Chemo-Enzymatic Synthesis Using Transketolase

Submitted by

Andrew Joseph Humphrey, B.A. (Oxon.)

A thesis submitted for the degree of

Doctor of Philosophy

at the University of Edinburgh,

This thesis is dedicated to Henry John Wyndham, scholar, teacher, and oblate of Prinknash Abbey, with thanks for unfailing friendship and support.
Abstract

The enzyme transketolase (TK) [E.C. 2.2.1.1] catalyses the stereoselective transfer of a two-carbon ketol unit from a donor substrate such as lithium hydroxypyruvate (LiHPA, i) to an α-hydroxyaldehyde (Figure A). Research into the use of TK from *Escherichia coli* as a process catalyst for asymmetric carbon-carbon bond formation has required the development of synthetic routes to novel acceptor substrates. The preparation of novel α-hydroxyaldehydes in enantiomerically pure form from chiral pool α-hydroxy- or α-amino acids, and of racemic α-hydroxyaldehydes of utility in the synthesis of natural product analogues, is described.

![Chemical Reaction](attachment:image.png)

**Figure A. Biotransformation catalysed by transketolase**

Formerly, biotransformations mediated by TK involved the use of an excess of aldehyde substrate in buffered aqueous solution. An alternative protocol is presented, in which the biotransformations are performed in unbuffered medium; the natural pH change during the biotransformation is offset by use of a pH autotitrator to maintain the solution pH at the process optimum of 7.0. The synthetic utility of the chiral triols produced from TK-mediated biotransformations has been demonstrated through the development of synthetic routes to the natural product nectrisine, ii, and the N-hydroxypyrrolidine sugar analogue iii from the triols iv and v, available *via* TK-mediated condensation of LiHPA with the appropriate α-hydroxyaldehyde.
I wish to begin by thanking my supervisor, Dr. Nick Turner, for unfailing encouragement, patience and enthusiasm over the past three years. His inspiration has been invaluable and his support generously given.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>AcOZ</td>
<td>p-Acetoxybenzyloxycarbonyl</td>
</tr>
<tr>
<td>A. niger</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>Ar</td>
<td>Aryl</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>2BG</td>
<td>2-Benzylxy-3-hydroxypropanal (2-O-benzylglyceraldehyde)</td>
</tr>
<tr>
<td>3BG</td>
<td>3-Benzylxy-2-hydroxypropanal (3-O-benzylglyceraldehyde)</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>B. sulfurescens</td>
<td>Beauvaria sulfurescens</td>
</tr>
<tr>
<td>Bu</td>
<td>n-Butyl</td>
</tr>
<tr>
<td>'Bu</td>
<td>tert-Butyl</td>
</tr>
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</table>
| 5BX          | (3S, 4R)-5-Benzylxy-1,3,4-trihydroxypentan-2-one  
               (5-O-benzyl-D-xylulose) |
<p>| Cho          | Choline ester |
| CoA          | Coenzyme A |
| CRL          | Candida rugosa lipase |
| DERA         | 2-Deoxyribose-5-phosphate aldolase |
| DHAP         | Dihydroxyacetone phosphate |
| DIBAL/DIBAL-H| Diisobutylaluminium hydride |
| 2,4-DNP      | 2,4-Dinitrophenylhydrazine |
| DMF          | N,N-Dimethylformamide |
| DMS          | Dimethyl sulfide |
| DMSO         | Dimethylsulfoxide |
| DNJ          | Deoxynojirimycin |
| E            | Enantiomeric ratio |
| E. coli      | Escherichia coli |
| e.e.         | Enantiomeric excess |
| EMR          | Enzyme membrane reactor |
| Et           | Ethyl     |
| EtBr         | 2-Bromoethyl |
| Eu(hfc)$_3$  | Tris-[3-(heptfluoropropylhydroxymethylene)-d-camphorato] europium (III) |</p>
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tr>
<td>FDP</td>
<td>Fructose 1,6-diphosphate</td>
</tr>
<tr>
<td>Fuc1P</td>
<td>L-fuculose 1-phosphate</td>
</tr>
<tr>
<td>GDA</td>
<td>3,3-Diethoxypropane-1,2-diol (glyceraldehyde diethyl acetal)</td>
</tr>
<tr>
<td>GlcNAc</td>
<td>N-Acetylglucosamine</td>
</tr>
<tr>
<td>Gly-DH</td>
<td>Glycerate dehydrogenase</td>
</tr>
<tr>
<td>Gly-gly</td>
<td>Glycyiglycine</td>
</tr>
<tr>
<td>GPO</td>
<td>Glycerolphosphate oxidase</td>
</tr>
<tr>
<td>Hep</td>
<td>Heptyl</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HLADH</td>
<td>Horse liver alcohol dehydrogenase</td>
</tr>
<tr>
<td>HMDS</td>
<td>Hexamethyldisilazide</td>
</tr>
<tr>
<td>HPA</td>
<td>β-Hydroxypropionic acid</td>
</tr>
<tr>
<td>KDO</td>
<td>2-Keto-3-deoxy-D-manno-octulosonate</td>
</tr>
<tr>
<td>KDPG</td>
<td>2-Keto-3-deoxy-6-phosphogluconate</td>
</tr>
<tr>
<td>KDPGGal</td>
<td>2-Keto-3-deoxy-6-phosphogalactonate</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>LiHPA</td>
<td>β-Hydroxypropionic acid, lithium salt</td>
</tr>
<tr>
<td>M</td>
<td>Molar concentration (mol dm$^{-3}$)</td>
</tr>
<tr>
<td>ManNAc</td>
<td>N-Acetylmannosamine</td>
</tr>
<tr>
<td>mCPBA</td>
<td>m-Chloroperbenzoic acid</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxymethyl</td>
</tr>
<tr>
<td>NAD$^+$</td>
<td>Nicotinamide adenine dinucleotide, oxidised form</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide, reduced form</td>
</tr>
<tr>
<td>NADP$^+$</td>
<td>Nicotinamide adenine dinucleotide phosphate, oxidised form</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate, reduced form</td>
</tr>
<tr>
<td>Neu5Ac</td>
<td>N-Acetylneuraminic acid</td>
</tr>
<tr>
<td>NMO</td>
<td>N-Methylmorpholine-N-oxide</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Phosphate</td>
</tr>
<tr>
<td>PFL</td>
<td><em>Pseudomonas fluorescens</em> lipase</td>
</tr>
<tr>
<td>PG</td>
<td>Protecting group</td>
</tr>
<tr>
<td>PhAc</td>
<td>Phenylacetamido</td>
</tr>
<tr>
<td>PPL</td>
<td>Porcine pancreatic lipase</td>
</tr>
<tr>
<td><em>P. putida</em></td>
<td><em>Pseudomonas putida</em></td>
</tr>
<tr>
<td>pTs</td>
<td>p-Toluenesulfonyl</td>
</tr>
<tr>
<td>RAMA</td>
<td>Rabbit muscle fructose 1,6-diphosphate aldolase</td>
</tr>
<tr>
<td>Rha1P</td>
<td>L-rhamnulose 1-phosphate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>SAEP</td>
<td>(S)-1-Amino-2-(1-ethyl-1-methoxypropyl)pyrrolidine</td>
</tr>
<tr>
<td>SLe(^x)</td>
<td>Sialyl Lewis(^x)</td>
</tr>
<tr>
<td>sp.</td>
<td>Species</td>
</tr>
<tr>
<td>TA</td>
<td>Transaldolase</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetra-n-butylammonium fluoride</td>
</tr>
<tr>
<td>TBDPS</td>
<td>tert-Butyldiphenylsilyl</td>
</tr>
<tr>
<td>TBS</td>
<td>tert-Butyldimethylsilyl</td>
</tr>
<tr>
<td>TDP</td>
<td>Tagatose 1,6-diphosphate</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-Tetramethylpiperidinyl-1-oxo</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TFAA</td>
<td>Trifluoroacetic anhydride</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>THP</td>
<td>Tetrahydropyranyl</td>
</tr>
<tr>
<td>TIM</td>
<td>Triosephosphate isomerase</td>
</tr>
<tr>
<td>TK</td>
<td>Transketolase</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>tosic</td>
<td>p-Toluenesulfonic</td>
</tr>
<tr>
<td>TPAP</td>
<td>Tetra-n-propylammonium perruthenate (VII)</td>
</tr>
<tr>
<td>TPP</td>
<td>Thiamine pyrophosphate</td>
</tr>
<tr>
<td>triflate</td>
<td>Trifluoromethanesulfonate</td>
</tr>
<tr>
<td>U</td>
<td>Enzyme activity international units</td>
</tr>
<tr>
<td>UCL</td>
<td>University College, London</td>
</tr>
<tr>
<td>YADH</td>
<td>Yeast alcohol dehydrogenase</td>
</tr>
<tr>
<td>Z</td>
<td>Benzyloxycarbonyl</td>
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Chapter One. Introduction

1.1. Chirality and Catalysis

“How would you like to live in Looking-Glass House, Kitty? I wonder if they’d give you milk in there? Perhaps Looking-Glass milk isn’t good to drink.” With these words Lewis Carroll’s Alice began her adventures through the world of the Looking-Glass; but it is unlikely that Carroll, for all his fascination with mirror images, could guess at the prophetic resonance in his heroine’s words. Some years later, Pasteur’s separation (or resolution) of the enantiomers of tartaric acid led the way to a general understanding that the vast majority of the chemicals associated with living systems possessed an asymmetric molecular structure, and it was established that two mirror-image forms (enantiomers) of the same asymmetric (or chiral) molecule invariably interacted differently - sometimes strikingly so - with biological systems. “No laboratory or cow has yet produced reversed milk,” wrote Martin Gardner in his annotations to Alice, first appearing in 1960; “but if the asymmetric structure of ordinary milk were to be reflected, it is a safe bet that this Looking-Glass milk would not be good to drink.”

The true founding father of biological chemistry was Emil Fischer. He was also something of a scientific prophet. In studying the selectivity of degradative reactions performed on racemic mixtures of sugars and their glycosides using fermenting yeast, Fischer announced to the scientific world that “the fermentative principle of yeast cells is an asymmetric agent which can attack only those sugars bearing a structure not too different from that of glucose.” To explain the selectivity of such “fermentative principles” (later renamed enzymes by F.W. Kühne) he invoked the “lock and key” picture, in which the degradative agent was pictured as a large chiral molecular array containing a cleft, shaped exactly so as to fit the substrate to be degraded (Figure 1.1). This precise recognition of one molecule by another is the reason for the phenomenal power of enzymatic catalysis, the precision of nucleic acid translation and replication, and the intricacy of metabolic regulation and the control of hormone-mediated cell growth, for instance.

Molecular recognition is, of course, more than a matter of locks and keys. The molecules of life are dynamic species, and the sites where small molecules bind to larger ones are seldom one hundred per cent selective for a single molecule, and no
other. However, in illustrating the precision of molecular recognition in biological systems, the "lock and key" hypothesis remains an image with long-enduring value.

The precise interaction between an enzyme and its substrate also gives a clue to the significance of catalysis in the processes of life. The function of an enzyme is to accelerate the conversion of one molecule (the substrate) into another, and so to control the chemistry of biosynthesis and metabolic degradation. The remarkable capacity of enzymes to accelerate the rates of such chemical processes (typically to anywhere between $10^5$ and $10^7$ times the rate at which they would occur in the absence of enzyme), and their equally remarkable capacity to control the chemo-, regio- and enantioselectivity of such reactions, is dependent on the precise binding of substrates in the active site of the enzyme, and the exploitation of the chemical functionality of those portions of the enzyme molecule (amino acid sidechains in the active site) in proximity to the reactant molecules to provide alternative chemical reaction pathways requiring much lower activation energies than the uncatalysed processes.3

Given the remarkable efficiency of biological molecules such as enzymes in the catalysis of chemical reactions in vivo, it is little wonder that their exploitation has long been of interest to the scientific community. In 1894 Fischer, in the face of widely-held prejudice that the behaviour of "fermentative principles" was a property mystically associated with life itself, asserted that "there is no difference between the activity of a cell and a chemical reagent". The truth of these statements was shown three years later when H. and E. Buchner observed the fermentation of sucrose by cell-free extracts of yeast,3 demonstrating that enzyme activity could be retained even outside a living and growing cell. Such were the beginnings of the science of biocatalysis.

The use of fermentation technology, and the parallel development of methods for the isolation of enzymes and their exploitation in chemical synthesis, has grown phenomenally since the days of Fischer. The interface between chemistry and biology has attained a deeper significance than ever, as advances in molecular biology have elucidated the course of many of the fundamental processes of life, shedding light on the molecular basis of diseases and hinting at opportunities for their successful treatment. Proteins, polypeptides, oligosaccharides, nucleic acids, and chiral small molecules analogous to those which participate in the essential processes of metabolism and signal transduction have assumed a new significance, as potential therapeutic agents or probes to investigate more fully the functions of life at the molecular level; and to produce such materials presents the synthetic chemist with challenges for which an understanding of biological systems and an ability to harness their power is highly desirable, if not essential.4 At the same time, more and more potentially useful enzymes are being discovered, isolated in stable forms, and made
available to the research chemist for laboratory work. Industry, ever anxious to develop economically viable technologies for the generation of fine and commodity chemicals, materials and pharmaceuticals, and under the more recent pressures of the drive towards "clean technology" and the more stringent requirements of registration for new pharmaceuticals, is increasingly looking towards biocatalysis as a rich and versatile option in the production of chiral chemicals on a large scale. Fischer's vision that "the difference between natural and artificial synthesis is completely eliminated" is slowly but inexorably being realised.

![Figure 1.1. Schematic representation of Fischer's "lock and key" principle](image)

**Figure 1.1. Schematic representation of Fischer's "lock and key" principle**

### 1.2. Enzyme Chemistry - Some Current Perspectives

#### 1.2.1. General Observations

In the last decade, an abundance of material has appeared in the literature concerning the structure and properties of a whole range of enzymes, and their isolation and exploitation. The use of biological systems (including isolated enzymes, whole cells such as yeasts, novel organisms generated through recombinant DNA technology, and catalytic antibodies) in organic synthesis has been subject to extensive review, and as the different applications of the more well-established enzymes can
readily be located in the literature, no attempt will be made here to reproduce such a plethora of information. The purpose of this section is to offer some insight into how the structural and mechanistic properties of the major classes of enzymes currently exploited on a synthetic scale (subdivided into hydrolytic, oxidative-reductive, and carbon-carbon bond forming enzymes) facilitate the exploitation of these enzymes, and to indicate some of the most recent applications of enzyme technology in synthesis, including possible future applications. Whitesides and Wong\textsuperscript{46} have emphasised that “the area of enzymatic catalysis in organic synthesis is, as are most areas of practical catalysis, fundamentally a \textit{process chemistry}.” Therefore this review seeks rather to describe the \textit{process} of the exploitation of biocatalysis than to discuss individual applications in detail. For this reason, the emphasis is on enzymes which have been (or are in the process of being) isolated, and perhaps stabilised or genetically modified, for use in organic synthesis, and on the features which make these biocatalysts attractive for use in process chemistry. It is hoped that an idea of the current limitations of enzymatic catalysis will also be presented, and some indications as to how industry and academia are working to circumvent these limitations.

\textbf{1.2.2. HYDROLYTIC ENZYMES}

\textit{1.2.2.1. Enzyme structure and function}

The most widely exploited enzymes to date, in the laboratory and in industry, are the hydrolytic enzymes: lipases, esterases, proteases, amidases \textit{etc}. Their function \textit{in vivo} is to catalyse the formation or hydrolysis of ester and amide bonds, such as those present in proteins, lipids and sugar conjugates. Mechanistically, the hydrolytic enzymes can be divided into four categories.\textsuperscript{6} \textit{Serine hydrolases} (e.g. chymotrypsin, subtilisin, lipase, penicillin acylase and pig liver esterase) possess a triad of catalytically active residues in the enzyme active site - serine, histidine, and (usually) aspartate - which catalyse the formation of an O-acyl intermediate covalently bound to the enzyme \textit{via} the serine residue. Nucleophilic displacement of this intermediate then releases the product (Scheme 1.2.1). The nucleophile may be water, as in the hydrolysis of amide bonds by proteases, or an alcohol, amine or thiol group. The catalytic mechanism of the \textit{cysteine hydrolases} (such as papain) is similar, except that a cysteine, rather than a serine, residue serves as the initial nucleophile. \textit{Metalloproteases} (e.g. thermolysin and carboxypeptidase A) employ a divalent zinc ion as a Lewis acid, which coordinates to the nucleophilic water molecule and to the carboxyl group of the
substrate, and no covalent intermediate is formed. The mechanism of aspartyl proteases (e.g. pepsin and HIV protease) is a general acid/general base catalytic process involving carboxylic acid residues in the active site, and may or may not involve the formation of a covalent intermediate.

Scheme 1.2.1. Mechanism of serine protease-catalysed amide hydrolysis

The use of hydrolytic enzymes to generate enantiomerically enriched materials by techniques such as the kinetic resolution of racemic chiral alcohols or esters, the desymmetrisation of esters of meso-diols or diacids by selective hydrolysis of a single ester function (or acylation of a single hydroxyl function), or the selective coupling of
amino acids or peptide fragments is now well-established; a great many examples of such processes can be found in the books by Wong and Whitesides and Drauz and Waldmann. Stabilised forms of many of the more frequently employed enzymes (such as immobilised lipases) are commercially available and can readily be used "off the shelf".

The use of water-immiscible organic solvents as media for serine or cysteine hydrolase-catalysed transesterifications is now well established. In such a system, a chiral secondary alcohol or a meso-diol acts as the nucleophile in the hydrolase-catalysed dissociation of an organic ester (the acyl donor), and undergoes kinetic resolution (Scheme 1.2.2) or desymmetrisation as a consequence. Such reactions often prove inconvenient or unsuccessful in aqueous media, owing to the frequently low solubility of the substrates and the occurrence of competing hydrolytic reactions. Use of an organic solvent system offsets these difficulties, frequently leading to improved yields and enantioselectivities, and also often conferring improved stability to the enzyme (which remains encased in a hydration sphere). However, in order to avoid the establishment of complex equilibria in such systems it is necessary that the acyl donor be a highly activated species (such as the ester of an oxime or trihaloacetic acid), or else (for instance) an enol ester (so that when the acyl-enzyme intermediate is formed, the released enol rapidly tautomerises and thus makes the reaction irreversible).

Scheme 1.2.2. Kinetic resolution of a secondary alcohol by irreversible enzymatic acylation
Lipases, which are among the most widely used of all the hydrolytic enzymes, catalyse the cleavage of esters of long-chain fatty acids. Lipases from a variety of sources have found wide applicability in organic synthesis, and have been extensively used for kinetic resolutions of chiral alcohols and desymmetrisations of meso-diols in organic solvents. In vivo lipases function at the interface between the enzyme and a large lipid droplet; in aqueous solutions with soluble substrates, lipases display little activity, but activity increases very markedly once the substrate concentration exceeds the critical micellar concentration. Sih et al. isolated two forms of the lipase from *Candida rugosa* (CRL) which differed significantly in their enantioselectivity towards chiral carboxylic acids (resolutions of racemic chiral carboxylic acids using this lipase had consistently demonstrated poor enantioselectivity, though resolutions of racemic chiral alcohols with excellent enantioselectivity had been observed and also demonstrated that the poorly enantioselective form of the enzyme could be converted to the more enantioselective form by treatment with sodium deoxycholate followed by precipitation and repeated purification. Kazlauskas later showed that a simple treatment of the crude lipase with propan-2-ol was sufficient to increase significantly its activity and enantioselectivity. The reason for these observations is that lipases tend to exist in two conformational forms, distinguished by the position of a helical “lid” above the hydrophobic crevice which contains the active site of the enzyme. In the “closed” conformation the lid partially covers the active site (though not completely in the case of CRL, so that accessibility of the active site is restricted, but not entirely blocked), whereas in the more hydrophobic “open” conformation (which is thus favoured by treatment with a less polar solvent than water, such as propan-2-ol) the active site is entirely exposed; moreover, the oxyanion-stabilising residues in the active site are re-orientated slightly, so as to form an “oxyanion hole” which binds the negatively charged tetrahedral intermediate (see Scheme 1.2.1) in a very precise orientation, thereby conferring high enantioselectivity to the reaction.

The crystal structures of several lipases are known, and a great many of them possess a helical “lid”. The equilibrium between “closed” and “open” forms of the lipases is responsible for the phenomenon of “interfacial activation”. Above the critical micellar concentration for catalysis, the enzyme is adsorbed on the surface of a lipid droplet; the environment close to the enzyme surface is therefore hydrophobic, and the more hydrophobic “open” conformation, which allows entry of lipid molecules to the active site and generates an “oxyanion hole” therein, is favoured. Interfacial activation was not, however, observed with crude CRL, presumably because the “lid” does not

*Formerly known as *Candida cylindracea*
close off the active site completely to the substrate, and (slow) diffusion of lipid into the active site is possible even in the less active “closed” form of the enzyme.

1.2.2.2. Active site models

Even when the X-ray structures of enzymes are known, exact prediction of the stereochemical outcome, or the enantioselectivity, of a given reaction is seldom straightforward. Some hydrolytic enzymes have demonstrated unusual lack (or even reversal) of expected enantioselectivity among a series of closely related substrates. In order to rationalise such behaviour, empirical models for the active sites of a number of enzymes have now been described, based on a summation of the known substrate selectivity data. One of the most detailed is that proposed by Jones for pig liver esterase in which a cubic space model of the active site is drawn, comprising two hydrophobic regions and two more polar regions. This active site model has successfully accounted for the enantioselectivity of the enzyme towards a huge range of substrates, and was found to have significant value as a tool for predicting enantioselectivity. Active site models for other hydrolytic enzymes, including chymotrypsin and *Mucor miehei* lipase, have also been proposed, and “Kazlauskas’ rules” have been proposed to account for the enantioselectivity of CRL.

**Figure 1.2. Kazlauskas’ rules for *Candida rugosa* lipase**

1.2.2.3. Hydrolase enzymes for novel chiral intermediates

Hydrolase enzymes have been extensively used in the generation of enantiomerically enriched (or pure) chiral alcohols, diols, esters, amides, peptides etc., many of which are valuable synthetic intermediates in the production of pharmaceuticals, materials or fine chemicals. New applications for hydrolase enzymes in the production of chiral chemicals are constantly being discovered, as are enzymes which catalyse novel and/or highly selective transformations. A few such recent applications are reported below.
Epoxides such as glycidaldehyde diethyl acetal (1) are extremely valuable chiral building blocks in organic synthesis. In addition to chemical methods such as the Sharpless asymmetric epoxidation reaction, several enzymatic methods exist for the synthesis of chiral epoxides. One of the most versatile methods is the cyclisation of chiral secondary alcohols derived from the kinetic resolution of racemic 2-acetoxy-3-substituted acetals and ethers using porcine pancreatic lipase (PPL) or LP-80 lipase (Scheme 1.2.3). Recently Furstoss et al. have reported the use of the epoxide hydrolase activity of the micro-organisms Aspergillus niger and Beauvaria sulfurescens as a route to enantiomerically enriched chiral aromatic epoxides and 1,2-diols from a racemic mixture of the appropriate epoxide.14.15 A. niger and B. sulfurescens appeared to be enantiocomplementary in the hydrolysis of racemic styrene oxide, hydrolysing the (R)- and (S)-epoxide respectively.14.15 For other aromatic epoxides, the substrate specificity of B. sulfurescens appeared to be wider than that of A. niger.15 It was also noticed that during the hydrolysis of racemic cis-1-phenylpropene oxide, both enantiomers were hydrolysed with different regioselectivities, leading to an almost enantiomerically pure diol product in an overall preparative yield of 85% (Scheme 1.2.4). These observations have enabled active site models for the fungal epoxide hydrolase enzymes to be proposed.

Nitrile hydrolase enzymes are also finding wide applicability in organic synthesis.16 Nitrile hydrolysis typically involves a nitrile hydratase which converts the nitrile to an amide, followed by an amidase which further hydrolyses the amide to a carboxylic acid; either or both may show selectivity. A recent synthesis of the lactone pharmacophore of the hypocholesteremic agents mevinolin and compactin utilised the nitrile hydrolase activities of two micro-organisms: first, a selective hydrolysis of the
Scheme 1.2.4. Selectivity of *Beauvaria sulfurescens* epoxide hydrolase

pro-\(S\) nitrile moiety of 3-(benzyloxy)glutaronitrile using *Brevibacterium* R312, and later a hydrolysis using immobilised *Rhodococcus* sp. SP 361 (Scheme 1.2.5). Other applications of these enzymes include the industrial preparation of acrylamide from acrylonitrile utilising the nitrile hydratase activity of *Pseudomonas* sp.

Both epoxide hydrolase and nitrile hydrolase systems presently necessitate whole-cell systems rather than isolated enzymes. However, some progress has been made in the isolation of epoxide hydrolases\(^{15,16}\) and nitrile hydratase.\(^6\) Whether these isolated enzymes will be of such general use as, for instance, isolated lipases remains to be seen.

Scheme 1.2.5. Chemoenzymatic synthesis of mevinolin lactone utilising nitrile hydrolase enzymes
1.2.2.4. Enzymatic protecting group strategies

Another area where hydrolytic enzymes are beginning to come to the fore is that of protecting group chemistry in the synthesis of highly functionalised molecules such as peptides, saccharides, and their conjugates. The use of lipases and proteases to acylate selective hydroxyl groups of carbohydrates, and of lipases, esterases and proteases to achieve selective unmasking of multiply acylated carbohydrates, has been reviewed by Roberts et al. Depending on the enzyme and solvent systems employed, selectivity for the primary or for a particular secondary hydroxyl group can often be attained in good yield. Thus, for example, a naturally occurring rhamnopyranoside of p-hydroxybenzaldehyde was synthesised in good yield using a selective acetylation catalysed by *Pseudomonas fluorescens* lipase (PFL) in the final step (Scheme 1.2.6).

