CH_3$)), 1.20 (3H, t, *J* 7.1 Hz, OCH$_2$$CH_3$), 4.15 (2H, q, *J* 7.1 Hz, OCH_2CH$_3$);

^{13}C NMR (125 MHz): δ 2.9 (Si(CH_2CH$_3$), 6.7 (Si(CH$_2$$CH_3$), 14.3 (OCH$_2$$CH_3$), 42.3 ($CH$(N$_2$)), 60.5 (O$CH_2CH_3$), 169.4 ($CO_2$Et);

7.2.3.3 *N,N'*-Dibenzyl-formamide (229)



229

Dibenzylamine (1.0 mL, 52 mmol) was heated at reflux at 60 °C in ethyl formate (4.3 mL, 53 mmol overnight. The solvent was removed *in vacuo* and the solid recrystallised from ether. This yielded the title compound as a crystalline solid (10.87 g, 93%)

m.p. (°C): 53-54, Lit[192] (53-54.5);

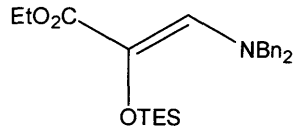
ν_{max} (KBr)/cm^{-1}: 3024s, 2931s, 1951s, 1651s (C=O);

^{1}H NMR (400 MHz): δ 4.26 (2H, s, NCH_2Ph), 4.41 (2H, s, NCH_2Ph), 7.16-7.35 (10H, m, Ar-*H*), 8.42 (1H, s CO*H*);

^{13}C NMR (100 MHz): δ 44.9 (NCH_2), 50.5 (NCH_2), 127.9, 128.4, 128.8, 129.0, 129.2, 135.9, 136.3 (Ar-*C*), 163.1 (N*C*OH);

***m/z* (EI):** 226 (MH$^+$, 100%), .134 (M – [$C_6H_5CH_2$]$^+$, 100), 91 ([$C_6H_5CH_2$]$^+$, 100).

7.2.3.4 3-Dibenzylamino-2-triethylsilanyloxy-acrylic acid ethyl ester (231)



231

N,N'-Dibenzyl-formamide (100 mg, 0.44 mmol) in dry toluene (4.0 mL) was added to a stirring solution of rhodium(II) acetate dimer (2.0 mg, 1.8 μmol) and ethyl diazo(triethylsilyl)acetate (105 mg, 0.46 mmol) in dry toluene (4.0 mL). The reaction mixture was heated at reflux for 12 h and monitored by TLC. The solvent was removed *in vacuo*, and purified by flash column chromatography (1:9, EtOAc: hexane). This yielded the title compound as an off-white solid (105 mg, 58%).

ν_{max} (film)/cm^{-1}: 3016s, 2954s, 2877s, 1728s (C=O), 1666s (C=C), 1218s (C-O);

^{1}H NMR (500 MHz): δ 0.64 (6H, q, *J* 7.8 Hz, Si(C*H*$_2$CH$_3$)), 0.90 (9H, t, *J* 7.8 Hz, Si(CH$_2$C*H*$_3$)), 1.27 (3H, t, *J* 5.7 Hz, OCH$_2$C*H*$_3$), 4.15 (2H, q, *J* 5.7 Hz, OC*H*$_2$CH$_3$), 4.42 (4H, s, NC*H*$_2$Ph), 6.94 (1H, s, NC*HC*(OTES)), 7.18-7.38 (10H, m, Ar-*H* and N*CH*CH(OTES));

^{13}C NMR (125 MHz): δ 5.7 (Si(*C*H$_2$CH$_3$)), 7.2 (Si(CH$_2$*C*H$_3$)), 14.8 (OCH$_2$*C*H$_3$), 54.3 (NCH$_2$Ph), 60.1 (O*C*H$_2$CH$_3$), 118.1 (N*C*HCH(OH)), 127.7 (NCH*C*H(OH)), 128.1, 128.8, 129.2, 133.2, 138.0 (Ar-*C*), 167.5 (*C*O$_2$Et);

***m/z* (EI):** 425 (M^+, 12%), 396 (M^+ - $[C_2H_5]^+$, 38), 91 ($[C_6H_5CH_2]^+$, 100).

7.2.3.5 Attempted Synthesis of aminopyruvate 5b using lactate oxidase (LAO) and pyruvate oxidase (PAO)

A 56-microwell titre plate format was used. Distilled water was used to make up solutions. Each well contained the following concentrations of reagents required for cofactor assay and colorimetric assay:

Cofactor assay (required cofactors and buffer):

Phosphate buffer (100 mM, pH 7.1);

Thiamine pyrophosphate (TPP), 0.6 mM;

Magnesium chloride (5 mM);

Flavin Adenine Dinucleotide (FAD), 0.02 mM.

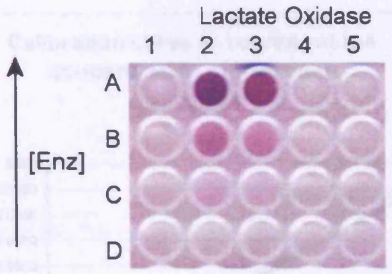
Colorimetric Assay (required enzyme and colouring agents):

4-aminoantipyrine (4-AA), 0.27 mg mL^{-1};

N-Ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline sodium salt dihydrate (TOOS), 0.4 mg mL^{-1};

Horseradish Peroxidase (HRP), 8 U mL^{-1}.

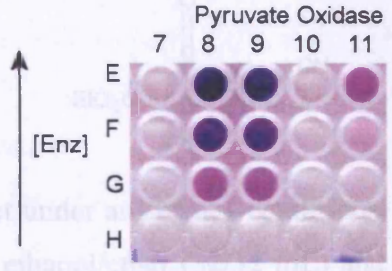
To the cofactor assay and colorimetric assay in each well, the enzymes (5.3 μL) in concentrations of 0 to 2 U mL^{-1} (A-D or E-H) were added (using serial dilution) for the unnatural substrate (*i.e.* DL-isoserine for either lactate oxidase and pyruvate oxidase). The enzyme concentration for the natural substrates (lactate for lactate oxidase and pyruvate for pyruvate oxidase) was added in 0 to 0.02 U mL^{-1} (A-D or E-H) using serial dilution. The concentration of substrates or hydrogen peroxide (0-0.1 mM, 75μL) added is represented in Figure 7.2.1, Figure 7.2.2 and Figure 7.2.3 to show the positive and negative controls.



1	2	3	4	5	Substrate
+	+	-	-	-	DL-Isoserine (10 mM, 120 μL)
-	+	+	-	-	Lactate (0.5 mM, 120 μL)
-	-	-	-	+	Pyruvate (0.5 mM, 120 μL)

Figure 7.2.1: Lactate oxidase micro wells with natural and unnatural substrates

Column 1 contained the unnatural substrate DL-isoserine, as indicated by the '+' in Figure 7.2.1. Column 2 was an inhibition test of DL-isoserine and lactate with lactate oxidase. Column 3 was a positive control containing the natural lactate substrate. Column 4 was a negative control containing only the cofactor and colorimetric assays. Column 5 was a test to see if pyruvate was accepted by lactate oxidase.



7	8	9	10	11	Substrate
+	+	-	-	-	DL-Isoserine (10 mM, 120 μL)
-	-	-	-	+	Lactate (0.5 mM, 120 μL)
-	+	+	-	+	Pyruvate (0.5 mM,120 μL)

Figure 7.2.2: Pyruvate oxidase micro wells with natural and unnatural substrates

Column 7 contained the unnatural substrate DL-isoserine, as indicated by the '+' in Figure 7.2.2. Column 8 was an inhibition test of DL-isoserine and pyruvate with pyruvate oxidase. Column 9 was a positive control containing the natural pyruvate substrate. Column 10 was a negative control containing only the cofactor and colorimetric assays. Column 11 was a test to see if pyruvate and lactate were accepted by pyruvate oxidase.

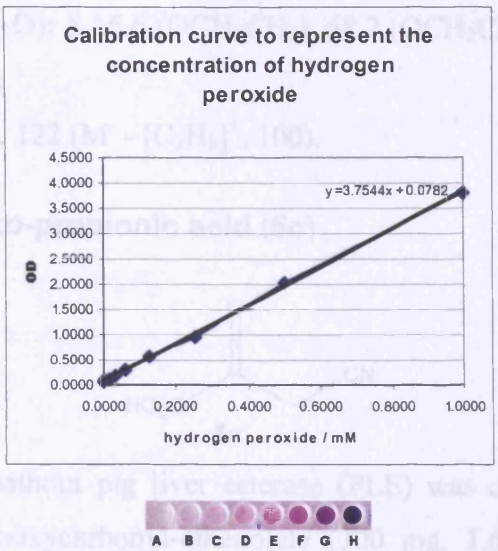
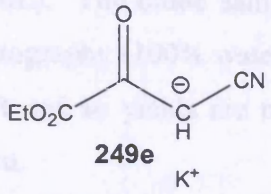


Figure 7.2.3: Calibration curve for concentration of hydrogen peroxide versus concentration found from micro well plate reader

7.2.4 Cyanopyruvate

7.2.4.1 Potassium 2-cyano-1-ethoxycarbonyl-ethenolate (249e)



249e

This reaction was carried out under anhydrous conditions. Potassium ethoxide (2.94 g, 0.03 mol) was dissolved in ethanol/ether (32/32 mL) and the mixture was cooled to 0 °C. Diethyl oxalate (9.35 g, 0.06 mol) was dissolved in ether (3 mL) and added to the reaction mixture, which was thereafter stirred for 30 min. A solution of acetonitrile (2.63 g, 0.06 mol) in ether (3 mL) was added and the reaction mixture warmed to room temperature and then stirred for 1 h. The precipitated solid was collected by filtration and was dried over phosphorous pentoxide to yield the title compound as an orange solid. The crude material was purified by reverse phase chromatography (100% water to remove excess potassium ethoxide) to yield the title compound as an orange solid (1.82 g, 16 %).

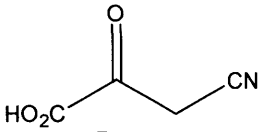
ν_{max} (KBr)/cm^{-1}: 3394s (C-OH), 2176s (C≡N), 1766s (C=O), 1635s (C=C), 1103 (C-O);

^{1}H NMR (400 MHz, D_2O): δ 1.20 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 4.13 (2H, q, *J* 7.2 Hz, OCH_2CH_3);

^{13}C NMR (100 MHz, D_2O): δ 15.6 (OCH_2CH_3), 68.2 (OCH_2CH_3), 125.7 (*C*≡N), 169.7 (*C*HCN), 172.3 (CO_2Et);

***m/z* (-CI):** 141 (M^-, 5%), 122 (M^- - $[C_2H_5]^+$, 100).

7.2.4.2 3-Cyano-2-oxo-propionic acid (5c)



5c

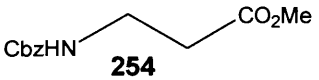
A control experiment without pig liver esterase (PLE) was conducted by dissolving potassium 2-cyano-1-ethoxycarbonyl-ethenolate (300 mg, 1.67 mmol) in phosphate buffer solution (pH 7.0, 100 mM, 3 mL). ^{13}C NMR was used to monitor the reaction over the course of 14 h. In phosphate buffer (pH 7.0, 100 mM, 3 mL), PLE (100 mg) was dissolved. To this was added potassium 2-cyano-1-ethoxycarbonyl-ethenolate (300 mg, 1.67 mmol). The reaction was monitored over the course of 14 h by ^{13}C NMR and by reverse phase TLC. The reaction mixture was filtered through a pad of C_{18} silica and washed through with water (10 mL). The crude sample (100 mg) was purified using preparative reverse-phase chromatography (100% water). It was not possible to remove phosphate as shown by ^{31}P NMR and so yields are unreliable. However, it was still possible to attempt characterisation.

ν_{max} (KBr)/cm^{-1}: 3294s (COOH), 2177s (CN), 1709s (C=O), 1564s

^{1}H NMR (400 MHz, D_2O) inter alia: δ 3.10 (1H, d, *J* 17.0 Hz, CH*H*CN), 3.16 (1H, d, *J* 17.0 Hz, C*H*HCN), See Appendix B;

^{13}C NMR (100 MHz, D_2O): δ 60.3 (*C*H(ONa)), 119.2 (*C*≡N), 165.5 (*C*HCN), 175.0 CO_2H);

7.2.4.3 Methyl 3-Benzyloxycarbonylamino-propionate (254)



254

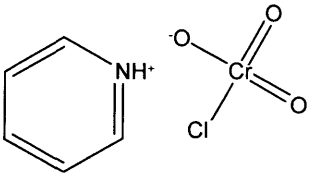
To a stirred solution of β-alanine methyl ester (4.50 g, 32.3 mmol) and TEA (4.5 mL, 33 mmol) in chloroform (70 mL) was added benzyl chloroformate (2.8 mL, 20 mmol). After a few minutes, TEA (5.4 mL, 39 mmol) was added and then after a few minutes again, was added benzyl chloroformate (2.8 mL, 20 mmol). The mixture was stirred for 2h. The mixture was then washed with water (3 x 100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by flash column chromatography (7:3, hexane:EtOAc) to yield the title compound as a colourless oil (6.54 g, 74 %).

ν_{max} **(film)/cm^{-1}:**3353s, 2953s, 1750s (C=O) 1699s (NCO), 1211 (C-O);

^{1}H NMR (500 MHz): δ 2.54 (2H, t, *J* 5.8 Hz, ($CH_2CH_2CO_2Me$)), 3.45 (2H, m, ($CH_2CH_2CO_2Me$)), 3.65 (3H, s, CO_2CH_3), 5.02 (2H, s, CO_2CH_2Ph), 5.29 (1H, s, N*H*), 7.23-7.38 (5H, m, Ar-*H*);

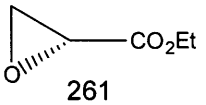
7.2.5 Phthalimido Pyruvate

7.2.5.1 Synthesis of Pyridinium chlorochromate



A 6M HCl solution (0.92 mmol) was added to a fully dissolved stirring solution of chromium trioxide (500 mg, 5.00 mmol) in water. This was cooled to 0 °C and to this was slowly added pyridine (0.39 mL, 5.00 mmol). The precipitated solution was recooled to 0 °C before suction filtration. The orange solid was dried *in vacuo*, to give the title compound (0.65 g, 65%).

7.2.5.2 Ethyl (R)-(+)-2,3-epoxypropanoate (Ethyl glycidate) (261)



261

(*S*)-(−)-2-Bromo-3-hydroxypropanoic acid (259)

L-Serine (52.5 g, 0.50 mol) and potassium bromide, KBr, (200 g, 1.70 mol) were dissolved in water (400 mL). Hydrobromic acid (48%, 123 mL, 1.09 mol) was added at room temperature and the mixture was cooled to −13 °C with stirring. Nitrogen was bubbled through the solution and sodium nitrite (42.8 g, 0.62 mol) was slowly added in small portions (*ca.* 5 g every 15 min). After each addition, the reaction mixture turned brown and the colour slowly faded, but the solution did not decolourise entirely. The total time required for addition of all the sodium nitrite was approximately 2.5 h. The solution was then allowed to warm to 0°C and the nitrogen purge was stopped. The mixture was then stirred for 6 h. Excess nitrogen oxides were removed by bubbling nitrogen through the solution for 1 h. The pale green solution was then extracted with ether (6 × 300 mL). The combined organic extracts were concentrated to 0.5 L by rotary evaporation and dried over anhydrous ($MgSO_4$). The ether was then evaporated *in*

vacuo. The pale yellow or green oil (82 g, 90%) was used immediately in the next reaction without further purification.[161]

Potassium (*R*)-(+)-2,3-epoxypropanoate (260)

The crude acid from the preceding step (74.5 g, 0.44 mol) was dissolved in absolute ethanol (300 mL) and cooled to -20°C. Under nitrogen, a filtered solution of potassium hydroxide (55.5 g, 0.85 mol) in absolute ethanol (300 mL) was slowly added. After 2 h, the mixture was allowed to warm to 0°C and stirred at this temperature for 14 h. The solution was filtered to remove precipitated salts. Half of the solvent was removed by rotary evaporation without warming and an additional crop of salt (2-4 g) was isolated by filtration. The combined salts were dried under vacuum to give 105 g of a 1:1 mixture of KBr and potassium glycidate.[161]

One third of this mixture (35 g) was extracted by refluxing in a mixture of 585 mL of absolute ethanol and 15 mL of water in a 1 L flask with good stirring for 45 min. After filtration of the hot suspension, the glycidate crystallized from the filtered solution to give 12-13 g of product. Another third of the 1:1 KBr: potassium glycidate mixture was heated at reflux with stirring in the mother liquors for 45 min. After the second batch of crystalline potassium glycidate was isolated by filtration, the procedure was repeated with the last portion of the KBr-glycidate mixture. The solids collected from the three hot filtrations were combined and extracted a fourth time with the same solution, then crystallized to give additional product. After drying, the total weight of recrystallised potassium glycidate was 35.5 g.[161]

m.p. (°C): 136-138, Lit[193] (135.5-137);

ν_{max} (nujol)/cm^{-1}: 2851s, 1614s (C=O);

^{1}H NMR (400 MHz): δ 2.83 (2H, d, *J* 6.3 Hz, CH_2O), 3.33 (1H d, *J* 6.3 Hz, C*H*CO_2K);

^{13}C NMR (100 MHz): δ 44.7 (*C*H_2O), 61.0 (*C*HO), 177.5 (*C*O_2K);

***m/z* (CI):** 103 (MH^+, 46%).

Ethyl (*R*)-(+)-2,3-epoxypropanoate (261)

A suspension of dry potassium glycidate (26.0 g of an 91:9 glycidate: KBr mixture, 0.19 mol), benzyltriethylammonium chloride (42.4 g, 0.19 mol), and ethyl bromide (76.0 g, 0.70 mmol) in dichloromethane (300 mL) was heated at reflux for 16 h with good stirring. The solvent and excess ethyl bromide was then slowly removed by rotary evaporation without warming, and the resulting viscous solid was triturated with anhydrous diethyl ether (3 × 100 mL) to extract the ethyl glycidate. The combined ethereal extracts were filtered and dried over anhydrous ($MgSO_4$). The solvent was slowly removed by rotary evaporation (without warming) and the residue was then distilled (2.4 mmHg; 40 °C) with 0 °C water circulating through the condenser to afford the title compound.[161] (8.80 g, 40%);

ν_{max} (neat)/cm^{-1}: 2985s, 1750 (C=O), 1736 (C=O), 1203s (C-O);

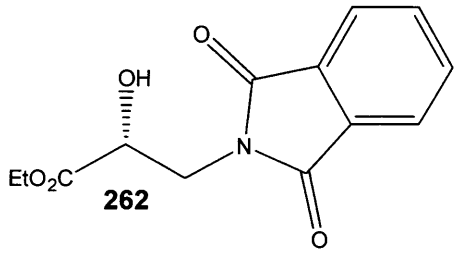
^{1}H NMR (400 MHz, D_2O): δ 1.29 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 2.94 (2H, t, *J* 6.5 Hz, CH_2CO), 3.42 (1H, d, *J* 4.1 Hz, *CH*CO), 4.25 (2H, q, *J* 6.5 Hz, OCH_2CH_3);

^{13}C NMR (100 MHz, D_2O): δ 13.9 (OCH_2CH_3), 46.1 (CH_2CO) 47.3 (*C*HCO), 61.6 (OCH_2CH_3), 169.1 (CO_2Et)

***m/z* (CI):** 117 (MH^+, 89%), 89 (M^+ - $[C_2H_5]^+$, 100);

***m/z* (+HRES):** 117.055019, (MH^+, $C_5H_8O_3$ requires 117.05517).

7.2.5.3 Ethyl 3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2-hydroxy-propionate (262)



262

Ethyl (*R*)-(+)-2,3-epoxypropanoate (220 mg, 1.89 mmol) in DMF (2 mL) was added dropwise to a potassium phthalimide (40 mg, 0.22 mmol)/phthalimide (220 mg, 1.50 mmol) mixture in DMF (50 mL). The reaction mixture was heated to 90 °C and stirred for 48 h. The reaction mixture was diluted with water (500 mL) and extracted with chloroform (3 x 100 mL). The organic layer was washed with water (50 mL), then brine (50 mL) and then dried (Na_2SO_4). The solvent was removed *in vacuo* and the solid recrystallised from chloroform to yield the title compound as colourless crystals (440 mg, 89 %).

m.p. (°C): 80-81, Lit[138] (82-83);

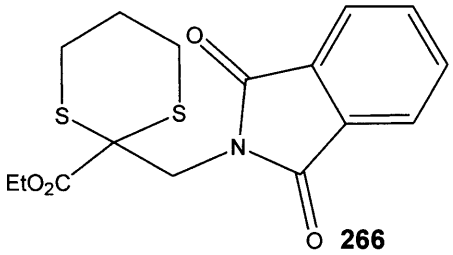
ν_{max} (KBr)/cm^{-1}: 3472s (C-OH), 1750 (C=O), 1735 & 1730s (C=O), 1103 (C-O);

^{1}H NMR (400 MHz): δ 1.26 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 3,12 (1H, d, O*H*), 4.03 (2H, dt, *J* 8.6, 4.5 Hz, CH_2NH), 4.21 (2H, dq, *J* 7.2, 2.3 Hz, OCH_2CH_3), 4.48 (1H, t, *J* 5.8 Hz, C*H*(OH))

^{13}C NMR (100 MHz): δ 13.9 (OCH_2CH_3), 41.0 (CH_2N), 62.3 (OCH_2CH_3), 68.3 (*C*H(OH)), 123.4, 131.3, 134.1 (Ar-*C*), 158 (N*C*O), 168.1 (CO_2Et);

***m/z* (FAB):** 264 (MH^+, 32%).

7.2.5.4 Ethyl 2-(1,3-Dioxo-1,3-dihydro-isoindol-2-ylmethyl)-[1,3]dithiane-2-carboxylate (266)



266

Under dry conditions, sodium hydride (6.94 g, 173 mmol of a 60 % mineral dispersion) was washed with pentane (3 x 20 mL). In a second round bottom flask, bromomethyl phthalimide (50.0 g, 208 mmol) and ethyl 1,3-dithiane-2-carboxylate (33.8 g, 173 mmol) was dissolved in DMF (200 mL). This was transferred to a dropping funnel by cannula. Sodium hydride was suspended in dry benzene (250 mL) at 5 °C. The DMF mixture was added to the sodium hydride suspension with caution over 2 h. On completion, the reaction mixture was allowed to warm to room temperature and was stirred for 18 h. The mixture was poured into water (1 L) and extracted with ether (5 x 500 mL). The combined extracts were dried (Na_2SO_4) and were then evaporated *in vacuo*. The residue was purified by recrystallisation, using EtOAc-light petroleum (1:2) to yield the title compound (22.89 g, 39%).

m.p. (°C): 145-146, Lit[160] (142-144);

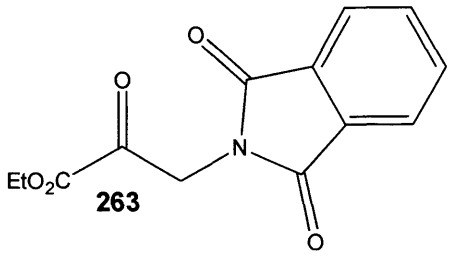
ν_{max} (film)/cm^{-1}: 2252s, 1776s (C=O), 1720s (C=O);

^{1}H NMR (400 MHz): δ 1.29 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 1.90 (2H, m, $SCH_2CH_2CH_2S$), 2.84-3.01 (4H, m, -$SCH_2CH_2CH_2S$), 4.23 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 7.64 - 7.81 (4H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 13.8 (OCH_2CH_3), 27.2 (-$SCH_2CH_2CH_2S$), 30.90 (-$SCH_2CH_2CH_2S$), 55.1 (OCH_2CH_3), 62.8 ($C(S(CH_2)_3S)$)), 123.5, 131.7, 134.1 (Ar-*C*), 167.9 (Ar(*C*=O)), 169.3 (CO_2Et);

***m/z* (+ES):** 374 (MNa^+, 100%).

7.2.5.5 Ethyl 3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2-oxo-propionate (263)



263

2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-[1,3]dithiane-2-carboxylic acid ethyl ester (19.3 g, 55.5 mmol) was dissolved in acetone (190 mL) and added to *N*-bromosuccinimide (68.4 g, 384 mmol) in acetone/water (97:3, 900 mL), maintained between –5 to 5°C. After 45 min, the reaction mixture was poured into hexane: dichloromethane (1:1, 600 mL) and aqueous sat. sodium bisulfite (1.6 L). The aqueous phase was extracted with hexane: dichloromethane (1:1, 2 x 600 mL). The combined organic layers were dried (Na_2SO_4) and the solvent then evaporated *in vacuo*. The residual phthalimide was removed by rinsing the residue with dichloromethane (70 mL) and concentrating the filtrate to give viscous oil. This was purified by flash column chromatography (100% $CHCl_3$) to yield the title compound as colourless crystals (8.80 g, 55 %).

m.p. (°C): 85-86, Lit[160] (89-90);

ν_{max} (KBr)/cm^{-1}: 1778 (C=O), 1722 (C=O), 1103 (C-O);

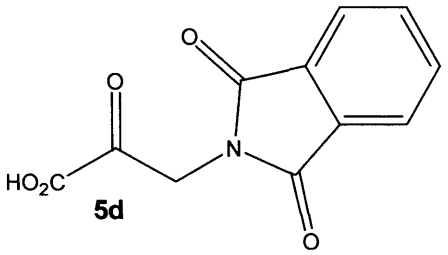
^{1}H NMR (400 MHz): δ 1.37 (3H, t, *J* 8.0 Hz, OCH_2CH_3), 4.35 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 4.41 (2H, s, CH_2N), 7.74 (2H, m, Ar-*H*), 7.87 (2H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 13.9 (OCH_2CH_3), 44.8 (CH_2N), 63.1 (OCH_2CH_3), 123.5, 123.7, 131.9, 134.3-134.2 (Ar-*C*), 159.0 (N(*C*O)), 167.2 (CO_2Et), 185.7 ($EtCO_2COCH_2N$);

***m/z* (+ES):** 284 (MNa^+, 21%), 262 (MH^+, 10);

***m/z* (+HRES):** 284.05372, (MNa^+, $C_{13}H_{11}NO_5Na^+$ requires 284.05294).

7.2.5.6 3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2-oxo-propionic acid (5d)



5d

(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2-oxo-propionic acid ethyl ester (261 mg, 1.00 mmol) was dissolved in methyl *tert* butyl ether (MTBE; 15 mL). To this was added a solution of pig liver esterase (PLE; 42 mg, 1000U) dissolved in phosphate buffer (pH 7.0; 100 mM, 15 mL). The mixture was stirred and periodically monitored by TLC for the disappearance of starting material. The MTBE layer was separated and the aqueous layer was washed with MTBE (3 x 75 mL). The pH was adjusted to 3 by dropwise addition of HCl and the aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried (Na_2SO_4) and then evaporated *in vacuo*. The mixture was recrystallised by layering with chloroform and hexane. White crystals emerged but were not the product, as determined by 1H NMR spectroscopy. The remaining oily residue was identified as the title compound and was sufficiently pure for use in further steps (100 mg, 40%).