Scheme 1.2.6. Selective protection in carbohydrate synthesis

The successful construction of peptide conjugates requires extremely mild conditions. Even mildly basic conditions can result in the cleavage of fatty acid, nucleotide or carbohydrate moieties from the peptide chain, while under acidic conditions glycosidic bonds are prone to anomerisation or cleavage. Therefore assembly of the peptide chain requires the mildest conditions, which are not always possible with conventional chemical synthesis. The *Ras* proteins, which play a crucial role in the regulation of cell growth, illustrate the difficulty. The C-terminal
lipohexapeptide (2) associated with human N-Ras protein possesses an acid-labile \( S \)-farnesylated cysteine methyl ester and a base-labile \( S \)-palmitoylated cysteine residue; therefore conventional peptide synthesis using acid-or base-labile protecting groups for the amino acid sidechains is impossible.\(^{22,23}\) The use of protecting groups which can be cleaved under the mildest conditions using hydrolytic enzymes can therefore be essential to a successful synthesis of such molecules, and a range of protecting groups and deprotection methodologies have been developed. A range of such protecting groups are listed in Table 1.1.\(^{20}\)

-----Cys(Pal)-Met-Gly-Leu-Pro-Cys(Far)OMe (2)

<table>
<thead>
<tr>
<th>Protecting group</th>
<th>Use</th>
<th>Cleavage conditions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylacetamido</td>
<td>( N )-terminal protection</td>
<td>Penicillin acylase</td>
<td>Stable to acid, base, hydrogenation. Can lead to some racemisation in conventional peptide synthesis</td>
</tr>
<tr>
<td>(PhAc) (ref. 24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( p )-Acetoxy-</td>
<td>( N )-terminal protection</td>
<td>Acetyl esterase, acetylcholinesterase, \textit{M. miehei} lipase</td>
<td>See Scheme 1.2.7 for cleavage mechanism. Used in first synthesis of N-Ras hexapeptide (2)</td>
</tr>
<tr>
<td>benzyloxy carbonyl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(AcOZ) (ref. 22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Bromoethyl</td>
<td>( C )-terminal protection</td>
<td>Lipase</td>
<td></td>
</tr>
<tr>
<td>(EtBr) (ref. 20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heptyl (Hep)</td>
<td>( C )-terminal protection</td>
<td>Lipase</td>
<td></td>
</tr>
<tr>
<td>(ref. 20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 2-(N\text{-morp} \text{holino}) )-</td>
<td>( C )-terminal protection</td>
<td>Lipase</td>
<td></td>
</tr>
<tr>
<td>ethyl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ref. 20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline (Cho)</td>
<td>( C )-terminal protection</td>
<td>Cholinesterase, chymotrypsin, papain</td>
<td>Enhances peptide solubility. Can be used as activating group in protease-catalysed peptide synthesis.</td>
</tr>
<tr>
<td>( \text{RCO}_2(\text{CH}_2)_2\text{NMe}_3^+ ) (ref. 23)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1. Enzyme-cleavable protecting groups for peptide synthesis
Scheme 1.2.7. Enzymatic deprotection of the $p$-acetoxybenzyloxy carbonyl (AcOZ) group

With the recent enormous growth of interest in methods for the synthesis of peptide conjugates and oligosaccharides using solid-phase “combinatorial” methodology, the challenge of developing linkers which can be cleaved under mild conditions has arisen. This is another area where enzyme technology may be of great importance, although to date relatively few examples of enzyme-cleavable linkers are reported in the literature. Elmore et al. have devised a linker containing a phosphodiester moiety for use in solid-phase peptide synthesis, wherein the phosphate group may be hydrolysed, and the peptide released from the support, by the use of phosphodiesterase; and Wong employed a phenylalanyl ester group, recognisable by chymotrypsin, in the solid-phase synthesis of a sialyl Lewis$^X$ (SLEx) glycopeptide. It is highly likely that similar applications of enzyme technology will be reported in the future.

1.2.2.5. Process chemistry

The transfer of enzyme technology from laboratory bench to industrial process is fraught with challenges. Economy of time, resources and finance are essential to produce a viable industrial process, and any or all of these factors can be a considerable encumbrance to the application of enzyme technology in industry. The costs of substrates, enzymes (and cofactors if necessary) and reactor operation and
design (if a new reactor has to be devised for the process) can all be limiting factors. In fact, hydrolytic enzymes possess a number of advantages: they are cofactor independent, many of them are readily available in large quantities and can be immobilised or otherwise stabilised for use in versatile chemical reactors, and they are often easy to remove and recycle once the process is finished.

A wide range of industrial processes making use of hydrolytic enzymes are now well-established. One example is the thermolysin-catalysed synthesis of aspartame (4) from N-benzyloxycarbonyl (Z) protected aspartic acid and phenylalanine methyl ester. Thermolysin is a robust metalloprotease which operates at neutral pH and retains significant activity even at elevated temperatures, and is therefore well suited to use in an industrial process. Moreover, it is entirely selective for L-amino acids (which means that cheap racemic mixtures of amino acids can be employed in the reactor), and for the α-carboxylate group of aspartic acid (Scheme 1.2.8), and no racemisation is observed in the process.

Scheme 1.2.8. Industrial preparation of aspartame (4)

Due to the cost of enzyme purification, crude enzyme preparations are most commonly used in industrial applications, but several disadvantages are inherent in this approach, most notably the fact that contaminants in the enzyme preparation can reduce
the enzyme lifetime, complicate downstream processing, and even lead to inconsistent or contradictory results in the process itself (for instance, the presence of other hydrolases as impurities in crude cell-free preparations of lipases etc. can lead to a lower enantioselectivity than expected, and to unpredictability in the effect of alterations in reaction conditions such as temperature and pH). Efficient, cheap methods for the purification and stabilisation of enzymes can therefore be essential to the operation of a viable biocatalytic process; they may additionally confer other advantages such as allowing the enzyme to be recycled. Enzyme immobilisation, i.e. attachment of the enzyme to a water-insoluble solid support, is the most common method of stabilising an enzyme, allowing the use of continuous packed bed reactors and enabling the catalyst to be recovered (often by the simple process of filtration) and recycled. Immobilised enzymes, however, do tend to lose activity over a period of time, and can degrade quite substantially by the physical process of stirring; moreover the specific activity of the immobilised enzyme is often considerably lower than in the crude cell-free preparation, as the amount of enzyme in an immobilised preparation is typically less than 5% by weight. An alternative to enzyme immobilisation is the use of cross-linked enzyme crystals (CLECs), which can be obtained by means of a simple purification step followed by crystallisation and treatment with a cross-linking agent such as glutaraldehyde. The resulting biocatalyst consists of a rigid, close-packed network of molecules, which are often water-insoluble, highly active, and significantly more stable to extremes of heat and pH and to the presence of exogenous proteases and water-miscible organic solvents than the crude enzyme preparations. Cross-linked crystals of thermolysin have been shown to be highly efficient catalysts in the preparation of aspartame, and crystals of CRL were shown to have significantly superior enantioselectivity to the more often used crude enzyme preparation in the hydrolysis of a range of esters of 2-arylpropanoic acids, including methyl ibuprofen (5), which was efficiently resolved on a 100 g scale (recrystallisation of (S)-ibuprofen (S)-6 gave material of > 99% enantiomeric excess (e.e.)). Given the low enantioselectivity of CRL in the resolution of chiral acids (vide supra), this represents a significant improvement in catalyst performance. Similar results for a range of hydrolases indicate that CLECs are potentially highly viable catalysts in large-scale industrial processes.

\[
\text{Me} - \text{OR} \\
\begin{align*}
\text{R} = \text{Me; } 5 \\
\text{R} = \text{H; } 6
\end{align*}
\]
Chemical modification of enzymes can also be invaluable in improving their performance as catalysts. For instance, pig liver esterase modified at surface lysine residues by chemical attachment of polyethylene glycol monomethyl ether units has demonstrated significantly improved activity in water-immiscible organic solvents over the native enzyme (which is all but inactive in such systems). The use of site-directed mutagenesis techniques to produce genetically modified enzymes may in future also enable the generation of biocatalysts with enhanced stability or activity in organic solvent systems, though the exploitation of such materials on a wide scale is not yet a reality.

Where immobilisation or cross-linking has not proved effective in the production of stabilised biocatalysts, an alternative is to utilise a homogeneous system with the enzyme enclosed within a membrane. The pioneering work of Wandrey et al. has enabled the development of enzyme membrane reactors (EMR) for the industrial scale preparation of a number of valuable chiral chemicals, e.g. the resolution of racemic N-acetyl-DL-amino acids using acylase I from Aspergillus oryzae enclosed in an ultrafiltration membrane through which substrate and product molecules can flow as necessary. The process can be applied to the synthesis of non-proteinogenic amino acids such as α-aminobutyric acid as well as proteinogenic amino acids, and has the advantage that the recovered N-acetyl-D-amino acids can be readily racemised, reducing chemical waste to a minimum.

Dealing with "waste" materials is of great importance to the viability of a chemical process. In an enzymatic kinetic resolution, only 50% of the desired material can be obtained, and requires separation from the remaining 50%. Often a use can be found for the unwanted material, which will also be enantiomerically enriched. Where an unwanted enantiomer is not directly useful, the ideal is for it to be converted to its enantiomer, or else racemised. Thus, the byproducts from the acylase-catalysed resolution of amino acids described above can readily be racemised by acetic anhydride in alkaline solution, or by using a racemase enzyme, and returned to the reactor. Even better is to use conditions which allow racemisation of the unreacted enantiomer in situ, allowing a so-called dynamic resolution of the starting material, with a theoretical maximum yield of 100%. Dynamic resolution of amino acids using hydantoinase has been exploited on an industrial scale, and can be performed in tandem with a second hydrolytic enzyme (carbamoylase) to release the free amino acid of desired configuration; as both D-and L-hydantoinases are known, this process can

---

* EMR systems have also been employed for the large-scale use of oxidative-reductive enzyme systems (see Section 1.2.3) and carbon-carbon bond forming enzyme systems (see Section 1.2.4) with considerable success.
be used for the synthesis of either D- or L-amino acids (Scheme 1.2.9). More recently, an application of the Lipozyme®-catalysed dynamic resolution of oxazolin-5(4H)-ones (azlactones) derived from racemic N-benzoylamino acids to the synthesis of enantiomerically pure amino acids (including the valuable chiral intermediate L-tert-leucine (7)) has been reported by Turner et al. (Scheme 1.2.9). Both hydantoin and azlactones racemise rapidly under the conditions employed in the biotransformation. An interesting new form of dynamic resolution process has recently been described for the resolution of chiral allylic alcohols using acetylcholinesterase or PFL in combination with a palladium (II) catalyst which racemises the allylic acetate starting material rapidly in aqueous buffer (Scheme 1.2.9). Simultaneous enzyme-and transition metal-catalysed reactions are unusual, and the scope for their wider implementation as tools in process chemistry is considerable.

a) amino acid synthesis using hydantoinase/carbamoylase

\[
\begin{align*}
\text{R-CO}_2\text{H} & \xrightarrow{\text{L-hydantoinase}} \text{D-CO}_2\text{H} \\
\text{HN-NH}_2 & \xrightarrow{\text{pH > 8}} \text{HN-NH}_2 \\
\text{L-carbamoylase} & \rightarrow \text{CO}_2\text{NH}_3 \\
\text{R-CO}_2\text{H} & \xrightarrow{\text{D-hydantoinase}} \text{L-CO}_2\text{H} \\
\text{HN-NH}_2 & \xrightarrow{\text{D-carbamoylase}} \text{CO}_2\text{NH}_3
\end{align*}
\]

b) Lipozyme-catalysed dynamic resolution of azlactones

\[
\begin{align*}
\text{H}_2\text{N-CO}_2\text{H} & \xrightarrow{i) \text{PhCOCl/NaOH}} \text{PhOCO-N} & \xrightarrow{\text{ii) Ac}_2\text{O}} \text{PhCO-} \\
\text{Lipozyme}^\circlearrowright/ \text{BuOH/ Et}_3\text{N} & \rightarrow \text{HN-CO}_2\text{Bu} \\
\text{L-tert-leucine} (7) & \xrightarrow{\text{H}_2\text{N-CO}_2\text{H}} \text{H}_2\text{N-CO}_2\text{H}
\end{align*}
\]
1.2.3. OXIDATIVE-REDUCTIVE ENZYMES

1.2.3.1. Enzyme structure and function

Redox enzymes have not been so widely exploited as hydrolytic enzymes in organic synthesis. A few large-scale processes exist, mostly involving whole-cell preparations of yeasts or micro-organisms rather than isolated enzymes; exploitation of free enzymes has often been hampered by the fact that a number of useful species are membrane-bound, and difficult to isolate, and all require redox-active cofactors, which have to be supplied in the reaction medium and recycled when isolated enzymes are employed. Those enzymes which have been exploited fall into two classes: metalloenzymes and nicotinamide-dependent oxidoreductases. The metalloenzymes are involved with the oxidation of relatively redox-inactive functionalities e.g. alkanes, carbon-carbon double bonds or heteroatoms, whereas the nicotinamide-dependent species take part in the oxidation and reduction of ketones and alcohols.

Metallo-oxidases of various kinds are known. The cytochrome P-450 monooxygenases contain a heme group, and catalyse the oxidation of alkanes (with oxygen insertion), carbon-carbon double bonds (forming a chiral epoxide), and heteroatoms (e.g. oxidation of sulfides to chiral sulfoxides) by molecular oxygen, with a high-valent \( \text{Fe}^\text{V}=\text{O} \) intermediate as the active species.\(^6\)\(^3\)\(^6\)\(^3\)\(^7\) The P-450 monooxygenase from \textit{Pseudomonas putida} involved in the oxidation of camphor (P-450\(_{\text{cam}}\)) has been overexpressed in \textit{Escherichia coli} and isolated, though as yet the use of the isolated enzyme as a synthetically useful catalyst has not been reported. \textit{Pseudomonas oleovorans} monooxygenase possesses a non-heme iron atom in the active site, and also functions \textit{via} an \( \text{Fe}^\text{V}=\text{O} \) active intermediate. The proposed catalytic cycle for these monooxygenases is shown in Scheme 1.2.10. Chloroperoxidase from \textit{Caldariomyces fumago} also makes use of a non-heme \( \text{Fe}^\text{V}=\text{O} \) intermediate,
though in this case the actual oxidant is not molecular oxygen but the hydroperoxide ion. The enzyme has been overexpressed and is commercially available, and has been found to be useful in the enantioselective epoxidation and halohydration of alkenes and the oxidation of sulfides; a radical mechanism has been proposed. Other metallo-oxidases include the Cu$^{II}$-dependent galactose oxidase, the Fe-dependent isopenicillin-N synthetase, and the arene dioxygenases (which add two oxygen atoms to each molecule of substrate, with molecular oxygen as the source) from *P. putida* which perform regio-and enantioselective dihydroxylation of aromatic rings, generating cis-cyclohexadienediols which are valuable intermediates in the synthesis of a huge range of chiral molecules, including sugar analogues.

![Scheme 1.2.10. Catalytic cycle for iron-dependent monooxygenases](image)

Scheme 1.2.10. Catalytic cycle for iron-dependent monooxygenases (the mechanism of the substrate oxidation step(s) is not yet clear)

The *alcohol dehydrogenase* enzymes are the most well-known examples of the nicotinamide-dependent oxidoreductases. Their function is dependent on the presence of *nicotinamide adenine dinucleotide* (NAD$^+$, 8) or its 2'-phosphorylated derivative (NADP$^+$, 9). The nicotinamide ring system readily accepts a proton and two electrons.
or a hydride ion, to form the 1,4-dihydronicotinamide derivatives NADH (10) and NADPH (11). The reversible hydride transfer from a reduced substrate to NAD\(^+\) or NADP\(^+\), or from NADH or NADPH to an oxidised substrate, is stereoselective, the nature of the selectivity varying from enzyme to enzyme. Most alcohol dehydrogenases, including those from horse liver (HLADH), yeast (YADH) and *Thermoanaerobium brockii*, follow “Prelog’s rule” (Scheme 1.2.11), transferring the pro-\(R\) hydride from the nicotinamide ring to the \(re\) face of a carbonyl substrate.

Scheme 1.2.11. Selectivity of alcohol dehydrogenase-catalysed ketone reductions

Alcohol dehydrogenases have been successfully employed in oxidative mode in the synthesis of \(\alpha\)-hydroxy-and \(\alpha\)-amino aldehydes with kinetic resolution of racemic
1,2-diols,\(^{39}\) and of chiral lactones by desymmetrisation of \textit{meso}-diols, and in reductive mode in the synthesis of chiral alcohols by reduction of the corresponding ketones.\(^{36}\) The enzymes have also proven useful in the synthesis of deuterium-or tritium-labelled alcohols and \(\alpha\)-hydroxyacids, utilising the precise nature of the deuterium or tritium transfer from a suitably generated nicotinamide cofactor (typically prepared through reduction of the oxidised species using \(l\)-labeled propan-2-ol). A cubic space model of the horse liver enzyme active site has been proposed by Jones,\(^{40}\) and shown to be valuable in predicting the stereochemical outcome of HLADH-catalysed reactions with new substrates.

Another class of NAD/NADP-dependent redox enzymes are the "Baeyer-Villigerases", monooxygenases which catalyse the regio-and enantioselective Baeyer-Villiger rearrangement of ketones.\(^{41,42,43}\) The ultimate oxidant in these systems is dioxygen, and the enzymes utilise a tightly-bound flavin group in addition to the nicotinamide cofactor; a flavin hydroperoxide adduct serves as the initial nucleophile, with the migrating C-C bond of the intermediate, the peroxodic (O-O) bond and a lone electron pair on the carbonyl oxygen of the substrate all adopting an antiperiplanar arrangement.\(^{41,43}\) Whole-cell cultures of \textit{Acinetobacter}\(^{41,44-45}\) strains NCIMB 9871 and TD63, and of \textit{Pseudomonas putida}\(^{42}\) strain NCIMB 10007 have exhibited "Baeyer-Villigerase" activity with striking regio- and enantioselectivity utilising a range of bi-\(^{41,42,46}\) and monocyclic\(^{42,44,47}\) ketones, the \textit{Acinetobacter} and \textit{Pseudomonas} strains being basically enantiocomplementary in their selectivity.\(^{42}\) A cubic model of the active site of \textit{Acinetobacter} monoxygenase has been proposed by Furstoss\(^{41}\) to explain the selectivity observed with bicyclic\(^{41,46}\) and monocyclic\(^{44,45}\) ketones.

1.2.3.2. Cofactor regeneration

The majority of biocatalytic reductions are performed using whole-cell systems.\(^{48}\) Yeasts especially are organisms which are adept at performing reductions on systems as diverse as ketones, aromatic nitro compounds, and carbon-carbon double bonds. Whole-cell systems can also be employed effectively in bio-oxidations such as Baeyer-Villiger reactions.\(^{41,42,44,46}\) Whole-cell biotransformations suffer from a number of disadvantages, however: notably the fact that the presence of other active enzymes within the cells can lead to side reactions and correspondingly poor yields of product. Isolation of the product from cell debris after the process can also be troublesome. One significant obstacle to be overcome in the harnessing of isolated redox enzymes for use in biocatalysis is the need to recycle cofactors. The nicotinamide cofactors are far too expensive to be used stoichiometrically on a synthetic scale; therefore methods have been developed to regenerate these cofactors.
with high efficiency once consumed. Cofactor recycling can, in fact, impart advantages to a process. Most importantly, it can drive reactions at equilibrium (or thermodynamically unfavourable reactions) to completion by coupling with a favourable regeneration process. It also eliminates product inhibition by the cofactor byproduct and can simplify considerably the reaction work-up.\textsuperscript{6,36}

At present, the best recycling systems are those which regenerate the cofactor via a second enzymatic reaction. NADH, for instance, can be regenerated from NAD\textsuperscript{+} by oxidation of propan-2-ol using an NAD\textsuperscript{+}-dependent alcohol dehydrogenase, or by oxidation of formate using formate dehydrogenase (Scheme 1.2.12). The formate dehydrogenase system is cheap and efficient, but enzyme activity is low, and the enzyme is specific for NAD\textsuperscript{+}. An alternative system utilises glucose and glucose dehydrogenase from \textit{Bacillus} sp, which is a highly active, robust enzyme; however, the byproduct (gluconate) may complicate product isolation. The glucose dehydrogenase system can also be used to recycle NADPH, as can the propan-2-ol/\textit{Thermoanaerobium brockii} alcohol dehydrogenase system; however, most alcohol dehydrogenases currently available are NAD\textsuperscript{+}-specific.

![Scheme 1.2.12. Some recycling systems for NAD(P)H](image)

Enzymatic methods for the recycling of NAD\textsuperscript{+} or NADP\textsuperscript{+} are shown in Scheme 1.2.13. The most commonly used systems are \(\alpha\)-ketoglutarate with glutamate dehydrogenase (though glutamate in the reaction mixture can complicate product isolation) and pyruvate with lactate dehydrogenase (NAD\textsuperscript{+}-specific), though other systems are also known.
1.2.3.3. Process chemistry

The usefulness of redox enzymes as catalysts for large-scale processes is hampered by a number of issues, notably enzyme availability (Section 1.2.3.1), cofactor recycling (Section 1.2.3.2), and the susceptibility of the enzymes to denaturing and product inhibition. However, as the scope of these enzymes to perform transformations with often unique selectivity (as in the case of the Baeyer-Villigerases for instance) is realised, a wide range of methods are currently in development for improving the synthetic utility of these enzymes.

New enzyme sources are constantly under investigation. Enzyme overexpression is making more and more potentially useful enzymes available, as shown recently in the case of the P-450\textsubscript{cam} monooxygenase, for instance.\textsuperscript{37} Isolated enzymes from synthetically useful whole-cell systems are also under extensive investigation, for instance the two purified Baeyer-Villiger monooxygenases (MO\textsubscript{1}, NAD\textsuperscript{+}-dependent, and MO\textsubscript{2}, NADP\textsuperscript{+}-dependent) from \textit{P. putida} NCIMB 10001,\textsuperscript{42,45} which were found to have very different substrate selectivities (MO\textsubscript{1} being a highly efficient catalyst for the oxidation of bicyclic ketones,\textsuperscript{42,49} MO\textsubscript{2} being more efficient for monocyclic ketones\textsuperscript{42,45}).

A number of industrially useful enzymatic redox processes are in operation. Commercial syntheses of the amino acids L-leucine and L-\textit{tert} leucine, for instance, consist of the reductive amination of \(\alpha\)-ketoacids using the NADH-dependent enzyme \textit{leucine dehydrogenase}, with formate/formate dehydrogenase to regenerate NADH.\textsuperscript{50} The enzyme is enclosed within a membrane, and in the case of L-leucine the cofactor is also retained within the membrane \textit{via} attachment to soluble polyethylene glycol, enabling the reactor to be operated continuously for over 3 months.

\textbf{Scheme 1.2.13. Some recycling systems for NAD(P)\textsuperscript{+}}

\[ \text{E}_1 = \text{glutamate dehydrogenase; E}_2 = \text{lactate dehydrogenase} \]
1.2.4. CARBON-CARBON BOND FORMING ENZYMES

1.2.4.1. General observations

The stereocontrolled formation of carbon-carbon bonds is of the foremost interest to research chemists, and although the technology exists for a wide range of asymmetric coupling reactions (such as stereocontrolled aldol condensations) to be performed in the laboratory, the high precision of the enzymes which catalyse carbon-carbon bond formation and their usefulness under mild conditions makes them potentially attractive catalysts in the synthesis of natural products, sugars and other highly sensitive molecules. Although the use of such enzymes in the laboratory-scale synthesis of a vast range of chiral molecules has been extensively documented, most are not yet widely exploited on a large scale. The fact that many of these enzymes require expensive co-substrates and can frequently experience severe product inhibition has to date limited their application. However, recent advances in biotechnology have made a number of these enzymes available in large quantities for the first time, making their application in large-scale processes an area of current interest.

1.2.4.2. Aldolases

Aldolases belong to a class of enzymes known as lyases. Their function in vivo is to catalyse the degradation of metabolites (sugars in the case of aldolases), but the reactions which they catalyse are reversible and so the enzymes can be used in vitro for the synthesis of carbon-carbon bonds. Aldolases, as the name implies, synthesise C-C bonds by means of an enzymatic aldol condensation between two substrates, the nucleophilic donor (usually a ketone) and the electrophilic acceptor (invariably an aldehyde). Type I aldolases, mainly found in animals and higher plants, operate by forming a Schiff base between an active site lysine residue and the donor, which subsequently adds stereospecifically to the acceptor; type II aldolases, mostly found in micro-organisms, are metalloenzymes utilising a divalent zinc ion in the active site as a Lewis acid co-catalyst (Scheme 1.2.14). Although most aldolases will accept a wide range of aldehydes as electrophilic substrates, their specificity for the nucleophilic component of the aldol condensation is fairly rigid. Table 1.2 lists the four main classes of aldolase, according to the preferred donor, with examples from each class (this is not an exhaustive list). Many aldolases have been exploited successfully in synthesis. Some representative examples appear in Section 1.3 below; others can be found in the numerous recent reviews.
Scheme 1.2.14. Mechanisms of aldolase-catalysed aldol condensation

<table>
<thead>
<tr>
<th>Donor</th>
<th>Examples</th>
<th>Natural substrate/product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydroxyacetone phosphate (DHAP) (12)</td>
<td>Fructose 1,6-diphosphate (FDP) aldolase [E.C. 4.2.1.13]</td>
<td><img src="image" alt="Fructose 1,6-diphosphate" /></td>
</tr>
<tr>
<td>DHAP (12)</td>
<td>L-Fuculose 1-phosphate (Fuc1P) aldolase [E.C. 4.1.2.17]</td>
<td><img src="image" alt="L-Fuculose 1-phosphate" /></td>
</tr>
<tr>
<td>DHAP (12)</td>
<td>L-Rhamnulose 1-phosphate (Rha1P) aldolase [E.C. 4.2.1.19]</td>
<td><img src="image" alt="L-Rhamnulose 1-phosphate" /></td>
</tr>
</tbody>
</table>
Table 1.2. Some synthetically useful aldolases (the BOLD bond indicates the carbon-carbon bond formed by the enzymatic aldol reaction)

The dihydroxyacetone phosphate (DHAP, 12) dependent aldolases have to date been the most widely exploited in synthesis. The FDP aldolase from rabbit muscle (RAMA) is commercially available; overexpressed FDP aldolase from *E. coli*......
has also been employed in synthesis, and microbial sources of the other three DHAP aldolases listed in Table 1.2 have also been reported. These four DHAP aldolases show effectively complementary diastereoselectivity (Scheme 1.2.15). The specificity of these aldolases for DHAP as the donor substrate is relatively rigid - only a small number of DHAP analogues were found to be (poor) substrates for RAMA - though a mixture of dihydroxyacetone and inorganic arsenate can serve effectively as a replacement for DHAP, and more recently the thio analogue of DHAP has been shown to be a synthetically versatile donor. The acceptor specificity is more flexible, a wide range of aldehydes being accepted as substrates (though species which are poorly soluble in aqueous buffer, such as aromatic aldehydes, tend to be non-substrates). FDP aldolase is highly stereoselective, with the two new chiral centres formed in the aldol reaction possessing the [3S, 4R] or “D-threo” stereochemistry in nearly every case.* Fuc1P and Rha1P aldolase similarly generate ‘D-erythro’ and “L-threo” diols, though a degree of relaxation in the selectivity of Rha1P aldolase with aliphatic aldehydes has been reported. TDP aldolase, by contrast, also showed unwanted FDP aldolase activity, which has to date limited its usefulness in synthesis; however, a recombinant enzyme was recently reported with significantly suppressed FDP aldolase activity which may be synthetically useful.

Scheme 1.2.15. Stereochemical preference of DHAP aldolases

* One recent report refers to a relaxed selectivity under thermodynamic control. (ref. 63)
The DHAP aldolases show varying degrees of selectivity for chiral centres in the acceptor substrate. Fuc1P and RhaiP aldolases show a high preference for the L-enantiomers of racemic mixtures of \( \alpha \)-hydroxylaldehydes, allowing kinetic resolution of the D-(R)-enantiomers (see Chapter Two). The presence of a negatively charged residue (such as phosphate or carboxylate) at least five bonds away from the aldehyde group of the acceptor was shown to have a profound influence on the kinetic diastereoselectivity of RAMA for \( \alpha \)-hydroxylated aldehydes, the formation of an ion pair between a negatively charged residue on the substrate and an active site lysine residue effectively anchoring the substrate in position and resulting in high kinetic selectivity (cf. the “oxyanion hole” in Candida lipase (vide supra)). Thus, under kinetic control, RAMA accepted D-glyceraldehyde-3-phosphate (16) with a 20:1 selectivity over the L-enantiomer, though with the unphosphorylated sugars little selectivity was observed. Thermodynamic differentiation between the enantiomers of a racemic aldehyde can also occur, if the products can reversibly cyclise to form stable hemiketals. When the reaction reaches equilibrium, a mixture of aldehydes and the two possible products will be present, and if one cyclic product is significantly more stable than its diastereomer (e.g. if there are fewer 1,3-diaxial interactions) it will predominate in the equilibrium mixture (see Scheme 1.2.16 for an example). However, where there is little or no difference in the stabilities of the cyclic products, an = 1:1 mixture of products will predominate.

![Scheme 1.2.16. "Thermodynamic resolution" using RAMA](image-url)
To date, the most extensively studied of the pyruvate aldolases has been N-acetylneuraminic acid aldolase, a type I aldolase which catalyses the reversible aldol reaction of N-acetylmannosamine (ManNAc) 17 and pyruvate 13 to form N-acetylneuraminic (or sialic) acid (Neu5Ac), 18, an important component in a number of biologically significant oligosaccharides and glycoconjugates such as sialyl Lewis\(^x\) (SLex), 3 (vide infra). The enzyme is specific for pyruvate, but accepts a range of 4-carbon and higher sugars as substrates. A free hydroxyl group at C-3 of the acceptor is necessary for reaction,\(^{65}\) the stereochemistry at C-2 is also critical, as ManNAc is an efficient substrate but N-acetylglucosamine (GlcNAc), 19, its C-2 epimer, is not. A wide range of configurations and substitution patterns at the remaining chiral centres of the substrate is tolerated. The stereochemical course of the reaction can follow one of two paths (Scheme 1.2.17): the normal mode involves si-face attack of the enamine intermediate on the acceptor, with the formation of an (S)-chiral centre as in the in vivo reaction. The “inverted” mode, which is observed partially or exclusively with some substrates (e.g. with D-arabinose 20 to form 2-keto-3-deoxy-D-manno-octulosonic acid (KDO), 21, as well as its expected C-4 epimer 22), involves re-face attack.\(^66\) Although the si-face attack is the kinetically preferred mode, under thermodynamic control the products of re-face attack can accumulate if the resulting (R)-product in its cyclic form is the more stable diastereomer. An active site model of the enzyme proposed by Wong\(^{65}\) suggests that in the kinetically disfavoured re-face attack, the substrate is forced into a “boatlike” conformation at the active site.

a) synthesis of N-acetylneuraminic acid\(^{65,66}\)

\[
\begin{align*}
\text{HO} & \quad \text{NHAc} \\
\text{HO} & \quad \text{OH} \\
\text{HO} & \quad \text{O} \\
\text{HO} & \quad \text{OH} \\
\text{NHAc} & \quad \text{OH} \\
\text{HO} & \quad \text{O} \\
\text{HO} & \quad \text{OH} \\
\end{align*}
\]

+ \quad \begin{align*}
\text{HO} & \quad \text{C} \\
\text{OH} & \quad \text{C} \\
\text{HO} & \quad \text{C} \\
\end{align*}

\[\text{Neu5Ac aldolase} \quad \text{AchN} \quad \text{OH} \quad \text{HO} \quad \text{OH} \quad \text{OH} \quad \text{CO}_2\text{H}
\]

b) synthesis of KDPG\(^{68,69}\)

\[
\begin{align*}
\text{P} & \quad \text{O} \\
\text{O} & \quad \text{H} \\
\text{OH} & \quad \text{C} \\
\text{HO}_2\text{C} & \quad \text{C} \\
\end{align*}
\]

+ \quad \begin{align*}
\text{HO} & \quad \text{O} \\
\text{HO}_2\text{C} & \quad \text{C} \\
\end{align*}

\[\text{KDPG aldolase} \quad \text{P} \quad \text{O} \quad \text{OH} \quad \text{C} \quad \text{C} \quad \text{CO}_2\text{H}
\]
c) synthesis of KDO\textsuperscript{66,67}

\[ \text{HO} \quad \text{HO} \quad \text{I} \quad \text{Neu5Ac} \quad \text{HO} \quad \text{HO} \]

\[ \text{aldolase} \quad \text{H01111} \quad \text{H01111} \quad \text{HO} \quad \text{OH} \]

\[ \text{CO}_2 \text{H} \quad \text{CO}_2 \text{H} \]

\[ \text{(21)} \quad \text{(20)} \quad \text{(22)} \]

Scheme 1.2.17. Reactions catalysed by pyruvate aldolases

Other pyruvate aldolases have also been exploited in organic synthesis, though less widely. KDO aldolase\textsuperscript{67} catalyses the condensation of pyruvate with D-arabinose 20 on the re-face to yield 21 (Scheme 1.2.17); several other sugars were found to be substrates, but the reactions were often under thermodynamic control and product mixtures resulted. 2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, which catalyses the condensation of pyruvate and D-glyceraldehyde-3-phosphate 16 to form KDPG 23, has been isolated from three microbial sources\textsuperscript{68,69} and shown to have a wide acceptor specificity, even accepting pyridine-carboxaldehydes as substrates (though simple aliphatic and aromatic aldehydes are not accepted, nor are aldehydes with bulky sidechains).\textsuperscript{69} The enzymes from \textit{E. coli} and \textit{P. putida} were highly selective for D-\textalpha-hydroxyaldehydes, and all three strains exhibited solely kinetic diastereoselectivity.\textsuperscript{69}

Two other aldolases which have recently emerged as useful biocatalysts are L-threonine aldolase\textsuperscript{70,71} and 2-deoxyribose-5-phosphate aldolase (DERA).\textsuperscript{63,72,73} Threonine aldolase uses glycine (14) as the donor, forming \(\alpha\)-amino-\(\beta\)-hydroxy acids with high stereocontrol at the \(\alpha\)-centre but a highly variable degree of control at the \(\beta\)-centre.\textsuperscript{70} DERA is unusual as it is the only known aldolase which utilises an aldehyde (acetaldehyde, 15) as the donor, forming a chiral centre of (R)-configuration. When an \(\alpha\)-hydroxyaldehyde is used as the substrate, the preference is for the (R)-enantiomer.\textsuperscript{73} The structure of the products is such that they can undergo sequential aldol reactions \textit{in vitro}, the reactions eventually ceasing when a stabilised cyclic product can form (Scheme 1.2.18).\textsuperscript{72,73}
In living cells, the link between the metabolic pathways of glycolysis, which degrades sugar molecules with the generation of chemical energy stored in the form of adenosine triphosphate (ATP), and the pentose phosphate pathway, which is used by the cell to provide small-molecule precursors for biosynthetic pathways and reducing power in the form of (reduced) NADPH, is mediated by the enzymes transketolase and transaldolase. These two enzymes provide a reversible link by catalysing the interconversion of sugar phosphate molecules. Transketolase (TK) [E.C. 2.2.1.1] is responsible for the transfer of a two-carbon ketol unit, C(O)CH₂OH, from a donor ketose such as D-xylulose 5-phosphate 24 to an acceptor aldose e.g. ribose 5-phosphate 25 or erythrose 4-phosphate 26 (Scheme 1.2.19). The enzyme requires magnesium ions and the cofactor thiamine pyrophosphate (TPP) (27) for its activity. The enzyme has been isolated from a variety of sources, and transketolase from yeast, spinach and E. coli have all been employed in synthesis (see Section 1.4).
Scheme 1.2.19. Reactions catalysed by transketolase in vivo

Transaldolase (TA) [E.C. 2.2.1.2] catalyses the transfer of the C1-C3 aldol unit from D-sedoheptulose 7-phosphate 28 to D-glyceraldehyde-3-phosphate 16 to produce D-fructose 6-phosphate 29 and D-erythrose 4-phosphate 26, functioning via a Schiff base intermediate. Although TA is commercially available, its substrate specificity has not been studied in detail.

1.2.4.4. Oxynitrilase

Oxynitrilases, or hydroxynitrile lyases, catalyse the reversible addition of hydrogen cyanide to an aldehyde. (R)-Oxynitrilase from bitter almonds [E.C. 4.1.2.10], and (S)-oxynitrilases [E.C. 4.1.2.11] from sorghum and *Hevea brasiliensis* have been isolated and employed in the stereospecific synthesis of carbon-carbon bonds. The co-substrate, HCN, tends to add non-stereospecifically to the substrate when reactions are performed in aqueous solution, reducing the enantiomeric purity of the isolated cyanohydrins; however, the extent of the non-enzymic process can be minimised by a choice of low operational pH and temperature. The enzymes can also be employed in solvents such as ethyl acetate and diisopropyl ether in the presence of minimal amounts of water, thereby suppressing the
non-enzymic reaction. Another alternative is to use acetone cyanohydrin as the HCN donor; a transcyanation reaction, rather than a direct addition of HCN, then occurs, and the problem of non-enzymic addition is avoided.

The substrate specificities of oxynitrilases have been examined for a small range of aldehydes. Almond (R)-oxynitrilase will accommodate a range of aliphatic and aromatic aldehydes, forming the (R)-cyanohydrins in high e.e. The enzyme is tolerant of a broad range of substrates containing chiral centres at remote positions on the sidechains, but a near-total loss of selectivity was observed with (R)-2-phenylpropanaldehyde as the substrate. (S)-Oxynitrilase from Hevea also appears to have a wide substrate specificity, though only aromatic aldehydes are tolerated by the enzyme from sorghum. The enzymes have been employed in the synthesis of chiral α-hydroxy carboxylic acids and the (R)-oxynitrilase has been employed in the kinetic resolution of (S)-ketone cyanohydrins and the formation of enantiomerically pure substituted heterocycles. A recent report also describes the use of (R,E)-2-hydroxy-3-pentenenitrile, available in ≈ 200 mmol batches from an (R)-oxynitrilase-catalysed addition, in the synthesis of enantiomerically pure α-hydroxy-β-amino acids, including N-benzoyl-(2S, 3S)-3-phenylisoserine, the amino acid component of the sidechain of Taxol. 

**Scheme 1.2.20. Synthesis of α-hydroxy-β-amino acids using oxynitrilase**
1.2.4.5. Anabolic carbon-carbon bond forming enzymes

An enormous class of carbon-carbon bond forming enzymes are involved in the biosynthesis of fatty acids, polyketides, terpenes and numerous other secondary metabolites.\textsuperscript{89} The majority of these synthase enzymes function by repetitive condensation of small molecule building blocks (such as acetate, malonate or propionate) activated in the form of coenzyme A (CoA) thioesters; the resulting carbon chains are then modified by reduction, cyclisation \textit{etc}. The mechanisms of action of these synthases, many of which consist of huge multienzyme complexes with each part of the complex catalysing a particular biosynthetic step, have been extensively investigated, as has the arrangement of the polyketide synthases on a genetic level.\textsuperscript{90} A number of organisms possessing genetically engineered polyketide synthases for the production of novel antibiotics have been recently reported, and the sequential arrangement of the synthase genes in the microbial genome has even made possible the selection of genes to produce new polyketides (a concept which one reviewer has colourfully described as “combinatorial biosynthesis”!\textsuperscript{91}). Isolated enzyme systems are not yet exploited, however; a number of obstacles will have to be surmounted, \textit{e.g.} the recycling of acyl CoA units, before any of these systems are usable as synthetically useful isolated enzyme systems.

1.2.4.6. Process chemistry

The efficiency of carbon-carbon bond forming enzymes in laboratory-scale synthesis has, on the whole, not yet been transferred to the large-scale industrial process. The fact that most syntheses employing DHAP-dependent aldolases are carried out under thermodynamic conditions means that reaction times are typically long (five days being not uncommon for mmol-scale laboratory transformations) with repeated additions of enzyme being necessary; the fact that mixtures of products often form under these conditions (\textit{vide supra}) can further complicate product isolation (chromatography is normal in laboratory-scale syntheses). An additional hindrance is the expense of the co-substrate DHAP. Chemical methods now exist which can produce multigramme quantities of high-purity DHAP;\textsuperscript{92} \textit{in situ} enzymatic methods (such as the cleavage of FDP in the presence of RAMA with subsequent isomerisation of glyceraldehyde-3-phosphate \textbf{16} using triosephosphate isomerase (TIM),\textsuperscript{29} or the oxidation of L-glycerol-3-phosphate using glycerolphosphate oxidase (GPO)\textsuperscript{93}) are often more convenient, but suffer from their own disadvantages, such as the expense of starting materials and the presence of extra components in the eventual equilibrium mixture. However, the versatility of the DHAP aldolases remains their greatest asset as potential process catalysts. The recently reported\textsuperscript{94} use of cross-linked crystals of
RAMA, which display exceptionally high stability and catalytic efficiency even in relatively high concentrations of water-miscible organic solvents, may indicate new opportunities for the application of this catalyst beyond the research laboratory.

The pyruvate aldolases may prove to be even more versatile than the DHAP aldolases as process catalysts. Pyruvate addition to an aldehyde produces a densely and differentially functionalised carbon skeleton (four different oxidation states of carbon being present in four contiguous carbon centres). The high commercial value of many of the products obtained from these aldolases, particularly Neu5Ac aldolase, has led to the development of efficient reactors for their synthesis. Wandrey’s enzyme membrane reactor for the multigramme synthesis of Neu5Ac utilises an epimerase to convert the cheap N-acetylglucosamine, 19 (a non-substrate for Neu5Ac aldolase) into the substrate ManNAc, 17, with an excess of pyruvate to drive the aldol condensation equilibrium in the direction of Neu5Ac (Scheme 1.2.21). The process was successfully scaled up by Glaxo Ltd., who have used simple solvent treatment and crystallisation steps to obtain highly pure Neu5Ac on a multi-kilogramme scale. To date the Glaxo process represents the most successful large-scale enzymatic carbon-carbon bond forming process. Other pyruvate aldolases, notably KDPG aldolase, however also have characteristics which may make them suitable large-scale process catalysts, including high stability in organic cosolvents and entirely kinetic, rather than thermodynamic, patterns of reaction. Oxynitrilases also have considerable potential as catalysts for large-scale processes. The enzymes lend themselves readily to immobilisation, and have been shown to be highly effective in packed bed reactors at high substrate concentrations and in an enzyme membrane reactor. The co-catalyst (HCN) is inexpensive and does not unduly complicate downstream processing. At present the major limitation on their exploitation is catalyst availability; the availability of recombinant oxynitrilases may well provide the key to their successful implementation as process catalysts.

![Scheme 1.2.21. Production of Neu5Ac in the enzyme membrane reactor](image-url)

* N-acetylneuraminic acid derivatives have found application as anti-influenza compounds, as well as in the synthesis of new glycoconjugates many of which have significant biological activity.
1.3. Enzymes in the Synthesis of Carbohydrates and Carbohydrate Mimetics

1.3.1. General Observations

Advances in the synthesis of complex carbohydrates have necessitated parallel advances in biocatalytic technology. The conditions most favourable for enzymatic processes (mild temperatures, aqueous solution, approximately neutral pH) are ideal, and often essential, for the synthesis of molecules containing labile functionalities and sensitive glycosidic linkages; furthermore, the stereochemical precision of enzymatic catalysis often makes it the only feasible method to access selectively functionalised carbohydrates. Figure 1.3 shows a representative carbohydrate, sialyl LewisX (3), which is the focus of a considerable body of current research. Conjugates of sialyl LewisX play a crucial part in a multitude of cell recognition phenomena, and are therefore important synthetic targets. Figure 1.3 suggests some processes in which enzymatic reactions could be invaluable (and indeed have often proven to be so). The scheme shown for sialyl LewisX is general: the techniques illustrated in Figure 1.3 could be applied to any such densely functionalised and sensitive molecule.

The use of glycosidases and glycosyltransferases to catalyse the synthesis of glycosidic linkages and of lipases etc. in the selective manipulation of functional groups has been reviewed recently. Some representative examples of carbohydrate synthesis by carbon-carbon bond forming enzymes appear below.

Enzymatic catalysis also comes into its own in the preparation of carbohydrate analogues, many of which have their own biological activity. Aza sugars, for instance, are highly potent inhibitors of glycosidases, and often possess antiviral activity. While “chiral pool” methods for the synthesis of sugar analogues are extremely valuable, in many cases chemoenzymatic methods have proven equally successful, if not more so. Carbon-carbon bond forming enzymes are often employed to insert the critical chirality in such molecules; other enzymes, such as lipases and oxidoreductases, may also be employed in the subsequent selective functionalisation of the molecule.
Selective protection and functionalisation of hydroxyl groups: lipases, esterases, proteases etc.

Solid phase/combinatorial applications: acylases etc.

Selective protection and functionalisation of hydroxyl groups: lipases, esterases, proteases etc.

Solid phase/combinatorial applications: acylases etc.

Figure 1.3. Enzyme chemistry applied to complex carbohydrates such as Sialyl Lewis (3)

1.3.2. APPLICATIONS OF CARBON-CARBON BOND FORMING ENZYMES

1.3.2.1. Rare sugars/deoxy sugars/higher sugars

DHAP aldolases have been used in the synthesis of sugars and their analogues in combination with over 100 aldehyde substrates. The pyruvate aldolases and DERA have also been extensively used in the synthesis of rare or modified sugars, including deoxy sugars. Both racemic aldehydes (typically from the ozonolysis of an olefin or the unmasking of an acetal) and enantiomerically pure aldehydes (from a lipase-catalysed resolution, e.g. of the epoxide precursors in Scheme 1.2.3 - the epoxides can be subsequently opened with a variety of nucleophiles without affecting the stereochemistry at the α-centre) have been employed. Recently a strategy for the synthesis of a diverse range of sugar structures has been reported, in which the
substrate is generated by Sharpless asymmetric dihydroxylation of a masked \( \alpha,\beta \)-unsaturated aldehyde. The combination of asymmetric dihydroxylation and aldol condensation using any of the four DHAP aldolases gives rise to a huge range of sugar structures of high enantiomeric and diastereomeric purity (Scheme 1.3.1).

\[
\begin{align*}
\text{Scheme 1.3.1. Combined dihydroxylation/enzymatic aldol condensation}
\end{align*}
\]

“Tandem aldol” approaches to diverse sugar structures have recently been reported, involving either sequential enzymatic aldol additions to a dialdehyde substrate\(^{100}\) or formation of a substrate for one enzyme by aldol condensation of an even simpler substrate catalysed by a complementary enzyme.\(^{63,100}\) Some examples of these approaches can be found in Scheme 1.3.2. “Tandem aldol” reactions of this type are extremely versatile, and have the potential to produce a huge range of diastereomeric sugar analogues by simple variation of the substituent(s) on the precursor aldehydes. It has been noted that this approach may be particularly suitable to the development of combinatorial libraries of sugars and their analogues.\(^{52}\)

a) DERA/FDP aldolase\(^{63}\)

\[
\begin{align*}
\text{Scheme 1.3.2. Combined dihydroxylation/enzymatic aldol condensation}
\end{align*}
\]
The ketoses which are the products of aldolase-catalysed reactions can be converted to aldoses by application of an isomerase \(^{60,101,102}\) or by use of “inversion” \(^{103}\) or “pseudoisomerisation” \(^{61}\) strategies (Scheme 1.3.3). In the former strategy, a half-protected dialdehyde such as 2,2-diethoxyacetaldehyde (31) is used as substrate; the ketone moiety of the product is reduced stereoselectively using a chemical reducing agent or a polyol dehydrogenase enzyme, and finally the aldehyde is unmasked. The product is an “inverted” aldose, with stereochemistry at the portion of the molecule remote from the anomeric centre controlled by the aldolase. In the latter strategy, the thio analogue 32 of DHAP is employed as the donor. Stereoselective reduction of the ketone moiety of the product is followed by hydrolysis of the thiophosphate ester, conversion to a thioether, oxidation, Pummerer rearrangement, and final deprotection.
with diisobutylaluminium hydride (DIBAL-H). The stereochemistry of the portion of
the molecule adjacent to the anomeric centre in the final product is controlled by the
aldolase, making these two techniques complementary.

a) “inversion” strategy

\[
\text{(i) DHAP/RAMA; (ii) Phosphatase; (iii) L-iditol dehydrogenase/NADH; (iv) deprotection}
\]

b) “pseudoisomerisation” strategy

\[
\text{Scheme 1.3.3. Ketose to aldose interconversions}
\]

1.3.2.2. Aza sugars/thio sugars

Polyhydroxylated pyrrolidines and piperidines (loosely known as aza sugars)
are readily accessible using aldolase methodology. Aldehydes possessing a suitably
protected amine group (e.g. an azide) have been employed in the synthesis of the
6-membered aza sugars deoxynojirimycin (DNJ, 33) and deoxymannojirimycin (34)
(Scheme 1.3.4). The racemic precursor, 3-azido-2-hydroxypropanal 35, yields
a mixture of diastereomeric final products; single enantiomers of 35 can be obtained by ring opening of glyceraldehyde diethyl acetal 1 (accessible as shown in Scheme 1.2.3) with sodium azide.\textsuperscript{53} Five-membered aza sugars can be accessed analogously, e.g. from 2-azido-3-hydroxypropanal 36\textsuperscript{105,106} (used as racemate) or (enantiomerically pure) 3-acetamido-2-azidopropanal.\textsuperscript{107} Castanospermine derivatives can be similarly obtained from suitably substituted sialic acids obtained \textit{via} Neu5Ac aldolase.\textsuperscript{108}

\textbf{a) six-membered aza sugars}\textsuperscript{53,104}

\begin{equation}
\begin{array}{c}
\text{(35)} \\
\begin{array}{c}
\text{N}_3 \\
\text{OH} \\
\text{O} \\
\text{P}
\end{array}
\end{array}
\begin{array}{c}
\text{O} \\
\text{P}
\end{array}
\xrightarrow{\text{FDP aldolase}}
\begin{array}{c}
\text{(33)} \\
\begin{array}{c}
\text{N}_3 \\
\text{OH} \\
\text{O} \\
\text{P}
\end{array}
\end{array}
\end{equation}

\textbf{b) five-membered aza sugars}\textsuperscript{105,106}

\begin{equation}
\begin{array}{c}
\text{(36)} \\
\begin{array}{c}
\text{HO} \\
\text{N}_3 \\
\text{P}
\end{array}
\end{array}
\begin{array}{c}
\text{O} \\
\text{P}
\end{array}
\xrightarrow{\text{FDP aldolase}}
\begin{array}{c}
\text{(34)} \\
\begin{array}{c}
\text{N}_3 \\
\text{OH} \\
\text{O} \\
\text{P}
\end{array}
\end{array}
\end{equation}

\textbf{Scheme 1.3.4. Aldolase-mediated routes to N-containing sugars}

\textit{Homooazasugars}, bearing an extra carbon substituent at C-1, have been accessed by a divergent synthesis involving Sharpless epoxidation, ring opening with azide ion, unmasking of a protected aldehyde moiety, and RAMA-catalysed aldol condensation.\textsuperscript{109} Depending on the choice of olefinic starting material and of (+)-or (-)-tartrate co-catalyst in the Sharpless epoxidation, both C-1 epimers of 1-homonojirimycin (37\textalpha, 37\textbeta) and of 1-homomannojirimycin (38\textalpha, 38\textbeta) can be accessed. (\textbf{Scheme 1.3.5}).
Scheme 1.3.5. Aldolase-mediated synthesis of homoazasugars

Thio sugars, in which the endocyclic oxygen atom is replaced with a sulfur atom, can be obtained in an analogous fashion from the ring opening of enantiomerically pure 1 with a nucleophilic thiol followed by aldolase-mediated condensation with DHAP.\textsuperscript{10,11}

1.3.2.3. Cyclitols

Cyclitols (carbocyclic sugar analogues) are also, in principle, accessible using aldolase enzymes, though there are fewer such reports in the literature. Two recent strategies for the construction of such compounds involve an aldolase-mediated condensation followed by an \textit{in situ} Horner-Wadsworth-Emmons cyclisation,\textsuperscript{112} and a post-enzymatic, Lewis acid-catalysed intramolecular nitroaldol reaction\textsuperscript{113} (Scheme 1.3.6). Other routes to important carbocycles such as (-)-shikimic acid have been envisaged,\textsuperscript{114} though not altogether successfully.
a) *via* Horner-Wadsworth-Emmons cyclisation\textsuperscript{112}

\[
\ce{EtO2C&CN &Et + CO2 &OH} \xrightarrow{\text{RAMA}} \ce{EtO2P&CN &OH &OH &OH &P}
\]

\[
\begin{array}{c}
\text{OH} \\
\text{OH} \\
\text{NC}
\end{array}
\]

b) *via* nitroaldol reaction\textsuperscript{113}

\[
\ce{O2N &OH &CO2 &OH} \xrightarrow{\text{RAMA}} \ce{O2N \text{OAc} &OAc &OAc &OAc &OAc &OAc}
\]

\[
\begin{array}{c}
\text{OH} \\
\text{OH} \\
\text{AcO}
\end{array}
\]

Scheme 1.3.6. Aldolase-mediated synthesis of cyclitols
1.4. Transketolase

1.4.1. Background and Biochemical Studies

Transketolase occupies a pivotal place in the metabolic regulation of biological systems. As a link between the glycolytic (degradative) and pentose phosphate pathways, it has a controlling rôle in the supply of ribose units for nucleoside biosynthesis, and (in micro-organisms) in the supply of erythrose 4-phosphate into the shikimate pathway for aromatic amino acid biosynthesis.\textsuperscript{115,116} Assays for transketolase have long been of clinical interest, since a number of conditions associated with thiamine deficiency have an impact on the \textit{in vivo} activity of TK.\textsuperscript{117}

Transketolase was first identified by Racker and co-workers in 1953, and purified from yeast.\textsuperscript{118} They were able to show the dependence of transketolase upon divalent metal cations (usually Mg\textsuperscript{2+}) and TPP (27) for its activity. In the course of this study it was shown that, in addition to the phosphorylated sugars involved in the biological reactions, the lithium salt of hydroxypyruvic acid (LiHPA, 39) was accepted by the enzyme as a donor substrate. The byproduct in this case was not another sugar molecule but carbon dioxide, rendering the reaction irreversible.\textsuperscript{118}

Subsequent investigations into the properties of yeast TK\textsuperscript{119} showed the enzyme to have a pH optimum of 7.6. The equilibrium constants for the biological reactions (\textbf{Scheme 1.2.19}) were found to be = 1.2 with ribose 5-phosphate as acceptor and = 10 with erythrose 4-phosphate as acceptor. Sulfate, phosphate, and oxidised TPP were found to inhibit the enzyme. Other sources of transketolase — notably spinach\textsuperscript{120,121} and \textit{Escherichia coli}\textsuperscript{78,116,122,123} — have been described.