ν_{max} (film)/cm^{-1}: 3279br (C-OH), 3019s, 1776 (C=O), 1722 (C=O), 1616m, 1600m, 1580m, 1469m;

^{1}H NMR (400 MHz): δ 4.93 (2H, s, C*H*$_2$N), 7.61 (2H, m, Ar-*H*), 7.83 (2H, m, Ar-*H*), 9.39-9.86 (br, 2H);

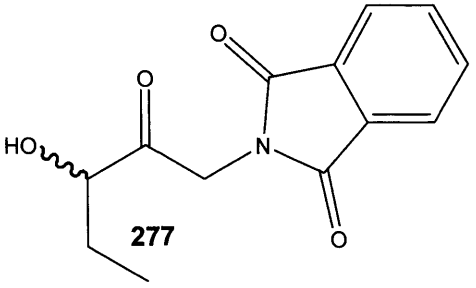
^{13}C NMR (100 MHz): δ 44.7 (*C*H$_2$N), 122.9, 132.6, 134.3 (Ar-*C*), 162.7 (Ar(*C*=O), 171.3 (*C*O$_2$H), 188 (*C*O);

***m/z* (CI):** 234 (MH$^+$, 4%);

***m/z* (+HRES):** 234.03912 (MH$^+$, $C_{11}H_7NO_5$ requires 234.04025).

7.2.6 Phthalimido pyruvate: Biomimetic reaction with propionaldehyde

7.2.6.1 2-(3-Hydroxy-2-oxo-pentyl)-isoindole-1,3-dione (277)



277

3-(1,3-Dioxo-indan-2-ylamino)-2-oxo-propionic acid (54 mg, 0.2 mmol) and propionaldehyde was mixed with water (5 mL). The pH was adjusted to 7 with saturated $NaHCO_3$. *N*-Methylmorpholine (25 μL, 0.2 mmol) was added and the pH was adjusted to pH 7 with 0.01 M HCl. The reaction was stirred for 48 h and the solvent ws then removed *in vacuo*. The residue was purified using preparative TLC (5:95 MeOH/dichloromethane) to yield the title compound (23 mg, 46%). Attempts were made to record a ^1H NMR spectrum, but unreliable signals were observed from excess propionaldehyde.

ν_{max} (film)/cm^{-1}: 3323br (C-OH), 3019s, 2927s, 1711m (C=O);

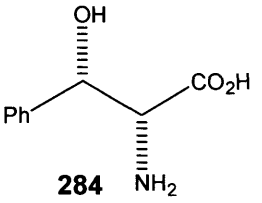
***m/z* (+CI):** 248 (MH^+, 95%);

***m/z* (+HRES):** 248.09187, (MH^+, $C_{13}H_{13}NO_4$ requires 248.09228).

7.3 Chiral HPLC standard syntheses

7.3.1 Unsubstituted aromatic *syn* standards

7.3.1.1 *Syn*-2-Amino-3-hydroxy-3-phenyl-propionic acid (284)



284

Benzaldehyde (2.01 g, 18.9 mmol), glycine (706 mg, 9.40 mmol) and KOH (1.06 g, 18.9 mmol) were dissolved in ethanol (70 mL) and the mixture was stirred at room temperature for 5 h. The Schiff's base product was hydrolysed using HCl (2M), with warming (40-50 °C). The precipitate formed, on cooling, was collected by filtration and recrystallised from water/ethanol to yield the title compound as colourless crystals (557 mg, 16%).

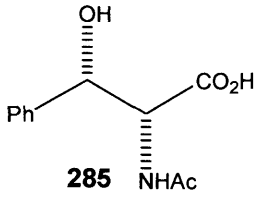
m.p. (°C): 177-180, Lit[194] (175);

ν_{max} (nujol)/cm^{-1}: 3614m (C-OH), 3371m (NH_2), 1641m (C=O);

^{1}H NMR (500 MHz, D_2O): δ 3.94 (1H, d, *J* 4.1 Hz, C*H*(NH_2)), 5.32 (1H, d, *J* 4.0 Hz, C*H*(OH)), 7.43-7.48 (5H, m, Ar-*H*);

^{13}C NMR (125 MHz, TFA-d): δ 55.7 (*C*H(NH_2)), 66.9 (*C*H(OH)), 120.4, 123.7, 124.8, 125.2, 130.0 (Ar-*C*), 169.6 (*C*O_2H);

7.3.1.2 *Syn*-2-Acetylamino-3-hydroxy-3-phenyl-propionic acid (285)



285

DL-*threo*-3-phenylserine (1.00 g, 5.51 mmol) was dissolved in water (10 mL). The pH was adjusted to 9, using NaOH (2M) and acetic anhydride (1.67 g, 16.2 mmol) was added. The pH of the solution was maintained at 9.0 by addition of NaOH (1M). The mixture was cooled after 1 h and slowly the aqueous layer was acidified using HCl to pH 2. A white solid precipitated and was collected. The aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and then evaporated *in vacuo* to yield a white solid. The white solids were rinsed with diethyl

ether and dried *in vacuo*. No further purification was necessary, yielding the title compound as a colourless solid (1.23 g, 68%).

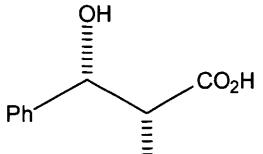
m.p. (°C): 177-178, Lit[164] (177-178);

^{1}H NMR (400 MHz): δ 1.82 (3H, s, NHCOC*H*$_3$), 4.62 (1H, d, *J* 3.4 Hz, C*H*(NHAc)), 5.24 (1H, d, *J* 3.4 Hz, C*H*(OH)), 7.22-7.34 (5H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 23.8 (NCO*C*H$_3$), 61.2 (*C*H(NHAc)), 75.1 (*C*H(OH)), 128.4, 130.6, 130.9, 142.1 (Ar-C), 175.8 (N*C*OCH$_3$), 176.5 (*C*O$_2$H);

***m/z* (CI):** 224 (MH$^+$, 35%), 206 (M$^+$ - OH), 107 ([C$_6$H$_5$C(OH)]$^+$).

7.3.1.3 *Syn*-2-Benzyloxycarbonylamino-3-hydroxy-3-phenyl-propionic acid (287)



287

DL-*threo*-3-phenylserine (1.00 g, 5.51 mmol) was dissolved in water (25 mL) and cooled to 0 °C. Sodium bicarbonate (1.15 g, 13.8 mmol) was added, before the dropwise addition of benzyl chloroformate (0.94 mL, 6.61 mmol). The reaction mixture was allowed to come to room temperature and stirred for 2 h. The aqueous layer was extracted with ether (3 x 30 mL), before acidification of the aqueous layer to pH 3.0. The acidified aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried (Na_2SO_4) and then evaporated *in vacuo* to yield a white solid (1.58 g, 94%). No further purification was necessary as determined by ^{1}H NMR spectroscopic analysis.

m.p. (°C): 80-82, Lit[195] (83-85);

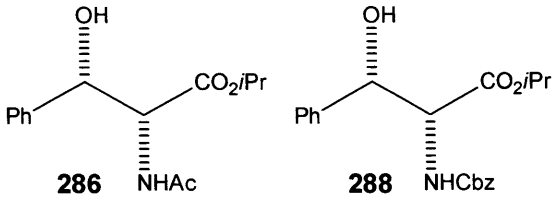
ν_{max} (KBr)/cm^{-1}: 3325s (C-OH), 3085br (C-OH), 1720s (C=O), 1226 (C-O);

^{1}H NMR (400 MHz): δ 4.68 (1H, d, *J* 9.3 Hz, C*H*(NHCBz)), 4.76-5.00 (2H, m, C*H*$_2$Ph), 5.39 (1H, s, C*H*(OH)), 6.00 (1H, d, *J* 9.3 Hz, N*H*), 7.18-7.35 (10H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 59.8 (*C*H(NHCbz), 67.1 (O*C*H$_2$Ph), 73.0 (*C*H(OH)), 125.9, 128.0, 128.3 (overlap), 128.6, 139.3 (Ar-*C*), 156.9 (N*C*O$_2$Bn), 171.5 (*C*O$_2$H);

***m/z* (FAB):** 328 (MNa$^+$, 12%).

7.3.1.4 Isopropyl *Syn*-acetylamino-3-hydroxy-3-phenyl-propionate (286) and Isopropyl *syn*-benzyloxycarbonylamino-3-hydroxy-3-phenyl-propionate (288)



286 **288**

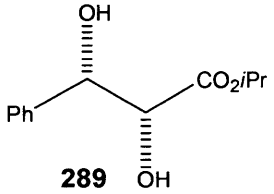
To a solution of ethanol (100 mL) containing acid (1.09 g, 3.50 mmol) was added caesium carbonate (583 mg, 1.78 mmol) in water (30 mL). The resulting mixture was evaporated and azeotroped with toluene to remove the majority of water from the reaction mixture. The resultant solid was dried *in vacuo* and dissolved in dry DMF (20 mL). Under anhydrous conditions, 2-iodopropane (17.9 mL, 178 mmol) was added to the reaction mixture. The mixture was left to stir at room temperature for 24 h. DMF was evaporated *in vacuo* and the residue was purified by flash column chromatography (1:9, acetone:toluene) to yield **286** (390 mg, 42%) and **288** (800 mg, 65%).

HPLC (286): Chiracel AD-H, 4.6mm x 250 mm, hexane/*i*PrOH, 85/15, 0.6 ml min^{-1}, 210 nm, 14.6 (2*R*,3*S*), 15.4 (2*S*,3*R*)

HPLC (288): Chiracel AD-H, 4.6mm x 250 mm, hexane/*i*PrOH, 85/15, 0.6 ml min^{-1}, 210 nm, 16.7 (2*R*,3*S*), 18.0 (2*S*,3*R*).

Full characterisation *vide infra*.

7.3.1.5 Isopropyl *syn*-2,3-Dihydroxy-3-phenyl-propionate (289)



289

Isopropyl cinnamate (3.45 mmol) was dissolved in acetone (10 mL). To this was added, with stirring, *N*-methylmorpholine oxide hydrate (3.78 mmol) and osmium tetroxide (0.39 mmol). The reaction was monitored by TLC and quenched by adding sodium bisulfite (6% wt/v, 10 mL). The mixture was extracted into ethyl acetate and magnesol® (5 g) was added with stirring for 10 min. The magnesol® was filtered and combined organic extracts were dried (Na_2SO_4). Then the solvent removed *in vacuo* and the crude was purified by flash column chromatography (4:6 EtOAc:hexane) to yield the title compounds.

(407 mg, 61 %);

ν_{max} **(film)/cm^{-1}:** 3425br (C-OH) , 1729s (C=O), 1216 (C-O);

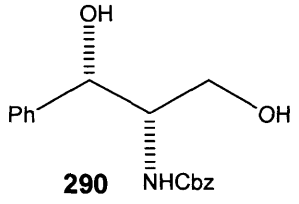
^{1}H NMR (500 MHz): δ 1.15 (3H, d, *J* 6.3 Hz, OCH(C*H*$_3$)$_2$), 4.27 (1H, d, *J* 3.7 Hz, C*H*(OH))), 4.91 (1H, d, *J* 3.6 Hz, C*H*(OH)), 5.03 (1H, sept, *J* 6.5 Hz, O*CH*(CH$_3$)$_2$), 7.18-7.39 (5H, m, Ar-*H*);

^{13}C NMR (125 MHz): δ *ca* 21.5 (OCH(*C*H$_3$)$_2$), 69.8 (O*C*H(CH$_3$)$_2$), 74.6 (*C*H(OH)), 74.8 (*C*H(OH)), 126.3, 127.8, 128.2, 128.5, 139.3 (Ar-*C*), 172.1 (*C*O$_2$*i*Pr);

***m/z* (FAB):** 244 (M$^+$, 28%), 207 (M$^+$ - [OH]).

HPLC: Chiracel AD-H, 2mm x 250 mm, hexane/*i*PrOH, 80/20, 0.6 ml min^{-1}, 210 nm, 20.2, 21.9 (*syn*-products)

7.3.1.6 Benzyl *Syn*-(2-Hydroxy-1-hydroxymethyl-2-phenyl-ethyl)-carbamate (290)



290

Lithium borohydride (1.40 g, 56.3 mmol) was added portionwise to a cooled solution of DL-*threo*-benzyloxycarbonylamino-3-hydroxy-3-phenyl-propionic acid isopropyl ester (4.64 g, 13.1 mmol) in THF/ethanol (1/9), with stirring. Once added, the reaction mixture was warmed to room temperature and left to stir for 48 h. To this mixture was added HCl (0.5 M, 200 mL), with caution. The aqueous layer was extracted with EtOAc (2 x 100 mL) and the combined organic layers dried (Na_2SO_4) and then evaporated *in vacuo*. The residue was purified by flash column chromatography (1:1, EtOAc:hexane) to yield the title compound as a colourless crystalline solid (3.25 g, 80%).

m.p. (°C): 100-101, Lit[196] (102-103);

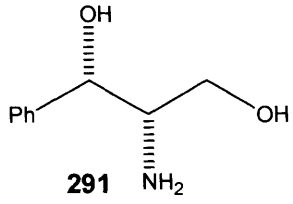
ν_{max} **(film)/cm^{-1}:** 3617s (C-OH), 3433br (C-OH), 3018s, 1706s (C=O), 1216s (C-O);

^{1}H NMR (400 MHz): δ 3.23 (1H, br, O*H*), 3.63 (2H, m, C*H*$_2$OH), 3.85 (1H, m, C*H*(NHCBz)), 4.76 (NC*H*$_2$Ph, s, 2H), 5.61 (1H, d, *J* 8.3 Hz, C*H*(OH)), 7.23-7.33 (10H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 57.6 (*C*H(NHCbz)), 63.4 (*C*H(OH)), 66.9 (O*C*H$_2$Ph), 73.6 (*C*H(OH)), 123.4, 125.6, 127.8, 128.3, 129.4, 134.1, 136.2, 140.8 (Ar-*C*), 157.0 (N*C*O$_2$Bn);

***m/z* (+ES):** 302 (MH$^+$, 100%), 284 (M - [H_2O]).

7.3.1.7 *Syn*-2-Amino-1-phenyl-propane-1,3-diol (291)



291

A mixture of Benzyl DL-*threo*-(2-Hydroxy-1-hydroxymethyl-2-phenyl-ethyl)-carbamate (3.05 g, 10.0 mmol), 10% Pd-C (1.18 g), cyclohexene (57 mL) and ethanol (114 mL) was heated at reflux with stirring for 3 h. The mixture was filtered through celite with ethanol (3 x 100 mL) and water (3 x 100 mL). The ethanol layer was evaporated *in vacuo* to yield the title compound as a white solid. No further purification was necessary. (1.25 g, 74%).

m.p. (°C): 115-17, Lit[197] (112-113);

ν_{max} (KBr)/cm^{-1}: 3394s (C-OH), 3309s (C-OH) 3055s (NH_2), 2862s, 2738s, 2669s, 1581 (C-N);

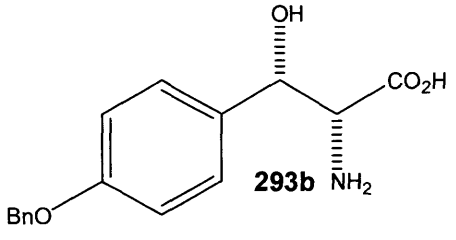
^{1}H NMR (400 MHz): δ 2.90 (1H, dd, *J* 9.9 Hz, C*H*(NH_2)), 3.50 (2H, dd, *J* 16.7, 5.0 Hz, C*H*$_2$OH), 4.61 (1H, d, *J* 5.2 Hz, C*H*(OH)), 7.13-7.43 (5H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 58.1 (*C*H(NH_2)), 64.6 (*C*H$_2$OH), 74.7 (*C*H(OH)), 126.2 (overlap), 127.7 (overlap), 128.5, 142.1 (Ar-*C*);

***m/z* (+ES):** 168 (MH^+, 94%), 335 (M, dimer, 20).

7.3.2 *p*-Substituted aromatic *syn* standards

7.3.2.1 *Syn*-2-Amino-3-[4-(benzyloxy)phenyl]-3-hydroxy-propionic acid (293b)



293b

To a solution of potassium hydroxide (5.61 g, 0.10 mol) and glycine (3.75 g, 0.05 mol) in ethanol (75 mL), was added a solution of *p*-benzyloxybenaldehyde (21.2 g, 0.10 mol) in ethanol (50 mL). The mixture was warmed until it was absolutely clear (*ca.* 60 °C) and the solution was allowed to stand at room temperature. A crystalline solid precipitated, which was filtered after 5 h and washed thoroughly with alcohol and ether. This product was the potassium salt of *N*-*p*-benzyloxybenzylidene-*p*-benzyloxyphenyl-

3-serine (**296**) and was obtained as a white powder (15.6 g yield average, 60-63%), m.p. 161-163 °C. The potassium salt **296** (41.6 g, 0.08 mole) was stirred vigorously with 1M HCl (400 mL) and the mixture was filtered immediately. The solid *p*-benzyloxybenzaldehyde was washed with 0.5M HCl (200 mL) and then with water (100 mL). It weighed 37.10 g and probably contained some *p*-benzyloxy-3-phenylserine. The acid filtrates were combined and on neutralization the title compound was obtained. A sample was recrystallized from a mixture of alcohol (6 parts), water (2 parts) and dimethylformamide (2 parts) to afford colourless crystals (17.7 g, 77.0%).

m.p. (°C): 190-192, Lit[169] (190-192);

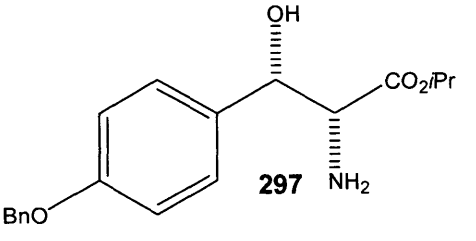
ν_{max} (KBr)/cm^{-1}: 3363br (C-OH), 3147s (NH_2), 1666s (C=O);

^{1}H NMR (400 MHz, DMSO-d6): δ 2.24 (1H, s, O*H*), 3.27 (1H, d, *J* 4.0 Hz, *CH*(NH_2)), 4.87 (1H, d, *J* 3.6 Hz, C*H*(OH)), 4.94 (2H, s, CH_2Ph), 6.81 (2H, d, *J* 8.5 Hz, Ar-*H*), 7.11-7.32 (7H, m, Ar-*H*);

^{13}C NMR (100 MHz, DMSO-d6): δ 59.2 (*C*H(NH_2)), 69.2 (O*C*H_2Ph)), 70.4 (*C*H(OH)), 114.3, 127.7 (overlap), 127.9, 128.5 (overlap), 137.3 (Ar-*C*), 157.6 (Ar (*C*-O));

***m/z* (+FAB):** 289 (MH_2^+, 8%).

7.3.2.2 Isopropyl *syn*-2-Amino-3-[4-(benzyloxy)phenyl]-3-hydroxy-propionate (297)



DL-*threo*-2-Amino-3- (4-benzyloxy-phenyl)-3-hydroxy-propionic acid (5.00 g, 17.4 mmol) was suspended in ^{i}PrOH (50 mL) and cooled to 0 °C. $SOCl_2$ (1.4 mL, 19.2 mmol) was added dropwise with stirring. The mixture was warmed to 40 °C. The reaction was monitored using TLC. The solvent was then removed *in vacuo*. An initial column ran in EtOAc removed all less polar organic components leaving behind unreacted carboxylic acid. A second column was run in (3:7 EtOAc:hexane) to yield the title compound (610 mg, 10 %).

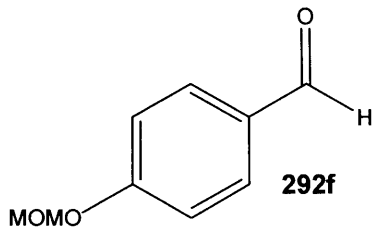
^{1}H NMR (400 MHz): δ 1.31 (6H, d (overlap), *J* 6.3 Hz, OCH(*C*H_3)$_2$), 4.21 (1H, d, *J* 5.3 Hz, C*H*(OH)), 5.08 (2H, s, CH_2Ph), 5.14 (1H, sept, *J* 6.3 Hz, C*H*(OH)), 5.56 (d, *J* 5.2 Hz, NH_2), 7.01 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.48-7.23 (7H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 21.6 (OCH(CH_3)$_2$), 61.0 (CH(NH$_2$)), 68.4 (OCH_2Ph), 69.0 (OCH(CH$_3$)$_2$), 72.3 (CH(OH)), 114.4, 127.5, 127.8, 128.1, 128.6, 129.6, 131.0, 133.9, 157.0 (Ar-C), 170.0 (CO_2iPr);

***m/z* (+ES):** 330 (MH$^+$, 24%), 312 (M – [H$_2$O], 100);

***m/z* (+HRES):** 330.16978, ([MH$^+$], $C_{19}H_{24}NO_4$ requires 330.17053).

7.3.2.3 4-(Methoxymethoxy)benzaldehyde (292f)



292f

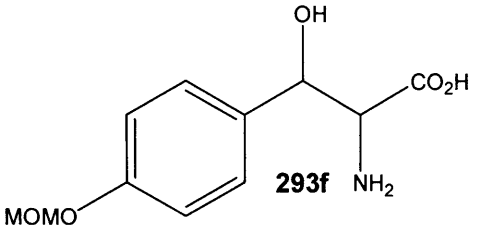
The reaction was performed under an inert atmosphere. 4-Hydroxybenzaldehyde (32 g, 0.25 mol) was dissolved in dichloromethane (500 mL). To this was added, *p*-toluenesulphonic acid monohydrate (250 mg) followed by dimethoxymethane (100 mL, 1.13 mol) and the mixture was heated at reflux under a soxhlet containing 150 g of molecular sieves (3Å) overnight. The solvent was removed *in vacuo* and the residue purified by vacuum distillation (0.5 Torr; 125-130 °C), yielding the title compound as an oil (10.31 g, 23%).

ν_{max} (neat)/cm^{-1}: 1685s (C=O), 1110 (C-O);

^{1}H NMR (400 MHz): δ 3.45 (3H, s, OCH_3), 5.19 (2H, s, OCH_2OCH$_3$), 7.08 (2H, d, J 8.8 Hz, Ar-H), 7.79 (2H, d, J 8.8 Hz, Ar-H), 9.87 (1H, s, COH);

^{13}C NMR (100 MHz): δ 56.6 (OCH_3), 94.3 (OCH_2OCH$_3$), 116.5, 131.0, 132.2, 162.4 (Ar-C), 191.1 (COH);

7.3.2.4 2-Amino-3-hydroxy-3-[4-(methoxymethoxy)phenyl]propionic acid (293f)



293f

Glycine (10.28 g, 0.14 mol) and 4-(methoxymethoxy)benzaldehyde (33.00 g, 0.20 mol) were added to a suspension of potassium hydroxide (15.37 g, 0.27 mol) in ethanol (300 mL) at room temperature, and the reaction mixture was stirred for 19 h. The solvent was evaporated, and the residue dissolved in water and acidified with HCl. The

aqueous solution was washed with EtOAc and the pH adjusted to 6.0 with $NaHCO_3$. A white solid was precipitated and collected by filtration to give the title compound, after recrystallisation to remove excess[173] glycine. The title compound was found to exist as a 1:1 (A: B) diastereomeric mixture (3.63 g, 11%).

m.p. (°C): 162-163, Lit[173] (164-166);

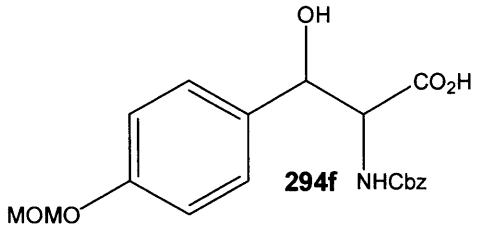
ν_{max} (KBr)/cm^{-1}: 3454br (C-OH), 3070s (NH_2), 2962s, 1635s (C=O), 1512s (C-N), 1234s (Ar-O);

^{1}H NMR (500 MHz, D_2O): δ 3.47 (6H, s, 6H, A + B, OCH_3), 3.89 (1H, d, *J* 5.5 Hz, A, $CH(NH_2)$), 4.06 (1H, d, *J* 5.3 Hz, B, $CH(NH_2)$), 5.19 (4H, s, A + B, OCH_2OCH_3 & CH(OH)), 7.11 (4H, m, A, Ar-*H*), 7.33 (4H, m, B, Ar-*H*);

***m/z* (+ES):** 242 (MH^+, 54), 180 (M^+ - $[MOM]^+$, 100%);

***m/z* (+HRES):** 242.10302, (MH^+, $C_{11}H_{15}NO_5$ requires 242.10285).

7.3.2.5 2-Benzyloxycarbonylamino-3-hydroxy-3-[4-(methoxymethoxy) phenyl]propionic acid (294f)



To a solution of 2-amino-3-hydroxy-3-[4-(methoxymethoxy)phenyl]propionic acid (935 mg, 3.86 mmol) in sat $NaHCO_3$ (100 mL) at 0 °C was added benzyl chloroformate (0.55 mL, 3.86 mmol). Once added, the mixture was allowed to warm to room temperature and was stirred for 1 h. The aqueous layer was extracted with ether (4 x 20 mL) and acidified to pH 3.0. Once acidified the aqueous layer was extracted with EtOAc (3 x 100 mL). The organic layers were combined, dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was subjected to flash column chromatography (5:95, methanol:$CHCl_3$), giving an inseparable 2:3 (A:B) diastereomeric mixture (1.04 g, 71%).

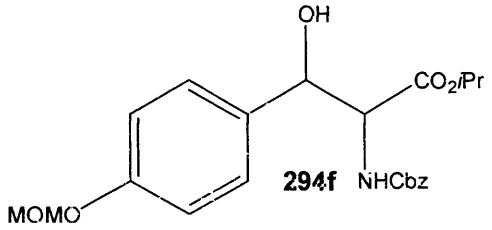
^{1}H NMR (500 MHz, CD_3OD): δ 3.30 (6H, s, A + B, OCH_3), 3.96 (1H, d, *J* 5.1 Hz, B, CH(NHCBz)), 4.42 (1H, d, *J* 9.1 Hz, A, CH(NCBz)), 4.99-5.02 (4H, m, A + B, OCH_2OCH_3), 5.43 (1H, d, *J* 5.1 Hz, B, CH(OH)), 5.62 (1H, d, *J* 9.3 Hz, 1H, A, CH(OH)), 6.75-7.29 (12H, m, Ar-*H*);

^{13}C NMR (125 MHz, CD_3OD): δ 56.2 (OCH_2OCH_3), *ca* 62 (CH(NHCBz)), 67.3 ($OCH(CH_3)_2$), 69.1 (NCO_2CH_2Ph), 95.4 (OCH_2OCH_3), 116.9, 128.33, 128.6, 128.9, 129.1, 129.4, 138.2 (Ar-*C*), 158.0 (NCO_2Bn), 177.3 (CO_2H);

***m/z* (+ES):** 398 (MNa^+, 100%);

***m/z* (+HRES):** 398.12211, ([MNa^+], $C_{19}H_{21}NO_7Na$ requires 398.12157).

7.3.2.6 Isopropyl 2-Benzyloxycarbonylamino-3-hydroxy-3-[4-(methoxymethoxy)phenyl]propioniate (294f)



2-Benzyloxycarbonylamino-3-hydroxy-3,[4-(methoxymethoxy)phenyl]propionic acid (1.00 g, 4.10 mmol) and benzyltriethylammonium chloride (940 mg, 4.10 mmol) were dissolved in dimethylacetamide (DMA) (40 mL). Potassium carbonate (14.9 g, 108 mmol) was added to the stirred solution, followed by 2-iodopropane (19.9 mL, 199 mmol). The mixture was stirred at 55 °C for 24 h. The reaction was allowed to cool, poured into water (100 mL) and was extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and then concentrated *in vacuo* to give a residue. The residue was purified by flash column chromatography (1:9, acetone: toluene), giving two inseparable diastereomers (*syn*-A+ *anti*-B) in a 1:1 ratio (604 mg, 54%). The mixture was further purified using preparative HPLC and derivatized (see **299**) to determine relative stereochemistry, ((isolated *syn*-A): 52 mg).

ν_{max} (film)/cm^{-1} (A+B): 3433s (C-OH), 3018s, 2927s, 1720 (C=O);

$\delta_{H syn\text{-}A}$ (500 MHz): 1.15 (3H, d, *J* 6.3 Hz, OCH(CH_3)), 1.22 (3H, d, *J* 6.2 Hz, OCH(CH_3)), 3.44 (3H, s, OCH_3), 4.49 (1H, dd, *J* 9.0, 4.3 Hz, C*H*(NHCBz)), 4.96-5.06 (5H, m, OCH_2OCH_3, CH_2Ph & C*H*(OH)), 5.58 (1H, d, *J* 9.0 Hz, N*H*), 6.96 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.21-7.35 (7H, m, Ar-*H*);

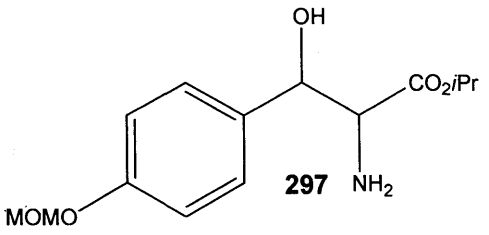
$\delta_{C syn\text{-}A:anti\text{-}B}$ (125 MHz): *ca* 21 (OCH(CH_3)$_2$), 55.9 (OCH_2OCH_3), ca 60 (*C*H(NHCBz)), *ca* 66 (OCH_2Ph), *ca* 69 (OCH(CH_3)$_2$), *ca* 73.5 (*C*H(OH)), 94.3 (OCH_2OCH_3), 115.9-116 (overlap), 124.1, 126.2, 127.8, 128.5, .128.6, 130.1, 131.6, 135.9 (Ar-*C*), 157.1 (NH*C*O_2Bn), 170.0 (*C*O_2*i*Pr)

***m/z* (FAB):** 440 (MNa^+, 100%);

***m/z* (+HRFAB):** 440.16995, (MNa^+, $C_{22}H_{27}NO_7Na$ requires 440.16851).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 20.2 (*anti*-B), 23.4 (*syn*-A) and 24.1 (*syn*-A), 26.4 (*anti*-B).

7.3.2.7 Isopropyl 2-Amino-3-hydroxy-3-[4-(methoxymethoxy)phenyl]propionate (297)



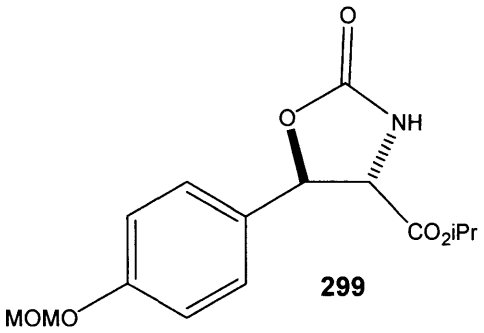
297

Isopropyl 2-Benzyloxycarbonylamino-3-hydroxy-3-[4-(methoxymethoxy)phenyl]propionate (50 mg, 0.1 mmol) was dissolved in methanol (1 mL). To this was added 5% Pd/C (10 mg). The mixture was hydrogenated at room temperature and pressure. The reaction was stirred over the weekend. The mixture was filtered through celite and was washed through with methanol (50 mL). The solvent was removed *in vacuo* and the residue purified using preparative TLC (95.5:2.5:2 dichloromethane/methanol/TEA) to yield the title compound as a 1:1 diastereomeric (A:B) mixture (27.5 mg, 81%).

^{1}H NMR $_{(A + B)}$ (400 MHz): δ 1.10 (3H, d, *J* 6.3 Hz, A, (OCH(C*H*$_3$)$_2$)), 1.15 (9H, m, A + B, OCH(C*H*$_3$)$_2$)), 3.46 (6H, s, OC*H*$_3$), 3.52 (1H, d, *J* 5.5 Hz, A, C*H*(NH$_2$)), 3.76 (1H, d, *J* 5.5 Hz, B, C*H*(NH$_2$)), 4.76 (1H, d, *J* 5.3 Hz, 1H, A, C*H*(OH)), 4.99 (3H, m, B, CH(OH) & A + B OC*H*(CH$_3$)$_2$), 5.14 (4H, s, A + B, OC*H*$_2$CH$_3$), 6.93-7.26 (8H, m, A + B Ar-*H*);

^{13}C NMR (A + B) (100 MHz): δ 21.7 (OCH(*C*H$_3$)$_2$), 56.2 (OCH$_2$O*C*H$_3$), 60.1 (*C*H(NHCbz)), 69.3 (O*C*H(CH$_3$)$_2$), 74.1 (*C*H(OH)), 94.6 (O*C*H$_2$OCH$_3$), 116.3 (overlap), 127.6, 127.9, 128.0, 133.4, 134.4 (Ar-*C*), 172.7 (*C*O$_2$*i*Pr);

7.3.2.8 Isopropyl 5-[4-(Methoxymethoxy)phenyl]-2-oxo-oxazolidine-4-carboxylate (299)



299

This reaction was performed under anhydrous conditions. Cooled to -10 to -15 °C, a solution of 2-Amino-3-hydroxy-3-(4-methoxymethoxy-phenyl)-propionic acid isopropyl ester (20.0 mg, 0.07 mmol) was dissolved in THF (1 mL) and TEA (28 μL,

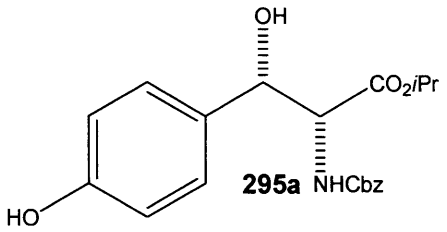
0.2 mmol). This mixture was added dropwise to a round bottom flask containing triphosgene (8.40 mg, 0.03 mmol) cooled to -10 to -15 °C. The reaction mixture was stirred for 1 h at low temp and was then stirred for 1.5 h at room temp. Triethylamine hydrochloride was filtered off and the filter cake washed with THF (10 mL). The filtrate was evaporated *in vacuo* and the residue was dissolved in EtOAc (5 mL). This was washed with sat. $NaHCO_3$ (1 x 5 mL). The aqueous layer was then extracted with EtOAc (3 x 5 mL). The organic layers were combined, dried (Na_2SO_4) and then evaporated *in vacuo*. The residue was then purified using preparative TLC (1:9, acetone: toluene). Two diastereomeric (*syn*-A) and (*anti*-B) products were separated and identified by ^1H NMR spectroscopic analysis (*syn*-8.40 mg + anti-10.3 mg, 94 %).

$\delta_{H syn\text{-}A}$ (400 MHz): 1.30 (6H, d, *J* 6.2 Hz, OCH(CH_3)$_2$), 3.46 (3H, s, OCH_3), 4.19 (1H, d, *J* 5.2 Hz, C*H*(NHCO)), 5.10-5.17 (3H, m, OCH_2OCH$_3$ & OC*H*(CH_3)2), 5.56 (1H, d, *J* 5.2 Hz, C*H*(OH)), 7.06 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.33 (2H, d, *J* 8.8 Hz, Ar-*H*);

***m/z* (+ES):** 310 (MNa^+, 89%), 266 (M – $[CH(CH_3)_2]^+$, 100);

***m/z* (+HRES):** 310.12778, (MNa^+, $C_{15}H_{19}NO_6Na$ requires 310.12851).

7.3.2.9 Isopropyl 2-Benzyloxycarbonylamino-3-hydroxy-3-[4-(hydroxyl)phenyl]-propionate (295a)



295a

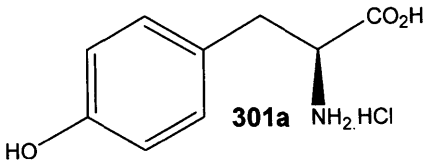
To a suspension of magnesium bromide (65 mg, 0.36 mmol) in ether (5 mL) was added Isopropyl *syn*-2-benzyloxycarbonylamino-3-hydroxy-3-[4-(methoxymethoxy)phenyl]-propionate (50 mg, 0.12 mmol), followed by 1-butanethiol (26 mg, 0.29 mmol). The reaction mixture was stirred and monitored by TLC. After 2 h, magnesium bromide was filtered and the solvent was then removed *in vacuo*. The residue was purified using preparative TLC (4:6 EtOAc: hexane), yielding the title compound as two inseparable (A: B, 1:1) diasteromers, (20 mg, 51%).

^1H NMR (500 MHz): δ 1.10 (12H, d, *J* 6.1 Hz, A + B, OCH(CH_3)$_2$), 4.49 (1H, dd, *J* 5.9, 3.15 Hz, A, C*H*(NHCBz)), 4.67 (1H, dd, *J* 4.0, 3.9 Hz, B, C*H*(NHCBz)), 5.05 (4H, m, A + B, CH_2Ph), 5.10 (2H, m, A + B, C*H*(OH)), 5.50 (1H, d, *J* 7.3 Hz, A, N*H*), 5.60 (1H, d, *J* 9.1 Hz, B, N*H*), 6.75-7.40 (16H, m, A + B, Ar-*H*);

***m/z* (FAB):** 396 (MNa^+, 100%);

***m/z* (+HRFAB):** 396.14278, (MNa^+, $C_{20}H_{23}NO_6Na$ requires 396.14230).

7.3.2.10 2-Amino-3-[4-(hydroxyl)phenyl]propionic acid hydrochloride (301a)



301a

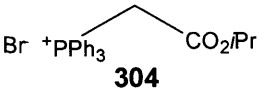
L-Tyrosine (100 mg) was dissolved in 2M HCl (2 mL) and was stirred for 30 min. The reaction mixture was lyophilised yielding the title compound (116 mg, 96%). The melting point was the same as the literature value (242 °C).

7.3.2.11 Preparation of Amberlite IRA-97 and testing with 301b

Amberlite IRA-97 was washed with copious amounts of water until the pH was neutral. Once neutral, L-tyrosine hydrochloride (100 mg, 0.45 mmol) was dissolved in water (10 mL). The Amberlite IRA-97 resin was added bead wise, until neutralisation was achieved. The mixture was lyophilised to regain L-tyrosine **300a** (59.3 mg, 71%).

7.4 Synthesis of isopropyl cinnamate esters

7.4.1.1 Isopropoxycarbonylmethyl-triphenyl-phosphonium bromide (304)



304

This reaction was carried out under inert conditions. Isopropyl bromoacetate (18.00 g, 67.3 mmol) was dissolved in toluene (200 mL). Triphenylphosphine (17.65 g, 67.3 mmol) was added and the mixture was stirred for 7 d. The precipitate was filtered and washed with toluene (3 x 100 mL), followed by hexane (3 x 100 mL). The solid was collected was dried *in vacuo* over phosphorus pentoxide to yield the title compound as a colourless solid (27.20 g, 94%).

^{1}H NMR (400 MHz, DMSO-d6): δ 0.97 (6H, d, *J* 6.3 Hz, OCH(CH_3)$_2$), 4.85 (1H, sept, *J* 6.2 Hz, OC*H*(CH_3)$_2$), 5.60 (2H, d, *J* 14.5 Hz, CH_2P), 7.72 - 7.91 (15H, m, Ar-*H*);

^{13}C NMR (75.4 MHz): δ 21.2 (OCH(CH_3)$_2$), 33.3 (CH_2P), 71.2 (OCH(CH_3)$_2$), 117.5, 126.7, 127.3, 128.2, 128.5, 135.1 (Ar-*C*), 163.2 (CO_2^{i}Pr);

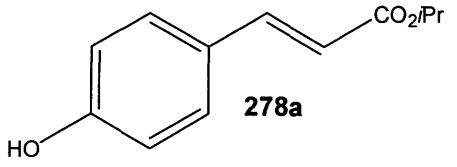
***m/z* (FAB)**: 363 (M^+, 100%);
***m/z* (+HRFAB):** 363.15090, (M^+, $C_{23}H_{24}PO_2$ requires 363.15138).

7.4.2 Wittig reactions in dichloromethane, aqueous THF and water: A general procedure

Isopropoxycarbonylmethyl-triphenyl-phosphonium bromide (1 equivalent) was dissolved either in dichloromethane (25 mL), aqueous THF (5:1, v/v, 25 mL), or water (25mL) and was gently shaken with NaOH (2M, 25 mL) in a separatory funnel for 5 min when using dichloromethane. The organic layer was separated, dried (Na_2SO_4) and concentrated *in vacuo*, only for dichloromethane. The aldehyde (0.95 equiv) was dissolved in dichloromethane (50 mL) and added to the crude phosphorus ylid. The reaction mixture was stirred vigorously for 12 h and concentrated *in vacuo* for the reaction containing dichloromethane. The residues were purified by flash column chromatography or vacuum distillation, as indicated.

For reactions containing aqueous THF and water, the aldehyde was mixed with the solvent (25 mL) and the phosphorus ylid created *in situ* was added to the aldehyde (0.95 equiv). These reaction mixtures were also stirred for 12 h and were monitored by TLC for the formation of cinnamate products. These reactions were extracted into dichloromethane (2 x 100 mL), dried (Na_2SO_4) and the solvent removed *in vacuo* to yield the crude cinnamate, which was purified either by flash chromatography or by vacuum distillation, as indicated.

7.4.2.1 Isopropyl 3-[4-(hydroxyl)phenyl]acrylate (278a)



278a

The residue was purified by flash column chromatography (2:1 EtOAc: hexane) to yield the title compound as gold oil

(1.66 g, 96 %). From using dichloromethane.

(2.10 g, 88%). From using aqueous THF

(2.01 g, 47%). From using water

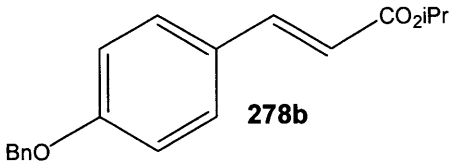
ν_{max} **(film)/cm^{-1}**: 3342br (C-OH), 3019s, 2984s, 1701 (C=O), 1653 (C=C), 1605s (Ar(C=C)), 1514s (Ar(C=C));

^{1}H NMR (500 MHz): δ 1.28 (1H, d, *J* 6.2 Hz, OCH(CH_3)$_2$), 5.12 (1H, sept, *J* 6.2 Hz, OC*H*(CH_3)$_2$), 6.27 (1H, d, *J* 15.9 Hz, ArCHC*H*CO_2*i*Pr), 6.88 (2H, d, *J* 8.5 Hz, Ar-*H*), 7.38 (2H, d, *J* 8.5 Hz, Ar-*H*), 7.61 (1H, d, *J* 15.9 Hz, ArC*H*CHCO_2*i*Pr);

^{13}C NMR (125 MHz): δ 22.2 ($OCH(CH_3)_2$), 68.4 ($OCH(CH_3)_2$), 116.3 ($ArCHCHCO_2iPr$), 127.0, 130.3 (Ar-*C*), 145.0 ($ArCHCHCO_2iPr$), 156.5 (Ar(*C*-O)), 168.0 (CO_2iPr);

***m/z* (FAB):** 165 (MH^+, 71%)

7.4.2.2 Isopropyl 3-[4-(benzyloxy)phenyl]acrylate (278b)



278b

Purified by flash column chromatography (1:9, EtOAc: hexane) to yield the title compound as colourless crystals

(1.41 g, 90%). From using dichloromethane

(1.41 g, 90%). From using aqueous THF

(2.43 g, 67%). From using water

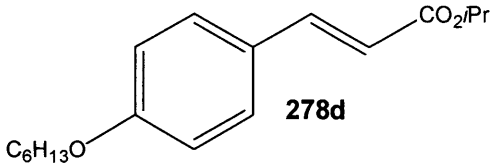
^{1}H NMR (400 MHz): δ 1.32 (6H, d, *J* 6.3 Hz, $OCH(CH_3)_2$), 5.11 (2H, s, CH_2Ph), 5.16 (1H, sept, *J* 6.3 Hz, $OCH(CH_3)_2$), 6.32 (1H, d, *J* 15.9 Hz, $ArCHCHCO_2iPr$), 6.98 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.26-7.48 (7H, m, Ar-*H*), 7.65 (1H, d, *J* 16.0, $ArCHCHCO_2iPr$);

^{13}C NMR (100 MHz): δ 21.9 ($OCH(CH_3)_2$), 67.5 (OCH_2Ph), 69.9 ($OCH(CH_3)_2$), 115.1 ($ArCHCHCO_2iPr$), 116.3, 127.4, 1278.1, 128.6, 129.6, 136.4 (Ar-*C*), 143.8 ($ArCHCHCO_2iPr$), 160.3 (Ar(*C*-O)), 166.7 (CO_2iPr);

***m/z* (FAB):** 297 (MH^+, 95%), 255 (MH^+ - $[CH_3CHCH_3]^+$, 100);

***m/z* (+HRFAB):** 297.14942, (MNa^+, $C_{19}H_{20}O_3$ requires 297.14906).

7.4.2.3 Isopropyl 3-[4-(Hexyloxy)phenyl]acrylate by aqueous Wittig reaction (278d)



278d

Purified by flash column chromatography (1:9, EtOAc: hexane) to yield the title compound as a colourless crystalline solid (2.80 g, 80%).

^{1}H NMR (400 MHz): δ 0.90 (m, 3H, $O(CH_2)_5CH_3$), 1.30 (6H, d, *J* 6.3 Hz, $OCH(CH_3)_2$), 1.32 (4H, m, $O(CH_2)_2CH_2CH_2CH_3$), 1.45 (2H, m, $O(CH_2)_3CH_2CH_2CH_3$), 1.77 (2H, quint, *J* 7.9 Hz, $OCH_2CH_2CH_2(CH_2)_2CH_3$), 3.96 (2H, t, *J* 6.8 Hz, $OCH_2(CH_2)_4CH_3$), 5.13 (1H, sept, *J* 6.3 Hz, $OCH(CH_3)_2$), 6.26 (1H, d, *J* 15.8 Hz,

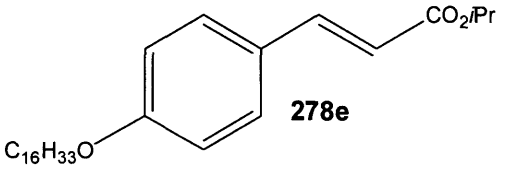
ArCHC*H*CO_2*i*Pr), 6.87 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.45 (2H, d. *J* 8.7 Hz, Ar-*H*), 7.63 (1H, d, 16.0 Hz, ArC*H*CHCO_2*i*Pr);

^{13}C NMR (100 MHz): δ 14.0 (O$(CH_2)_5CH_3$), 22.1 (OCH$(CH_3)_2$), 22.8, 26.4, 30.6, 32.5 ($(CH_2)_4CH_3$), 67.6 (OCH$(CH_3)_2$), 68.2 (O$CH_2(CH_2)_4CH_3$), 114.9, 128.8, 129.2 (Ar-*C*), 116.2 (ArCH*C*HCO_2*i*Pr), 144.2 (Ar*C*HCHCO_2*i*Pr), 161.0 (Ar(*C*-O)), 167.0 (*C*O_2*i*Pr);

***m/z* (+ES):** 291 (MH^+, 54%), 231 (MH^+ – [$(CH_3)_2$CHOH]);

***m/z* (+HRES):** 291.19500, (MH^+, $C_{18}H_{26}O_3$ requires 291.19547).

7.4.2.4 Isopropyl 3-[4-(hexadecyloxy)phenyl]acrylate (278e) by aqueous Wittig reation



278e

Purified by flash column chromatography (1:9 EtOAc: hexane) to yield the title compound as a colourless solid (2.50 g, 80 %);

ν_{max} (film)/cm^{-1}: 3019s, 2926s, 2854s, 1699 (C=O), 1634s (C=C), 1108 (C-O);

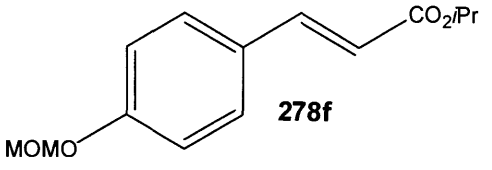
^{1}H NMR (500 MHz): δ 0.86 (3H, m, O$(CH_2)_{15}CH_3$), 1.27-1.29 (31H, m, $OCH_2(CH_2)_{15}CH_3$ & OCH$(CH_3)_2$), 1.41 (2H, m, O$(CH_2)_4CH_2(CH_2)_{10}CH_3$), 1.45 (2H, m, O$(CH_2)_3CH_2(CH_2)_{11}CH_3$), 1.76 (2H, quint, *J* 7.5, O$(CH_2CH_2(CH_2)_{13}CH_3)$), 3.95 (2H, t, *J* 6.8 Hz, O$CH_2(CH_2)_{14}CH_3$), 5.11 (1H, sept, *J* 6.5 Hz, OC*H*$(CH_3)_2$), 6.26 (1H, d, *J* 15.9 Hz, ArCHC*H*CO_2*i*Pr), 6.90 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.43 (2H, d. *J* 8.7 Hz, Ar-*H*), 7.61 (1H, d, *J* 16.0 Hz, ArC*H*CHCO_2*i*Pr);

^{13}C NMR (125 MHz): δ 14.2 (O$(CH_2)_{15}CH_3$), 21.9 (OCH$(CH_3)_2$), 22.6, 25.9, 29.1, 29.3 (overlap), 29.5 (overlap), 29.6 (overlap), 31.9 ($OCH_2(CH_2)_{15}CH_3$), 67.4 (OCH$(CH_3)_2$), 68.1 (O$CH_2(CH_2)_4CH_3$), 115.1, 127.0. 127.2 (overlap) (Ar-*C*), 116.0 (ArCH*C*HCO_2*i*Pr), 144.0 (Ar*C*HCHCO_2*i*Pr), 160.8 (Ar(*C*-O)), 166.8 (*C*O_2*i*Pr);

***m/z* (EI):** 430 (MH^+, 25%), ($[CH_3(CH_2)_{11}]^+$, 100%);

***m/z* (+HRES):**, 430.34494, (MH^+, $C_{28}H_{46}O_3$ requires 430.34415).

7.4.2.5 Isopropyl 3-[4-(methoxymethoxy)phenyl]acryate (278f)



278f

Purified by vacuum distillation (0.5 - 0.6 mmHg; 160 – 165 °C) to afford the title compound as a yellow oil

(2.17 g, 75%). From using DCM

(2.80 g, 93%). From using aqueous THF

(1.56 g, 63%). From using water.