The ketol condensation reaction catalysed by transketolase represents the addition of an acyl anion equivalent or “active glycolaldehyde” unit\textsuperscript{124} to the carbonyl group of an appropriate aldehyde. This high activation energy process is mediated by the cofactor, TPP,\textsuperscript{124,125} which is easily deprotonated at the 2-position of the thiazolium ring.\textsuperscript{125} The first step of the reaction is nucleophilic condensation of the anion of TPP with the carbonyl group of the ketol donor as shown in \textbf{Scheme 1.4.1} (LiHPA shown as donor). Decarboxylation (or release of a sugar byproduct \textit{in vivo}) results in the formation of a stabilised carbanion which then performs a nucleophilic addition to the acceptor aldehyde, and the final condensation product is released with the regeneration of the anion of TPP. The sequence of events at the active site of the enzyme is thus:
Donor in - byproduct out - acceptor in - condensation product out
- a so-called “Ping-Pong Bi-Bi” mechanism.

Scheme 1.4.1. Catalytic cycle for TPP-mediated carbon-carbon bond formation

1.4.2. CARBON-CARBON BOND FORMATION USING TRANSKETOLASE

Transketolase-catalysed carbon-carbon bond formation is both stereospecific, in
that the new chiral centre formed in the product has the (S)-configuration (the same
configuration as C-3 of the natural donor substrates), and stereoselective, in that
although D-(R)-glyceraldehyde 3-phosphate (R)-16 is accepted by the enzyme, its
enantiomer is not.\textsuperscript{119} Subsequent \textit{in vitro} studies of the reactivity of transketolase from
yeast,\textsuperscript{75,77} spinach\textsuperscript{75} and \textit{E. coli}\textsuperscript{78} have confirmed the generality of this behaviour, and
shown that transketolase-mediated condensation of an $\alpha$-substituted aldehyde with LiHPA produces chiral triols with the $[3S, 4R]$ or "D-threo" stereochemistry. The specificity of carbon-carbon bond formation is near-total with TK from yeast and E. coli, producing an (S)-chiral centre, though with spinach TK some loss of enantiospecificity has been observed with certain substrates, notably $\alpha$-unsubstituted substrates.\textsuperscript{75,126} Non-phosphorylated, as well as phosphorylated, substrates are accepted by the enzyme; the best substrates for TK are $\alpha$-hydroxyaldehydes of $(R)$-configuration, though $\alpha$-oxo-and $\alpha$-unsubstituted aldehydes are also accepted.\textsuperscript{75,77,78} The generalised TK-catalysed reaction \textit{in vitro}, with HPA as donor, is shown in \textbf{Scheme 1.4.2}.

The stereochemistry of product formation with TK is the same as that obtained with FDP aldolase. Both enzymes accept a wide range of aldehydes as substrates, besides the natural ones. However, TK adds a two-carbon unit, rather than a three-carbon unit; it produces unphosphorylated products, which makes the course of reaction easier to follow and can simplify product isolation; moreover, its selectivity for $(R)$-$\alpha$-hydroxyaldehydes means that TK can be employed in the kinetic resolution of racemic substrates, forming a single condensation product, whereas FDP aldolase would tend to produce diastereomeric mixtures of products.\textsuperscript{75,76} TK therefore appears to possess a number of advantages as a useful catalyst for asymmetric carbon-carbon bond formation. It does, however, share a number of the disadvantages of FDP aldolase: namely, availability of the catalyst and expense of the necessary co-substrate (LiHPA). Any attempt to develop TK as a viable process catalyst must therefore address these factors.

\begin{center}
\textbf{Scheme 1.4.2. Generalised TK-catalysed reaction}
\end{center}
1.4.3. Transketolase from *Escherichia coli*

1.4.3.1. The transketolase project

Methodology for enzymatic carbon-carbon bond formation on a large scale is much less well-developed than that required for other large-scale enzymatic processes. The use of EMR technology in combination with *e.g.* Neu5Ac aldolase\(^95\) and oxynitrilase\(^82\) has begun to make such processes viable, but only a handful of processes are so far reported. The transketolase project, of which the work reported in this thesis forms a part, aims to take a collaborative, multi-disciplinary approach\(^127\) to the development of a biocatalytic process which can eventually be operated on a large scale. The interaction between the various disciplines involved in the transketolase project is illustrated in Figure 1.4.

The first limitation to be overcome in the development of a biocatalytic process is availability of the enzyme. The high cost of yeast transketolase and the awkwardness of extracting appreciable quantities of enzyme from spinach has led to a recent interest in bacterial sources of transketolase, from which large quantities of enzyme can in principle be produced via overexpression and fermentation technology. An ideal enzyme for study therefore was transketolase from *E. coli*.

The gene encoding transketolase in *E. coli* was originally cloned by Frost\(^115,116\) into a low copy number vector, as part of an investigation into regulation of the flux of material into the shikimate pathway for aromatic amino acid biosynthesis in bacteria. The plasmid containing the *tkt* gene (the major gene responsible for transketolase production in *E. coli*) was labelled pKD112A\(^115\). Subsequent genetic manipulation of this construct\(^78,122\) produced plasmids pQR182 and pQR183, which enabled the production of TK with a fourfold increase in specific activity when small-scale fermentations were performed using bacteria (*E. coli* strain JM107) transformed with this plasmid. Overexpression of TK by the organism was controlled by a transketolase promoter in the *tkt* sequence. However, these constructs did not perform well in larger-scale (20 dm\(^3\)) fermentations,\(^122,127\) and improved constructs were developed.

---

\(^{*}\) The project represents a collaboration between chemists, structural biochemists, biochemical engineers, enzymologists and molecular biologists based at the universities of Edinburgh and Exeter and University College, London.
The next generation of constructs were pQR700 and pQR701, which proved to be stable in 20 dm$^3$ fermentations and highly amenable to large-scale enzyme production. In a 1000 dm$^3$ fed-batch fermentation, *E. coli* JM107 transformed with pQR700 produced 4 g dm$^{-3}$ soluble intracellular transketolase. Given that the specific activity of the enzyme was shown to be approximately 23 U mg$^{-1}$, this figure represents $\approx 0.028$ mol enzyme, or approximately 43% of the total intracellular protein produced by the organism. Simple downstream processing produced a
partially purified (approx. 70%) extract of TK which was highly suited to laboratory-scale preparative biotransformations. Further improvements to the expression system for *E. coli* transketolase have subsequently been made, in the hope of developing *E. coli* strains which could be grown without the selective pressure of antibiotic in the fermenter. Subsequent genetic work has been directed towards modification of the protein itself, with addition of polylysine tails to assist enzyme immobilisation studies, and selective modification of active site residues. In parallel work, Sprenger *et al.* have developed constructs to overexpress transketolase in *E. coli* and obtain the enzyme in highly purified form.

1.4.3.2. *Structural features of E. coli* transketolase

The high expression of transketolase in JM107/pQR700 has enabled quantities of highly pure enzyme to be prepared from which crystals have been grown for X-ray structural studies. The enzyme has been shown to be a homodimer, with a molecular weight of ≈ 73 kDa per subunit. The holoenzyme possesses two active sites, located between the two subunits, with residues from each subunit participating in binding and catalysis. There is ≈ 43% sequence homology between the *E. coli* and yeast enzymes (the yeast enzyme also forms a homodimer of comparable size). Although modelling studies have given some indication as to the binding of the TPP-donor complex in the active site, the mode of binding of the acceptor is still unclear.

1.4.3.3. *Substrate specificity*

The specificity of *E. coli* TK with respect to various acceptor aldehydes has been probed by means of an enzyme-linked assay. The principle of the assay is shown in Figure 1.5. Aliquots of a small-scale reaction mixture containing the enzyme, cofactors and substrates in buffer are removed at regular intervals, and the consumption of LiHPA in the reaction mixture with time is monitored via an enzyme-linked depletion of NADH (10). NADH has a strong UV absorption at 340 nm, the change in the magnitude of which after addition of the linking enzyme (*glycerate dehydrogenase* (Gly-DH), which reduces hydroxypyruvate to glycerate in the presence of NADH) is proportional to the concentration of LiHPA in the reaction mixture.

Substrate specificity results for a variety of aldehydes, both sugars and non-sugar-like molecules, are reported in Table 1.3. The results are reported in

---

† Unless otherwise specified, the transketolase employed in the biotransformations described in this thesis is transketolase extracted from JM107/pQR700.
Figure 1.5. The hydroxypyruvate assay

the form of a rate parameter $v_{rel}$ representing the initial rate of reaction relative to glycolaldehyde (40) (the best acceptor) which has a $v_{rel}$ of 100%. The overwhelming preference of the enzyme for (R)-$\alpha$-hydroxylated aldehydes is clear from this data.
α-Oxo-substituted and α-unsubstituted aldehydes are also accepted as substrates, but the selectivity for (R)-α-hydroxyaldehydes is almost total, as demonstrated by the relative rates for D-, L- and racemic glyceraldehyde (41).

\[
\begin{array}{ccc}
\text{HO} & \text{OH} & \text{Me} \\
(40) & 100 & 21 \\
(\text{R})-41 & 66 & (\pm)-41 \\
(\text{S})-41 & 0 & \\
\text{OH} & \text{OH} & \text{Ph} \\
13 & 4 & 30 \\
\end{array}
\]

Table 1.3. Substrate specificity of transketolase from *E. coli* JM107/pQR183

1.4.3.4. Sources of hydroxypyruvate

A variety of phosphorylated ketosugars have been shown to function as donor substrates in TK-catalysed reactions, the key stereochemical requirement being the (S)-configuration at the 3-position. However the fact that these donors produce equilibrium mixtures restricts their usefulness in synthesis. Of the analogues of HPA
so far investigated, none have been accepted by the enzyme.\textsuperscript{77} Thus, at present any practical development of methodology for the use of TK requires hydroxypyruvate as the donor substrate; and the high cost and difficulty of preparation of LiHPA present a serious limitation to the large-scale use of TK.\textsuperscript{128}

HPA can be formed by the action of D-amino acid oxidase on D-serine; this technique has been used for \textit{in situ} generation of HPA in transketolase-based biotransformations.\textsuperscript{133} However, the enzyme requires molecular oxygen for its activity, which may have a destabilising effect upon other enzymes, substrates or cofactors; moreover, the byproduct, H\textsubscript{2}O\textsubscript{2}, has to be constantly removed by the action of catalase. The presence of unreacted L-serine in the reaction mixture when racemic serine is used as precursor may also complicate work-up. This method has reportedly been used with some success in an EMR with transketolase;\textsuperscript{134} however it was felt for the purposes of this project that a custom chemical synthesis of HPA was more appropriate.

The literature synthesis of LiHPA from bromopyruvic acid\textsuperscript{135} suffers from modest yield (23\% for 2.5 g) and high dilution. A slight modification of the reported procedure\textsuperscript{128} yielded essentially pure LiHPA monohydrate in 51\% yield on a 25 g scale. Thus a supply of co-substrate was readily available for synthetic use. An analogous synthesis of the more water-soluble potassium salt proceeded in lower yield (36\%) but at effectively half the dilution required for LiHPA.

1.4.3.5. \textit{Process development}

The design and optimisation of reactors for transketolase-catalysed biotransformations is currently under study at University College London.\textsuperscript{127} Investigation of the enzyme stability, and the behaviour of substrates and products under possible reaction conditions, along with knowledge of the rate equation for the reaction enables a rational approach to reactor design.\textsuperscript{136} Factors under consideration\textsuperscript{137} include reaction monitoring, the maintenance of an optimum pH, the evolution of carbon dioxide during the reaction, and the possible advantages and disadvantages of enzyme immobilisation and \textit{in situ} product removal.\textsuperscript{127}

1.4.4. \textbf{AIMS OF THIS PROJECT}

Although transketolase-based methodology has been used extensively in the synthesis of unusual sugars, including D-\textit{glycero-D-ido-octulose},\textsuperscript{119}
3-deoxy-D-arabino-heptulosonic acid\textsuperscript{15} and D-sedoheptulose,\textsuperscript{138} the potential of TK to produce a range of novel homochiral compounds by reaction with functionalised \textit{non-sugar} aldehydes has yet to be realised. Whitesides,\textsuperscript{77} Demuyck\textsuperscript{75} and Effenberger\textsuperscript{76,104,110} have reported a range of preparative-scale TK reactions using a variety of readily available racemic \(\alpha\)-hydroxyaldehydes, with concomitant kinetic resolution of the (S)-aldehydes. Doubly \textsuperscript{13}C-labelled sugars,\textsuperscript{133} deoxysugars\textsuperscript{75,139} and sugars with synthetically useful functional groups such as olefins,\textsuperscript{77} thiols\textsuperscript{110} and thioacetals\textsuperscript{140} have also been prepared (\textbf{Scheme 1.4.3}). However, only a handful of these potentially useful synthetic intermediates have been used in the synthesis of interesting products. Despite promising early examples of the usefulness of TK, such as the synthesis of the five-membered aza sugar 1,4-dideoxy-1,4-imino-D-arabinitol \textsuperscript{42} from racemic 3-azido-2-hydroxypropanal \textsuperscript{35} by Effenberger and co-workers,\textsuperscript{104} and Whitesides’ TK-based synthesis\textsuperscript{141} of the beetle pheromone (\(+\)-exo-brevicomin \textsuperscript{43} (\textbf{Scheme 1.4.3}), its use as a versatile catalyst has not been generally demonstrated.

The aim of this thesis is to demonstrate the utility of TK as a catalyst in the chemoenzymatic synthesis of potentially valuable, enantiomerically pure molecules, making use of the ready availability of both TK and hydroxypyruvate as described above. The following chapters are subdivided into investigations into the synthesis of novel acceptor substrates (\textbf{Chapter Two}), development of methodology for TK-based biotransformations (\textbf{Chapter Three}), and the use of transketolase-derived chiral triols in the synthesis of natural product analogues (\textbf{Chapter Four}).

\textbf{a) \textsuperscript{13}C-labelled sugars}\textsuperscript{133}

\begin{align*}
\text{\textsuperscript{*}OH} & \quad \text{D-amino acid oxidase} \quad \text{\textsuperscript{*}OH} \\
\text{CO}_{2}\text{H} & \quad \text{L-serine} \quad \text{\textsuperscript{*}OH} \\
\text{\textsuperscript{*}NH_2} & \quad \text{TK/Mg}^{2+}/\text{TPP} \quad \text{\textsuperscript{*}OH} \\
\end{align*}

\textbf{b) unsaturated sugars}\textsuperscript{77}

\begin{align*}
\text{\textsuperscript{OH} + \textsuperscript{O}_2C} & \quad \text{TK/Mg}^{2+}/\text{TPP} \quad \text{\textsuperscript{OH} \textsuperscript{O}} \\
\text{C} & \quad \text{CO}_2 \quad \text{\textsuperscript{OH} \textsuperscript{O}} \\
\end{align*}
c) **deoxysugars**\(^{139}\)

\[
\text{O} \quad \text{S} \quad \text{OH} \quad \text{O}_{2}\text{C} \quad \text{OH} \quad \text{OH} \quad \text{TK/Mg}^{2+}/\text{TPP} \quad \text{CO}_{2} \\
\text{OH} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \text{OH}
\]

**Furaneol**

---

d) **sugars with thioacetal groups**\(^{140}\)

\[
\text{O} \quad \text{OEt} \quad \text{Et} \quad \text{OEt} \quad \text{TK/Mg}^{2+}/\text{TPP} \quad \text{CO}_{2} \\
\text{ OH} \quad \text{S} \quad \text{OH} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \text{OH}
\]

---

e) **aza sugars**\(^{104}\)

\[
\text{N} \quad \text{OH} \quad \text{TK/Mg}^{2+}/\text{TPP} \quad \text{HPA} \quad \text{CO}_{2} \\
\text{O} \quad \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH}
\]

(42)

---

f) **(+)-exo-brevicomin**\(^{141}\)

\[
\text{OH} \quad \text{TK/Mg}^{2+}/\text{TPP} \quad \text{HPA} \quad \text{CO}_{2} \\
\text{O} \quad \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH}
\]

(43)

---

**Scheme 1.4.3. Some synthetic applications of transketolase**
Chapter Two
2.1. A Route to Enantiomerically Pure α-Hydroxyaldehydes

2.1.1. α-Hydroxyaldehydes: A Literature Survey

Scheme 2.1.1. Some synthetically useful transformations of glycidaldehyde diethyl acetal (1)

The best substrates for *E. coli* transketolase are α-hydroxyaldehydes (see Section 1.4.3.3 and Table 1.3). The enzyme is selective for aldehydes with (R)-configuration at the α-carbon, and therefore can be used to resolve a racemic mixture of α-hydroxyaldehydes. Racemic mixtures of α-hydroxyaldehydes are readily available from simple precursors which can be generated on a multigramme scale in a research laboratory. Glycidaldehyde diethyl acetal, 1, for instance, can readily be synthesised by epoxidation of acrolein diethyl acetal, and ring-opened with a variety of nucleophiles (Scheme 2.1.1) to yield a β-functionalised 2-hydroxypropanal acetal or (if a Grignard reagent is employed as the nucleophile) an α-hydroxyacetal with extended carbon chain. Simple unmasking of the acetal under acidic conditions releases the free α-hydroxyaldehyde. Alternatively, an allylic
alcohol may be employed; cleavage of the olefin by ozonolysis followed by work-up in the presence of dimethyl sulfide (DMS) generates an $\alpha$-hydroxyaldehyde directly.\textsuperscript{77} These methods have been extensively used to generate sugarlike, non-sugar precursors in the synthesis of carbohydrate analogues using aldolases, since $\alpha$-hydroxyaldehydes are also very effective substrates for aldolases.

Although in most cases the products of TK-catalysed reactions are easily separable from resolved $\alpha$-hydroxyaldehydes using chromatography,\textsuperscript{76,77} the use of racemic mixtures of aldehydes does have a number of implications for the possible large-scale use of TK in synthesis. Product isolation by chromatography on a large scale is tedious, and in most industrial processes is prohibitively expensive. Moreover, $\alpha$-hydroxyaldehydes tend to be highly reactive compounds; they are susceptible to oligomerisation in aqueous solution, which can lead to the formation of gelatinous suspensions, further complicating work-up procedures; they may also have a strong inhibitory effect upon the enzyme, by condensation with active residues \textit{e.g.} lysine (Schiff base formation) on the protein surface, leading to denaturation. In principle, therefore, an inexpensive \textit{general} method for the production of novel $\alpha$-hydroxyaldehyde substrates \textit{in enantiomerically pure form} would be an advantage to the development of TK as a process catalyst. Stoichiometric amounts of substrates could be employed in the biotransformation, and simple TLC or HPLC analysis could be used to follow the course of a reaction.

Methods for the synthesis of enantiomerically pure $\alpha$-hydroxyaldehydes are relatively sparse in the literature; many of the reported procedures are actually syntheses of $O$-protected derivatives. The high reactivity of $\alpha$-hydroxyaldehydes and their relative instability is to blame for the paucity of successful methods for the preparation of isolated enantiomerically pure $\alpha$-hydroxyaldehydes.\textsuperscript{60,142} Their tendency towards oligomerisation can make isolating and characterising the compounds extremely difficult. Furthermore, the lability of the $\alpha$-proton means that $\alpha$-hydroxyaldehydes are prone to ready isomerisation. Successful synthesis of homochiral $\alpha$-hydroxyaldehydes therefore will necessitate methods for their handling and characterisation which avoid racemisation. Three general methods are reported in the literature for the synthesis of such compounds: they are chiral auxiliary, chiral pool and enzymatic methods.
Scheme 2.1.2. Mukaiyama's bicyclic aminal approach to the synthesis of homochiral \(\alpha\)-hydroxyaldehydes

The most extensively used of the chiral auxiliary techniques is Mukaiyama's bicyclic aminal method.\(^{143,144}\) The method involves addition of an organometallic reagent to an \(\alpha\)-ketoaldehyde in which the aldehyde is masked as an aminal derived from L-proline (Scheme 2.1.2). Phenylglyoxal \((44, R^1 = \text{Ph})\) was originally employed as the precursor, but variously substituted \(\alpha\)-ketoaminals can be formed by condensation of the prolinediamine \(45\) with methyl glyoxylate followed by attack by a
Grignard reagent on the resulting aminal-ester.\textsuperscript{144} Yields of the key precursors can be increased by conversion of the intermediate aminal-ester to the Weinreb amide (RCON(OCH\textsubscript{3})CH\textsubscript{3}) before the first addition of Grignard reagent.\textsuperscript{145} The bicyclic aminal intermediates are formed as the \textit{exo} diastereomers exclusively. Attack of a carbon nucleophile, such as a Grignard reagent\textsuperscript{143} or a zinc enolate,\textsuperscript{144} to the \(\alpha\)-ketoaminal occurs with extremely high diastereoselectivity, since the orientation of the incoming nucleophile can be controlled by chelation, depending on the nature of the metal ion employed.\textsuperscript{145} This methodology has been extensively used in the preparation of chiral \(\alpha\)-hydroxyaldehyde intermediates in the synthesis of natural products such as (-)-malyngolide,\textsuperscript{144} and is compatible with sensitive functionality such as phosphonate groups;\textsuperscript{145} the major disadvantage is that the method is generally employed for the synthesis of tertiary \(\alpha\)-hydroxyaldehydes, whereas most aldehydes of interest as substrates for TK are secondary \(\alpha\)-hydroxyaldehydes.