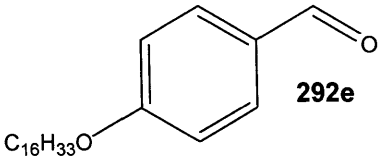
ν_{max} (neat)/cm^{-1}: 2979s, 2937s, 1735s (C=O), 1708s, 1635s (C=C), 1109 (C-O);

^{1}H NMR (400 MHz): δ 1.28 (6H, d, *J* 6.3 Hz, OCH(C*H*$_3$)$_2$), 3.46 (3H, s, OC*H*$_3$), 5.11 (1H, sept, *J* 6.3 Hz, OC*H*(CH$_3$)$_2$), 5.18 (s, 2H, OC*H*$_2$OCH$_3$), 6.27 (1H, d, *J* 16.0 Hz, (ArCHC*H*CO$_2$*i*Pr), 7.10 (2H. d, *J* 8.8 Hz, Ar-*H*), 7.45 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.60 (1H, d, *J* 16.0 Hz, ArC*H*CHCO$_2$*i*Pr);

^{13}C NMR (100 MHz): δ 22.3 (OCH(*C*H$_3$)$_2$), 56.5 (OCH$_2$O*C*H$_3$), 68.0 (O*C*H(CH$_3$)$_2$), 94.5 (O*C*H$_2$OCH$_3$), 116.9 (ArCH*C*HCO$_2$*i*Pr), 116.7, 116.9, 117.1, 128.6, 129.9, 130.2 (Ar-*C*), 144.2 (Ar*C*HCHCO$_2$*i*Pr), 159.1 (Ar(*C*-O)), 167.2 (*C*O$_2$*i*Pr);

***m/z* (EI):** 250 (MH$^+$, 100).

7.4.2.6 4-Hexadecyloxy-benzaldehyde (292e)



292e

This reaction was carried out under an inert atmosphere. Potassium carbonate (28.3 g, 205 mmol) was suspended in DMF (30 mL). This was treated with 4-hydroxybenzaldehyde (5.00 g, 40.9 mmol) and 1-bromohexadecane (12.5 mL, 40.9 mmol). The suspension was stirred vigorously at room temperature for 30 min and was quenched with water (150 mL). The aqueous layer was extracted with dichloromethane (2 x 75mL) and organic layers were washed with water (2 x 75 mL). The organic layers were combined, dried (Na_2SO_4) and were then evaporated *in vacuo*. The residue was purified by flash column chromatography (100% dichloromethane) to yield the title compound as a yellow solid (11.30 g, 80%)

m.p. (°C): 120-122, Lit[198] (121-122);

ν_{max} (KBr)/cm^{-1}: 2927s, 2854s, 1685 (C=O), 1600s;

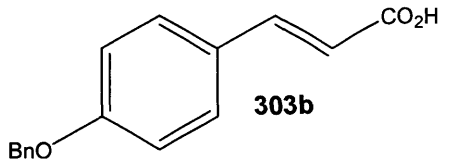
^{1}H NMR (500 MHz): δ 0.87 (3H, m, O(CH$_2$)$_{15}$C*H*$_3$), 1.33-1.38 (26H, m, O(*CH*$_2$)$_{15}$CH$_3$), 1.40 (2H, m, OCH$_2$CH$_2$CH$_2$C*H*$_2$(CH$_2$)$_{11}$CH$_3$), 1.77 (2H, quint, *J* 6.8 Hz, OCH$_2$C*H*$_2$CH$_2$(CH$_2$)$_{12}$CH$_3$), 4.09 (2H, t, *J* 6.7 Hz, OC*H*$_2$(CH$_2$)$_{14}$CH$_3$), 6.94 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.79 (2H, d, *J* 8.8 Hz, Ar-*H*), 9.92 (1H, s, CO*H*);

^{13}C NMR (75.4 MHz): δ 14.0 ($O(CH_2)_{15}CH_3$), 22.5, 25.8, 28.9, 29.4, 29.6 (overlap), 31.8 ($O(CH_2)_{15}CH_3$), 68.2 ($OCH_2(CH_2)_{14}CH_3$), 114.6-164.1 (Ar-*C*), 190.7 (*C*OH);

***m/z* (+ES):** 347 (MH^+, 100%).

7.4.3 Other methods towards the synthesis of cinnamates

7.4.3.1 3-[4-(Benzyloxy)phenyl]acrylic acid (303b)



303b

3-[4-(Hydroxy)phenyl]acrylic acid (5.00 g, 30.0 mmol) was dissolved in an ethanolic solution (100 mL) of NaOH (62 mL, 1M). The reaction mixture was stirred for 5 min before the addition of benzyl bromide (3.7 mL, 31 mmol). A precipitate was formed, but stirring was commenced for 48 h. The precipitate was filtered and recrystallised from methanol/water. The solid was dried *in vacuo* over phosphorus pentoxide for 48 h, yielding the title compound as a colourless solid (2.56 g, 33%).

m.p. (°C): 209-211, Lit[199] (208-209);

ν_{max} (nujol)/cm^{-1}: 2924s, 2853s, 1667 (C=O), 1624 (C=C);

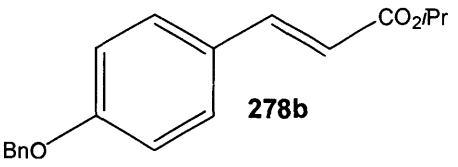
^{1}H NMR (400 MHz, THF-d8): δ 5.23 (2H, s, CH_2Ph), 6.37 (1H, d, *J* 15.9 Hz, ArCH*CH*CO_2H), 7.06 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.30-7.56 (7H, m, Ar-*H*), 7.63 (1H, d, *J* 15.9 Hz, Ar*CH*$CHCO_2$H);

^{13}C NMR (100 MHz, THF-d8): δ 69.4 (OCH_2Ph), 115.1 (ArCH*C*HCO_2H), 114.

9, 127.8, 128.0, 128.5, 129.5, 129.8, 131.8, 136.8 (Ar-*C*), 136.8 (Ar*C*$HCHCO_2$H), 163.7 (CO_2H);

***m/z* (EI):** 254 (M^+, 100%).

7.4.3.2 Isopropyl 3-[4-(benzyloxy)phenyl]acrylate (278b)



278b

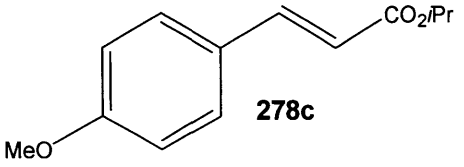
3-[4-(Benzyloxy)phenyl]acrylic acid (1.00 g, 3.93 mmol) was suspended in 2-propanol. Acetyl chloride (0.3 mL, 0.3 equiv.) was added dropwise. Concentrated sulphuric acid (4 drops) was also added and the reaction mixture was then heated at reflux for 12 h. The residual solid was filtered and the filtrate evaporated *in vacuo*. The residue was taken up in EtOAc (100 mL) and washed with sat. $NaHCO_3$ (4 x 100 mL), water (100

mL) and brine (100 mL). The organic layer was collected, dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The residue was purified by flash column chromatography (5:95 EtOAc: hexane) to yield the title compound as a colourless solid (1.00 g, 86%); Characterised *vide supra*

7.4.3.3 General procedure for Fischer esterification

Cinnamic acid (1 equiv) was suspended in 2-propanol (100mL). Sulfuric acid (4 drops) was also added and the reaction mixture was heated at reflux for 12 h. The residual solid was filtered and the filtrate evaporated *in vacuo*. The residue was taken up in EtOAc (2 x 100 mL) and washed with sat. $NaHCO_3$ (4 x 100 mL), water (100 mL) and brine (100 mL). The organic layer was collected, dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The residues were purified by column chromatography (1:9 ether:hexane) to yield the following compounds:

7.4.3.4 3-[4-(Methoxy)phenyl]acrylate (278c)



278c

Yield: 5.90 g, 98%;

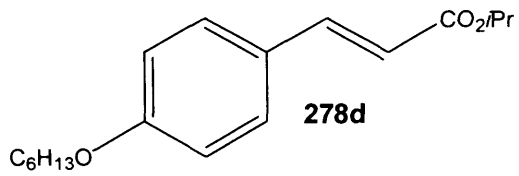
ν_{max}/cm^{-1} (KBr): 2977s, 2939s, 2839s, 1697s (C=O), 1621s (C=C);

^{1}H NMR (400 MHz): δ 1.30 (6H, d, *J* 6.3 Hz, $OCH(CH_3)_2$), 3.83 (3H, s, O*Me*), 5.12 (1H, sept, *J* 6.3 Hz, $OCH(CH_3)_2$), 6.29 (1H, d, *J* 15.9 Hz, $ArCHCHCO_2iPr$), 6.90 (2H, d, *J* 8.5 Hz, Ar-*H*), 7.45 (2H, d, *J* 8.5 Hz, Ar-*H*), 7.64 (1H, d, *J* 15.9 Hz, $ArCHCHCO_2iPr$);

^{13}C NMR (100 MHz): δ 21.1 ($OCH(CH_3)_2$), 55.3 (OCH_3), 60.2 ($OCH(CH_3)_2$), 114.2, 127.2, 129.6 (Ar-*C*), 115.7 ($ArCHCHCO_2iPr$), 144.2 ($ArCHCHCO_2iPr$), 161.3 (Ar (*C*-O)), 167.2 (CO_2iPr);

***m/z* (EI):** 221 (MH^+, 82%), 205 ($M - [CH_3]^+$), 161 ($M^+ - [HOCH(CH_3)_2]$).

7.4.3.5 Isopropyl 3-[4-(hexyloxy)phenyl]acrylate (278d) by Fischer esterification

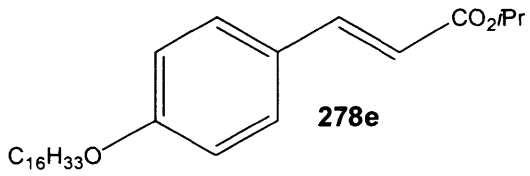


278d

Yield: 585 mg, 50 %;

Characterised *vide supra*

7.4.3.6 Isopropyl 3-[4-(hexadecyloxy)phenyl]acrylate (278e) by Fischer esterification



278e

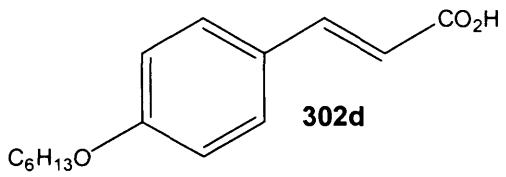
Yield: 894 mg, 20 %;

Characterised *vide supra*

7.4.4 General procedure for phenolic alkylation of cinnamic acids

3-[4-(Hydroxy)phenyl]acrylic acid (5.00 g, 30.4 mmol), potassium iodide (57.2 mg, 0.30 mmol) and potassium hydroxide (5.13 g, 91.4 mmol) were dissolved in ethanol/water (75/25 mL) and was then heated to reflux for 1 h. 1-Bromoalkane (30.4 mmol) was then added and the reaction mixture was heated at reflux for 24 h. The solvent was removed *in vacuo* and the residue was stirred vigorously with HCl (1M, 100 mL). The crude material was filtered and recrystallised from dichloromethane to yield the following compounds.

7.4.4.1 3-[4-(Hexyloxy)phenyl]acrylic acid (303d)



302d

3-[4-(Hydroxy)phenyl]acrylic acid (5.00 g, 30.4 mmol), potassium iodide (57.2 mg, 0.30 mmol) and potassium hydroxide (5.13 g, 91.4 mmol) were dissolved in ethanol/water (75/25 mL) and were heated at reflux for 1 h. 1-Bromohexane (4.27 mL,

30.4 mmol) was added and the reaction mixture was heated at reflux for 24 h. The solvent was then removed *in vacuo* and the residue stirred vigorously with HCl (1M, 100 mL). The crude material was filtered and recrystallised from dichloromethane to yield the title compound as colourless crystals (3.25 g, 43%).

m.p. (°C): 150, Lit[200] (150-151);

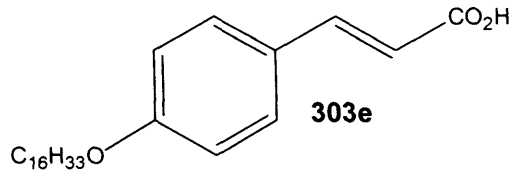
^{1}H NMR (400 MHz): δ 0.90 (m, 3H, $O(CH_2)_5CH_3$), 1.25 (8H, m, $OCH_2(CH_2)_4CH_3$), 3.96 (2H, t, *J* 6.8 Hz, $OCH_2(CH_2)_4CH_3$) , 6.32 (1H, d, *J* 15.8 Hz, $ArCHCHCO_2H$), 6.89 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.47 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.63 (1H, d, 16.0 Hz, $ArCHCHCO_2H$);

^{13}C NMR (75.4 MHz, DMSO-d6): δ 13.8 ($(CH_2)_5CH_3$), 21.6-30.8 ($OCH_2(CH_2)_4CH_3$), 67.5 ($OCH_2(CH_2)_4CH_3$), 114.7-129.8 (Ar-*C*), 114.7 ($ArCHCHCO_2H$), 143.4 ($ArCHCHCO_2H$), 160.3 (Ar(*C*-O)), 168.9 (CO_2H);

***m/z* (FAB):** 249 (MH^+, 25%);

***m/z* (+HRFAB):** 249.14829, (MH^+, $C_{15}H_{20}O_3$ requires 249.14906).

7.4.4.2 3-[4-(Hexadecyloxy)phenyl] acrylate (303e)



303e

White solid (894 mg, 20 %).

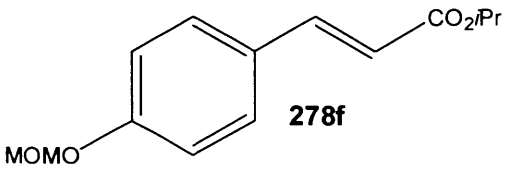
m.p. (°C): 120-121, Lit[199] (118-119);

ν_{max} (nujol)/cm^{-1}: 3434br (C-OH), 2852s, (nujol), 2725s, 2670s, 1676s (C=O), 1633s (C=C), 1604s (C=C), 1556s, (C=C), 1110(C-O);

^{1}H NMR (500 MHz): δ 0.86 (3H, m, $(O(CH_2)_{15}CH_3)$), 1.23 (26H, m, $OCH_2CH_2(CH_2)_{13}CH_3$)), 1.41 (2H, m, $OCH_2CH_2CH_2CH_2(CH_2)_{11}CH_3$), 1.45 (2H, m, $OCH_2CH_2CH_2CH_2(CH_2)_{11}CH_3$), 1.76 (quint, *J* 7.8 Hz, 2H, OCH_2CH_2 $(CH_2)_{13}CH_3$)), 3.95 (2H, t, *J* 6.5 Hz, $OCH_2(CH_2)_{15}CH_3$)), 6.27 (1H, d, *J* 15. 9 Hz, $ArCHCHCO_2H$), 6.89 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.46 (2H, d, *J* 8.7 Hz, Ar-*H*), 7.73 (1H, d, *J* 16.0 Hz, $ArCHCHCO_2H$);

^{13}C NMR (125 MHz): δ 14.2 ($(CH_2)_5CH_3$), 22.6, 26.6 (overlap), 28.6 (overlap), 30.3 (overlap), 31.9 ($OCH_2(CH_2)_{15}CH_3$), 68.1 ($OCH_2(CH_2)_{14}CH_3$), 114.8-129.6 (Ar-*C*), 114.2, 126.5, 130.0 ($ArCHCHCO_2H$), 146.7 ($ArCHCHCO_2H$), 161.3 (Ar(*C*-O)), 171.9 (CO_2H);

7.4.4.3 3-(4-Methoxymethoxy-phenyl)-acrylic acid isopropyl ester (278f)

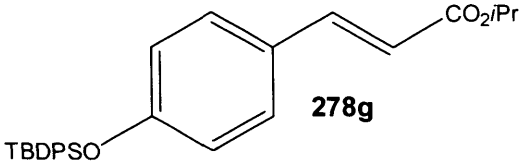


278f

1-Chloromethyl methyl ether (0.28 mL, 3.64 mmol) was added to a solution of Isopropyl 3-[4-(hydroxyl)phenyl]acrylate (500 mg, 2.42 mmol) and diisopropylethylamine (DIPEA) (0.42 mL, 2.42 mmol) in dichloromethane (25 mL). The mixture was stirred overnight and the solvent removed *in vacuo*. The crude was partitioned between EtOAc (50 mL) and water (50 mL). The organic layer was washed with sat. $NaHCO_3$ (3 x 60 mL). The organic layers were combined, dried ($MgSO_4$) and then evaporated *in vacuo*. The residue was purified by flash column chromatography (2:8 EtOAc: hexane) to yield the title compound as a colourless oil (860 mg, 24%).

Characterised *vide supra*

7.4.4.4 Isopropyl 3-[4-(*tert*-butyl-diphenyl-silanyloxy)phenyl]acrylate (278g) by phenolic silylation



278g

This reaction was carried out under an inert atmosphere. Isopropyl 3-[4-(Hydroxy)phenyl]acrylate (3.00 g, 14.6 mmol) and imidazole (1.90 g, 16.0 mmol) were dissolved in DMF (60 mL). Once dissolved, tert-butyldiphenylchlorosilane (4.15 mL, 16.0 mmol) in DMF (25 mL) was added. The reaction mixture was left to stir for 48 h, before another 2 equiv of tert-butyldiphenylchlorosilane (8.30 mL, 32.0 mmol) in DMF (25 mL) were added dropwise. The reaction mixture was stirred for another 48 h and analysis by TLC showed no further reaction. The reaction mixture was poured into 1M HCl (60 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were dried (Na_2SO_4) and then evaporated *in vacuo*. The residue was purified by flash column chromatography (1:1, dichloromethane: hexane) to yield the title compound as colourless crystals (4.60 g, 65%).

ν_{max} (KBr)/cm^{-1}: 3018s, 2932w, 1701m (C=O), 1635s (C=C), 1600s (C=C), 1108 (C-O);

^{1}H NMR (500 MHz): δ 1.08 (9H, s, $SiC(CH_3)_3$), 1.29 (d, *J* 6.3 Hz, $OCH(CH_3)_2$), 5.12 (1H, sept, *J* 6.3 Hz, $OCH(CH_3)_2$), 6.22 (1H, d, *J* 16.0 Hz, $ArCHCHCO_2iPr$), 6.78 (2H,

d, *J* 8.8 Hz, Ar-*H*), 7.27 (2H, d, *J* 8.5 Hz, Ar-*H*), 7.34-7.46 (6H, m, Ar-*H*), 7.54 (1H, d, *J* 16.0 Hz, ArC*H*CHCO$_2$H), 7.68-7.73 (4H, m, Ar-*H*);

^{13}C NMR (125 MHz): δ 19.7 (SiC(CH$_3$)$_3$), 22.2 (OCH(CH$_3$)$_2$), 26.7 (SiC(CH$_3$)$_3$), 67.8 (OCH(CH$_3$)$_2$), 116.7 (ArCHCHCO$_2$H), 120.4, 127.9, 128.2, 129.7, 128.2, 129.7, 130.4, 132.8, 135.7 (Ar-*C*), 144.3 (ArCHCHCO$_2$H), 157.8 (Ar*C*-O), 167.1 (*C*O$_2$*i*Pr);

***m/z* (FAB):** 467 (MNa$^+$, 18%), 387 (M$^+$ - [C(CH$_3$)]$^+$, 100);

***m/z* (+HRFAB):** 467.20274, (MNa$^+$, $C_{28}H_{32}NaO_3Si$ requires 467.20183).

7.5 Sharpless Asymmetric Aminohydroxylation reactions

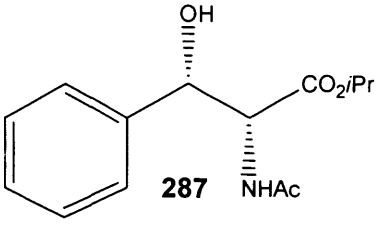
7.5.1 Homogeneous Sharpless asymmetric aminohydroxylations with unsubstituted cinnamate esters

All yields cited within this section are quoted for both regioisomers and are not isolated yields.

7.5.1.1 General procedure for Sharpless asymmetric aminohydroxylations with *N*-bromoacetamide and $(DHQ)_2AQN$ under homogeneous conditions, with added acetamide and calculated reaction volumes (for isopropyl cinnamate, an example).

In 0.85 mL of an aqueous solution of $LiOH{\cdot}H_2O$ (42.8 mg, 1.02 mmol), a solution of osmium tetroxide was added (11.4 mg, 0.05 mmol, 1 mL), with stirring. After addition of ^{n}PrOH (1.85 mL), $(DHQ)_2AQN$ (42.8 mg, 0.05 mmol) was added, and the mixture was stirred for 10 min. After the chiral ligand was dissolved completely, the mixture was immersed in a cooling bath set to 4 °C. After addition of *trans*-cinnamate (1.00 mmol), *N*-bromoacetamide (165 mg, 1.20 mmol) and acetamide (59 mg, 1.0 mmol) were added in one portion (which resulted in an immediate colour change from yellow to green) and the mixture was vigorously stirred at the same temperature. After completion of the reaction, the catalyst was filtered and was then washed with dichloromethane. Saturated aqueous sodium sulfite and 1N HCl were added to the filtrate and the mixture was stirred for an additional hour. The organic product was extracted with dichloromethane and after removing the solvent *in vacuo*, the crude product was purified by chromatography on silica gel with EtOAc/hexanes.

7.5.1.2 Isopropyl (2*R*,3*S*)-2-acetylamino-3-hydroxy-3-phenyl-propionate (287)



287

Purified using preparative TLC (dichloromethane: methanol, 98-90:2%→10%) to yield the title compound (40.3 mg, 15%).