Other chiral auxiliary-based methods for the synthesis of enantiomerically pure \(\alpha\)-hydroxyaldehydes have also been reported (Scheme 2.1.3). Eliel \textit{et al.}\textsuperscript{146} employed the anion of an enantiomerically enriched six-membered oxathiane auxiliary to add diastereofacially to benzaldehyde; subsequent oxidation of the resulting hydroxyl group was followed by a facially selective, chelation controlled addition of a Grignard reagent to yield the optically enriched \(O\)-methylated \(\alpha\)-hydroxyaldehyde \textit{46} after cleavage of the oxathiane ring. Aggarwal and co-workers\textsuperscript{147} have more recently employed \textit{trans}-1,3-dithiane-1,3-dioxide \textit{47} as a chiral acyl anion equivalent. Addition to an aldehyde proceeds with high (> 95\%) diastereoselectivity; after hydroxyl protection, the auxiliary can be removed by Pummerer rearrangement and transthiolesterification, and the resulting thioesters converted to aldehydes by reduction with triethylsilane over palladium/charcoal.\textsuperscript{148} \(\alpha,\beta\)-Unsaturated \textit{cis}-dioxolanones such as \textit{48}, formed from mandelic acid, have also been employed to exert diastereocontrol in the addition of carbon nucleophiles to precursors of \(\alpha\)-hydroxyaldehydes;\textsuperscript{149} ozonolysis of the olefinic bond then produced \(O\)-protected \(\alpha\)-hydroxyaldehydes of high \textit{e.e.}; the process does however invariably involve a significant degree of side reactions, notably allylic addition. A slightly different technique involved the \(2,3\)-sigmatropic Wittig rearrangement of metalated (S)-1-amino-2-(1-ethyl-1-methoxypropyl)pyrrolidine (SAEP) hydrazones,\textsuperscript{150} yielding \(\alpha\)-hydroxyaldehydes of high enantiomeric purity, though some loss of optical integrity at the \(\alpha\)-chiral centre was observed during ozonolytic cleavage of the hydrazone moiety; these \(\alpha\)-hydroxyaldehydes were not isolated in unprotected form but as the \(O\)-silylated derivatives.
a) 1,3-oxathiane as chiral auxiliary

\[ \text{Me}^{(S)}_\text{S} \quad \text{i) nBuLi} \quad \text{Me}^{(R)}_\text{S} \quad \text{DMSO/} \quad \text{Me}_\text{MgI} \]

\[ \text{Me}_\text{Ph} \quad \text{ii) PhCHO} \quad \text{Et}_3\text{N} \quad \text{MeO} \quad \text{Me} \quad \text{OH} \]

\[ \text{MeO}_\text{Ph}^{(S)} \quad \text{i) NaH/CH}_3\text{I} \quad \text{Me}^{(R)}_\text{S} \quad \text{ii) deprotection} \]

b) trans-1,3-dithiane-1,3-dioxide as chiral auxiliary

\[ \text{R}_\text{C}^- \text{H} \quad \text{i) NaHMDS/} \quad \text{R}^- \text{CHO} \quad \text{TFAA} \quad \text{LiOH/} \quad \text{EtOH} \quad \text{EtS}^- \]

\[ \text{R}^- \text{OTHP} \quad \text{i) protection} \quad \text{(THP)} \quad \text{R}_{\text{OTHP}} \quad \text{Dimeric intermediates} \]

\[ \text{R}^- \text{OTHP} \quad \text{Omp} \quad \text{OH} \]

\[ \text{R}^- \text{SEt} \quad \text{O} \quad \text{O} \]

\[ \text{R}^- \text{O} \quad \text{CO} \quad \text{CO}_2\text{H} \]

c) cis-dioxolanone as chiral auxiliary

\[ \text{R}^- \text{MgBr} \quad \text{Ph} \quad \text{R}^- \text{CO}_2\text{H} \quad \text{O}_3 \quad \text{R}^- \text{CO}_2\text{H} \]
The naturally occurring “chiral pool” has provided a great many novel, chiral \( \alpha \)-hydroxyaldehydes, usually in protected form, as intermediates in the synthesis of natural products etc. Sugars (naturally occurring \( \alpha \)-hydroxylated carbonyl compounds) have frequently been the source of chirality in these building blocks. Small molecules such as hydroxyl-protected lactate\(^{151-153}\) and glycerate\(^{154}\) esters and amides have been frequently employed as precursors to protected \( \alpha \)-hydroxyaldehydes such as (S)-O-benzyllectaldehyde 49\(^{153}\) and (R)-and (S)-2-O-benzylglyceraldehyde (2BG) 50,\(^{154}\) the aldehyde being formed by reduction of the carboxylate derivative using diisobutylaluminium hydride\(^{151,152}\) (DIBAL-H)\(^*\) or sodium bis (2-methoxyethoxy)aluminium hydride (“Vitride”\(^*\)),\(^{153}\) or by periodate cleavage of a reduced tartrate derivative\(^{154}\) (Scheme 2.1.4). Hydrolysis of dichloromethyl-substituted sugars has more recently been employed as an effective route to higher sugar-based \( \alpha \)-hydroxyaldehydes.\(^{155}\) Zinc-mediated cleavage of halo-substituted pentose rings (the Vasella reaction\(^{156}\)) has also been used by Whitesides et al., to prepare \( \gamma,\delta \)-unsaturated hydroxyaldehydes in high enantiomeric purity.\(^{77}\) Finally, selective oxidation has occasionally been employed to good effect, in some cases, partial loss of optical integrity was observed when DIBAL reduction of the ester was employed, e.g. in Baker’s attempted synthesis of O-benzyllectaldehyde 49 (ref. 151)
e.g. Wenger’s use of the Pfitzner-Moffat oxidation of the primary hydroxyl group of a tartrate-derived chiral 1,2-diol in the first synthesis of the amino acid component of cyclosporine.\textsuperscript{157} These techniques are illustrated in Scheme 2.1.4.

Amino acids are also a valuable source of chiral starting materials, but have been employed relatively infrequently in the preparation of enantiomerically pure $\alpha$-hydroxyaldehydes. Larchevêque and Petit\textsuperscript{158,159} have prepared ethyl (R)- and (S)-glycidate \textsuperscript{51} from D- and L-serine \textsuperscript{52}; ring opening of the epoxide with an organocopper reagent, silyl protection of the hydroxyl group, and reduction with DIBAL yielded protected homochiral $\alpha$-hydroxyaldehyde intermediates for the syntheses of leukotrienes\textsuperscript{158} and an insect pheromone.\textsuperscript{159}

a) protected lactaldehyde derivatives\textsuperscript{151}

\[
\begin{align*}
\text{Methyl L-lactate} & \xrightarrow{\text{OPG}} \text{OPG-OMe} & \text{DIBAL-H} & \xrightarrow{\text{PG}} \text{OPG} \\
& & & \text{PG = TBS, TBDPS, MOM}
\end{align*}
\]

b) $O$-benzyl lactaldehyde (49)\textsuperscript{153}

\[
\begin{align*}
\text{Ethyl L-lactate} & \xrightarrow{i) \text{Me}_2\text{NH} \quad ii) \text{BnCl/base}} \text{OPG} & \xrightarrow{\text{Vitride}} \text{OPG} \\
& \text{OCH}_2\text{Ph} & \text{OCH}_2\text{Ph} (49)
\end{align*}
\]

c) 2-$O$-benzylglyceraldehyde (50)\textsuperscript{154}

\[
\begin{align*}
\text{EtO}_2\text{C} & \xrightarrow{i) \text{PhCHO/pTsOH} \quad ii) \text{LiAlH}_4/\text{AlCl}_3} \text{OH} & \xrightarrow{\text{NaIO}_4} \text{OH} \\
& \text{PhCH}_2\text{O} & \text{PhCH}_2\text{O} (50)
\end{align*}
\]

d) Vasella ring cleavage\textsuperscript{77}

\[
\begin{align*}
\text{D-ribose} & \xrightarrow{\text{I}} \text{OMe} & \xrightarrow{\text{Zn/HOAc}} \text{OH} & \xrightarrow{\text{H}^+} \text{H}_{2}\text{O} \\
& \xrightarrow{\text{H}_{2}\text{O}} \text{OH}
\end{align*}
\]
e) selective oxidation

\[ (+)-\text{Diethyl tartrate} \rightarrow \text{Moffat oxidation} \rightarrow \text{selective oxidation} \]

f) from ethyl (R)-glycidate

\[
\begin{align*}
\text{HO} & \rightarrow \text{CO}_2\text{Et} & \text{ii) protection} \\
\text{NH}_2 & \rightarrow \text{CO}_2\text{Et} & \text{i) NaNO}_2/\text{HBr} \\
\text{CO}_2\text{H} & \rightarrow \text{OPG} & \text{ii) KOH} \\
\text{(51)} & \rightarrow \text{OPG} & \text{iii) Et}_2\text{SO}_4 \\
\end{align*}
\]

DIBAL-H

Scheme 2.1.4. “Chiral pool” syntheses of α-hydroxyaldehydes

Enzymatic methods for the preparation of homochiral α-hydroxyaldehydes have the advantage that the aldehydes are invariably formed in unprotected form. Transketolase itself has been employed as a tool in the preparation of (S)-α-hydroxyaldehydes by kinetic resolution, though the yields of recovered aldehyde were modest. Fuculose 1-phosphate and rhamnulose 1-phosphate aldolases will resolve (R)-α-hydroxyaldehydes as they are selective for the (S)-enantiomers of these substrates. Horse liver alcohol dehydrogenase has been employed in the synthesis of (S)-α-hydroxyaldehydes by selective oxidation of the primary OH group of a range of 3-substituted propane-1,2-diols; only the (S)-alcohols are oxidised by the enzyme. The lipase-catalysed resolution of 3-halo-2-hydroxyacetals, used by Wong and co-workers in the preparation of both enantiomers of glycidaldehyde diethyl acetal 1 (Scheme 1.2.3), also allows access to homochiral α-hydroxyaldehydes, which can be generated directly from 1 by ring opening and acetal unmasking; this technique was used extensively in the preparation of aldolase substrates for aza sugar synthesis (see Section 1.3.2.2 and references therein). More recently the availability of enantiomerically pure cyanohydrins from the reactions of (R)‐ and (S)‐oxynitrilases has enabled protected α-hydroxyaldehydes to be prepared by reduction of the nitrile functionality using DIBAL.
2.1.2. A “CHIRAL POOL” APPROACH TO ENANTIOMERICALLY PURE $\alpha$-HYDROXYALDEHYDES

2.1.2.1. General observations

Most literature methods for the preparation of $\alpha$-hydroxyaldehydes report rather complex behaviour for these compounds. Dimerisation or oligomerisation of both free and $\alpha$-protected materials is common; moreover, the high reactivity of the carbonyl and $\alpha$-carbon atoms render the materials highly prone to decomposition, loss of optical purity or rearrangement to the often more thermodynamically stable ketols. Thus, most unprotected $\alpha$-hydroxyaldehydes prepared as substrates for RAMA and TK have tended to be formed in situ, rather than being isolated and characterised. It is noteworthy that the most effective preparations of $\alpha$-hydroxyaldehydes in unprotected form tend to utilise slightly acidic conditions for the unmasking of hydroxyl and aldehyde groups. The most likely decomposition routes for these compounds would involve $\alpha$-deprotonation (leading to enolisation and rearrangement or side reaction), which is presumably suppressed under mildly acidic conditions. It is to be expected that neutral or mildly acidic conditions would also suppress racemisation via enolate formation.

The method which we envisioned for the synthesis of homochiral $\alpha$-hydroxyaldehydes was a “chiral pool” method, beginning with enantiomerically pure $\alpha$-hydroxy carboxylic acids. Many of these materials are commercially available, or else can readily be synthesised, either from enzymatic reduction of $\alpha$-keto acids, or from diazotisation of $\alpha$-amino acids followed by ring-opening of the intermediate $\alpha$-lactones with water as the nucleophile (Scheme 2.1.5). Since the latter process consists of two sequential S$_2$2 displacements, it proceeds for many amino acids with complete or near-complete retention of configuration at the $\alpha$-centre. Derivatisation of the carboxylic acid moiety, followed by reduction with an appropriate reagent (e.g. DIBAL) ought in principle to provide a high-yielding route to $\alpha$-hydroxyaldehydes under conditions mild enough to ensure retention of optical integrity at the $\alpha$-centre. Similar schemes have been reported in the syntheses of enantiomerically pure protected $\alpha$-aminoaldehydes (which are also highly reactive, sensitive compounds) via reduction of carboxylic acid esters and mixed anhydrides and it was verified in both these cases that the reduction step did not interfere with the chirality at the $\alpha$-carbon.

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7 These results have been published in the Journal of the Chemical Society, Chemical Communications, 1995. (ref. 161)

63
Scheme 2.1.5. \(\alpha\)-Substitution of enantiomerically pure \(\alpha\)-amino acids

2.1.2.2. Synthesis of 2-hydroxy-3-phenylpropanal

The effectiveness of the proposed synthetic scheme was first explored using the amino acid phenylalanine, 53, as chiral starting material. The choice of phenylalanine was dictated by the fact that very few aldehydes bearing aromatic rings have been found to be effective substrates for other carbon-carbon bond forming enzymes (e.g. RAMA\(^6\)), making substrates such as 2-hydroxy-3-phenylpropanal 54 a useful probe for investigating the synthetic utility of TK. DL-Phenylalanine was easily converted to (\(\pm\))-2-hydroxy-3-phenylpropanoic acid 55 by diazotisation with sodium nitrite in aqueous sulfuric acid and displacement with water, although to optimise the yield of 55 it was necessary to balance the need for a strongly acidic system (to solubilise the starting material) with conditions sufficiently mild to avoid decomposing the sodium nitrite as it was added. Overnight stirring in 1.2 molar sulfuric acid was found to be optimum for this transformation. Esterification of acid 55 either under acid-catalysed conditions in refluxing methanol or using dropwise addition of thionyl chloride to a solution of 55 in cold methanol yielded the methyl ester 56, which, however, was not reduced by DIBAL-H at \(-78^\circ\mathrm{C}\), although vigorous effervescence was observed in the solution; instead, starting material was recovered in 80% yield after quenching with aqueous acid, solvent extraction, and chromatography. Presumably the reducing agent first deprotonated the \(\alpha\)-hydroxyl group, with the emission of hydrogen and the formation of a stabilised aluminate complex, from which the starting material was regenerated by reprotonation in the work-up.

Consideration was therefore given to the use of a suitable hydroxyl protection step prior to the DIBAL reduction. A range of \(\alpha\)-hydroxyl protecting groups have been employed in the syntheses of \(\alpha\)-hydroxyaldehydes by reductive methods.\(^{142, 151-153}\) For the purpose of this synthesis a protecting group was desired which could be installed
without affecting the chirality at the $\alpha$-carbon, and which could be removed, preferably under mildly acidic conditions, without loss of optical integrity in the product. At first the tert-butyldimethylsilyl (TBS) group was chosen. This group was easily installed by treating compound ($\pm$)-56 with TBS trifluoromethanesulfonate (triflate) in anhydrous $N,N$-dimethylformamide (DMF) in the presence of imidazole. The resulting $\alpha$-silyloxyester 57 was efficiently reduced to the aldehyde 58 using DIBAL at -78°C, but the TBS group was not removed using Corey’s conditions (hydrolysis in a 3:1:1 solution of acetic acid/water/tetrahydrofuran (THF)). Attention therefore turned to the much more labile trimethylsilyl (TMS) group, which had been employed by Hayashi et al. in the synthesis of unprotected $\alpha$-hydroxyaldehydes in excellent yield. The TMS ether 59 was formed by treatment of a solution of 56 and imidazole in dry THF with chlorotrimethylsilane, and purified by chromatography on silica neutralised by pre-treatment with a 2% solution of triethylamine in petrol, since a degree of hydrolysis of the TMS ether was observed when the compound was purified using untreated silica. DIBAL reduction at -78°C, followed by acidic quenching (2 M HCl), yielded an intermediate $\alpha$-silyloxyaldehyde which was isolated by solvent extraction but not purified further. The crude silyloxyaldehyde was redissolved in 3:1:1 acetic acid/water/THF; concentration of the reaction mixture in vacuo followed by chromatography yielded $\alpha$-hydroxyaldehyde ($\pm$)-54 in 69% yield (Scheme 2.1.6).

The efficacy of the synthetic sequence having been established, the preparation of (R)-54 was now undertaken, starting from D-(R)-phenylalanine (R)-53. Diazotisation and displacement to yield (R)-55, esterification, TMS protection, reduction with DIBAL and final deprotection using Corey’s conditions yielded enantiomerically pure $\alpha$-hydroxyaldehyde (R)-54; the yield of the reduction/deprotection sequence tended to increase as the reactions were scaled up (best yield: 0.6 g of (R)-54, representing 95% yield from (R)-59). Equally important was the fact that no racemisation of any of the intermediates was detected (the methyl ester 56 and its silylated derivative 59 were examined by $^1$H chiral shift NMR analysis using tris-[3-(heptafluoropropylhydroxymethylene)-$d$-camphorato] europium (III) (Eu(hfc)$_3$) as chiral shift agent). The optical integrity of the $\alpha$-hydroxyaldehyde was confirmed by conversion to the diethyl acetal 60; $^1$H chiral shift NMR study confirmed that the acetal was > 95% enantiomerically pure, establishing that no racemisation of the substrate had taken place at any stage in the synthesis. The $^1$H chiral shift NMR spectra of racemic and (R)-60 can be found in Figure 2.1.
Scheme 2.1.6. A general route to chiral $\alpha$-hydroxyaldehydes

Methods and reagents: (i) $\text{NaN}_3$/$\text{H}^+$; (ii) $\text{MeOH/MeOH}^+$; (iii) silyl protection; (iv) DIBAL-H/78°C; (v) deprotection; (vi) $\text{EtOH/Ambleryst H}^+$ resin
Figure 2.1. $^1$H NMR chiral shift spectra of racemic and (R)-acetal 60
2.1.2.3. Synthesis of $\alpha$-hydroxyphenylacetaldehyde (mandelaldehyde)

$\alpha$-Hydroxyphenylacetaldehyde (or mandelaldehyde), 61, illustrates powerfully the high reactivity of $\alpha$-hydroxylaldehydes and the need for mild conditions in their synthesis. Its use as a possible synthetic intermediate had long been of interest in this group, but previous attempts to synthesise racemic 61 had failed owing to isomerisation of the $\alpha$-hydroxyaldehyde to the more stable ketol under the conditions employed. The rearrangement has been shown to proceed via an enediol intermediate under even mildly basic conditions. Free racemic $\alpha$-hydroxyphenylacetaldehyde (in dimeric form) has been successfully synthesised by the acid-catalysed hydrolysis of a dimethyl acetal, as shown in Scheme 2.1.7, but most attempted syntheses have generated only the rearrangement product, 2-hydroxyacetophenone. To the best of our knowledge, the synthesis of enantiomerically pure 61 has never been reported.

Commercially available mandelic acid, 62, was employed as starting material for the synthesis of 61. The sequence described above (formation of the methyl ester 63, TMS protection (forming $\alpha$-silyloxyester 64), reduction and deprotection) was employed to prepare racemic, pure (R)- and pure (S)-$\alpha$-hydroxyphenylacetaldehyde 61 (Scheme 2.1.6), $^1$H chiral shift NMR analysis once again indicating that no racemisation occurred at any point in the sequence. Yields were in general slightly lower than in the synthesis of 54 (see Table 2.1), perhaps reflecting the greater instability of these intermediates. Conversion of (R)-61 to the diethyl acetal 65, followed by chiral shift NMR, however confirmed that $\alpha$-hydroxyphenylacetaldehyde had been synthesised in enantiomerically pure (e.e. > 95%) form.

Scheme 2.1.7. Acid-catalysed synthesis of mandelaldehyde (61)

† The author is grateful to Hitesh Sanganee for pointing out this procedure.

* Preparation of mandelic acid from phenylglycine was reported to occur with complete racemisation at the $\alpha$-carbon, (ref. 162) the reaction presumably proceeding via a stabilised, planar benzyl carbocation ($S_n$1 reaction) rather than an $\alpha$-lactone intermediate.
<table>
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<tr>
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<th>Structure</th>
<th>Protecting group</th>
<th>Deprotection conditions</th>
<th>Yield for steps</th>
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<td></td>
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<td>(v)</td>
</tr>
<tr>
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<td>AcOH/H₂O/THF</td>
<td>55 77 58 69 81</td>
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<td>AcOH/H₂O/THF</td>
<td>73 82a 82 95 63</td>
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<td>AcOH/H₂O/THF</td>
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<tr>
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<td></td>
<td>TBS</td>
<td>TBAF/THF</td>
<td>68 55 73 7 –</td>
</tr>
</tbody>
</table>

Footnotes: (a) using MeOH/H⁺; (b) using SOCl₂/MeOH (product was slightly impure); (†) aldehyde recovered from biotransformation mixture and derivatised (see Chapter Three).

Table 2.1. Synthesis of α-hydroxyaldehydes

2.1.2.4. Synthesis of (±)-2-hydroxy-3-methylbutanal

In attempting to prepare the aliphatic α-hydroxyaldehyde 66, using valine (67) as starting material, an unexpected difficulty was encountered. DL-Valine was converted in high yield to the α-hydroxyacid 68 and esterified, but attempts to convert ester 69 to the TMS ether resulted in no product being isolated. Since the methyl ester 69 itself had already been observed to be unexpectedly volatile, it was assumed that the even more volatile TMS ether had simply evaporated when the reaction mixture was concentrated *in vacuo* on a rotary evaporator. The more bulky TBS protecting group was therefore employed instead; TBS ether 70 was synthesised from 69, TBS triflate and imidazole in anhydrous DMF, and reduced using DIBAL-H; but as with compound 58, it was found that the resulting α-silyloxyaldehyde was not amenable to
hydroxyl deprotection under Corey’s conditions. At first, only very low yields (< 10 mg) of a product were obtained whose $^{13}$C spectrum contained a signal at $\delta$ 63 ppm which corresponded to a methylene group, as shown by DEPT analysis. It therefore appeared that small amounts of the over-reduced product, the diol (Me$_2$CH.CH(OH).CH$_2$OH, 71), had been isolated, while the $\alpha$-silyloxyaldehyde had disappeared (the $\alpha$-silyloxyaldehyde isolated after the DIBAL reduction could be expected to be reasonably volatile, and probably evaporated during lyophilisation of the deprotection mixture, for which heating on a rotary evaporator at 40°C for = 30 mm was necessary). Thus deprotection of the crude product obtained from the DIBAL reduction using tetra-$n$-butylammonium fluoride (TBAF) was attempted. A complex mixture of products was observed by TLC, from which a very low yield of the desired $\alpha$-hydroxyaldehyde 66 was eventually isolated after careful chromatography (Scheme 2.1.6).

2.1.2.5. Isolation and characterisation of $\alpha$-hydroxyaldehydes

$^1$H NMR analysis of a solution of aldehyde (±)-2-hydroxy-3-phenylpropanal 54 (initially isolated as a clear oil) in chloroform showed a complicated mixture of peaks which could not be readily assigned. Bearing in mind the tendency of $\alpha$-hydroxyaldehydes to oligomerise (and that in non-hydroxylic solvents such as chloroform the extent of oligomerisation was likely to be high) several other solvent systems were employed in an effort to characterise the aldehyde. Of these solvents, dimethylsulfoxide (DMSO), methanol, and 3:1 methanol/D$_2$O all failed to dissociate the aldehyde sufficiently for monomers to be seen. However, when a sample of (±)-54 was treated with = 1 ml D$_2$O, a white amorphous solid began to settle out of solution. $^1$H NMR analysis of the supernatant showed the monomeric aldehyde clearly (mainly in hydrated form, as shown by the characteristic gem-diol doublet at $\delta$ = 5.1 ppm, though a small aldehyde peak at $\delta$ 8.9 could also be seen). The contrast between the spectrum of (±)-54 in organic and aqueous solutions is shown in Figure 2.2.

Aldehydes 54 and 61 were found to be only sparingly soluble in water, though both dissolved (presumably in a variety of oligomeric forms) in a range of organic solvents. The racemic aldehydes gradually solidified in moist air, but the enantiomerically pure aldehydes could be kept for several days as stable oils at room temperature. Aliphatic aldehyde 66 was somewhat more water-soluble than its aromatic counterparts. In D$_2$O, freshly prepared solutions of all three aldehydes contained a mixture of free aldehyde, hydrate, and a variety of oligomeric forms, with the time taken to reach equilibrium varying between a couple of hours (for valine
derivative 66, which formed a 2:1 mixture of hydrate and free monomeric aldehyde (Figure 2.3)) to ten days (for α-hydroxyphenylacetaldehyde 61, which eventually comprised a single species in solution, either a hydrated monomer or a C₂-symmetric dimer as previously reported¹⁶⁰,¹⁶⁷ (Figure 2.4)) at room temperature.
2.1.3. ENZYME ASSAYS

The substrate specificity of TK with a particular aldehyde as substrate can be measured by means of an assay\textsuperscript{[31]} to follow the rate of consumption of hydroxypyruvate in an \( \approx 7.5 \) \( \mu \)mol-scale biotransformation mixture containing the aldehyde of interest (see \textbf{Section 1.4.3.3}). The principle of the assay is that HPA in the reaction mixture can be reduced to glycerate by the enzyme glycerate dehydrogenase (Gly-DH) using NADH (10) as cofactor; the NADH is oxidised to NAD\(^+\) in the process, and the absorbance of the solution to ultraviolet light at 340 nm (the region where NADH strongly absorbs) will change. Thus, addition of Gly-DH to a cuvette containing NADH and a solution of an aliquot from the reaction mixture removed and quenched at a given time (t) will cause a decrease in the UV absorbance of the solution at 340 nm, the magnitude of which (\( \Delta A \)) is directly related to the concentration of hydroxypyruvate in the cuvette (\textbf{Scheme 2.1.8} and \textbf{Figure 1.5}). The relationship between \( \Delta A \) and the concentration of hydroxypyruvate ([HPA]) in
solution is determined for each new set of substrate specificity assays by treatment of solutions of hydroxypyruvate (in buffer) of known concentration and a standard amount of NADH with Gly-DH, and plotting AA against hydroxypyruvate concentration.

\[
\begin{array}{c}
\text{(39)} \\
\begin{array}{c}
\Theta \\
O_2C
\end{array}
\begin{array}{c}
\text{OH} \\
\text{C=O}
\end{array} \\
\text{Gly-DH} \\
\text{NADH} \\
\text{NAD}^+ \\
\text{D-(R)-glycerate}
\end{array}
\]

Scheme 2.1.8. Reduction of hydroxypyruvate by Gly-DH

Figure 2.4. $^1$H NMR spectrum of aldehyde (±)-61 in D$_2$O

Substrate specificity assays were conducted for the novel aldehydes (±)-54 and (±)-66 (racemates were initially employed to test the efficacy of these aldehydes as substrates). A solution of partially purified E. coli transketolase (activity ≈ 200 U cm$^{-2}$) was added to a mixture of the substrate, cofactors and buffer (glycylglycine, pH 7.6)
in a 1 cm³ Eppendorf tube, and aliquots extracted periodically and assayed for HPA remaining as described above. Control experiments (one without LiHPA, to detect natural decomposition of HPA under the assay conditions, and one without enzyme, to confirm that no non-enzymatic reaction took place) were conducted in parallel with the substrate specificity assays.