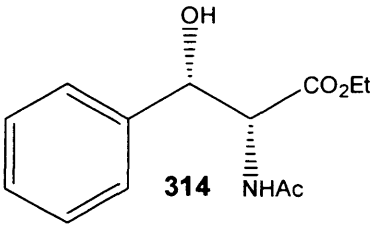
ν_{max} **(nujol)/cm^{-1}:** 3329br (C-OH), 1719s (C=O), 1650s (C=O);

^{1}H NMR (400 MHz, CD_3OD): δ 1.07 (3H, d, *J* 7.2 Hz, OCH(C*H*$_3$)), 1.14 (3H, d, *J* 6.3 Hz, OCH(CH$_3$), 1.94 (3H, s, COC*H*$_3$), 4.69 (d, *J* 3.9 Hz, O*H*), 5.09 (1H, q, *J* 6.3 Hz, C*H*(NHAc)), 5.19 (1H, d, *J* 3.9 Hz, C*H*(OH)), 7.12-7.38 (5H, m, Ar-*H*);

^{13}C NMR (400 MHz, CD_3OD): δ 16.8 (OCH$_2$*C*H$_3$), 24.5 (NCO*C*H$_3$), 62.7 (*C*H(NHAc), 64.8 (O*C*H$_2$CH$_3$), 76.6 (*C*H(OH)), 126.2, 127.8, 128.1, 128.5, 140.3 (Ar-*C*), 174.2 (N*C*OCH$_3$), 175.8 (*C*O$_2$*i*Pr);

***m/z* (CI):** 252 (MH$^+$, 95%), 234 (M – [OH]), 121 ([C$_6$H$_5$CH(OH)CH$_2$]$^+$).

7.5.1.3 Ethyl (2*R*,3*S*)-2-acetylamino-3-hydroxy-3-phenyl-propionate (314)



314

Purified using preparative TLC (6:4 hexane:ethyl actetate) to yield the title compound (47.1 mg, 20%);

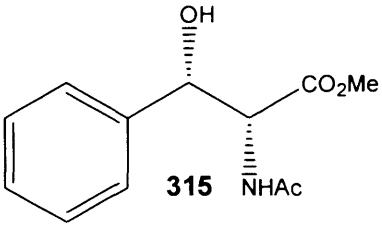
ν_{max} **(KBr)/cm^{-1}:** 3454br (C-OH), 1699s (C=O);

^{1}H NMR (400 MHz): δ 1.23 (t, *J* 7.1 Hz, 3H), 1.92 (3H, s, COC*H*$_3$), 4.81 (1H, dd, *J* 4.7, 3.9 Hz, C*H*(NHAc)), 5.20 (1H, d, *J* 3.9 Hz, C*H*(OH)), 7.25-7.40 (5H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 21.7 (OCH(*C*H$_3$)$_2$), 23.1 (NCO*C*H$_3$), 54.8 (*C*H(NHAc), 69.6 (OCH(CH$_3$)$_2$), 73.9 (*C*H(OH)), 129.7, 131.3, 131.5, 144.6 (Ar-*C*), 170.2 (N*C*OCH$_3$), 172.4 (*C*O$_2$*i*Pr);

***m/z* (FAB):** 252 (MNa$^+$, 90%), 176 (M – [H$_2$O], 100), 121 ([C$_6$H$_5$CH(OH)CH$_2$]$^+$, 40).

7.5.1.4 Methyl (2*R*,3*S*)-2-acetylamino-3-hydroxy-3-phenyl-propionate (315)



315

Purified using preparative TLC (6:4 hexane:ethyl actetate) to yield the title compound as colourless crystals (36.4 mg, 16%);

^{1}H NMR (400 MHz, CD_3OD): δ 1.99 (3H, s, NHCOC*H*$_3$), 4.01 (3H, s, O*Me*), 4.71 (1H, dd, *J* 4.7, 3.9 Hz, C*H*(NHAc)), 5.19 (1H, d, *J* 3.9 Hz, C*H*(OH)), 7.15-7.35 (5H, m, Ar-*H*);

***m/z* (CI):** 237 (M^+, 8 %);

7.5.1.5 General procedure for Sharpless asymmetric aminohydroxylations with *N*-bromoacetamide and $(DHQ)_2AQN$ under homogeneous conditions on a 5 mmol scale.

In 150 mL of an aqueous solution of $LiOH{\cdot}H_2O$ (273 mg, 6.50 mmol), potassium osmate dihydrate was added (81 mg, 4.4 mol %), with stirring. A 1-propanol (100 mL) solution containing $(DHQ)_2AQN$ (171 mg, 4 mol %) and *trans* isopropyl cinnamate (951 mg, 5.00 mmol). The two solutions were combined and the mixture was immersed in a cooling bath set at 4 °C. *N*-bromoacetamide (966 mg, 7.00 mmol was added in one portion (which resulted in an immediate colour change from brown to green) and the mixture was vigorously stirred at the same temperature. After completion of the reaction, the catalyst was filtered and washed with dichloromethane. Saturated aqueous sodium sulfite and 1N HCl were added to the filtrate and the mixture was stirred for an additional hour. Organic product was extracted with dichloromethane. After removing the solvent, the crude product was purified by chromatography on silica gel with EtOAc/hexanes.

7.5.1.6 Isopropyl (2*R*,3*S*)-2-acetylamino-3-hydroxy-3-phenyl-propionate (287)

Product was purified by flash column chromatography (4:6, EtOAc: hexane) to yield the title compound (130 mg, 11%).

Characterised *vide infra*.

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 8.84 and 9.74 (2*R*,3*S*) & (2*S*,3*R*).

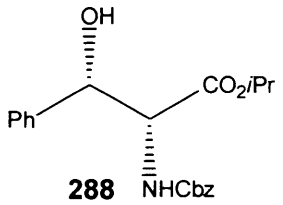
7.5.1.7 General procedure for homogeneous Sharpless asymmetric aminohydroxylations with benzyl carbamate and $(DHQ)_2AQN$ under homogeneous conditions

A reaction flask was charged with NaOH (2.98 mL, 1.02 M) and diluted with water (4.5 mL). Potassium osmate dihydrate (14.7 mg, 0.04 mmol) was dissolved in a separate vial with an aliquot of the alkaline solution (0.5 mL). With vigorous stirring, 1-propanol (4 mL) was added, followed by benzyl carbamate (469 mg, 3.10 mmol). 1,3-dichloro-5,5′-dimethylhydantoin (300 mg, 1.52 mmol) was then added in small portions. Once added and completely dissolved, a 1-propanol solution (3.5 mL)

containing cinnamate (1.00 mmol) and $(DHQ)_2AQN$ (42.8 mg, 0.05 mmol) was added, followed by the aqueous solution of potassium osmate dihydrate.

After 2 h, sodium bisulfite (500 mg) was added. The aqueous phase was separated and then extracted wih EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residues were purified by flash column chromatography (1:9, acetone:toluene).

7.5.1.8 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-hydroxy-3-phenyl-propionate (288)



288

Yield: 239 mg, 66%;

ν_{max} (film)/cm^{-1}: 3435s (C-OH), 3018s (C-H), 1713s (C=O);

^{1}H NMR (500 MHz): δ 1.16 (3H, d, *J* 6.3 Hz, OCH(C*H*$_3$)), 1.23 (3H, d, *J* 6.8 Hz, OCH(C*H*$_3$)), 4.54 (1H, dd, *J* 9.1, 2.5 Hz, C*H*(NHCbz)), 5.00 (2H, s, C*H*$_2$Ph), 5.05 (1H, sept, *J* 6.3 Hz, OC*H*(CH$_3$)$_2$), 5.19 (1H, s, C*H*(OH)), 5.50 (1H, d, *J* 9.1 Hz, N*H*), 7.23-7.35 (10H, m, Ar-*H*);

^{13}C NMR (125 MHz): δ *ca* 21.6 (OCH(*C*H$_3$)$_2$), 58.4 (*C*H(NCbz), 66.9 (O*C*H$_2$Ph), 69.7 (O*C*H(CH$_3$)$_2$), 73.5 (*C*H(OH)), 126.1, 127.9, 128.1 (overlap), 128.4 (overlap), 136.2, 139.6 (Ar-*C*), 155.6 (N*C*O$_2$CH$_2$Ph), 170.0 (*C*O$_2$*i*Pr);

Elemental Analysis: Found C, 65.64, H, 6.41, N, 3.79, %. $C_{21}H_{27}NO_6$.MeOH requires C, 64.77, H, 6.99, N, 3.60, %.

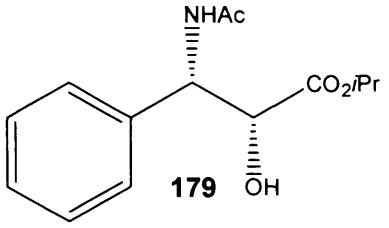
***m/z* (CI):** 358 (MH$^+$, 42%), 340 (M – [H$_2$O]), 314 (M$^+$ - [CH(CH$_3$)$_2$]$^+$), 107 ([C$_6$H$_5$C(OH)]$^+$);

***m/z* (+HRES):** 358.16476, ([MH$^+$], $C_{20}H_{23}NO_5$, requires 358.16545

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 34.6 (2*R*,3*S*).

$[\alpha]_D^{20}$ = + 35.6 (c 0.5, $CHCl_3$).

7.5.1.9 Synthesis of Isopropyl (2*R*,3*S*)-3-acetylamino-2-hydroxy-3-phenyl-propionate (179) by *N*-bromoacetamide and $(DHQ)_2PHAL$ under homogeneous conditions on a 5 mmol scale.



179

In 150 mL of an aqueous solution of $LiOH \cdot H_2O$ (273 mg, 6.50 mmol), $K_2[OsO_2(OH_4)].2H_2O$ was added (81 mg, 4.4 mol %), with stirring. A ^tBuOH (100 mL) solution containing $(DHQ)_2PHAL$ (156 mg, 4.00 mol %) and *trans* isopropyl cinnamate (951 mg, 5.00 mmol). The two solutions were combined and the mixture was immersed in a cooling bath set to 4 °C. *N*-bromoacetamide (966 mg, 7.00 mmol was added in one portion (which resulted in an immediate colour change from brown to green) and the mixture was vigorously stirred at the same temperature. After completion of the reaction, the catalyst was filtered and then washed with dichloromethane. Saturated aqueous sodium sulfite and 1N HCl were added to the filtrate and the mixture was stirred for an additional hour. The organic product was extracted with dichloromethane. After removing the solvent, the crude product was purified by column chromatography (4:6, EtOAc: hexane) to yield the title compound as a white solid (1.04 g, 79%).

m.p. (°C): 111-112, Lit[201] (108-109);

ν_{max} **(KBr)/cm^{-1}:** 3332br (C-OH), 3019s, 2931s, 1712s (C=O), 1651s;

^{1}H NMR (500 MHz): δ 1.26 (6H, d, *J* 7.4 Hz, $OCH(CH_3)_2$), 1.98 (3H, s, $COCH_3$), 3.19 (1H, d, *J* 3.8 Hz, O*H*), 4.46 (1H, s, C*H*(OH)), 5.09 (1H, sept, *J* 6.3 Hz, $OCH(CH_3)_2$), 5.54 (1H, dd, *J* 9.3, 2.2 Hz, C*H*(NHAc)), 7.24-7.38 (5H, m, Ar-*H*);

^{13}C NMR (125 MHz): δ 21.3 ($OCH(CH_3)_2$), 23.3 ($NCOCH_3$), 54.7 (*C*H(NHAc)), 60.7 ($OCH(CH_3)_2$), 74.9 (*C*H(OH)), 127.2, 128.1, 128.9, 139.2 (Ar-*C*), 169.8 ($NCOCH_3$), 172.7 (CO_2iPr);

***m/z* (FAB):** 288 (MNa^+, 100%);

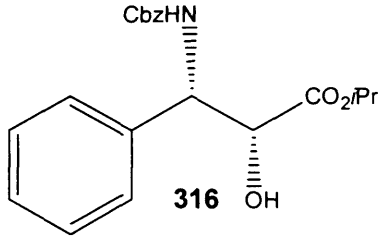
HPLC: Chiracel AD-H, 2 mm x 250 mm, hexane/*i*PrOH, 60/40, 20 ml min^{-1}, 210 nm, 17.7 (2*R*,3*S*).

7.5.1.10 General procedure for homogeneous Sharpless asymmetric aminohydroxylations with benzyl carbamate and $(DHQ)_2PHAL$.

A reaction flask was charged with NaOH (2.98 mL, 1.02 M) and diluted with water (4.5 mL). Potassium osmate dihydrate (14.7 mg, 0.04 mmol) was dissolved in a separate vial with an aliquot of the alkaline solution (0.5 mL). With vigorous stirring, 1-propanol (4 mL) was added, followed by benzyl carbamate (469 mg, 3.10 mmol). 1,3-dichloro-5,5'-dimethyl hydantoin (300 mg, 1.52 mmol) was added in small portions. Once added and completely dissolved, a 1-propanol solution (3.5 mL) containing cinnamate (1.00 mmol) and $(DHQ)_2PHAL$ (38.9 mg, 0.05 mmol) was added, followed by the aqueous solution of potassium osmate dihydrate.

After 2 h, sodium bisulfite (500 mg) was added. The aqueous phase was separated and then extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residues were purified by flash column chromatography (1:9, acetone: toluene).

7.5.1.11 Isopropyl (2*R*,3*S*)-3-benzyloxycarbonylamino-2-hydroxy-3-(4-phenyl)-propionate (316)



316

Yield: 285 mg, 80%;

ν_{max} (KBr)/cm^{-1}: 3535s (C-OH), 3019s (C-H), 1717s (C=O);

^{1}H NMR (500 MHz): δ 1.20 (3H, d, *J* 6.1 Hz, OCH(CH_3)), 1.23 (3H, d, *J* 6.9 Hz, OCH(CH_3), 3.29 (1H, d, *J* 3.9 Hz, O*H*), 3.76 (3H, s, OCH_3), 4.65 (1H, s, C*H*(OH)), 4.99-5.09 (3H, m, O*CH*$(CH_3)_2$ & CH_2Ph), 5.22 (1H, d, *J* 9.2 Hz, C*H*(NHCbz)), 5.70 (d, *J* 9.6 Hz, N*H*), 6.86 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.26-7.30 (7H, m, Ar-*H*);

^{13}C NMR (75.4 MHz): δ 21.6 (OCH(CH_3)$_2$), 55.2 (OCH_3), 55.9 (*C*H(NHCbz)), 66.9 (OCH$(CH_3)_2$), 70.7 (NCO_2CH_2Ph), 73.6 (*C*H(OH)), 113.9, 126.3, 127.1, 127.2, 127.8, 128.6, 128.7, 129.8, 130.0, 136.5, 139.3 (Ar-*C*), 159 (NCO_2Bn), 172.3 (CO_2*i*Pr);

Elemental Analysis: Found C, 66.64, H, 6.44, N, 3.83, %. $C_{20}H_{23}NO_5$ requires C, 67.21, H, 6.49, N, 3.83 %;

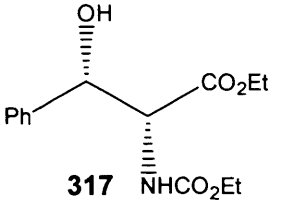
***m/z* (+ES):** 388 (MH^+, 100 %), 370 (M – OH);.

m/z (+HRES): 410.15618, (MNa^+, $C_{21}H_{25}NO_6Na$ requires 410.15741).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 18.7 (2*R*,3*S*).

$[\alpha]_D^{20}$ = + 32.8 (c 0.5, $CHCl_3$)

7.5.1.12 Large Scale synthesis of Ethyl (2*R*,3*S*)-2-ethoxycarbonylamino-3-hydroxy-3-phenyl-propionate (317)



317

A reaction flask was charged with NaOH (794 mg in water (25 mL), 19.7 mmol). Potassium osmate dihydrate (23.9 mg, 0.07 mmol) was dissolved in a separate vial with an aliquot of the alkaline solution (0.5 mL). With vigorous stirring, 1-propanol (12.5 mL) was added, followed by ethyl carbamate (1.78 g, 20.0mmol) and 1,3-dichloro-5,5′-dimethylhydantoin (1.94 g, 9.85 mmol) was added in small portions. Once added and completely dissolved, a 1-propanol solution (12.5 mL) containing cinnamate (6.50 mmol) and $(DHQ)_2AQN$ (68.5 mg, 0.08 mmol) was added, followed by the aqueous solution of potassium osmate dihydrate. After 2 h, sodium bisulfite (2 g) was added. The aqueous phase was separated and then extracted with EtOAc (3 x 100 mL). The combined organic layers were dried (Na_2SO_4) and then evaporated. The residue was purified by flash column chromatography (6:4:1, hexane:dichloromethane:methanol) to yield the title compound as a colourless solid (250 mg, 14%).

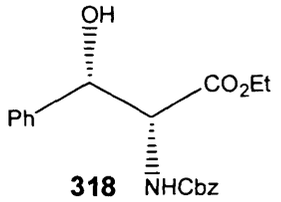
ν_{max} **(film)/cm^{-1}**: 3435br (C-OH), 3018s, 2983m, 2931m, 1720s (C=O);

^{1}H NMR (400 MHz): δ 1.15 (3H, t, *J* 7.2 Hz, $NCO_2CH_2CH_3$), 1.22 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 4.02 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 4.20 (2H, q, *J* 7.1 Hz, $NCO_2CH_2CH_3$), 4.55 (1H, dd, *J* 4.1, 9.1 Hz, *CH*(NCO_2Et)), 5.23 (1H, s, *CH*(OH)), 5.42 (1H, d, *J* 9.1 Hz, N*H*), 7.26-7.37 (5H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ 13.9 ($NCO_2CH_2CH_3$), 14.3 (OCH_2CH_3), 56.2 ($NCO_2CH_2CH_3$), 59.8 (OCH_2CH_3), 61.6 (*C*H($NHCO_2Et$)), 73.4 (*C*H(OH)), 125.9, 126.7, 127.8, 128.3, 128.5, 139.8 (Ar-*C*), 155.9 (NCO_2Et), 170.7 (CO_2Et);

m/z **(ES):** 282 (MH^+, 20%).

7.5.1.13 Ethyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-hydroxy-3-phenyl-propionate (318) by varying oxidants: 1,3-dichloro-5,5'-dimethylhydantoin and dichloroisocyanuric acid



318

A reaction flask was charged with sodium hydroxide (2.98 mL, 1.02 M) and diluted with water (4.5 mL). Potassium osmate dihydrate (14.7 mg, 0.04 mmol) was dissolved in a separate vial with an aliquot of the alkaline solution (0.5 mL). With vigorous stirring, 1-propanol (4 mL) was added, followed by benzyl carbamate (469 mg, 3.10 mmol) and 1,3-dichloro-5,5'-dimethyl hydantoin (300 mg, 1.52 mmol) or dichloroisocyanuric acid sodium salt (334 mg, 1.52 mmol), in small portions. Once added and completely dissolved, a 1-propanol solution (3.5 mL) containing cinnamate (1.00 mmol) and $(DHQ)_2AQN$ (42.8 mg, 0.05 mmol) was added, followed by the aqueous solution of potassium osmate dihydrate. After 2 h, sodium bisulfite (500 mg) was added. The aqueous phase was separated and then extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and then evaporated. The residues were purified by flash column chromatography (6:4:1, hexane:dichloromethane:methanol).

Yield: 163 g, 48 %; (from 1,3-dichloro-5,5-dimethylhydantoin);

Yield: 56 mg, 16 %; (from dichloroisocyanuric acid sodium salt):

ν_{max} **(KBr)/cm^{-1}:** 3363br (C-OH), 2978s, 1723s (C=O);

^{1}H NMR (500 MHz): δ 1.24 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 4.21 (2H, q, *J* 6.9 Hz, OCH_2CH_3), 4.60 (1H, dd, *J* 8.5, 2.4 Hz, C*H*(NHCBz)), 5.02 (1H, s, C*H*(OH)), 5.26 (1H, s, N*H*), 7.21 - 7.42 (5H, m, Ar-*H*);

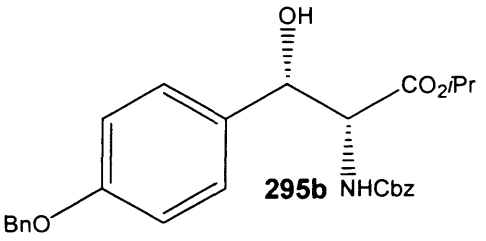
^{13}C NMR (125 MHz): δ 14.1 (OCH_2CH_3), 56.5 (*C*H(NHCBz)), 61.9 (NCO_2CH_2Ph), 67.1 (OCH_2CH_3), 73.5 (*C*H(OH)), 126.1, 126.9 (overlap), 128.1 (overlap), 128.2, 128.3, 128.5, 128.8, 136.3, 139.7 (Ar-*C*), 158.2 (NCO_2CH_2Ph), 170.7 (CO_2Et);

***m/z* (FAB):** 366 (MNa^+, 31%).

7.5.2 Homogeneous Sharpless asymmetric aminohydroxylations with *p*-substituted isopropyl cinnamate esters

All yields cited within this section are quoted for both regioisomers and are not isolated yields. The general procedures 7.5.1.7 and 7.5.1.10 were used for $(DHQ)_2AQN$ **136a** and $(DHQ)_2PHAL$ **137a**, respectively.

7.5.2.1 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(benzyloxy)phenyl]-3-hydroxy-propionate (295b)



295b

m.p. (°C): 85-86;

Yield: 353 mg, 76%;

ν_{max} (film)/cm^{-1}: 3435m (C-OH), 3019s, 1718s (C=O), 1611m (Ar(C=C)), 1585m (Ar(C=C)), 1454m (Ar(C=C)), 1104 (C-O);

^{1}H NMR (400 MHz): δ 1.17 (3H, d, *J* 6.3 Hz, OCH(C*H*$_3$)), 1.23 (3H, d, *J* 6.8 Hz, OCH(C*H*$_3$)), 4.53 (1H, dd, *J* 9.0, 2.5 Hz, C*H*(NHCbz)), 5.00 (4H, m NCO_2CH_2Ph & CH_2Ph), 5.05 (1H, sept, *J* 6.3 Hz, OC*H*$(CH_3)_2$), 5.19 (1H, s, C*H*(OH)), 5.56 (1H,d, *J* 9.0 Hz, N*H*), 6.93 (2H, d, *J* 8.6 Hz, Ar-*H*), 7.18-7.39 (12H, m, Ar-*H*);

^{13}C NMR (100 MHz): δ *ca* 21.6 (OCH(*C*$H_3)_2$), 58.4 (*C*H(NCbz), 66.9 (OCH_2Ph), 69.7 (OC*H*$(CH_3)_2$), 70.0 (NCO_2CH_2Ph) 73.5 (*C*H(OH)), 114.9, 127.5, 128.1 (overlap), 128.5, 128.6, (overlap), 131.5, 136.4, 137.0 (Ar-*C*), 158.6 (NCO_2CH_2Ph), 170.0 (*C*O_2*i*Pr);

Elemental Analysis: Found C, 68.70, H, 6.28, N, 3.04, %. $C_{27}H_{29}NO_6$ requires C, 69.96; H, 6.31; N, 3.02 %;

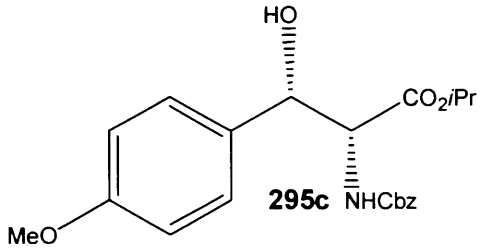
***m/z* (+ES):** 486 (MNa^+, 10%), 446 (M^+ - OH, 20), 402 (MH^+ - $OCH(CH_3)_2$,100);

***m/z* (+HRFAB):** 486.18967, ([MNa^+], $C_{27}H_{29}NO_6Na^+$, 486.18925);

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 70/30, 20 ml min^{-1}, 210 nm, 25.7 (2*R*,3*S*);

$[\alpha]_D^{20}$ = + 43.1 (c 0.5, $CHCl_3$).

7.5.2.2 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(methoxy)phenyl]-3-hydroxy-propionate (295c)



Yield: 232 mg, 60%;

ν_{max} **(film)/cm^{-1}:**3019s, 2400m, 1723 (C=O), 1623m (C=O), 1514s, 1216s (C-O);

^{1}H NMR (500 MHz): δ 1.20 (3H, d, *J* 6.2 Hz, OCH(C*H*$_3$)), 1.23 (3H, d, *J* 6.2 Hz, OCH(C*H*$_3$), 3.79 (3H, s, OC*H*$_3$), 4.52 (1H, dd, *J* 9.0, 4.0 Hz, C*H*(NHCbz)), 4.99-5.12 (3H, m, OC*H*(CH$_3$)$_2$ & C*H*$_2$Ph), 5.15 (1H, s, C*H*(OH)), 5.61 (d, *J* 9.0 Hz, N*H*), 6.86 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.26-7.30 (7H, m, Ar-*H*);

^{13}C NMR (75.4 MHz): δ 21.6 (OCH(*C*H$_3$)$_2$), 55.2 (O*C*H$_3$), 55.9 (*C*H(NHCbz)), 66.9 (O*C*H(CH$_3$)$_2$), 70.7 (NCO$_2$*C*H$_2$Ph), 73.6 (*C*H(OH)), 113.9, 127.9, 128.1, 128.5, 131.2, 136.4 (Ar-*C*), 159 (N*C*O$_2$Bn), 172.3 (*C*O$_2$*i*Pr);

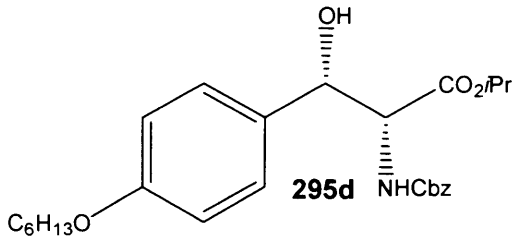
***m/z* (+FAB):** 388 (MH$^+$, 100 %), 370 (M – OH);.

***m/z* (+HRFAB):** 410.15858, ([MNa$^+$], $C_{21}H_{25}NO_6Na$ requires 410.15795);

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 24.4 (2*R*,3*S*);

$[\alpha]_D^{20}$ = + 28.1 (c 0.5, $CHCl_3$)

7.5.2.3 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(hexyloxy) phenyl]-3-hydroxy-propionate (295d)



Yield: 341 mg, 75%;

ν_{max} **(film)/cm^{-1}:** 3434s (C-OH), 3017s, 2933s, 1717s (C=O);

^{1}H NMR (500 MHz): δ 0.86-0.91 (3H, m, O(CH$_2$)$_5$C*H*$_3$), 1.16 (3H, d, *J* 6.3 Hz, OCH(C*H*$_3$)), 1.23 (3H, d, *J* 6.3 Hz, OCH(C*H*$_3$)), 1.53 (6H, m, O(CH$_2$)$_2$(C*H*$_2$)$_3$CH$_3$), 1.81 (2H, quint, *J* 6.2 Hz, OCH$_2$C*H*$_2$CH$_2$(CH$_2$)$_2$CH$_3$), 3.91 (2H, t, *J* 6.6 Hz, OC*H*$_2$(CH$_2$)$_4$CH$_3$), 4.43 (1H, dd, *J* 9.0, 4.1 Hz, C*H*(NHCBz)), 5.03 (2H, s, C*H*$_2$Ph), 5.13

(1H, s, C*H*(OH)), 5.50 (1H, d, *J* 9.0 Hz, N*H*), 6.83 (2H, d, *J* 8.8 Hz, 1H), 7.22-7.36 (m, 1H);

^{13}C NMR (100 MHz): δ 14.0 ($O(CH_2)_5CH_3$), 21.6 ($OCH(CH_3)_2$), 22.6, 26.1, 29.4, 29.7, ($OCH_2(CH_2)_4CH_3$), 59.1 (*C*H(NHCbz)), 66.9 ($OCH(CH_3)_2$), 68.0 (OCH_2Ph), 69.5 (*C*H(OH)), 73.6 ($OCH_2(CH_2)_4CH_3$), 114.4, 114.5, 127.2, 127.7, 128.4, 131.3, 155.4 (Ar-*C*), 158.5 (NCO_2Bn), 169.3 (CO_2iPr);

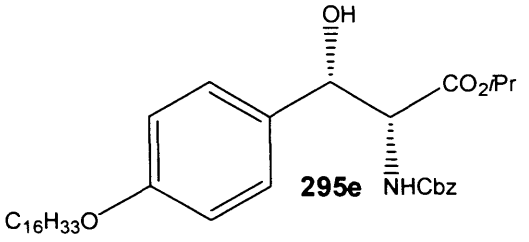
***m/z* (+ES):** 458 (MH^+, 11%), 440 (M – [OH],), 396 (M – [*i*PrOH],), 307 (M – $[Cbz]^+$);.

***m/z* (+HRFAB):** 480.23718 (MNa^+, $C_{26}H_{35}NO_6Na$ requires 480.23726);

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 85/15, 20 ml min^{-1}, 210 nm, 26.9 (2*R*,3*S*);

$[\alpha]_D^{20}$ = + 51.0 (c 0.5, $CHCl_3$)

7.5.2.4 Isopropyl (2*R*,3*S*)-2-Benzyloxycarbonylamino-3-[4-(hexadecyloxy) phenyl]-3-hydroxy-propionate (295e);



295e

Yield: 205 mg, 34 %;

^{1}H NMR (400 MHz): δ 0.91 (3H, m, $O(CH_2)_{15}CH_3$), 1.28-1.29 (31H, $OCH_2(CH_2)_{15}CH_3$ & $OCH(CH_3)_2$), 3.92 (2H, t, *J* 6.6 Hz, $OCH_2(CH_2)_{14}CH_3$), 4.52 (1H, dd, *J* 8.4, 3.5 Hz, C*H*(NHCbz)), 4.93-5.31 (4H, m, CH_2Ph, $OCH(CH_3)_2$ & C*H*(OH)), 5.56 (1H, d, *J*8.2 Hz, N*H*), 6.85 (2H, d, *J*8.4 Hz, Ar-*H*), 7.24-7.40 (7H, m, Ar-*H*);

^{13}C NMR (75.4 MHz): δ 14.1 ($O(CH_2)_{15}CH_3$), 21.6 ($OCH(CH_3)_2$), 21.6, 22.7, 26.1, 29.4, 29.7, 31.9 (overlap) ($O(CH_2)_{15}CH_3$), 55.6 ($OCH_2(CH_2)_{14}CH_3$), 61.0 (*C*H(NH(Cbz)), 66.9 ($OCH(CH_3)_2$), 68.0 (OCH_2Ph), 69.1 (*C*H(OH)), 114.4, 114.5, 127.3, 127.9, 128.0, 128.5, 131.5, 155.5 (Ar-*C*), 158.0 (NCO_2Bn), 172.6 (CO_2iPr);

***m/z* (FAB):** 620 (MNa^+, 100%);

***m/z* (+HRFAB):** 620.39042, ([MNa^+], $C_{36}H_{55}NO_6Na^+$ requires 620.39269);

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 8.1 (2*R*,3*S*);

$[\alpha]_D^{20}$ = + 43.2 (c 0.5, $CHCl_3$)

7.5.2.5 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-hydroxy-3-[4-(methoxymethoxy) phenyl]propionate (295f)

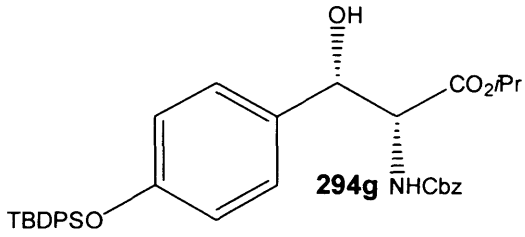
Yield: 353 mg, 76%;

Characterised *vide supra*

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 25.9 (2*R*,3*S*);

$[\alpha]_D^{20}$ = + 16.5 (c 0.5, $CHCl_3$)

7.5.2.6 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(*tert*-butyl-diphenyl-silanyloxy)-phenyl]-3-hydroxy-propionate (295g)



Yield: 246 mg, 40 %;

^{1}H NMR (500 MHz): δ 1.09 (9H, s, $SiC(CH_3)_3$), 1.12 (3H, d, *J* 6.2 Hz, $OCH(CH_3)$), 1.20 (3H, d, *J* 6.3 Hz, $OCH(CH_3)$), 4.45 (1H, dd, *J* 9.1, 4.9 Hz, CH(NHCbz)), 4.96-5.05 (4H, m, $OCH(CH_3)_2$, CH(OH) & CH_2Ph), 5.46 (1H, d, *J* 9.1 Hz, NH), 6.71 (2H, d, *J* 8.6 Hz, Ar-H), 7.07 (2H, d, *J* 8.6 Hz, Ar-H), 7.25-7.34 (9H, m, Ar-H), 7.36-7.44 (2H, m, Ar-H), 7.66-7.71 (2H, m, Ar-H);

^{13}C NMR (75.4 MHz): δ 19.4 ($SiC(CH_3)_3$), 21.5 ($OCH(CH_3)_2$), 26.5 ($SiC(CH_3)_3$), 55.1 (CH(NHCbz)), 66.9 (($OCH(CH_3)_2$)), 70.7 (NCO_2CH_2Ph), 73.4 (CH(OH)), 115.9, 119.6, 127.3 (overlap), 128.1, 128.4, 129.9, 132.0, 132.7, 135.5, 155.6 (Ar-C), 155.5 (NCO_2Bn), 172.3 (CO_2iPr);

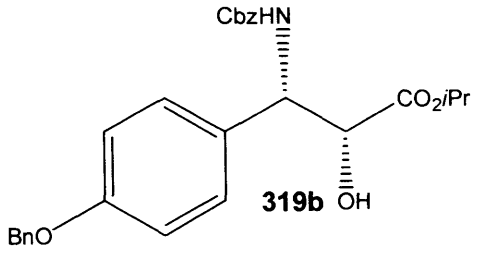
***m/z* (FAB):** 634 (MNa^+, 100%)

***m/z* (+HRFAB):** 634.25836, (MNa^+, $C_{36}H_{41}NO_6SiNa^+$ requires 634.26007).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 9.3 (2*R*,3*S*);

$[\alpha]_D^{20}$ = - 67.7 (c 0.5, $CHCl_3$).

7.5.2.7 Isopropyl (2*R*,3*S*)-3-benzyloxycarbonylamino-2-hydroxy-3-[4-(benzyloxy)phenyl]propionate (319b)



319b

Yield: 371 mg, 80%;

^{1}H NMR (500 MHz): δ 1.20 (3H, d, *J* 6.1 Hz, OCH(C*H*$_3$)), 1.23 (3H, d, *J* 6.9 Hz, OCH(C*H*$_3$), 3.29 (1H, d, *J* 3.9 Hz, O*H*), 3.76 (3H, s, OC*H*$_3$), 4.65 (1H, s, C*H*(OH)), 4.99-5.09 (3H, m, OC*H*(CH$_3$)$_2$ & C*H*$_2$Ph), 5.22 (1H, d, *J* 9.2 Hz, C*H*(NHCbz)), 5.70 (d, *J* 9.6 Hz, N*H*), 6.86 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.26-7.30 (7H, m, Ar-*H*);

^{13}C NMR (75.4 MHz): δ 21.6 (OCH(*C*H$_3$)$_2$), 55.2 (O*C*H$_3$), 55.9 (*C*H(NHCbz)), 66.9 (O*C*H(CH$_3$)$_2$), 70.7 (NCO$_2$*C*H$_2$Ph), 73.6 (*C*H(OH)), 114.9, 127.4, 128.0, 128.5, 128.6, 131.5, 136.4, 136.9, (Ar-*C*), 159 (N*C*O$_2$Bn), 172.3 (*C*O$_2$*i*Pr);

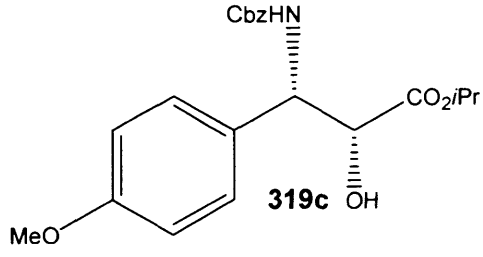
***m/z* (+ES):** 486 (MNa$^+$, 10%), 446 (M – [H$_2$O], 20), 402 (MH$^+$ - OCH(CH$_3$)$_2$,100);

***m/z* (+HRES):** 486.18889, (MNa$^+$, $C_{27}H_{29}NO_6Na^+$, 486.18871);

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 18.3 (2*R*,3*S*);

$[\alpha]_D^{20}$ = + 54.1 (c 0.5, $CHCl_3$)

7.5.2.8 Isopropyl (2*R*,3*S*)-3-benzyloxycarbonylamino-2-hydroxy-3-[4-(methoxy)phenyl]propionate (319c)



319c

Yield: 188 mg, 48%;

ν_{max} (KBr)/cm^{-1}: 3434s (C-OH), 3019s, 2838w, 1724 (C=O);

^{1}H NMR (500 MHz): δ 1.20 (3H, d, *J* 6.1 Hz, OCH(C*H*$_3$)), 1.23 (3H, d, *J* 6.9 Hz, OCH(C*H*$_3$), 3.29 (1H, d, *J* 3.9 Hz, O*H*), 3.76 (3H, s, OC*H*$_3$), 4.65 (1H, s, C*H*(OH)), 4.99-5.09 (3H, m, OC*H*(CH$_3$)$_2$ & C*H*$_2$Ph), 5.22 (1H, d, *J* 9.2 Hz, C*H*(NHCbz)), 5.70 (d, *J* 9.6 Hz, N*H*), 6.86 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.26-7.30 (7H, m, Ar-*H*);

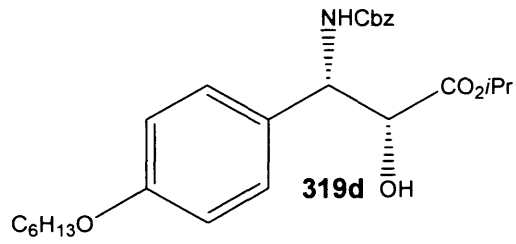
^{13}C NMR (75.4 MHz): δ 21.6 ($OCH(CH_3)_2$), 55.2 (OCH_3), 55.9 (*C*H(NHCbz)), 66.9 ($OCH(CH_3)_2$), 70.7 (NCO_2CH_2Ph), 73.6 (*C*H(OH)), 113.9, 128.0, 128.1, 128.4, 131.2, 155.6, (Ar-*C*), 159 (NCO_2Bn), 172.3 (CO_2iPr);

***m/z* (+ES):** 388 (MH^+, 100 %), 370 (M – OH);.

m/z (+HRES): 410.15618, (MNa^+, $C_{21}H_{25}NO_6Na$ requires 410.15741).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 18.7 (2R,3S).

7.5.2.9 Isopropyl (2*R*,3*S*)-3-benzyloxycarbonylamino-2-hydroxy-3-[4-(hexyloxy)phenyl]propionate (319d)



319d

Yield: 300 mg, 65%;

ν_{max} (film)/cm^{-1}: 3433 br (C-OH), 3019s, 2985m, 2935m, 1718 (C=O), 1512s;

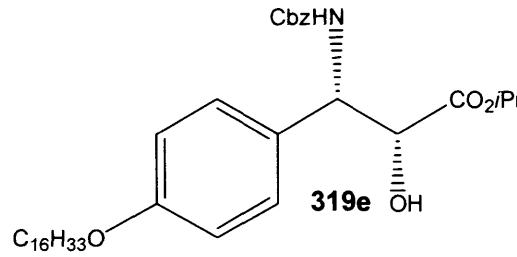
^{1}H NMR (400 MHz): δ 0.85 (3H, m, $O(CH_2)_5CH_3$), 1.14 (3H, d, *J* 6.2 Hz, $OCH(CH_3)$), 1.23 (3H, d, *J* 6.1 Hz, $OCH(CH_3)$), 1.27 (4H, m, $O(CH_2)_3(CH_2)_2CH_3$), 1.38 (2H, m, $O(CH_2)_3CH_2CH_2CH_3$), 1.72 (2H, quint, *J* 7.1 Hz, $OCH_2CH_2(CH_2)_3CH_3$), 3.88 (2H, t, *J* 6.8 Hz, $OCH_2(CH_2)_4CH_3$), 4.29 (1H, s, C*H*(OH)), 4.75 (2H, s, CH_2Ph), 5.03 (1H, m, $OCH(CH_3)_2$), 5.14 (1H, d, *J* 9.1 Hz, C*H*(NHAc)), 5.84 (1H, d, *J* 9.4 Hz, N*H*), 6.81 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.21-7.31 (7H, m, Ar-*H*);

***m/z* (ES):** 458 (MH^+, 15%); 307 (MH^+ - $[NHCbz]^+$, 100);

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 13.4 (2*S*,3*R*);

$[\alpha]_D^{20}$ = + 44.4 (c 0.5, $CHCl_3$)

7.5.2.10 Isopropyl (2*R*,3*S*)-3-benzyloxycarbonylamino-3-[4-(hexadecyloxy)phenyl]-2-hydroxy-propionate (319e)



319e

Yield: 34.2 g, 6 %;

ν_{max} **(KBr)/cm^{-1}:** 3359br (OH), 3024s (C-H), 1710 (C=O);

^{1}H NMR (500 MHz): δ 0.88 (m, 3H $O(CH_2)_{15}CH_3$), 1.19 (3H, d, *J* 5.6 Hz, $OCH(CH_3)$), 1.23 (23H, m, $OCH(CH_3)$ & $O(CH_2)_5(CH_2)_{10}CH_3$), 1.41 (m, 2H, $O(CH_2)_5CH_2(CH_2)_9CH_3$), 1.46 (4H, m, $O(CH_2)_3CH_2CH_2(CH_2)_{10}CH_3$), 1.73 (2H, m, $O(CH_2CH_2(CH_2)_{13}CH_3$), 3.91 (2H, t, *J* 6.5 Hz, $OCH_2(CH_2)_{14}CH_3$), 4.37 (1H, s, CH(OH)), 4.97-5.09 (3H, m, CH_2Ph & $OCH(CH_3)_2$), 5.20 (d, *J* 8.8 Hz, CH(NHCbz)), 5.57 (1H, d, *J* 9.1 Hz, NH), 6.84 (2H, d, *J* 8.8 Hz, Ar-H), 7.21-7.34 (7H, m, Ar-H);

^{13}C NMR (75.4 MHz): δ 14.1 ($O(CH_2)_{15}CH_3$), 21.8 ($OCH(CH_3)_2$), 22.7, 26.6, 29.2, 29.4, 29.7, 31.9 ($O(CH_2)_{15}CH_3$), 55.6 ($OCH_2(CH_2)_{14}CH_3$), 61.0 (CH(NH(Cbz)), 67.4 ($OCH(CH_3)_2$), 67.5 (OCH_2Ph), 68.1 (CH(OH)), 114.0, 126.1, 126.3, 127.1, 128.2, 155.5 (Ar-C), 158.0 (NCO_2Bn), 172.8 (CO_2*i*Pr);

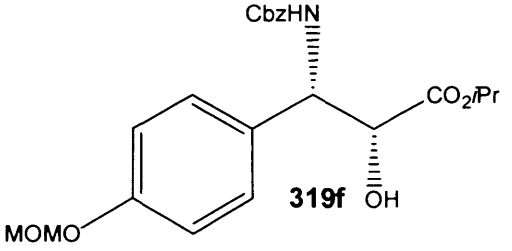
***m/z* (FAB):** 620 (MNa^+, 100%);

***m/z* (+HRFAB):** 620.39042, (MNa^+, $C_{36}H_{55}NO_6Na$ requires 620.39269).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 8.8 (2*S*,3*R*);

$[\alpha]_D^{20}$ = + 55.1 (c 0.5, $CHCl_3$)

7.5.2.11 Isopropyl (2*R*,3*S*)-3-benzyloxycarbonylamino-2-hydroxy-3-[4-(methoxymethoxy)phenyl]propionate (319f)



319f

Yield: 62 mg, 15%;

^{1}H NMR (500 MHz): δ 1.19 (3H, d, *J* 6.2 Hz, $OCH(CH_3)$), 1.26 (3H, d, *J* 6.2 Hz, $OCH(CH_3)$), 3.44 (s, OCH_3), 4.39 (1H, s, CH(OH)) 4.97-5.06 (5H, m, $OCH(CH_3)_2$, OCH_2OCH_3 & NCH_2Ph), 5.21 (1H, d, *J* 9.2 Hz, CH(NHCbz), 5.60 (d, *J* 9.2 Hz, NH), 6.99 (2H, d, *J* 8.8 Hz, Ar-H), 7.26-7.36 (7H, m, Ar-H);

^{13}C NMR (75.4 MHz): δ 21.4 ($OCH(CH_3)_2$), 56.9 (OCH_2OCH_3), 59.8 (CH(NHCBz)), 67.5 (OCH_2Ph), 69.2 ($OCH(CH_3)_2$), 73.2 (CH(OH)), 94.5 (OCH_2OCH_3), 115.6, 116.3, 126.3, 127.2, 129.8, 144.4, 160.0 (Ar-C), 158.7. ($NHCO_2Bn$), 167.1 (CO_2*i*Pr)

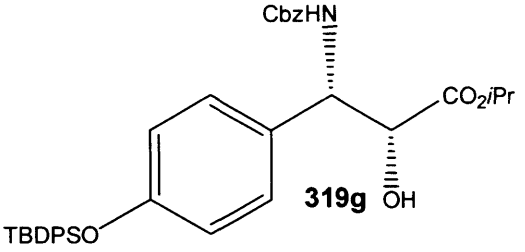
***m/z* (+ES):** 418 (MH^+, 26%), 357 (MH^+ – $[(CH_3)_2CHO]^-$) or (M^+ - $[OCH_2OCH_3]$);

***m/z* (+HRFAB):** 440.16994, (MNa^+, $C_{22}H_{27}NO_7$ requires 440.16851).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 22.9 (2*S*,3*R*);

$[\alpha]_D^{20}$ = + 61.0 (c 0.5, $CHCl_3$)

7.5.2.12 Isopropyl (2*R*,3*S*)-3-benzyloxycarbonylamino-3-[4-(*tert*-butyl-diphenyl-silanyloxy)-phenyl]-2-hydroxy-propionate (319g)



319g

Yield: 301 mg, 49 %;

ν_{max} **(film)/cm^{-1}**: 3435m (C-OH), 3016s, 2932s, 2859s, 1717 (C=O), 1106 (C-O);

^{1}H NMR (400 MHz): δ 1.09 (9H, s, SiC(CH_3)$_3$), 1.17 (3H, d, *J* 6.3 Hz, OCH(CH_3)), 1.25 (3H, d, *J* 6.3 Hz, OCH(CH_3)), 4.35 (1H, s,C*H*(OH)), 4.96-5.10 (3H, m, CH_2Ph & OC*H*(CH_3)$_2$), 5.15 (1H, d, *J* 9.1 Hz, C*H*(NHCbz)), 5.54 (1H, d, *J* 9.8 Hz, N*H*), 6.72 (2H, d, *J* 8.8 Hz, Ar-*H*), 7.05-7.75 (17H, m, Ar-*H*);

^{13}C NMR (75.4 MHz): δ 19.4 (Si*C*(CH_3)$_3$), 21.5 (OCH(*C*H_3)$_2$), 26.5 (SiC(*C*H_3)$_3$), 55.1 (*CH*(NHCbz)), 66.9 ((OCH(*CH*$_3$)$_2$)), 70.7 (NCO$_2$*CH*$_2$Ph), 73.4 (*CH*(OH)), 119.6-135.5 (Ar-*C*), 155.5 (N*CO*$_2$Bn), 172.3 (*CO*$_2$*i*Pr);

***m/z* (FAB):** 634 (MNa^+, 100%), 387 (M – [HSi(Ph)2(C(CH_3)$_2$)]$^+$) .

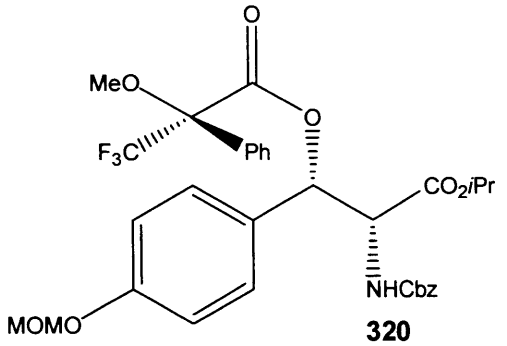
***m/z* (+HRFAB):** 634.26121, ([MNa^+], $C_{36}H_{41}NO_6SiNa^+$ requires 634.26007).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 18 ml min^{-1}, 210 nm, 11.5 (2*R*,3*S*);

$[\alpha]_D^{20}$ = - 40.8 (c 0.5, $CHCl_3$)

7.5.3 Mosher's Ester synthesis

7.5.3.1 (2*R*,3*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic acid 2-benzyloxycarbonylamino-2-isopropoxycarbonyl-1-(4-methoxymethoxy-phenyl)-ethyl ester (320)



320

In a round bottomed flask was placed (2*R*,3*S*)-2-Benzyloxycarbonylamino-3-hydroxy-3-(4-methoxymethoxy-phenyl)-propionic acid isopropyl ester (33.0 mg, 0.08 mmol), DCC (18.0 mg, 0.08 mmol) and DMAP (0.76 mg, 6.20 μmol) in dichloromethane (1 mL). This was stirred for a couple of minutes before the (*S*)-MTPA (20.0 mg, 0.08 mmol) was added to the reaction mixture. The reaction mixture was stirred for 48 h. The crude product was loaded directly onto silica and purified by flash chromatography (100% dichloromethane) to yield the title compound as an oil in (21 mg, 53%).

^{1}H NMR (400 MHz): δ 1.12 (3H, *J* 6.3 Hz, (OCH(C*H*$_3$)), 1.21 (3H, *J* 6.3 Hz, (OCH(C*H*$_3$)) 3.46 (3H, s, OC*H*$_3$), 3.48 (3H, s, OC*H*$_3$), 4.73 (1H, dd, *J* 8.0, 5.1 Hz, C*H*(NHCBz)), 4.99-5.02 (3H, m, (OC*H*(CH$_3$)$_2$, OC*H*$_2$OCH$_3$), 5.12 (2H, s, CH$_2$Ph), 5.39 (1H, d, *J* 12 Hz), 6.91 (2H, *J* 8.4 Hz, Ar-*H*), 7.00 (2H, *J* 8.4 Hz, Ar-*H*), 7.23-7.39 (10H, m, Ar-*H*);

***m/z* (+ES):** 633 (MH$^+$, 12%), 482 (MH$^+$ - [Cbz], 100%);

***m/z* (+HRES):**, 656.20857 (MNa$^+$,C$_{32}$H$_{34}$F$_3$NO$_9$ requires 656.20779).

7.5.4 Heterogeneous Catalysis

7.5.4.1 General method for the preparation Amberlite XAD-4, XAD-7 & XAD-16–OsO_4 anchored resins

Amberlite XAD4, 16 and XAD7 (10.0 g) was weighed and pretreated by soxhlet extraction for three days in refluxing methanol. The resins were dried under vacuum for a further three days. To a suspension of XAD4, 7 & 16 resins (5.00 g) in ^{t}BuOH/H_2O (1:1 v/v, 95 mL) was added 0.98 wt % OsO4 (50 mL). This was stirred for 24 h. When the resins were filtered, the filtrate was clear, indicating the osmium had been successfully anchored. Each resin contains 0.35 mmol of OsO_4/g.[120]

7.5.4.2 General procedure for Sharpless asymmetric aminohydroxylations with *N*-bromoacetamide and $(DHQ)_2AQN$ or under heterogeneous conditions with XAD4 and 7–OsO_4 resins.