The best known substrate for TK is glycolaldehyde, HOCH₂CHO (40), which is also used as the standard against which reaction rate values ($v_{rel}$) for other substrates are determined. A solution that is 25 mM with respect to glycolaldehyde shows essentially complete consumption of HPA after 12 minutes at room temperature. The rate, $v$(HOCH₂CHO), which is measured for substrate specificity determination is given by the initial slope of the hydroxypyruvate decay curve, and by comparing the slopes of decay curves the relative rate values ($v_{rel}$) can be calculated. Assay of (±)-2-hydroxy-3-methylbutanal 66 in this manner showed this substrate to have a $v_{rel}$ of 5% compared with glycolaldehyde (see Figure 2.5 for the HPA decay curve). The low solubility of (±)-2-hydroxy-3-phenylpropanal 54, however, meant that an accurate $v_{rel}$ value for this substrate was impossible to obtain. The observation of a “lag phase” of roughly 30 min duration after initial preparation of the reaction mixture, during which time no depletion of hydroxypyruvate was observed at all, suggested that the solution concentration of substrate when depletion of HPA began was well below the concentration of the standard (glycolaldehyde) and that the solution equilibrium was not yet established. Substantial depletions in HPA concentration were observed, however, over a 3-hour period, suggesting that transketolase accepted 54 as a substrate despite its low solubility (Figure 2.6).

In early published studies on *E. coli* transketolase, the hydroxypyruvate assay had proven extremely valuable as a gauge of the effectiveness of TK as a general catalyst. Almost all of the substrates investigated initially, however, were sugars or sugarlike molecules, or at least relatively soluble at the dilutions employed in the assay. The inconclusive results obtained with (±)-54 as substrate, however, cast some doubt on the validity of the hydroxypyruvate assay in determining the general effectiveness of a given substrate in the biotransformation. Since many potentially useful α-hydroxyaldehyde substrates might be expected to suffer from the same problems of poor solubility as 54, it was unlikely that their performance as acceptor substrates on a preparative scale could be deduced from assay results. Attention therefore shifted to the preparative-scale biotransformation of aldehydes using TK, as described in Chapter Three.
Figure 2.5. Substrate specificity assay with Me₂CH.CH(OH)CHO (66) (conditions: 25 mM aldehyde, 7.5 mM LiHPA)

Figure 2.6. Substrate specificity assay with PhCH₂CH(OH)CHO (54) (conditions: 25μmol aldehyde (semisolid), 7.5 mM LiHPA)
2.2. α-Hydroxyaldehyde Synthetic Intermediates

2.2.1. 3,3-Diethoxy-2-hydroxypropanal

2.2.1.1. Synthetic targets - nectrisine

The [3S, 4R] stereochemistry of the chiral triols derived from the transketolase-mediated condensation of LiHPA and an α-hydroxyaldehyde makes these compounds extremely valuable as intermediates in the synthesis of sugar analogues, for instance aza sugars. One such synthetic target of interest to this group was the iminosugar FR900483, also known as nectrisine, which was isolated from the fungus Nectria lucida in 1988. Potent biological activity was reported for this compound, both as an inhibitor of α-glucosidase and α-mannosidase, and as an immunoactivator. Nectrisine was shown to have the structure 72 by extensive NMR study and a total synthesis starting from D-glucose. Two later syntheses of nectrisine were reported by Danishefsky, one derived from D-glucal using a Vasella cleavage of the sugar ring as the key step, and one from D-arabinose utilising a Mitsunobu inversion to achieve the desired stereochemistry at C-4 (Scheme 2.2.1). All of these “chiral pool” methods involved an extensive sequence of protection and deprotection steps.

a) from D-glucose

\[
\begin{align*}
\text{D-glucose} & \xrightarrow{\text{HON \BnO}} \xrightarrow{\text{CF}_3 \text{O} \text{Ph}_3} \xrightarrow{\text{N H BnO}} \text{(+ C-5 epimer)} \\
\text{(72)} & \text{i) NaIO}_4 \quad \text{ii) H}_2\text{Pd-C/HCO}_2\text{H} \quad \text{iii) NaOH}
\end{align*}
\]
b) from D-glucal\textsuperscript{171}

\begin{align*}
\text{TBSO} & \rightarrow \text{Br} \\
\text{BnO} & \rightarrow \text{Zn} \\
\text{EtOH} & \rightarrow \text{in} \\
\text{H}_2/\text{Pd}-\text{Al}_2\text{O}_3 & \rightarrow \text{AcO} \\
\text{N}_3 & \rightarrow \text{Hydrolysis}
\end{align*}

\begin{align*}
\text{HO} & \rightarrow \text{Mitsunobu} \\
\text{OH} & \rightarrow \text{as (b) above}
\end{align*}

\begin{align*}
\text{HO} & \rightarrow \text{OH}
\end{align*}

\begin{align*}
\text{N}_3 & \rightarrow \text{Triol 74 could in principle be obtained via either of two routes: an FDP aldolase-catalysed condensation of dihydroxyacetone phosphate 12 with 2,2-diethoxyacetaldehyde (31), or a transketolase-catalysed condensation of lithium}
\end{align*}

\begin{align*}
\text{Scheme 2.2.1. Syntheses of nectrisine}
\end{align*}

Recently, this group\textsuperscript{114} and others\textsuperscript{140} have proposed an alternative retrosynthetic analysis of nectrisine, as shown in Scheme 2.2.2. The target material is derived from cyclisation of the \(\gamma\)-aminoaldehyde 73, which in turn could be generated from a chiral triol such as 74 with the “D-threo” configuration at C-3 and C-4 and a masked aldehyde group at C-5. Triol 74 could in principle be obtained via either of two routes: an FDP aldolase-catalysed condensation of dihydroxyacetone phosphate 12 with 2,2-diethoxyacetaldehyde (31), or a transketolase-catalysed condensation of lithium.
hydroxypyruvate 39 with the (S)-enantiomer\(^{\dagger}\) of the chiral dialdehyde synthon 3,3-diethoxy-2-hydroxypropanal (75). The former method, which had been reported by Whitesides\(^{103}\) in the development of the “inversion” strategy for enzymatic aldose synthesis (see Section 1.3.2.1), was studied in this group by Janette Sawden,\(^{114,172}\) the key condensation product 74 was generated in modest yield (100 mg, 40%) using PAMA, and this compound employed to produce milligramme quantities of silyl protected nectrisine in a four-step procedure starting from 74 (see Chapter Four). However, the key aldolase-catalysed reaction was not amenable to scale-up, and good yields of triol 74 could only be obtained after exhaustive extraction of the reaction mixture.

\[
\text{(72)} \xrightarrow{\text{Aldolase}} \text{(73)} \xrightarrow{\text{Transketolase}} \text{(74)}
\]

Scheme 2.2.2. Retrosynthetic analysis of nectrisine (72)

\(^{\dagger}\) The configuration of the active enantiomer of a chiral \(\alpha\)-hydroxyaldehyde (the “hydroxyl-up” configuration when the molecule is drawn in the manner shown in Scheme 1.4.2) is normally assigned the (R)-configuration according to the Cahn-Ingold-Prelog rules. However, in this case the rules assign a higher priority to the diethyl acetal moiety than to the aldehyde group, and so the assignment of absolute configuration for the active enantiomer of substrate 75 is (S).
Thus it was proposed that a chemo-enzymatic synthesis of nectrisine using a TK-catalysed condensation as the key step might circumvent the problems of scale-up that were experienced in the RAMA-catalysed reaction, and allow the generation of sufficient material for a study of the final deprotection of silylated nectrisine to be undertaken. The key factor in making such a synthesis feasible, however, would be the availability of the novel aldehyde-acetal 75.

2.2.1.2. Synthetic routes towards (±)-3,3-diethoxy-2-hydroxypropanal

Three possible synthetic routes to the racemic aldehyde (±)-75 were envisaged, all starting from the readily available (±)-glyceraldehyde diethyl acetal (GDA), (±)-76, which can be prepared via dihydroxylation of acrolein diethyl acetal 77 using N-methyl-morpholine-N-oxide (NMO) and catalytic osmium tetroxide, or via acid-catalysed acetalisation of DL-glyceraldehyde 41. These possible routes, illustrated in Scheme 2.2.3, were as follows:

- Selective oxidation of the primary hydroxyl group of GDA using an appropriate reagent. However, oxidation of a primary hydroxyl group in the presence of a secondary is not trivial, very few methods are reported in the literature;
- Addition of a vinyl organometallic reagent to 2,2-diethoxyacetaldehyde (31), followed by double bond cleavage (ozonolysis). A preliminary study of this route was undertaken in this group by Mark Liebster,166 however it was found that yields were mediocre, and that protection of the hydroxyl group prior to ozonolysis was necessary. The final deprotection proceeded in poor yield to give a few milligrammes of the desired product, and no success at scale-up or improved deprotection methodology was reported.
- Cyanosilylation of aldehyde 31 using trimethylsilyl cyanide, followed by reduction with DIBAL following the protocol of Hayashi et al.142 However, it was by no means certain that the acid-sensitive diethyl acetal moiety would survive the necessary acidic hydrolysis in working-up the reaction.

It was reported that dihydroxylation of acrolein diethyl acetal 77 generated (±)-GDA 76 in high yield;166 however, a repeat of this reaction on ≈ 5 g scale gave mediocre yields of product (21%). Preparation of (±)-76 by acetal formation in ethanol using Amberlyst® H+ resin as catalyst generated GDA in excellent (82% after chromatography) yield on a 1.5 g scale. Scale-up of the reaction produced 19.5 g (86%) of (±)-GDA after stirring for 5 days in ≈ 500 cm³ ethanol; the end-point could

---

8 The successful synthesis of the dimethyl acetal analogue of 75 by ozonolysis of the hydroxyl-unprotected precursor was, however, recently reported to the author. (ref. 173)
be estimated since glyceraldehyde formed a cloudy suspension in ethanol whereas GDA produced a clear solution. The product was purified by filtration, neutralisation of the (acidic) solution by treatment with Amberlite® OH⁻ ion-exchange resin that had been pre-washed in 2 M NaOH, and concentration in vacuo. ¹H NMR analysis showed a small amount of glyceraldehyde as contaminant, but otherwise the GDA thus formed was essentially pure and no further purification was attempted. Pure (±)-GDA could also be obtained by hydrogenation of (±)-3-O-benzylglyceraldehyde diethyl acetal 78 (vide infra) over palladium-charcoal (47% yield), though optimisation of this reaction was not attempted.

2,2-Diethoxyacetaldehyde (31) is formed from (±)-GDA by cleavage of the diol with periodate. Once again, although excellent yields ¹⁶⁶ were reported for this reaction, on a smaller scale only a modest amount of the desired product could be synthesised (26% yield).

Aldehyde 31 was readily converted to the α-trimethylsilyloxy nitrile (±)-79 by treatment with trimethylsilyl cyanide in CH₂Cl₂ in the presence of a catalytic quantity of zinc iodide.¹⁷⁴,¹⁷⁵ Reduction of the nitrile with DIBAL-H was followed by quenching with 2 M HCl, vigorous stirring in order to hydrolyse the imine which is the first formed reduction product (Scheme 2.2.4), and extraction with ethyl acetate; the resulting solution appeared by GCMS analysis to contain two fractions, but these could not be unequivocally identified (since the functional groups corresponding to

Scheme 2.2.3. Proposed routes to (±)-3,3-diethoxy-2-hydroxypropanal
starting material, intermediate and product - nitrile, imine and aldehyde respectively - would be expected to give very similar mass spectral profiles) - however it appeared that one fraction contained a silyl group and one did not. Evaporation of the extracts in vacuo yielded a murky yellow oil which was dissolved in water and stirred in the presence of acidic ion-exchange resin (Amberlyst®). TLC analysis of the reaction mixtures showed two fractions and a significant streak to the baseline, implying that a significant degree of deprotection of the acetal moiety had indeed occurred. Extraction with EtOAc followed by solvent evaporation yielded 7 mg of an oil which did not appear, by ¹H NMR or GCMS, to be the desired aldehyde; a mass spectral peak of m/z 158 suggested that the isolated material may have contained the unprotected cyanohydrin [M - H⁺], but NMR analysis showed the product to be very impure.

It thus appeared that the reduction/deprotection route of Hayashi et al. had failed to yield the desired product, presumably because of the sensitive nature of the acetal moiety, and attention turned to alternative syntheses of aldehyde 75.

Scheme 2.2.4. Proposed cyanosilylation/reduction route to
(±)-3,3-diethoxy-2-hydroxypropanal

2.2.1.3. Selective oxidations

A limited number of methods are mentioned in the literature for the oxidation of a primary hydroxyl group in the presence of a secondary. Many of the most general, versatile methods of oxidation regularly employed by organic chemists (such as the Swern and related oxidations) are reported to oxidise primary and secondary alcohols with more or less equal ease (though in a few isolated examples - Wenger's synthesis of the cyclosporine amino acid moiety being one such - a degree of selectivity has been reported). Although selective enzymatic oxidations of primary hydroxyl groups using horse liver alcohol dehydrogenase are well-known the resulting α-hydroxyaldehydes have the wrong configuration for reaction with
transketolase. Of the remaining methods, oxidations mediated by ruthenium complexes\textsuperscript{178,179} and oxoammonium ions\textsuperscript{180-185} appeared most likely to provide the desired selectivity in the oxidation of (±)-GDA \textsuperscript{76}.

It is reported\textsuperscript{178} that stoichiometric quantities of tris-(triphenylphosphine) ruthenium (II) chloride will preferentially oxidise the primary hydroxyl group of a 1,2-diol. However, stirring of a mixture of the ruthenium complex (700 mg) and GDA (1 mmol) in toluene yielded no product at all; only large amounts of a ruthenium complex were eluted when the reaction mixture was filtered, concentrated and purified by chromatography. Similarly, oxidation using NMO and catalytic quantities of the oxidant tetra-n-propyl-ammonium perruthenate (VII) (TPAP)\textsuperscript{179} yielded only small amounts of a product which appeared (by \textsuperscript{1}H NMR) to contain a trace of the desired aldehyde \textsuperscript{75}, but in a very impure form; a repeat of the reaction using fresh reagents showed rapid decomposition of the starting material, and no product at all was isolated.

The use of oxoammonium ions as mild oxidising agents, both stoichiometrically in acid solution\textsuperscript{181} and catalytically in a biphasic mixture with sodium hypochlorite\textsuperscript{180,182,183} or sodium bromite\textsuperscript{184} as the co-oxidant is well documented. The active oxoammonium ion is generated by oxidation (in basic conditions) or disproportionation (in acidic conditions) of the sterically hindered 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) radical, \textsuperscript{80}; oxidation of an alcohol by the oxoammonium ion (Scheme 2.2.5) leads to reduction to the nitroxide anion, from which TEMPO is regenerated, and the catalyst recycled, by oxidation with NaOCl (in the catalytic mode) or by synproportionation (in the stoichiometric mode). In the catalytic, two-phase system, the reaction has been reported to be most favourable at 0°C and with bicarbonate buffer (pH = 8.6), these conditions ensuring an even distribution of hypohalous acid between the organic and aqueous phases.\textsuperscript{180}

Widely differing reports have appeared in the literature regarding the extent of oxidation of alcohols mediated by TEMPO, and the selectivity of the reaction for primary over secondary alcohols. The outcome of the reaction appears to be highly dependent on the conditions employed and the nature of the substrate,\textsuperscript{185} so that in basic biphasic conditions it is reported that the reaction is more rapid with primary hydroxyl groups than with secondary,\textsuperscript{180} and Skarzewski \textit{et al.} have achieved selective oxidation of a range of primary-secondary diols, including 1,2-diols, using TEMPO and NaOCl.\textsuperscript{182} Under acidic conditions, however, little or no selectivity for primary hydroxyl groups over secondary has been observed, and 1,2-diols were found to be very unreactive.\textsuperscript{181} These findings have led de Nooy\textsuperscript{185} and others to propose
alternative mechanisms for the TEMPO-mediated oxidation of alcohols to aldehydes in acidic and basic conditions (Scheme 2.2.5).

Scheme 2.2.5. Proposed mechanism for TEMPO-mediated oxidation of primary alcohols

The reported efficiency of the selective oxidation of primary-secondary diols to hydroxyaldehydes\(^{182}\) prompted investigation into the preparation of (±)-3,3-diethoxy-2-hydroxypropanal (75) by this route. However, it was also noted that the high hydrophilicity of 1,2-diols can make selective oxidation of such substrates difficult or impossible using this method;\(^{180}\) presumably this is because the key redox steps occur between the alcohol, oxoammonium ion and hypohalous acid in the organic phase of the mixture.

Initial attempts to oxidise (±)-GDA (1 mmol) selectively using sodium hypochlorite and TEMPO in a biphasic mixture of dichloromethane and saturated aqueous sodium bicarbonate (with KBr and tetra-n-butyrammonium chloride as oxidation and phase-transfer co-catalysts respectively) produced a small quantity (9 mg) of a clear oil (seen by TLC as two closely-running, smeared spots in the \(R_f = 0.5-0.7\) region [EtOAc (neat) as eluant]) whose \(^1\)H NMR spectrum was entirely
consistent with the presence of pure (±)-75. However, traces of other products were also seen by TLC, including a significant baseline spot (possibly the result of overoxidation of the product to the carboxylic acid), and a high proportion of unreacted starting material was also observed to be present in the organic extracts from the reaction. This was expected to be due to the high hydrophilicity of (±)-GDA, so considerable effort was exerted to optimise the conditions of reaction and increase the proportion of (±)-GDA in the organic phase. Although a yield of 14% (22 mg material) was eventually achieved (using 1:1 toluene/ethyl acetate instead of CH₂Cl₂), this yield could not be increased further, and a threefold scale-up of the reaction decreased the yield to 7% (33 mg).

A more significant (10 mmol) scale-up of the reaction (with NaBr and benzyl trimethyl ammonium chloride as co-catalysts), using ultrasound to ensure intimate mixture of the two phases, however succeeded in producing a single batch yield of 110 mg (7%) of aldehyde 75. Further scale-up using vigorous magnetic stirring followed by continuous extraction of the reaction mixture with ethyl acetate produced 400 mg (17%) of aldehyde 75 in a single batch, with starting material recovered in typically ≈ 20% yield. Although such a process represented a somewhat uneconomical synthesis of this molecule (and therefore imposed a natural constraint on the feasibility of a transketolase-based synthesis of nectrisine, using this substrate at least), the availability of the starting material (±)-GDA in multigramme batches meant that the key intermediate was available in sufficient quantities to allow investigation of the biotransformation of aldehyde 75 with transketolase and to allow further work on the synthesis of nectrisine following Janette Sawden's earlier work. These steps are reported in Chapters Three and Four respectively.

2.2.1.4. Kinetic resolution of (±)-glyceraldehyde diethyl acetal

Since aldehyde (±)-75 was synthesised in only modest yield, it was considered that the efficiency of a biotransformation using this aldehyde as substrate could be increased greatly if enantiomerically pure, rather than racemic, 75, could be used in the biotransformation. It is reported that TEMPO-catalysed oxidation of chiral

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† GCMS analysis of a solution of aldehyde 75 showed three fractions, all with almost identical mass spectra including a peak at m/z 161 [M - H⁺]. The variation in relative intensities of these peaks in samples of 75 prepared freshly and isolated from biotransformation mixtures (see Chapter Three) suggested that one fraction corresponded to the free aldehyde, one to the hydrate (as observed in the ¹H NMR spectrum in D₂O) and one to an oligomeric form, probably the dimer. Such an explanation would be consistent with the observed appearance of the otherwise pure aldehyde when visualised on a TLC plate.
α-substituted primary alcohols can be achieved without loss of enantiomeric purity;\textsuperscript{183} thus consideration was given to the preparation of an enantiomerically enriched source of the precursor, glyceraldehyde diethyl acetal (76), which ought to yield enantiomerically enriched \((S)\)-75 after oxidation with sodium hypochlorite and TEMPO. The expense of homochiral glyceraldehyde as a starting material led to the search for a lipase-catalysed kinetic resolution of \((\pm)-76\) (see Section 1.2.2). A screen of eight hydrolytic enzymes was attempted with \((\pm)\)-GDA as acyl acceptor. M ixtures containing \((\pm)\)-GDA, vinyl butyrate, a constant amount of a standard inert reagent (\(n\)-decane) and a catalytic amount of the enzyme in toluene were incubated at 30°C, and aliquots abstracted periodically and analysed by GCMS (to determine the extent of reaction, by reference to the \(n\)-decane peak) and chiral GC on a Chirasil-Dex-CB\textsuperscript{\textregistered} column (to determine the e.e. of the unreacted diol).\textsuperscript{\textdagger} Of these enzymes, two (PPL and lipase OF\textsuperscript{\textregistered}) were completely unreactive; two (Novozyme\textsuperscript{\textregistered} and \(M.~miehei\) lipase) rapidly and completely acylated both enantiomers of \((\pm)\)-GDA; and the enantiomeric ratio (E) values\textsuperscript{186} for the other enzymes were all \(= 2\). An E-value close to unity is an indication that poor resolution was achieved; thus, it appeared that a good enzymatic resolution of \((\pm)\)-GDA could not be found.

Scheme 2.2.6. Attempted kinetic resolution of \((\pm)\)-GDA (76)

2.2.2. 3-O-BENZYLGLYCERALDEHYDE

2.2.2.1. Synthesis of \((\pm)\)-3-O-benzylglyceraldehyde\textsuperscript{*}

The final α-hydroxyaldehyde substrate employed in this thesis was \((\pm)\)-3-benzylxyo-2-hydroxypropanal, or 3-O-benzylglyceraldehyde (3BG), 81. As with substrate 75, 3BG possesses functionality at the 3-position (in this case, a masked hydroxyl group) which makes this aldehyde potentially useful as an intermediate in the synthesis of chiral products. An alternative retrosynthesis of

\textsuperscript{\textdagger} The e.e. assay for compound 76 was devised by Christel Ries and Catherine Rippé at Chiroscience Ltd.

\textsuperscript{*} Work carried out by Mark E.B. Smith of this group.
nectrisine, for instance, could be envisaged (Scheme 2.2.7) using the triol 5-O-benzyl-D-xylulose (82), derived from TK-mediated condensation of 3BG and hydroxypyruvate, as intermediate. In this case, an extra (oxidative) step is necessary to raise the oxidation level of C-5 to that of an aldehyde prior to cyclisation. (±)-3BG was first synthesised by Effenberger from (±)-glycidaldehyde diethyl acetal (1) and shown to be an effective substrate for yeast TK. The procedure employed by M'erk Smith of this group for the synthesis of (±)-3BG was analogous to that employed by Effenberger.

Scheme 2.2.7. Retrosynthetic analysis of nectrisine involving 3-O-benzylglyceraldehyde (81)

Glycidaldehyde diethyl acetal, (±)-1, was synthesised using Wong’s conditions: epoxidation of acrolein diethyl acetal (77) using H₂O₂ mediated by benzonitrile and KHCO₃ (Scheme 2.2.8). Ring-opening of 1 (exclusively at C-3) with the anion of benzyl alcohol yielded 3BG diethyl acetal, (±)-78, which was purified by distillation. Using this procedure, 80 g of acetal 78 could be obtained in a single batch, indicating that use of (±)-3BG presents an advantage over (±)-75, available only in ~400 mg batches, as a synthetic intermediate. Acetal 78 provided a convenient medium for long-term storage of 3BG, from which the aldehyde could be unmasked in quantitative yield by hydrolysis in aqueous solution at pH = 1. As with aromatic aldehydes 54 and 61, (±)-3BG possessed limited water-solubility, forming a cloudy, oligomeric suspension in aqueous medium.
**Scheme 2.2.8. Synthesis of (±)-3-O-benzylglyceraldehyde (81)**

2.2.2.2. **Studies towards the synthesis of (R)-3-O-benzylglyceraldehyde**

As a possible route to the formation of enantiomerically pure (R)-3BG for use in the biotransformation, the preparation and ring-opening of ethyl (R)-glycidate (R)-51, as described by Larchevêque and Petit, was investigated (Scheme 2.1.4). Since ring-opening of epoxide 51 using organocuprates and nitrogen nucleophiles was reported to occur with exclusive attack at the less hindered position, generating a β-functionalised α-hydroxyester, it was hoped that ring-opening of (R)-51 with the anion of benzyl alcohol would yield ethyl (R)-3-O-benzylglycerate (R)-83 directly (cf. the synthesis of 3BG acetal 78 above, which occurs with complete regioselectivity for the less hindered carbon). Silyl protection, reduction with DIBAL, and deprotection ought then to yield (R)-81 directly as described in Section 2.1.2.

To investigate the regioselectivity of the proposed ring-opening, racemic ethyl glycidate 51 was prepared by Robert Gormley according to literature precedent. Diazotisation of DL-serine (±)-52 and ring-opening of the α-lactone intermediate with bromide ion yielded 2-bromo-3-hydroxypropanoic acid (±)-84, which was cyclised using KOH in ethanol. Potassium glycidate (±)-85 was isolated by crystallisation, and treatment of the salt with diethyl sulfate in the presence of 18-crown-6 gave ethyl glycidate (±)-51 in 23% overall yield from serine (Scheme 2.2.9). However, attempts at ring-opening epoxide (±)-51 using the sodium salt of benzyl alcohol under a variety of conditions did not yield the desired C-3 substitution product (±)-83. In THF at 0°C, a 17% yield of the side product, ethyl 2-benzyloxypropenoate (86), formed by C-2 attack followed by elimination, was obtained, while at low temperatures a transesterification product was the only product isolated (high...  

† Work carried out by Robert H. Gormley as a 4th year Honours project in this group, under the supervision of the author.
temperatures tended to decompose the material). Lewis acid-catalysed ring-opening reactions proved equally unsuccessful, with boron trifluoride etherate producing a complex mixture of products, and the somewhat milder titanium isopropoxide yielding no products except those of transesterification.