In 3 mL of an aqueous solution of $LiOH{\cdot}H_2O$ (42.8 mg, 1.02 mmol), XAD-4-Os complex (111 mg, 0.04 mmol) was suspended with stirring. After addition of ^{n}PrOH (6 mL), $(DHQ)_2AQN$ (42.8 mg, 0.05 mmol) was added, and the mixture was stirred for 10 min. After chiral ligand was dissolved completely, water (6 mL) was added subsequently, and the mixture was immersed in a cooling bath set to 4 °C. After addition of *trans*-cinnamate (1.00 mmol), *N*-bromoacetamide (152 mg, 1.10 mmol) was added in one portion (which resulted in an immediate colour change from brown to green) and the mixture was vigorously stirred at the same temperature. After completion of the reaction, the catalyst was filtered and washed with dichloromethane. Saturated aqueous sodium sulfite and 1N HCl were added to the filtrate and the mixture was stirred for an additional hour. The organic product was then extracted with dichloromethane. After removing the solvent, the crude product was purified by chromatography on silica gel (4:6 EtOAc: hexane).

7.5.4.3 Isopropyl (2*R*,3*S*)-2-acetylamino-3-hydroxy-3-phenyl-propionate (286)

Yield: 110 mg, 9 % (XAD4, **286**);

Yield: 116 mg, 9 % (XAD7, **286**);

Characterised *vide supra*.

7.5.4.4 General procedure for Sharpless asymmetric aminohydroxylations with *N*-bromoacetamide and $(DHQ)_2PHAL$ or under heterogeneous conditions with XAD4 and 7–OsO_4 resins.

In 3 mL of an aqueous solution of $LiOH{\cdot}H_2O$ (42.8 mg, 1.02 mmol), XAD-4-Os complex (111.1 mg, 0.04 mmol) was suspended with stirring. After addition of ^{t}BuOH (6 mL), $(DHQ)_2PHAL$ (38.9 mg, 0.05 mmol) was added, and the mixture was stirred for 10 min. After chiral ligand was dissolved completely, water (6 mL) was added subsequently, and the mixture was immersed in a cooling bath set to 4 °C. After addition of *trans*-cinnamate (1.00 mmol), *N*-bromoacetamide (151.8 mg, 1.10 mmol) was added in one portion (which resulted in an immediate colour change to green) and the mixture was vigorously stirred at the same temperature. After completion of the

reaction, the catalyst was filtered and washed with dichloromethane. Saturated aqueous sodium sulfite and 1N HCl were added to the filtrate and the mixture was stirred for an additional hour. Organic product was extracted with dichloromethane. After removing the solvent, the crude product was purified by chromatography on silica gel (4:6 EtOAc: hexane)

7.5.4.5 Isopropyl 3-Acetylamino-2-hydroxy-3-phenyl-propionate

Yield: 126 mg, 47 % (XAD4, **179**);

Yield: 1.04 g, 79 % (XAD4, 130, 5 mmol scale, **179**);

Yield: 183 mg, 69 % (XAD7, **179**);

Yield: 320 mg, 32 % (XAD7, **179**);

Characterised *vide supra.*

7.5.4.6 General procedure for Sharpless asymmetric aminohydroxylations with benzyl carbamate and $(DHQ)_2AQN$ under heterogeneous conditions with XAD-4, XAD-7 and XAD-16–OsO_4 resins.

A reaction flask was charged with NaOH (2.98 mL, 1.02 M) and diluted with water (4.5 mL). With vigorous stirring, 1-propanol (4 mL) was added, followed by benzyl carbamate (469 mg, 3.10 mmol). 1,3-dichloro-5,5′-dimethyl hydantoin (300 mg, 1.52 mmol) was added in small portions. Once added and completely dissolved, a 1-propanol solution (3.5 mL) containing cinnamate (1 mmol) and $(DHQ)_2AQN$ (42.8 mg, 0.05 mmol) was added, followed by chosen XAD-OsO_4 resin (115 mg, 0.05 mmol).
After 2 h, sodium bisulfite (500 mg) was added. The XAD-OsO_4 resin was filtered and washed with EtOAc (20 to 30 mL). The aqueous phase was separated and then extracted with EtOAc (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residues were purified by flash column chromatography (1:9, acetone: toluene).

7.5.4.7 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-hydroxy-3-phenyl-propionate from XAD-4-OsO_4, XAD-7-OsO_4-& XAD-16-OsO_4 Sharpless AA

Yield: 220 mg, 62%; (XAD-4-OsO_4, **288**);

Yield: 311 mg, 88%; (XAD-7-OsO_4, **288**);

Yield: 189 mg, 50%; (XAD-16-OsO_4, **288**).

Characterised *vide supra.*

7.5.4.8 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(benzyloxy) phenyl]-3-hydroxy-propionate (295b) from XAD4-OsO_4, XAD7-OsO_4-& XAD16-OsO_4 Sharpless AA.

Yield: 220 mg, 62%; (XAD4-OsO_4, **295b**) ;

Yield: 311 mg, 88%; (XAD7-OsO_4, **295b**);

Yield: 189 mg, 50%; (XAD16-OsO_4, **295b**).

Characterised *vide supra.*

7.5.4.9 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(methoxy)phenyl]-3-hydroxy-propionate (295c)

Yield: 254 mg, 67%; (XAD7-OsO_4, **295c**);

Characterised *vide supra.*

7.5.4.10 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(hexyloxy) phenyl]-3-hydroxy-propionate (295d) from XAD4-OsO_4, XAD7-OsO_4-& XAD16-OsO_4 Sharpless AA.

Yield: 170 mg, 37 %; (XAD4-OsO_4, **295d**);

Yield: 150 mg, 33 %; (XAD7-OsO_4, **295d**);

Yield: 200 mg, 44 %; (XAD16-OsO_4, **295d**).

Characterised *vide supra.*

7.5.4.11 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(hexadecyloxy)phenyl]-3-hydroxy-propionate (295e) by XAD-7-OsO_4 Sharpless AA

Yield: 204 mg, 35 %; (XAD-7-OsO_4, **295e**);

Characterised *vide supra.*

7.5.4.12 Isopropyl (2*R*,3*S*)-3-benzyloxycarbonylamino-2-hydroxy-3-[4-(methoxymethoxy)phenyl]propionate (295f)

Yield: 258 mg, 62 %; (XAD7-OsO_4, **295f**);

Characterised *vide supra.*

7.5.4.13 Isopropyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-[4-(*tert*-butyl-diphenyl-silanyloxy)-phenyl]-3-hydroxy-propionate (295g)

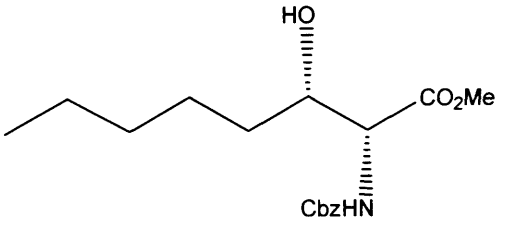
Yield: 251mg, 41%; (XAD7-OsO_4, **295g**);

Characterised *vide supra.*

7.5.5 Sharpless Asymmetric Aminohydroxylation (AA) using methyl trans-2-octenoate

This section used both homogeneous and hetereogenous Sharpless AA reactions, as before and the methods used are stated in the yield.

7.5.5.1 Methyl (2*R*,3*S*)-2-benzyloxycarbonylamino-3-hydroxy-octanate (325)



Yield: 113 mg, 35 % (using homogeneous method 7.5.1.7);

ν_{max} (film)/cm^{-1}: 3435s (C-OH), 3019s, 2956m, 2931m, 1734s (C=O);

^{1}H NMR (500 MHz): δ 0.88 (3H, m, $(CH_2)_4CH_3$)), 1.29 (6H, m, $CH_2(CH_2)_3CH_3$), 1.38 (2H, m, $CH_2(CH_2)_3CH_3$), 3.03 (1H, d, *J* 4.6 Hz, OH)), 3.77 (3H, s, OCH_3), 4.12 (1H, t, *J* 5.0 Hz, C*H*(NHCbz)), 4.39 (1H, d, *J* 9.1 Hz, C*H*(OH)), 5.14 (2H, s, CH_2Ph), 5.49 (1H, d, *J* 9.1 Hz, N*H*), 7.29-7.39 (5H, m, Ar-*H*);

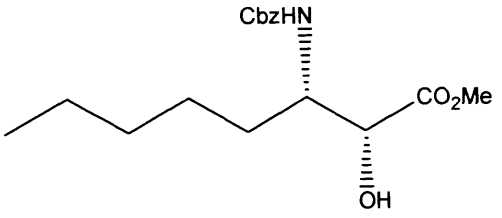
^{13}C NMR (75.4 MHz): δ 13.9 ($(CH_2)_4CH_3$), 22.5-33.6 ($(CH_2)_4CH_3$), 52.4 (OCH_3), 58.7 (*C*H(NHCbz)), 66.7 (OCH_2Ph), 71.8 (*C*H(OH)), 127.9-138.1 (Ar-*C*), 155.3 (NCO_2Bn), 171.2 (CO_2Me);

***m/z* (CI):** 325 (MH^+, 48%), 280 (M^+ - $[CH_3CH_2CH_2]^+$).

***m/z* (+HRES):** 324.18064, (MH^+, $C_{17}H_{25}NO_5$ requires 324.18109).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 11.8 (2*R*,3*S*).

7.5.5.2 Methyl (2*R*,3*S*)-3-benzyloxycarbonylamino-2-hydroxy-octanate (327)



Yield: 188 mg, 58 % (using homogeneous method 7.5.1.10);

^{1}H NMR (500 MHz): δ 0.88 (3H, m, $(CH_2)_4CH_3$), 1.30 (6H, m, $CH_2(CH_2)_3CH_3$), 1.38 (2H, m, $CH_2(CH_2)_3CH_3$), 3.03 (1H, d, *J* 4.6 Hz, O*H*), 3.74 (3H, s, OCH_3), 4.05 (1H, dt, *J*8.6, 6.5 Hz, C*H*(NHCbz)), 4.18 (1H, d, *J* 4.6 Hz, C*H*(OH)), 5.06 (2H, s, CH_2Ph), 7.29-7.38 (5H, m, Ar-*H*);

^{13}C NMR (75.4 MHz): δ 13.9 ($(CH_2)_4CH_3$), 22.5-33.6 ($(CH_2)_4CH_3$), 52.5 (OCH_3), 58.2 (*C*H(NHCbz)), 66.7 (OCH_2Ph), 71.8 (*C*H(OH)), 127.9-138.1 (Ar-*C*), 155 (NCO_2Bn), 171.2 (CO_2Me);

***m/z* (CI):** 325 (MH^+, 48%), 280 (M^+ - $[CH_3CH_2CH_2]^+$).

***m/z* (+HRES):** 324.18117, (MH^+, $C_{17}H_{25}NO_5$ requires 324.18109).

HPLC: Chiracel AD-H, 20mm x 250 mm, hexane/*i*PrOH, 80/20, 20 ml min^{-1}, 210 nm, 20.8 (2*R*,3*S*).

7.5.5.3 Methyl (2*R*,3S)-2-benzyloxycarbonylamino-3-hydroxy-octanoate by XAD7-OsO_4 Sharpless AA (325)

Yield: 113 mg, 335 % (using hetereogenous method 7.5.4.6);

Characterised *vide supra*.

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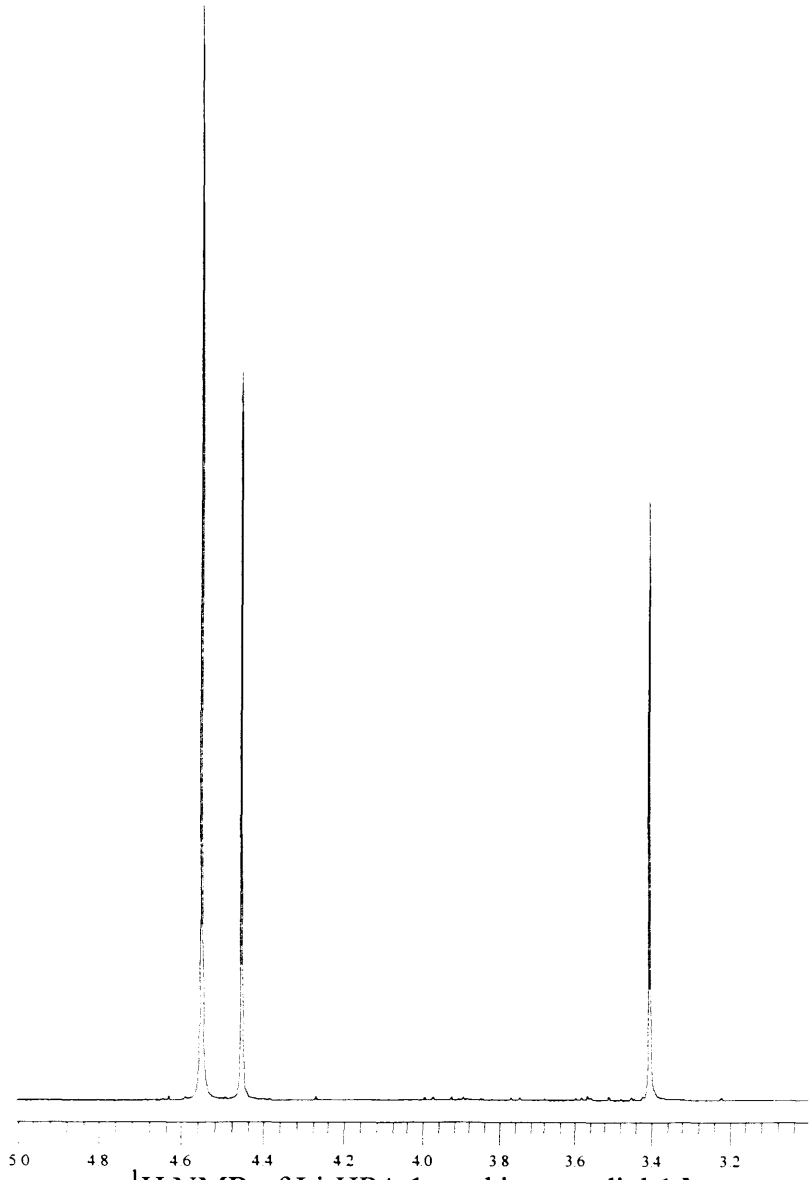
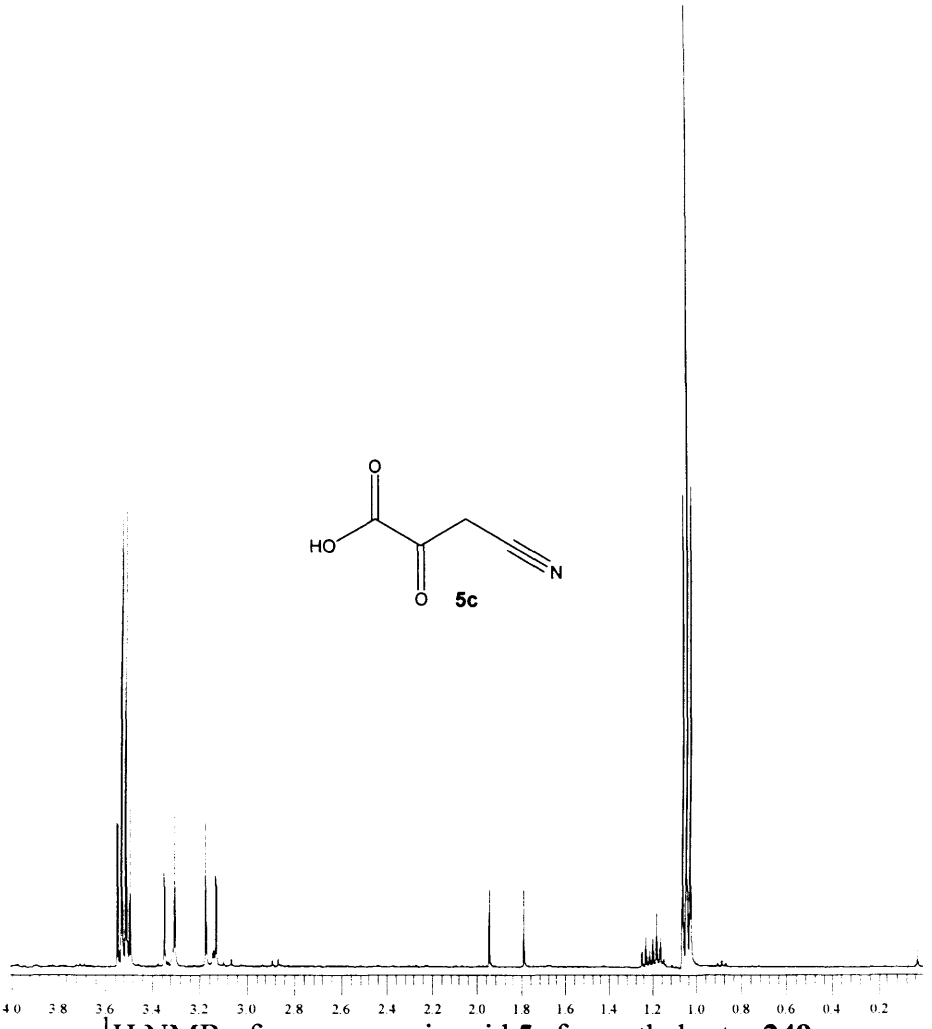
Appendix A

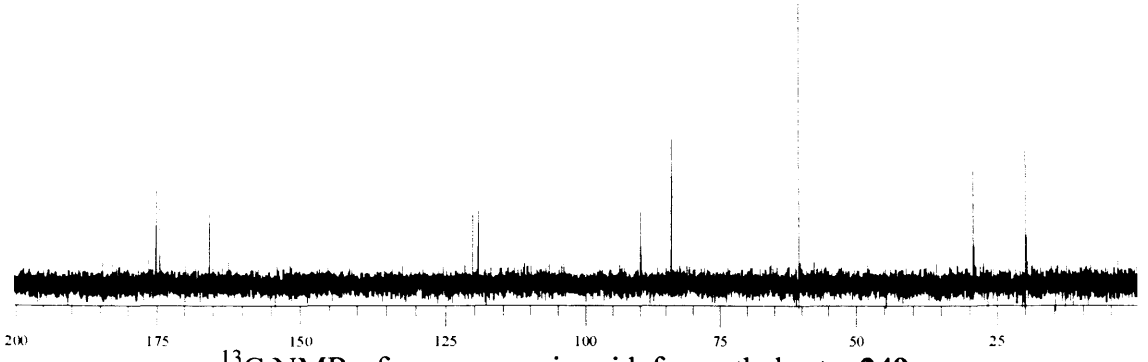


^{1}H NMR of Li-HPA **1c** and its *gem*-diol **1d**

Appendix B

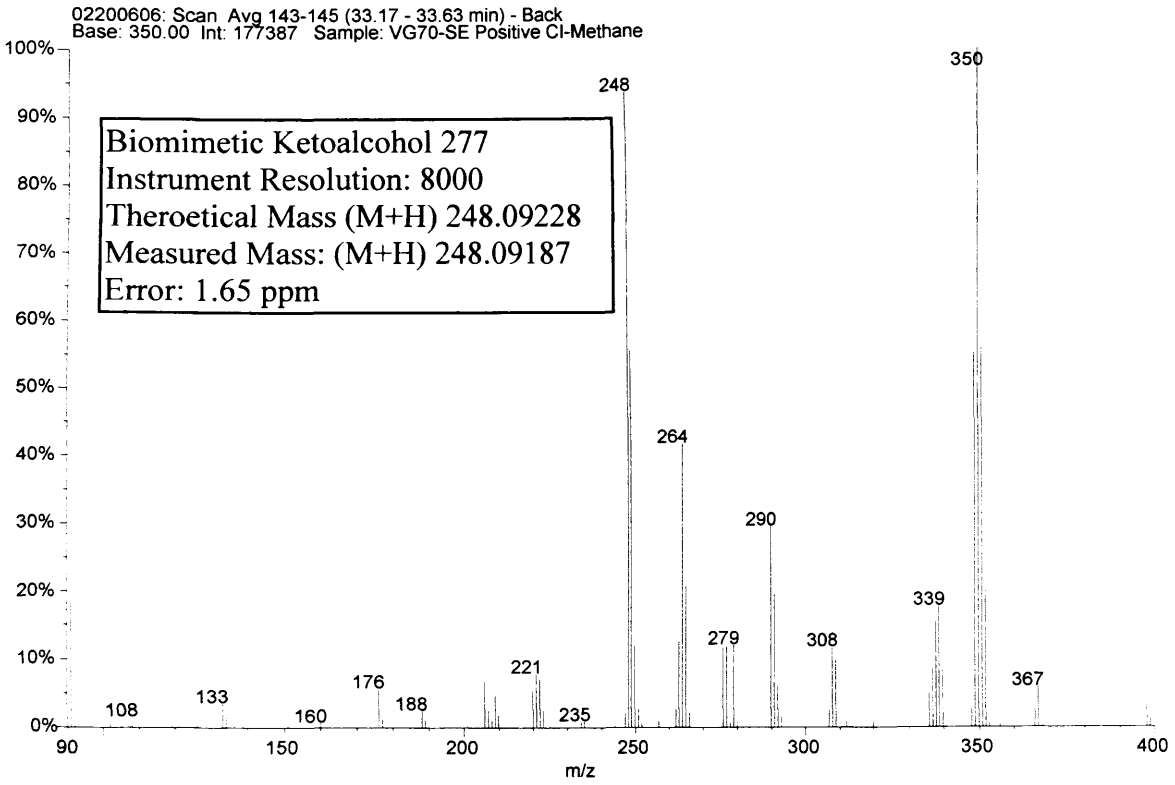


^{1}H NMR of cyanopyruvic acid **5c** from ethyl ester **249e**

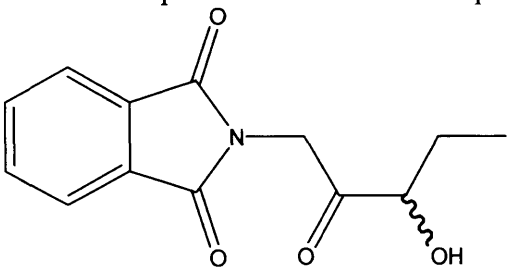


^{13}C NMR of cyanopyruvic acid from ethyl ester **249e**

Appendix C

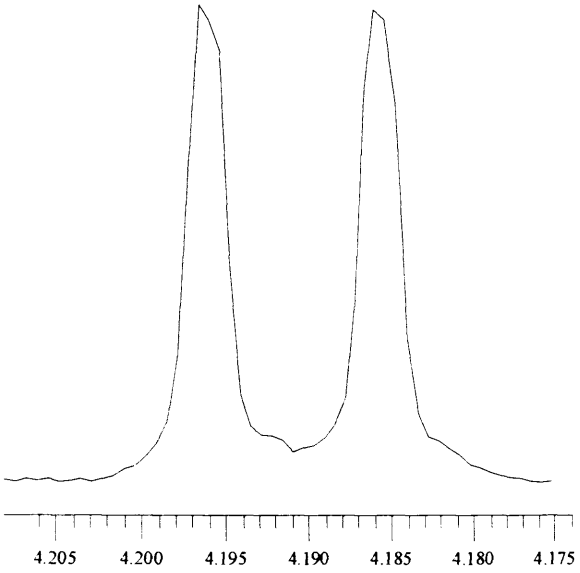


High Res Mass spectrum for ketoalcohol product

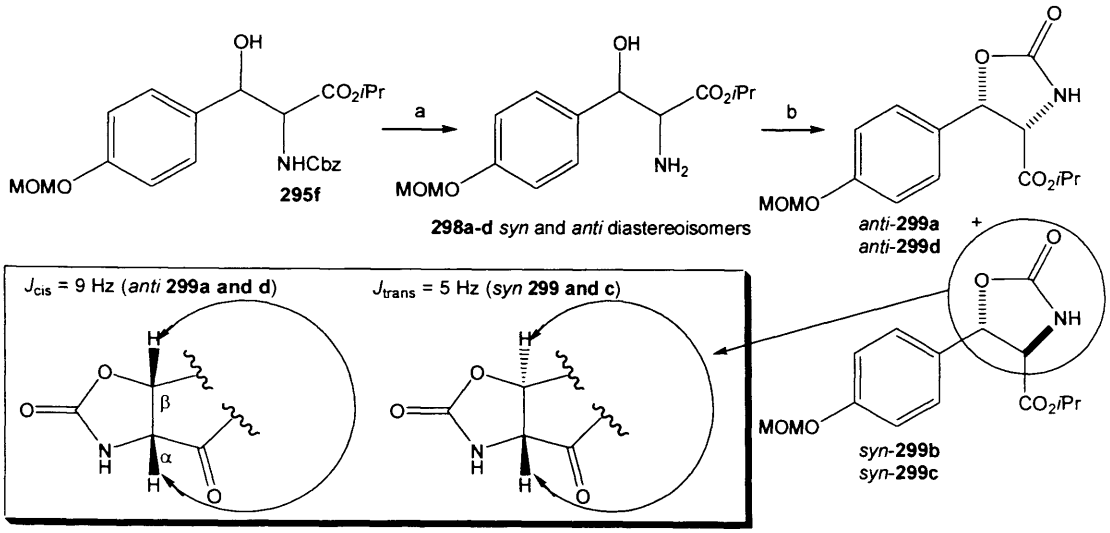


277

Appendix D

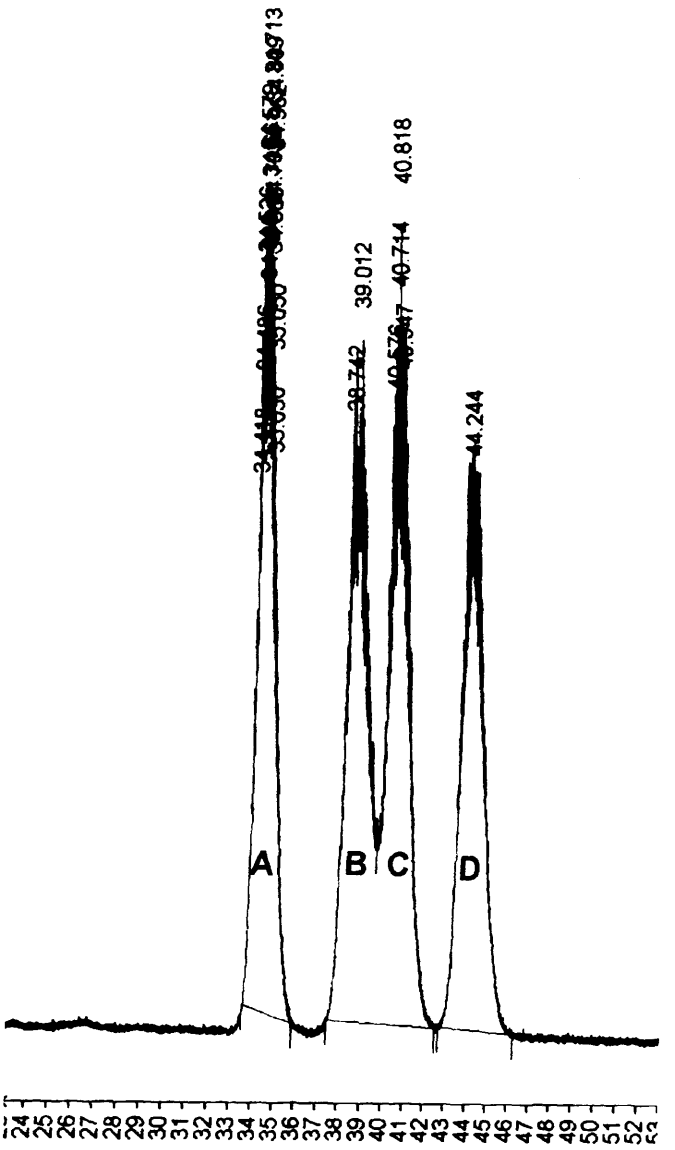


1H NMR for *syn*-oxazolidinone **299d** J 5.5 Hz

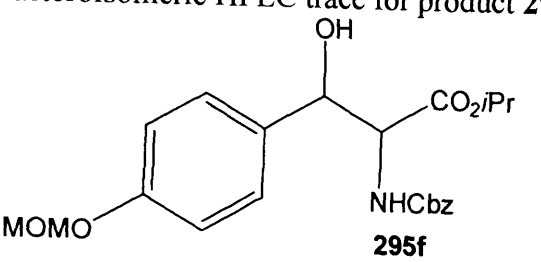


Determination of the relative *syn* and *anti* stereocenters of **295f** by derivatization to oxazolidinones **299a-d** for ^{1}H NMR spectroscopic analyses. Reagents and conditions: a) Pd/C (5%), H_2, room temperature, methanol (46% for **298a-d**); b) triphosgene, Et_3N, Ar, THF, -10 °C to room temp, (avg 42% yield for **299a-d**)