Thus it appeared that ethyl (R)-glycidate was not a viable intermediate in the synthesis of (R)-3BG. Other routes to this substrate in enantiomerically pure form were envisaged, e.g. from the diethyl acetal (R)-1 prepared after lipase-catalysed resolution (Scheme 1.2.3), however time constraints did not permit an investigation of this route.

\[
\begin{align*}
\text{(52)} & \xrightarrow{\text{NaNO}_2/\text{HBr}} \text{(84)} \\
\text{(84)} & \xrightarrow{\text{KOH}} \text{(85)} \\
\text{(83)} & \xrightarrow{\text{Et}_2\text{SO}_4/18\text{-crown-6}} \text{(51)}
\end{align*}
\]

Scheme 2.2.9. Ethyl glycidate (51) as a possible synthetic intermediate

2.3. An α-Nitrogen Substituted Potential Acceptor Substrate

2.3.1. (±)-2-Azido-3-Hydroxypropanal: Synthesis

2.3.1.1. Whitesides' route

The vast majority of published literature regarding the substrate specificity of TK involves α-hydroxyaldehydes as acceptor substrates.75-78,161 α-Unsubstituted aldehydes have also been employed, though less frequently;75,126,128 however no preparative-scale reactions of α-heteroatom-substituted aldehydes have been reported. Gordon Hobbs78 reported the use of the N-protected aldehyde 87 in substrate specificity studies using the hydroxypyruvate assay, for which a \( v_{rel} \) of 5% was recorded. However, since the error in measuring changes of hydroxypyruvate
concentration was found in our hands to be appreciable at high dilution and low rates of HPA depletion, the exact meaning of such a reported value was unclear.

Nonetheless, the potential use of α-N-substituted aldehydes as transketolase substrates was of considerable interest, as any products thereby formed (chiral amino alcohols) may also prove to be valuable synthetic intermediates. A suitable aldehyde to be employed as a “test substrate” was the protected α-amino aldehyde, (±)-2-azido-3-hydroxypropanal (36), which had been prepared by Whitesides\(^{106}\) and Wong\(^{105}\) as an intermediate in the aldolase-mediated syntheses of five-membered aza sugars. Wong’s method for the preparation of this aldehyde used the periodate cleavage of an azido-triol (Scheme 2.3.1); the aldehyde was not isolated but employed in situ in aldolase-catalysed condensations. Whitesides employed an ozonolysis of the azido-alcohol 88 formed by regioselective ring-opening of the cinnamaldehyde-derived epoxide 89 with sodium azide (Scheme 2.3.1). In initial studies towards the formation of 36, reproduction of Whitesides’ route was attempted.

Epoxide (±)-89 (20 mmol) was prepared by attack of dimethylsulfonium methylide on cinnamaldehyde (90). The ylid was prepared by treatment of trimethylsulfonium iodide with sodium hydride in a mixture of DMSO and THF at 0°C. Addition of cinnamaldehyde to the mixture resulted in the formation of 89 in 66% yield. Problems were encountered in the purification of 89, owing to the difficulty of removing DMSO completely from the material. Distillation proved impractical on the scale at which the reaction was performed, so a process of repeated chromatography on silica and heating of the product to 80°C on a Kugelrohr apparatus was necessary to remove the last traces of DMSO.

\[ \text{a) via periodate cleavage}^{105} \]

\[ \text{HO} = \text{C} = \text{CH}_2 \text{OH} \rightarrow \text{HO} \text{CH} = \text{CH}_2 \text{OH} \rightarrow \text{HO} \text{CH} = \text{CH}_2 \rightarrow \text{HO} \text{CH} = \text{CH}_2 \text{OH} \text{N}_3 \rightarrow \text{HO} \text{CH} = \text{CH}_2 \text{OH} \text{N}_3 \text{NaIO}_4 \rightarrow \text{HO} \text{CH} = \text{CH}_2 \text{OH} \text{N}_3 \]

(36)
More significant difficulties were encountered in the ring-opening of the epoxide, however, for which Whitesides reported a yield of 91%. Stirring of a solution of 89 and sodium azide in 2:1 acetone/water initially gave only very low yields (= 10%) of the desired product, and an appreciable amount of another more polar fraction. $^1$H NMR of the more polar fraction suggested that an alternative reaction path was being followed, involving substitution of azide at the allylic position, with concomitant rearrangement of the double bond. The $^1$H NMR spectrum of epoxide 89 shows characteristic olefinic peaks at $\delta$ 5.9 (double doublet) and 6.8 (doublet) with very large coupling constants (16 Hz) between them, as would be expected for a trans-substituted C=C bond. The corresponding peaks are clearly visible also in the spectrum of azido-alcohol 88 at $\delta$ 6.2 and 6.7. In addition to these peaks, however, the $^1$H NMR spectrum of the more polar fraction showed multiplets at $\delta$ 5.2 and 5.9 with very small coupling constant, suggesting that the fraction consisted of a mixture of cis and trans isomers, and that some degree of double bond rearrangement was occurring, presumably through attack of azide at the allylic position.

Numerous variations on the conditions employed by Whitesides were attempted in order to improve the yield of the desired product 88, but none proved successful. These included addition of an acidic salt (ammonium chloride) to the solution and changing the organic cosolvent from acetone to methanol, but the former method had no appreciable effect on the proportions of products isolated, and the latter produced a different product altogether, evidently by allylic attack of methanol rather than azide as the nucleophile. On one occasion a 56% yield of azido-alcohol 88 was obtained under
conditions identical to those reported by Whitesides but for the scale of the reaction (1.7 mmol), but this could not be repeated.‡

Ozonolysis of azido-alcohol 88 (120 mg) in CH₂Cl₂/methanol rapidly produced a blue solution; work-up with dimethyl sulfide followed by solvent evaporation and chromatography yielded 28 mg of a clear oil. The composition of the oil could not be confirmed unequivocally, since the product decomposed very rapidly when dissolved in 0.5 cm³ of D₂O and transferred to an NMR tube. Effervescence was observed in the tube and only a very poor spectrum could be obtained; NMR analysis of the solution a few days later showed nothing but decomposition products. In the light of subsequent studies on (±)-36 (vide infra) it would appear that aldehyde 36 was formed by ozonolysis of 88, though it decomposed too rapidly for characterisation. However, the difficulties experienced elsewhere in the synthesis suggested that an alternative strategy for the synthesis of (±)-36 be sought.

2.3.1.2. Synthesis of (±)-2-azido-3-hydroxypropanal by a reductive method

Aldehyde (±)-36 was eventually synthesised successfully via a route based on the reduction of a DL-serine-derived ester using DIBAL-H (cf. Section 2.1.2) (Scheme 2.3.2). DL-Serine (52) was converted to 2-bromo-3-hydroxypropanoic acid (±)-84 via diazotisation in the presence of potassium bromide. Formation of the methyl ester 91 was followed by nucleophilic substitution of bromide with sodium azide in the presence of the phase transfer catalyst “Aliquat 336” (methyl trioctyl ammonium chloride). Attempts to reduce ester 92 directly with DIBAL-H (conditions: 0.7 mmol 92, 1 mmol DIBAL-H, toluene, -78°C) led to no identifiable products being isolated; so the β-hydroxyl group was protected as the TBS ether using TBS triflate in DMF in the presence of imidazole. Reduction of the protected ester 93 with DIBAL-H proceeded smoothly, yielding aldehyde 94 in 51% yield after chromatography.†

Deprotection of aldehyde 94 was accomplished using Corey’s conditions, i.e. hydrolysis in 3:1:1 acetic acid/water/THF for 24 hours at RT. Solvent evaporation and chromatography of the resulting oil yielded a fraction which appeared as a smudge or a scattering of spots, Rₜ 0.3-0.5, by TLC analysis [Et₂O (neat)]. This fraction yielded aldehyde (±)-36 as a clear oil which was dissolved to 50 mM concentration in

‡ Attempts at the University of Exeter to reproduce Whitesides’ work have suggested that the purity of the epoxide 89 is critical for the success of the reaction. (ref. 191) The poor yields and side products observed in most of the ring-opening reactions reported here may have been a result of trace contamination with DMSO, which is very difficult to remove completely.

† More conveniently, the aldehyde 94 was deprotected without prior purification.
D₂O and refrigerated. It was notable that hydrolytic cleavage of the TBS group from the TBS-protected secondary alcohol precursors of aldehydes 54 and 66 (vide supra) had proven unsuccessful, whereas the primary TBS ether moiety of aldehyde 94 was entirely removed within 24 hours under the same conditions.

Scheme 2.3.2. Synthesis of (±)-2-azido-3-hydroxypropanal (36) via DIBAL reduction

Freshly prepared samples of (±)-36 were stored routinely as dilute (50 mM) solutions in D₂O, and kept in a refrigerator. The reason for this was that the compound proved to be quite unstable in pure form or in concentrated solution, and at room temperature. Decomposition times ranged from about 10 days at 50 mM concentration (storage below 4°C) to a matter of hours in 0.5 M solution at room temperature. The compound also decomposed rapidly when a dilute sample was concentrated on a rotary evaporator. Decomposition of the aldehyde was evident both physically (the solution turned yellow) and by ¹H NMR analysis (which showed several peaks below δ 5.0 and a broad mass of uncharacterisable signals in the δ 3.4-4.0 region). A clue to the
mechanism of decomposition of \((\pm)-36\) was obtained from the observation that slow effervescence occurred when a concentrated solution of \((\pm)-36\) in D_2O was left to stand at room temperature, possibly indicating spontaneous loss of nitrogen from the \(\alpha\)-azide group, leading to the formation of a reactive nitrene intermediate, which (after proton abstraction from the solvent) could polymerise rapidly and irreversibly.

2.3.1.3. Characterisation of \((\pm)-2\text{-azido}-3\text{-hydroxypropanal}\)

On first dissolving aldehyde \((\pm)-36\) in D_2O, \(^1\text{H} \text{NMR}\) analysis showed a number of peaks in the \(\delta 3.5-4.0, 4.1-4.4\) and 5.0-5.5 ppm regions (Figure 2.7). On leaving a solution of \((\pm)-36\) to stand for 6 h in D_2O, the oligomers which were assumed to be initially present disappeared, and the monomeric hydrated (gem-diol, with characteristic CH(OD)₂ doublet at \(\delta \approx 5.2\) ppm) form of 2-azido-3-hydroxypropanal was seen to be the only species present in solution.

Figure 2.7. \(^1\text{H} \text{NMR spectra of } (\pm)-2\text{-azido}-3\text{-hydroxypropanal (36) in D}_2\text{O}\)
2.3.2. **Enzyme Assays with (±)-2-Azido-3-Hydroxypropanal**

When the hydroxypyruvate assay was first employed to assess the specificity of TK from *E. coli* with soluble aldehydes as substrates, it was typical to employ a large excess of the aldehyde (typically 100 mM, compared with 7.5 mM LiHPA in the reaction vessel). With the poorly soluble aldehydes described in Section 2.1, such conditions could not be effectively employed. (±)-2-Azido-3-hydroxypropanal 36 was found to be significantly more soluble than these aldehydes; however, its instability in concentrated solution made assays using this substrate at high dilution essential. Assays were eventually performed using 10 mM (±)-36, related to a solution of 10 mM glycolaldehyde as standard.

The rate of depletion of hydroxypyruvate in assays with (±)-36 was found to be very low. Measured \( v_{rel} \) values varied between zero (mostly) and 4% compared with glycolaldehyde - consistent data was difficult to obtain because at such low conversion rates, background noise in the assay often obscured (or completely overwhelmed) the actual depletion of hydroxypyruvate. Experiments using significantly increased quantities of TK did not accelerate the rate sufficiently for any useful data to be obtained, and it was finally concluded that (±)-2-azido-3-hydroxypropanal is a very poor substrate for *E. coli* transketolase.

At present, the molecular basis for the reluctance of TK to accept \( \alpha \)-substituted aldehydes is not clear. Presumably, in the natural substrates, a favourable interaction (probably a hydrogen-bonding interaction) between the (R)-hydroxyl group of the \( \alpha \)-hydroxylaldehyde and a suitably positioned residue in the enzyme active site accounts for the tight binding of the acceptor, and the consequent high kinetic selectivity of the enzyme. The reluctance for \( \alpha \)-nitrogen-substituted aldehydes to be accepted presumably implies that steric factors, as well as electronic ones, play a part in the binding of the acceptor substrate. However, since little information has been available to date regarding the binding of the acceptor substrate, the nature of this interaction remains uncertain. Identification of the residue, or residues, directly involved in the substrate binding might, however, allow site-directed mutagenesis studies to be undertaken, which potentially could modify the catalyst to accept bulkier substituents in the \( \alpha \)-position of the acceptor substrates, for instance protected nitrogen atoms.
Chapter Three
3.1. Transketolase Activity Assay

The concentration of an active enzyme in a sample is measured through a quantity known as the activity of the enzyme. Enzyme activity is measured in units (U): one unit of enzyme is defined as that quantity which will produce one micromole of product per minute from a given transformation when the enzyme is functioning at its maximum velocity. For historical reasons the activity of solutions of *E. coli* TK have been determined at 35°C, at which temperature the rates of TK-catalysed reactions are at a near-maximum within the limits of stability of the enzyme.

The activity of a sample of transketolase solution may be determined by means of the multi-enzyme cascade system shown in Scheme 3.1.1, first developed by Heinrich and co-workers. The assay functions by reproducing in a cuvette one of the natural reactions catalysed by TK *in vivo* (namely, the transfer of the ketol unit of xylulose-5-phosphate (24) to D-ribose-5-phosphate (25)) and coupling this reaction to an NADH-mediated oxidation (in this case, of dihydroxyacetone phosphate (12) to glycerophosphate), with the result that the rate of change of UV absorbance of the solution at 340 nm can be measured and related directly to the activity of TK in the solution. Conditions must be chosen such that the TK-catalysed step is the rate-limiting step in the reaction cascade. Studies using aliquots of yeast TK of known activity to calibrate the assay showed it to be reliable for cuvette concentrations of TK between 0.001 and 0.03 U cm⁻¹. At higher concentrations the TK-catalysed reaction ceases to be the rate-limiting step in the cascade; at lower concentrations background oxidation of NADH overwhelms the depletion due to the enzyme.

Assay cocktails containing NADH and all the enzymes and cofactors with the exception of TK and ribose-5-phosphate (which is used to initiate the reaction) may be prepared well in advance of the assay and stored by freezing. Addition of a small aliquot of a dilute solution of TK (prepared with a known dilution factor from the sample whose activity is to be determined), glycylglycine buffer (pH 7.6) and ribose-5-phosphate initiates the reaction, which may be monitored by scanning the UV absorbance of the solution over a 10-minute period, after allowing for an initial "lag

† From the point of view of the biotransformation process, however, it is often more advantageous to perform biotransformations at room temperature, so as to avoid decomposition of hydroxypyruvate or unnatural aldehyde substrates.
phase” for the concentrations of reactants in the early enzymatic steps to build up to a steady level. After this initial phase, a linear depletion of NADH with time is observed; the rate of depletion of NADH in this period correlates directly with the activity of transketolase in the solution.

The biotransformations described in this chapter all employed clarified extracts of transketolase from *E. coli* JM107/pQR700 obtained from fermentations as described in Section 1.4.3.1. The liquors obtained after centrifugation of this material were regularly assayed for transketolase activity as described above, and shown to have activities in the range ≈ 140-220 U cm⁻³ at the assay temperature of 35°C.

![Scheme 3.1.1. Transketolase activity assay enzyme cascade](image)

**Scheme 3.1.1. Transketolase activity assay enzyme cascade**
3.2. Preparative Biotransformations using *E. coli* Transketolase

3.2.1. General Methodology

In the initial stages of the transketolase project, preparative-scale biotransformations were performed in glycylglycine (Gly-gly) buffer at pH 7.6 or 7.0\(^\ddagger\) by incubation at 35°C in an orbital shaker at 200 rpm. However, in these reactions a considerable (usually threefold) excess of aldehyde was necessary to ensure a reasonable yield of product. HPLC analysis of biotransformation mixtures containing glycolaldehyde as substrate, performed at UCL, indicated that a significant proportion of the aldehyde substrate was deactivated by Schiff's base formation with the buffer salt.\(^{127,128}\)

The TK-mediated condensation of an α-hydroxylaldehyde with LiHPA (39) consumes a mole of H\(^+\) ions per mole of aldehyde consumed (Scheme 3.2.1). It follows, therefore, that a TK-catalysed condensation performed in *unbuffered aqueous medium* will be accompanied by an increase in pH corresponding to the consumption of reactants. An alternative protocol for the biotransformation is therefore to perform the biotransformation in unbuffered aqueous solution, making use of a pH autotitrator to maintain the reaction pH at the process optimum (7.0) by addition of a strong acid (Figure 3.1).\(^{128}\) Such a procedure prevents inactivation of the substrate by buffer, and also avoids the interference of large quantities of buffer salts in the work-up procedure. Furthermore, the volume of acid of known molarity added to the biotransformation mixture over a given time period can be used to estimate the extent of the reaction (and thus to estimate the end-point) since an equivalent of protons are consumed per equivalent of aldehyde; however, the pH change during the reaction is partially offset by the dissolution of the byproduct, CO\(_2\),\(^{128}\) so that only an approximate end-point can be determined in this manner. More recent preparative biotransformations were performed in a pH stat at pH 7.0 and room temperature (or thermostatted at 25°C).

\(^\ddagger\) The pH optimum for yeast TK had been shown by Racker to be 7.6. (ref. 119) Sprenger’s studies of *E. coli* TK indicate that a slightly higher pH may be optimum for reactions catalysed by this enzyme (ref. 123); however from the viewpoint of the biotransformation process, it was found to be most advantageous to operate at pH 7.0. This “process optimum pH” conferred maximal stability to LiHPA and aldehyde substrates in the biotransformation, as well as to the enzyme.
Scheme 3.2.1. A natural pH change in the biotransformation

pH Controlled biotransformations using transketolase have also been employed by Whitesides and co-workers. Their methodology involved direct titration of free hydroxypyruvic acid into the biotransformation mixture. Although this method was found to be useful and reproducible, it was found to require more than a stoichiometric quantity of HPA to provide a stable pH in solution; the necessity of preparing free HPA by ion exchange prior to use in the biotransformation (since free HPA is not amenable to storage) was also found to be a disadvantage. A more straightforward approach is to use strong mineral acid (typically 1.0 M HCl) as titrant. Unless otherwise noted, pH controlled biotransformations reported in this chapter employed 1.0 M HCl to maintain a pH of 7.0.

Although several different methods were studied early in the project for isolating the products from biotransformation mixtures, all appeared to involve some degree of unavoidable mechanical losses of product. Of these methods the most favourable was addition of silica to the reaction mixture, followed by lyophilisation (either on a rotary evaporator or by freeze-drying), dry loading of the resultant powder onto a silica column, and chromatography. This was the method of choice employed in this report, although it was recognised that mechanical losses of product were still occurring, since the isolated yields of product were lower than the theoretical yields suggested by pH autotitrator readings and HPLC analysis.
3.2.2. Biotransformation Results

3.2.2.1. Biotransformation of 2-hydroxy-3-phenylpropanal 54°

Biotransformation of enantiomerically pure (R)-2-hydroxy-3-phenylpropanal (R)-54 (1 mmol) with transketolase (= 200 U) was first attempted in gly-gly buffer using 2:1 aldehyde/LiHPA. The relatively water-insoluble aldehyde quickly settled on the bottom of the reaction flask as a white gum; however incubation at 35°C in an orbital shaker for 18 h produced a pale yellow, translucent solution from which 87 mg (44%) of a pale yellow oil was isolated, with 1H and 13C NMR spectra consistent with the expected structure, 95. A single diastereomer was present, as evidenced by the 13C NMR spectrum, and assumed to have the (3S, 4R) configuration. A repeat of the reaction using the pH autotitrator using 1:1 aldehyde/LiHPA gave a 45% yield of triol 95 (Scheme 3.2.2); the autotitrator reading implied that the reaction had proceeded almost quantitatively, and no trace of the poorly soluble aldehyde could be seen in the reaction vessel or by TLC before work-up. Thus it appeared that transketolase was a useful biocatalyst even for the conversion of relatively insoluble aldehydes.

Initial attempts to scale up the biotransformation using the pH autotitrator (4 mmol, 200 U TK) were hampered by the small volume of the available reaction vessel. The reaction mixture was worked-up after 40 h (after which time the pH autotitrator reading implied ≈ 45% conversion, and a significant amount of aldehyde remained as an insoluble gum on the bottom of the reaction vessel) and a 25% yield of product isolated. 81 mg of unreacted aldehyde (13%) was also isolated after

* These results have been published in the Journal of the Chemical Society, Chemical Communications, 1995. (ref. 161)
chromatography, and in order to determine the configurational stability of the aldehyde under biotransformation conditions, this recovered aldehyde was converted to the diethyl acetal (R)-60 (62%) and analysed by $^1$H chiral shift NMR (see Section 2.1.2.2). Comparison with racemic material showed the recovered aldehyde to be essentially enantiomerically pure (e.e. > 95%), i.e. it was confirmed that there was no racemisation of the substrate under the conditions of the biotransformation.

Subsequent attempts at scaling-up this reaction using the facilities of the biotransformation laboratory at Chiroscience Ltd. were rather more successful. A 4 mmol scale biotransformation of (R)-54 (1:1 aldehyde/LiHPA, 400 U TK, 60 cm$^3$ H$_2$O, stir for 46 h at RT) showed apparent quantitative consumption of the substrate and yielded 450 mg (54%) product.

![Scheme 3.2.2. Biotransformation of (R)-2-hydroxy-3-phenylpropanal (54)](image)

3.2.2.2. Biotransformation of $\alpha$-hydroxyphenylacetaldehyde 61*

It is well-known that the $\alpha$-protons of most carbonyl compounds are relatively labile under conditions of acidic, basic or enzymatic catalysis. Gratifyingly, aldehyde (R)-54 had proven to be configurationally stable under biotransformation conditions. Of potentially greater interest, however, were aldehydes such as $\alpha$-hydroxyphenylacetaldehyde (61), in which the $\alpha$-proton was known to be especially labile (the enolate derived from this molecule being stabilised by conjugation with the aromatic ring, providing a facile route to rearrangement of this molecule$^{160,166}$) and in which the possibility therefore exists for an enzyme-catalysed racemisation of the inactive enantiomer of this aldehyde, leading to a possible dynamic resolution of the racemic aldehyde (which would allow a theoretical 100% conversion of the racemic substrate) (see Section 1.2.2.5). Thus, biotransformations of aldehyde 61 were initially attempted with racemic material, in order to ascertain whether a dynamic resolution would occur in the biotransformation.

* These results have been published in the Journal of the Chemical Society, Chemical Communications, 1995. (ref. 161)
Initial biotransformation of (±)-61 in gly-gly buffer (Scheme 3.2.3) (1.5:1 aldehyde/LiHPA, 100 U TK, 35°C) yielded a mediocre 26 mg of an oily compound (37% yield). This material appeared to consist of an inseparable mixture of two products (in a roughly 5:1 ratio as suggested by $^{13}$C NMR), though the nature of the minor product was unclear. At first it was considered possible that some relaxing of the selectivity of the enzyme for the (R)-configuration at the α-centre had occurred, resulting in the production of diastereomeric triols; if, as suggested in Section 2.3.2, unfavourable steric interactions are responsible for the kinetic selectivity of the enzyme, these might be minimised with 61 as substrate, since the molecule can adopt a configuration which would be near-planar apart from the α-hydroxyl group. To test this hypothesis, biotransformation of enantiomerically pure (S)-61 was attempted (pH stat, 1:1 aldehyde/LiHPA). However, there was no apparent consumption of the substrate even after 18 h, and the (S)-aldehyde was recovered in 53% yield, and derivatised as the diethyl acetal (S)-65; $^1$H chiral shift NMR showed that the e.e. of the recovered aldehyde was > 95%, implying that once again there was no racemisation of the substrate at pH 7.0, and evidently no dynamic resolution.

Other explanations for the contaminant in biotransformation of the racemic material were also considered. Mark Smith of this group had observed an unexpected, non-enzymatic rearrangement of the product of biotransformation of phenylacetaldehyde, PhCH$_2$CHO (96), with TK; however no analogous processes were observed in biotransformation of the pure (R)-enantiomer (R)-61, or with enantiomerically pure (R)-54. A more likely explanation is that significant rearrangement of the substrate occurred during biotransformation of (±)-61 at 35°C, and that substrate rearrangement products accounted for the contaminant observed in this biotransformation (though the nature of the contaminant could not be unequivocally identified by NMR).

Biotransformation of the (R)-enantiomer of 61 (Scheme 3.2.3) using the pH autotitrator (1:1 aldehyde/LiHPA) yielded 58 mg (44%) of a product which was poorly soluble in CDCl$_3$; $^1$H and $^{13}$C NMR analysis in D$_2$O suggested that a single diastereomer, 97, was the predominant product (there were traces, < 5%, of a contaminant in the $^1$H NMR spectrum, possibly a substrate rearrangement byproduct - it was notable that the degree of contamination in this case was significantly lower than when the biotransformation had been performed at 35°C).
Scheme 3.2.3. Biotransformation of α-hydroxyphenylacetaldehyde (61)

3.2.2.3. Biotransformation of (±)-3,3-diethoxy-2-hydroxypropanal 75

Condensation of 2.5 mmol aldehyde (±)-75 with LiHPA in the presence of transketolase (= 2:1 (rac)-aldehyde/HPA, 400 U TK, 40 h stir at RT) using the pH autotitrator (Scheme 3.2.4) produced 157 mg (56% yield based on active (S)-aldehyde) of the desired triol 74 as a clear viscous oil. The product of this biotransformation was of particular interest since it had hitherto been assumed that the products of TK-catalysed reactions had the D-threo stereochemistry, but not conclusively proven for E. coli transketolase that this was the case. Since the triol 74 had previously been synthesised using RAMA\(^{103,114,172}\) - an enzyme for which the stereoselectivity has been proven - and characterised, a product of the TK reaction with the same properties (especially NMR spectra and optical rotation) as the product of the RAMA-catalysed reaction can be assumed to have the same absolute stereochemistry.

Although the spectral and physical properties of the triol 74 obtained from this biotransformation were very similar to those reported for the product of the RAMA-catalysed reaction, one or two small discrepancies were noted; in particular the optical rotation observed for the product obtained using TK was -12° (reported: -18°). Given the high hydrophilicity of triol 74, however, and the consequent likelihood of any sample being contaminated by trace amounts of water, this discrepancy was not considered severe.

The resolved (R)-aldehyde was routinely recovered from biotransformation mixtures and purified, giving isolated yields typically = 10% (based on total amount of aldehyde in the biotransformation mixture). Chiral GC analysis of one such sample, separated on a Chirasil-Dex-CB\(^\circledast\) column, and comparison with a sample of the racemate\(^\dagger\) showed the aldehyde to have an e.e. of 78%. It thus appeared that the biotransformation had not proceeded to completion, possibly owing to inhibition (poisoning) of the enzyme by the unreacted aldehyde.