Appendix E

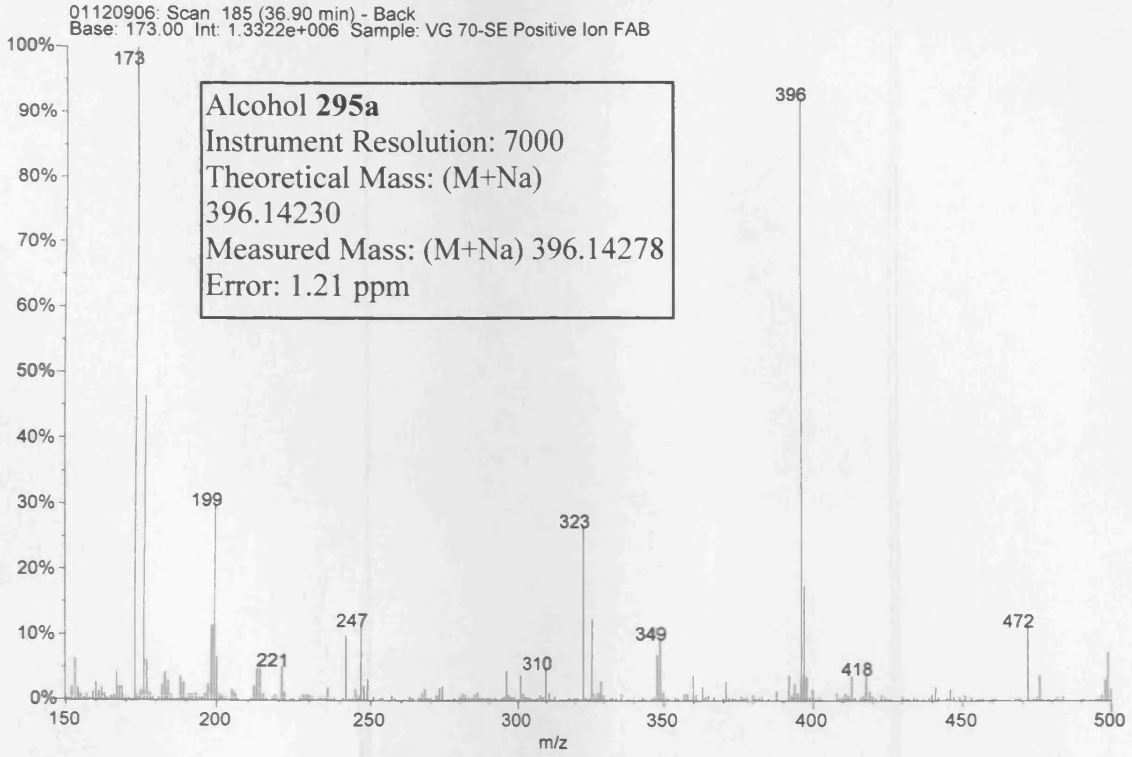


1:1 diasteroisomeric HPLC trace for product **295f**

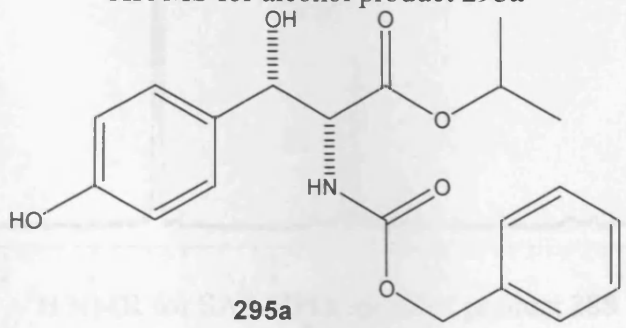


295f

Appendix F

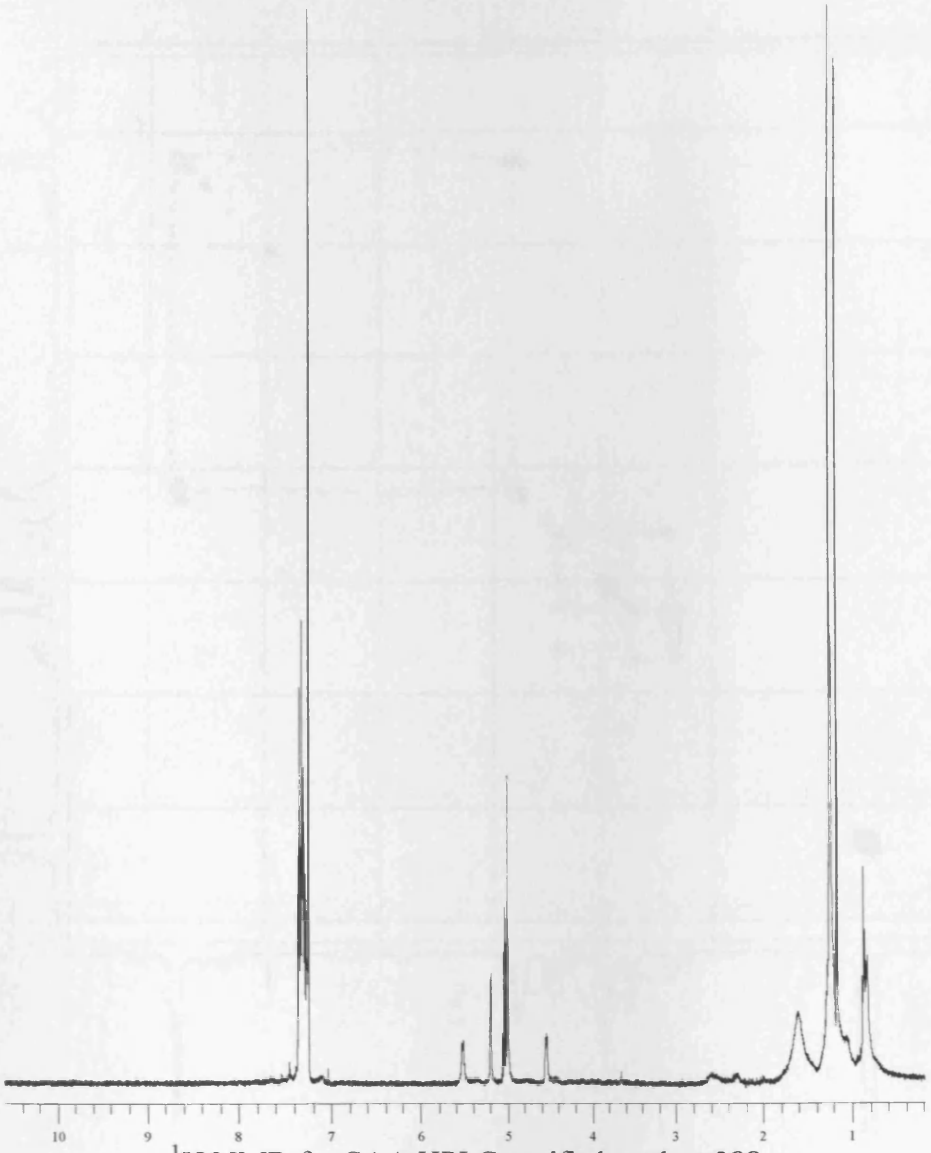


HR MS for alcohol product **295a**

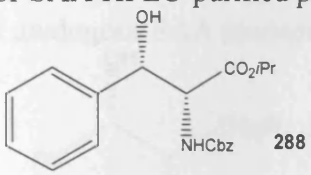


295a

Appendix G

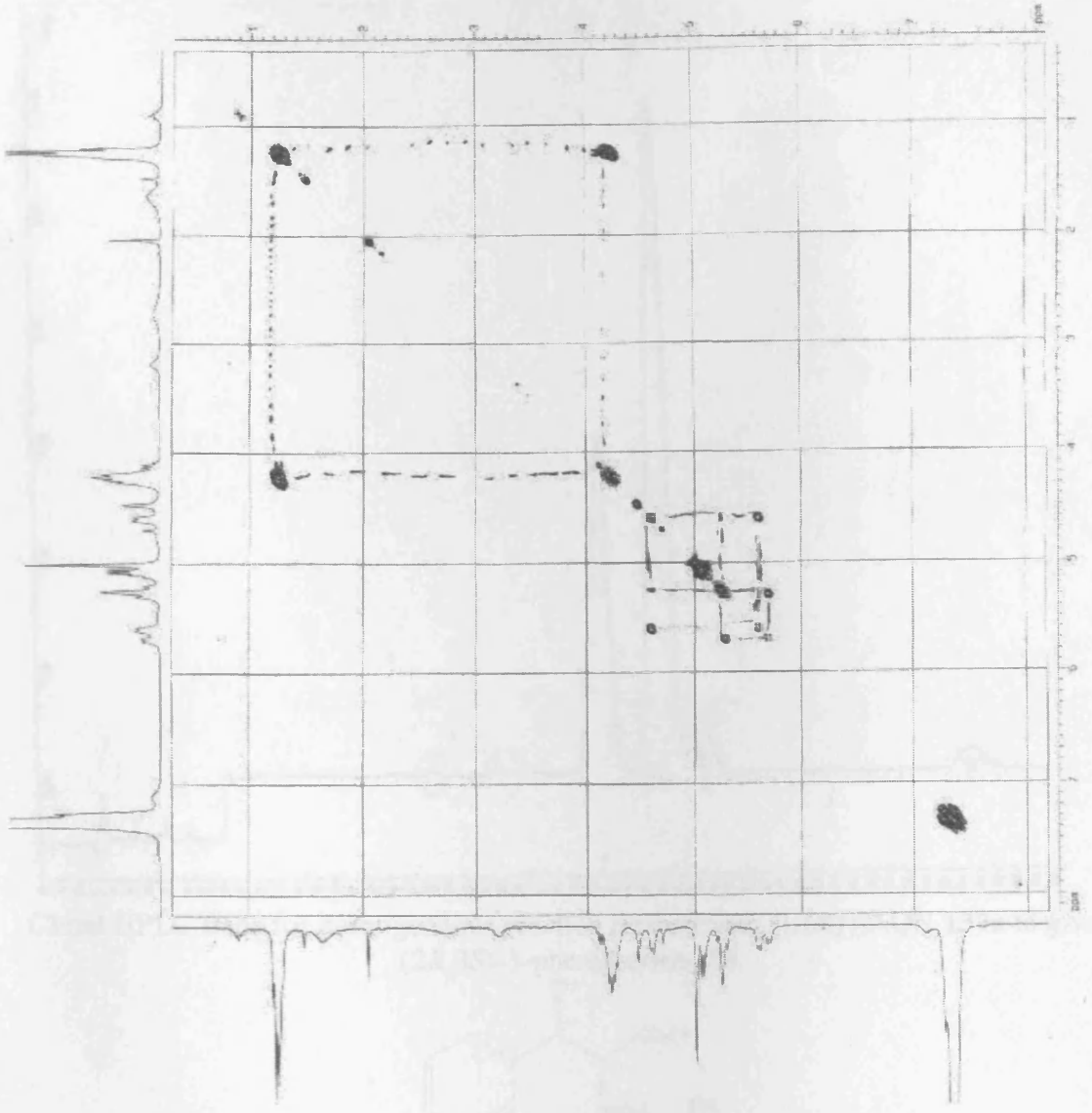


^{1}H NMR for SAA HPLC-purified product **288**

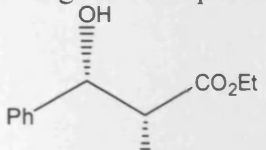


288

Appendix H

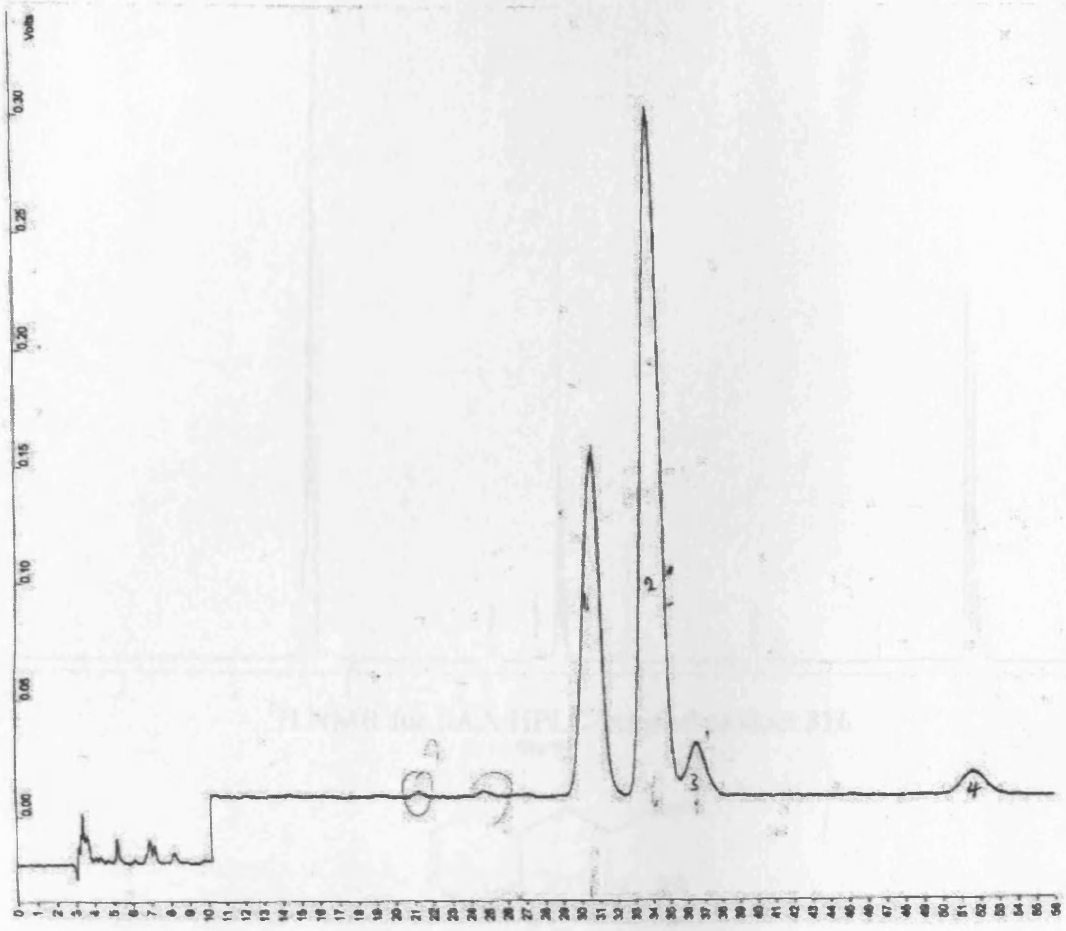


^{1}H NMR COSY for analogous SAA product with an ethyl ester

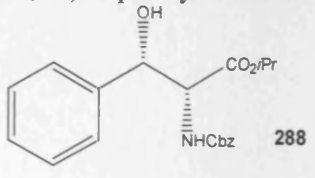


318

Appendix I

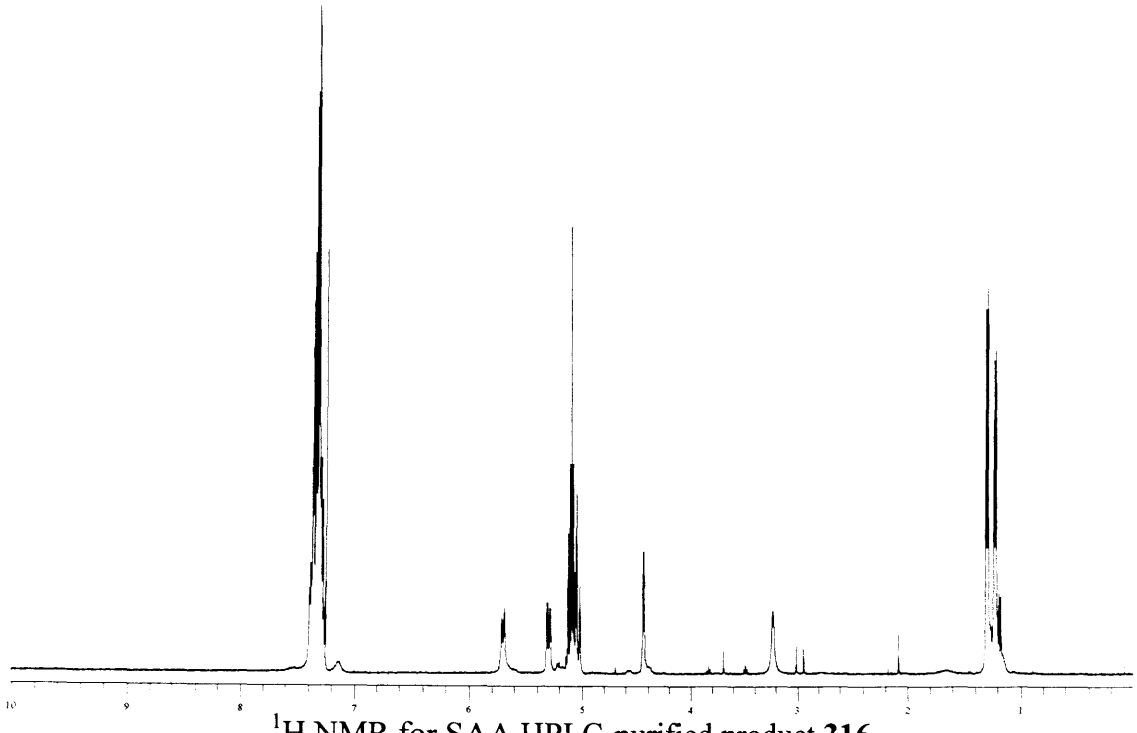


Chiral HPLC trace for homogeneous reaction mixture with $(DHQ)_2AQN$ **137a** to give (2*R*,3*S*)-3-phenylserine **288**

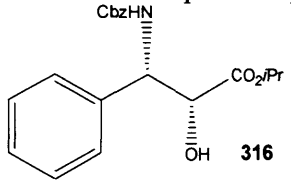


288

Appendix J

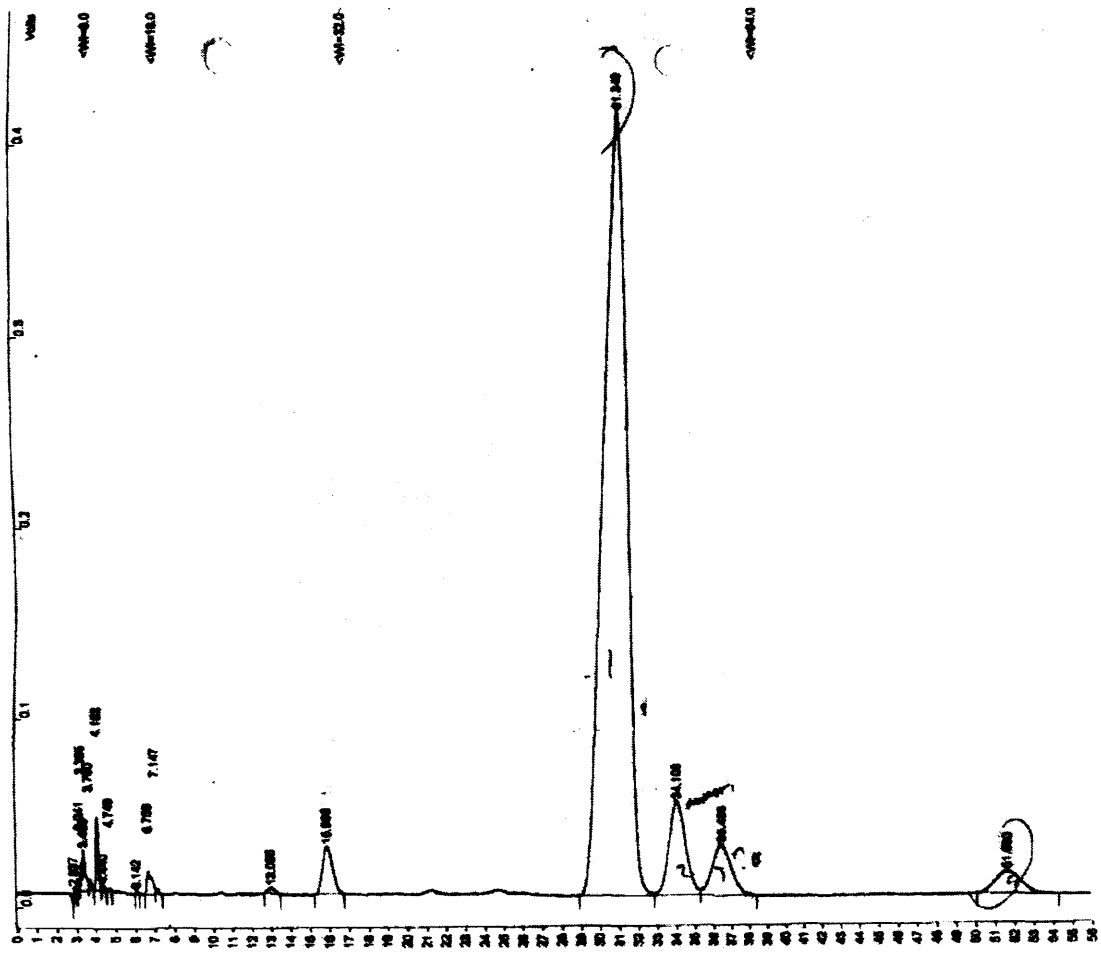


^{1}H NMR for SAA HPLC-purified product **316**

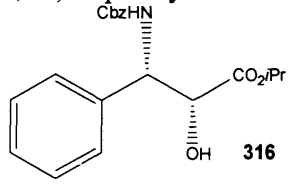


316

Appendix K



Chiral HPLC trace for homogeneous reaction mixture with $(DHQ)_2PHAL$ **136a** to give (2*R*,3*S*)-3-phenylserine **316**



316