\(\dagger\) The e.e. assay for this compound was devised by Christel Ries and Catherine Rippé at Chiroscience Ltd.
3.2.4. Biotransformation of (±)-3,3-diethoxy-2-hydroxypropanal (75)

3.2.4. Biotransformation of (±)-3-O-benzylglyceraldehyde 81

3-O-Benzylglyceraldehyde 81 was formed in situ on an ≈ 15 mmol scale by deprotection of the acetal precursor (±)-78 at pH 1.0 using the autotitrator to maintain a constant pH in the reaction vessel. Stirring for 2-3 days, depending on room temperature, released the aldehyde (±)-81 in near-quantitative yield as a cloudy suspension which was diluted to 500 cm$^3$ volume before biotransformation. Condensation with LiHPA at pH 7.0 (autotitrator, 1.0 M HCl as titrant) in the presence of transketolase (= 600 U) for 24 h (Scheme 3.2.5) yielded 5-O-benzyl-D-xylulose (82) and resolved (S)-3BG ((S)-81), demonstrating once more the efficiency of TK in accepting relatively water-insoluble substrates. In one such experiment aldehyde (S)-81 was recovered, derivatised as the diethyl acetal (S)-78, and analysed by chiral GC on a Chirasil-Dex-CB column; an e.e. of 72% was observed, although this analysis probably slightly underestimated the enantiomeric purity of the material, since the sample of recovered (S)-81 was observed by $^1$H NMR to be slightly contaminated with unreacted racemic acetal (±)-78. Subsequent studies at UCL, using in situ HPLC analysis of this biotransformation, have indicated that the reaction does proceed almost to completion. It has also been shown that addition of a small amount (5 vol %) of THF in the final stages of the reaction solubilises the remaining aldehyde sufficiently to allow the reaction to reach completion, without adversely affecting the performance of the enzyme. Using this procedure an isolated yield of 1.2 g (78%) 5-O-benzyl-D-xylulose 82 was obtained in a single batch.

† The e.e. assay for this compound was devised by Christel Ries and Catherine Rippé at Chirosience Ltd.
Subsequent process development studies on this substrate, carried out by Mark Smith in this group, have allowed this biotransformation to be performed on a ≈ 25 g scale, with near-quantitative isolated yield of product.\(^{187}\)

\[
\text{Ph} \quad \text{O} \quad \text{OH} \\
(\pm)-81
\]

\[
+ \quad \text{CO}_2 \\
\text{Ph} \quad \text{O} \quad \text{OH} \\
(3S,4R)-82
\]

\[
\text{TK/Mg}^{2+}/\text{TPP} \\
p\text{H 7.0}
\]

Scheme 3.2.5. Biotransformation of (±)-3-\textit{O}-benzylglyceraldehyde (81)

Biotransformation results for these substrates are summarised in Table 3.1.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
<th>Conditions &amp; yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-54</td>
<td>(3S,4R)-95</td>
<td>Gly-gly buffer: 44% pH stat: 54%</td>
</tr>
<tr>
<td>(R)-61</td>
<td>(3S,4R)-97</td>
<td>pH stat: 44%</td>
</tr>
<tr>
<td>(±)-75</td>
<td>(3S,4S)-74</td>
<td>pH stat: 56%</td>
</tr>
<tr>
<td>(±)-81</td>
<td>(3S,4R)-82</td>
<td>pH stat: 78%</td>
</tr>
</tbody>
</table>

Table 3.1. Biotransformation results using \textit{E. coli} transketolase
Chapter Four
Chapter Four. The Use of Transketolase-derived Chiral Triols in the Synthesis of Novel Chiral Products

4.1. The Synthesis of Nectrisine

4.1.1. Development of a Synthetic Route to Nectrisine

Initial studies towards the synthesis of nectrisine 72 were undertaken by Janette Sawden in this group, who employed a RAMA-catalysed condensation between DHAP and 2,2-diethoxyacetaldehyde 31 to generate the chiral triol 74 which was the key intermediate in the synthesis (Scheme 2.2.2). As noted in Chapter Two, the RAMA-catalysed route was useful for the production of triol 74 on a 50-100 mg scale, but did not prove amenable to scale-up. Elaboration of triol 74 en route to nectrisine then required reductive insertion of nitrogen at C-2 of triol 74, removal of the acetal moiety at C-5 to unmask an aldehyde, and cyclisation, with whatever additional protection/deprotection steps were required. Hydroxyl protection was considered a likely necessity to enable modification of triol 74 to be performed in organic solvent systems, and to allow a wide choice of possible reducing agents for the ketone moiety.

Of the possible hydroxyl protecting groups for triol 74, it was eventually decided to employ silyl protection. A wide range of other protecting groups were investigated, with the following results:

- Acetonides of the related chiral triol 98 were synthesised, however it was found that epimerisation at C-3 occurred very readily under the conditions of formation and that the result was an inseparable mixture of diastereomeric acetonides. Whitesides had observed the formation of a mixture of products in the attempted formation of the acetonide of triol 99 during his transketolase-catalysed synthesis of (+)-exo-brevicomin, however using ZnI₂ in anhydrous acetone had enabled the formation of a single acetonide 100, without epimerisation, as desired (Scheme 4.1.1). The same conditions applied to triol 98, however, were unsuccessful, and in general it was considered that the acetonide group was not a useful protecting group for the vicinal diol moiety of triols with reactive functionality at C-5.
- Acetyl, benzoyl and pivaloyl protecting groups for the secondary hydroxyl groups of triols 74 and 98 were found to be highly susceptible to migration on reduction of the ketone moiety or unmasking of the differentially protected primary hydroxyl group.

- Benzyl ethers of triol 74 could not be synthesised.

### Scheme 4.1.1. Whitesides' synthesis of acetonides of triol 99

The tert-butyldimethylsilyl (TBS) group was chosen as the hydroxyl-protecting group, owing to its relative stability and the fact that the silyl derivatives could be synthesised without epimerisation. However, surprisingly fierce conditions were found to be necessary to protect the hydroxyl groups of triol 74. Treatment of a solution of 74 and imidazole (6 equivalents) with TBS triflate (4.5 equivalents) resulted in the protection of only the primary hydroxyl group, yielding diol 101 in 90% yield. A stronger base, triethylamine, was necessary to allow protection of the secondary hydroxyl groups in addition (forming fully protected derivative 102) (Scheme 4.1.2).

Three schemes were envisaged for the insertion of the nitrogen functionality at C-2. Reduction of the ketone moiety of monosilylated 101, followed by nitrogen insertion via nucleophilic displacement or Mitsunobu inversion, was unsuccessful, the
Scheme 4.1.2. Janette Sawden's synthesis of protected derivatives of triol 74

The former approach producing a mixture of uncharacterisable products and the latter requiring differential protection of the secondary hydroxyl groups which resulted in protecting group migration. Similarly, reduction of trisilylated 102 was observed to occur with significant silyl migration to the newly formed alcohol at C-2. The second approach was reductive amination \(^*\) of the ketone moiety of monoprotected 101 using ammonium acetate to form an imine and sodium cyanoborohydride\(^{193}\) at pH = 6 to reduce the imine to an amino group; this approach failed, and a mixture of C-2 epimeric alcohols was isolated, implying either that the intermediate imine could not be formed or that hydrolysis of this intermediate was considerably more rapid than reduction by the cyanoborohydride anion. The final approach was conversion of the ketone moiety at C-2 to an oxime (or an oxime ether), and reduction of the oxime. Oximes 103 and 104 were readily synthesised from mono- and trisilylated 101 and 102, respectively, as were the corresponding benzyl ethers. However, almost all of these compounds proved inert to reduction using LiAlH\(_4\), borane-THF, sodium borohydride and divalent nickel, and catalytic hydrogenation over rhodium. Reduction of the trisilylated oxime 104 using hydrogenation over fresh Raney\(^\circ\) nickel, however, was successful, and a diastereomeric mixture of amines 105 (the desired precursor) and 106, in a 3:2 ratio, was formed. Cyclisation of the mixture of amines was accomplished by acetal unmasking using the electrophilic reagent, iodonitromethane,\(^{194}\) yielding a separable mixture of trisilylated nectrisine 107 and its C-2 epimer\(^\dagger\) 108 (Scheme 4.1.3); nuclear Overhauser effect (nOe) studies revealed that, as expected, the major diastereomer was 107, in which all substituents are \textit{trans}.

\(^*\) A reductive amination strategy was also proposed by Demuynck and co-workers (ref. 140) but no results, successful or otherwise, were reported.

\(^\dagger\) Numbering from the heteroatom in the ring as the 1-position.
Scheme 4.1.3. Preliminary synthesis of silylated nectrisine (107)

4.1.2. Validation of the Synthetic Route Using Transketolase

4.1.2.1. (±)-3,3-Diethoxy-2-hydroxypropanal as precursor

The formation of triol 74 by a transketolase-mediated condensation, with (±)-3,3-diethoxy-2-hydroxypropanal 75 as the acceptor substrate providing C-3 to C-5 of triol 74, is discussed in Section 3.2.2.3. Although the yields of 74 obtained using the TK-based route were not significantly higher than those obtained using the
RAMA-catalysed condensation, it appeared that the ease of aldehyde preparation and biotransformation would make sufficient material available to allow the synthesis of silylated nectrisine to be repeated and validated. Comparison of the spectral properties of the intermediates formed following the TK-based and RAMA-based routes would provide additional proof of the [3S, 4R] stereoselectivity of *E. coli* TK.

Silylation of triol 74 (82 mg, 0.37 mmol) using TBS triflate (1.7 mmol) and triethylamine (2.2 mmol) produced the trisilylated derivative 102 in 63% yield. Analysis of the crude reaction mixture by TLC, however, revealed the presence of two byproducts in addition to the major product: a relatively non-polar fraction ($R_f = 0.8$ [CH$_2$Cl$_2$ (neat)]) which separated easily from the desired product ($R_f = 0.45$) by chromatography, and a fainter spot slightly more polar than the desired product. It was considered that the relatively harsh conditions necessary to install the secondary hydroxyl protecting groups (*vide supra*) were more than likely responsible for the mixture of products formed in the reaction. The presence of a faint slightly polar spot on the TLC plate suggested a small degree of incomplete silylation of the triol (this fraction could not be isolated). The non-polar fraction was assumed to correspond to one or other of the possible silyl enol ethers, 109 and 110, which might be formed as products of over-reaction in the presence of excess base and TBSOTf. A small amount (12 mg) of this material was isolated and subjected to $^1$H NMR analysis; the results were inconclusive, since the isolated material was impure (probably contaminated with the byproduct, TBSOH), however the presence of an AB quartet-like signal at $\delta \approx 4.6$ ppm (corresponding to the protons at C-1 in the desired product 102) and the absence of a doublet at $\delta 4.4$ (corresponding to 3-H in 102) may suggest that the non-polar fraction contained the silyl enol ether 109. The reported lability of 3-H of some related triols (*vide supra*) might also imply that silyl enol ether formation is induced by enolisation at C-3, rather than at C-1.

The isolated trisilylated material 102, however, showed no evidence of epimerisation at C-3. $^1$H NMR and optical rotation data ($[\alpha]_D +8.7^\circ$, compared to +9$^\circ$) were in close agreement with those reported,$^{172}$ and only a single diastereomer was evident according to the $^1$H NMR spectrum.
Although a yield of 93% had been reported for the formation of \textbf{102} on a comparable scale to that reported herein,\textsuperscript{172} no higher yield than 74\% was ever obtained for the silylation of triol \textbf{74}, presumably owing to the formation of side products. The best single batch yield obtained from this reaction was 223 mg (69\%). Oxime \textbf{104} (as a mixture of (E)-and (Z)-double bond isomers) was, however, cleanly formed by stirring of \textbf{102} in methanol at reflux in the presence of hydroxylamine hydrochloride and KHCO\textsubscript{3} (Scheme 4.1.4), though considerably longer reaction times (3-5 hours) than those reported were found to be necessary. An 82\% yield of oximes \textbf{104} was obtained on a small scale (0.25 mmol); the best single batch yield recorded was 130 mg (73\%).

It was found that compounds \textbf{102} and \textbf{104} were particularly prone to decomposition in chloroform solution when submitted for NMR analysis. The reason for this instability was unclear, though partial unmasking of the diethyl acetal moiety by the traces of acid present in commercial supplies of chloroform-\textit{d} may have been responsible. Refrigeration was also found to be necessary for these compounds, which were isolated as clear or pale yellow oils after chromatography, and even in the refrigerator their lifetimes were short, typically less than one week.

\textit{4.1.2.2. Oxime reduction}

Initial attempts to reduce oximes (E)-and (Z)-\textbf{104} (0.26 mmol) by hydrogenation over freshly purchased Raney\textsuperscript{\textregistered} nickel (suppliers: Aldrich Chemical Co.) for 21 h resulted in the formation of a more polar spot which stained deep blue-black with resorcinol staining on a TLC plate (R\textsubscript{f} = 0.03-0.10 [petrol: EtOAc (9:1)]). Filtration through Celite\textsuperscript{\textregistered}, followed by chromatography, gave an inseparable mixture of diastereomeric amines \textbf{105} and \textbf{106} in 64\% overall yield. The diastereomer ratio was judged to be 3:2, as previously reported, by examination of the integrals of the 5-H doublets observed at \(\delta\) 4.55 and 4.63 in the \textit{\textsuperscript{1}H NMR} spectrum.

Unfortunately, the reduction of oximes \textbf{104} proved extremely difficult to reproduce. Janette Sawden had observed that the use of fresh Raney\textsuperscript{\textregistered} nickel was essential for a successful reaction.\textsuperscript{172} A second attempt at the reduction of oximes \textbf{104} using the original batch of Raney\textsuperscript{\textregistered} nickel indeed resulted in no reaction; a new batch yielded amines \textbf{105} and \textbf{106} in 51\% yield, but was successfully employed only twice before it too became useless. Subsequent supplies of Raney\textsuperscript{\textregistered} nickel purchased from Aldrich all accomplished the reduction in very low yield, or not at all; it transpired that all the purchased material had been supplied by Aldrich from the same batch. Raney\textsuperscript{\textregistered} nickel purchased from other sources, however, proved no more effective.
Scheme 4.1.4. Synthesis of silylated nectrisine

The unreliability of the oxime reduction inevitably presented a major obstacle to the successful synthesis of nectrisine, and severely limited the availability of material for subsequent studies. The oxime silyl ether 111, readily synthesised in 76% (92 μmol) yield from 104 by treatment with triethylamine and TBSOTf, also proved to be completely inert to Raney® nickel-catalysed hydrogenation. It thus appeared likely that an alternative synthetic scheme for amine 105 (or a closely related compound) would be essential for a successful synthesis of nectrisine.
4.1.2.3. Cyclisation and attempted deprotection

The meagre supply of amine 105 available from the Raney® nickel-catalysed hydrogenation was employed in small-scale cyclisation studies. The mixture of diastereomeric amines was readily cyclised by treatment with iodonitrtrimethylsilane in anhydrous CH₂Cl₂; the reaction proceeds via electrophilic unmasking of the protected aldehyde, followed by cyclisation (internal imine formation) as shown in Scheme 4.1.5. Janette Sawden originally reported the synthesis of the diastereomeric imines 107 and 108 in a 5:1 ratio (cf. the 3:2 ratio of the precursor amines 105 and 106), which was explained by a rapid cyclisation of amine 105 to form the all-trans product 107, but a much slower cyclisation of 106, since the resulting cyclic imine has a considerably more hindered cis arrangement of substituents at C-2 and C-3. A repeat of the reaction on a 36 mg (64 μmol) scale, with stirring for 1 h after addition of TMSI, led to the formation of cyclic imines 107 and 108 in almost quantitative total yield (60% 107, 37% 108 - an approximately 3:2 diastereomeric ratio). The

![Scheme 4.1.5. Mechanism for the iodonitrtrimethylsilane-mediated cyclisation of amines 105 and 106](image_url)
diastereomers were readily separable by chromatography on silica, and $^1$H NMR analysis showed that the major diastereomer was identical to that obtained via the RAMA-based route.

Unfortunately, only milligramme quantities of silylated nectrisine (107) were available for deprotection studies. Janette Sawden had previously attempted the desilylation of 107 using tetra-n-butylammonium fluoride (TBAF); however, a multitude of products were observed by TLC, and although partial purification of the mixture was possible by means of sequential ion-exchange chromatography, the product could not be unequivocally identified as containing nectrisine (72) (though an imine resonance at $\delta = 7.8$ in the $^1$H NMR spectrum, and a molecular ion in the mass spectrum consistent with the presence of nectrisine, were both observed). Alternative deprotection strategies (AcOH/H$_2$O/THF, fluoride resin, and HF/acetonitrile) were also reported to yield no product, though the exact nature of what was formed remained unclear. Decomposition in the presence of appreciably basic reagents such as TBAF was the most likely cause of poor deprotection results (TBAF was found to decompose $\alpha$-hydroxyaldehydes such as 66 quite significantly (see Chapter Two)). Another possibility is the existence of nectrisine in more than one form in solution. pH-Dependent equilibria for N-containing heterocycles, especially cyclic imines (such as $\Delta^1$-pyrroline-2-carboxylic acid and 4-epi-nectrisine), are well known, although Hashimoto and co-workers reported that the thermodynamically stable "all-trans" arrangement of substituents in nectrisine strongly disfavoured the existence of such equilibria.

The apparent failure of basic deprotection conditions to yield nectrisine in characterisable form encouraged us to study the acidic deprotection of 107, albeit on a small (= 25 mg) scale. Stirring of a solution of 107 in 3:1:1 AcOH/H$_2$O/THF for 7 days (Scheme 4.1.6) appeared to result in a progressive removal of the TBS groups, as judged by TLC; after 7 days, however, TLC analysis revealed only a faint streak to the baseline [EtOAc (neat)]. Evaporation of the solution to dryness in the presence of silica, followed by dry loading onto a silica column and chromatography, yielded a few milligrammes of a sticky off-white solid which could not be characterised further. Use of 9:1 trifluoroacetic acid (TFA)/water appeared to cleave the TBS groups much more rapidly (= 18 h). Evaporation to dryness yielded 5 mg of a red-brown, waxy solid, from which a white amorphous mass settled out on treatment with D$_2$O. As similar behaviour was observed with chiral $\alpha$-hydroxyaldehydes (see Chapter Two), it was considered possible that oligomeric material was present; after allowing a short time for equilibration of the mixture, the supernatant was analysed by $^1$H NMR. Silyl impurities were present, as were a series of poorly resolved multiplets
in the δ 3.4, 3.5, 3.7 and 4.0 regions, as reported by Hashimoto and co-workers for nectrisine (Figure 4.1). A very poorly defined signal at δ = 7.8 was also present. However, the material isolated was clearly impure, and there was not sufficient material available to confirm that the mixture contained nectrisine. Nor was it clear whether the observed white gelatinous precipitate was due to oligomeric forms of the cyclic imine, or merely to impurities.

Figure 4.1. 'H NMR spectrum of material isolated from the deprotection of imine 107 (D₂O)

Scheme 4.1.6. Attempted deprotection of nectrisine
4.2. 5-O-Benzyl-D-Xylulose as a Synthetic Intermediate‡

4.2.1. An Alternative Synthetic Route for Nectrisine

4.2.1.1. Development of a route from 5-O-benzyl-D-xylulose

The failure of the above synthetic scheme to yield sufficient material for a laboratory-scale synthesis of nectrisine led to a consideration of alternative routes to the synthesis of this natural product. As mentioned in Section 2.2.2.1, an alternative starting material for the synthesis is (±)-3-O-benzylglyceraldehyde (3BG), 81. This aldehyde has the advantage that it is available in multigramme quantities (Section 2.2.2.1) and that its biotransformation to 5-O-benzyl-D-xylulose (5BX), 82, is both efficient and can be accomplished easily on a 2-3 g scale (with the potential for considerable further scale-up) (Section 3.2.2.4). Thus, 5BX (82) was a readily available chiral synthon for an alternative route to nectrisine.

At first it was envisaged that the reaction scheme illustrated in Scheme 4.1.4 could be adapted for use with 5BX as starting material. The sequence of silylation, oxime formation, and reduction (when this step was successful) had been shown to yield silylated nectrisine 107 effectively, and this approach was adopted in the formal scheme devised for a synthesis of nectrisine via 5BX (Scheme 4.2.1). The addition of a debenzylation step, followed by subsequent oxidation of the free alcohol moiety, in principle would provide an aldehyde which is formally equivalent to the masked aldehyde/oxime 104. Formation of the diethyl acetal would yield 104, and create a "link point" between the two synthetic routes, hopefully providing sufficient material for an investigation into alternative means of accomplishing the troublesome oxime reduction step.

‡ The work reported in this Section was performed in collaboration with Dr. Mark E.B. Smith of this group. Specific contributions by Dr. Mark Smith are reported as such in the text.
Scheme 4.2.1. Proposed complementary routes to the synthesis of nectrisine

4.2.1.2. Initial studies

Initial investigation of this scheme was carried out by Mark Smith of this group. Silylation of 5BX using TBSOTf and triethylamine yielded the trisilylated derivative 112, from which the oxime 113 was efficiently synthesised by stirring with hydroxylamine hydrochloride and KHCO₃ in refluxing methanol (Scheme 4.2.1). Debenzylation of oxime 113 using catalytic hydrogenation in the presence of 5%...
palladium on charcoal (supplier: BDH Ltd.), proceeded cleanly to yield alcohol 114, however all attempts to oxidise the alcohol moiety of 114 to an aldehyde were unsuccessful. Reduction of the oxime moiety of 114 by hydrogenation over Raney nickel, however, proceeded cleanly; unfortunately, attempts to protect the resultant amine moiety and subsequently oxidise the free alcohol to an aldehyde proved fruitless, and this route was abandoned.

As an alternative strategy, the oxime silyl ether 115 was prepared, once again by treatment of oxime 113 with TBSOTf and triethylamine, and debenzylated by hydrogenation over palladium-charcoal to yield the silylated intermediate 116 (Scheme 4.2.2). No silyl group migration was observed in the synthesis of primary alcohol 116. Studies on the oxidation of alcohol 116 are reported below.

Scheme 4.2.2. Formation of oxime silyl ethers

4.2.2. VALIDATION OF THE PROPOSED SYNTHETIC ROUTE

4.2.2.1. Synthesis of key intermediates derived from 5BX

The initial steps of the proposed synthesis were easily reproduced. Treatment of a solution of 5BX (82) in dry CH$_2$Cl$_2$ with triethylamine (6 equivalents, or two per OH group) and TBSOTf (4.5 equivalents), followed by stirring for 30 min, produced trisilylated derivative 112 in modest (40%) yield; TLC indicated that as well as the trisilylated derivative, products of incomplete silylation were present in the mixture, as well as a silyl enol ether (assumed to be 117 or 118) which was not isolated.

* No evidence of silyl group migration in the synthesis of alcohol 114 was observed.
Reduction of the proportion of base used for the silylation (to 1.6 equivalents per OH) produced a much cleaner reaction mixture, and enabled 112 to be isolated in a much improved 83% yield (2.3 g).

Oxime 113 (as a 2:1 mixture of (E)- and (Z)-geometric isomers) was obtained in good (71%) yield by stirring of a mixture of 112, hydroxylamine hydrochloride and KHCO₃ in methanol at reflux for 1 h (it was notable that this reaction was considerably more rapid than that of the diethyl acetal analogue 104). Treatment of oxime 113 with TBS triflate and triethylamine formed the silyl ether 115 in almost quantitative yield. The short reaction times for these first three steps, and the straightforward chromatographic purification of the products, meant that the first three steps in the sequence could feasibly be accomplished during a single day in the laboratory.

Debenzylation of oxime ether 115 proved surprisingly problematic. Hydrogenation using catalytic amounts of 5% palladium on charcoal (supplier: BDH) resulted in extremely variable reaction times (from 22 h to 3-4 days), and addition of extra quantities of catalyst at 24 hour intervals was often found to be necessary for the reaction to reach completion. Using 10% palladium-charcoal obtained from Aldrich (both dry catalyst and Degussa-type) as catalyst resulted in no debenzylation of compound 115; palladium hydroxide and platinum (IV) oxide were likewise found to be ineffective for the hydrogenation of 115. The most effective catalyst proved to be 10% palladium-charcoal obtained from BDH, which resulted in complete debenzylation of 115 within 24 h under an atmosphere of H₂, though appreciable amounts of catalyst (40-50 weight %) were found to be necessary to obtain a rapid, efficient conversion. The alcohol 116 was isolated by filtration of the reaction mixture through Celite® (though copious washing with ethyl acetate was found to be necessary to desorb the material) and concentration in vacuo. The crude alcohol was found to be > 90% pure to ¹H and ¹³C NMR analysis (¹H NMR suggesting an ≈ 1:1 mixture of double bond isomers), and could be isolated in near-quantitative yield as a clear or yellow oil which slightly solidified after prolonged evacuation on a high-vacuum line. Alternatively, chromatography produced highly pure alcohol 116 as a white semisolid, though the slight reduction in yield after chromatography (typically to ≈ 60-70%) meant that the alcohol was more often stored and used as the crude extract.
The reasons for the relative inertness of compound 115 to catalytic hydrogenation is not entirely clear. It seems likely that either the starting material or the resultant alcohol 116 had a poisoning effect upon the catalyst, hence the necessity to use a substantial proportion by weight of catalyst to achieve an efficient reaction. The strong adsorption of the alcohol 116 to the catalyst may be evidence that catalyst poisoning by the product was taking place. Alternatively, trace silyl impurities in the silyl ether 115 may have been responsible for catalyst poisoning. The preponderance of silyl groups in the substrate presumably also presents a significant degree of steric hindrance to hydrogenation; it is likely that similar effects contribute to the inertness of the diethyl acetal substituted oxime 104 to catalytic hydrogenation (vide supra).

4.2.2.2. Oxidation of alcohol 116

Small-scale oxidation of the alcohol moiety of 116 under Swern conditions (oxalyl chloride/DMSO, followed by treatment with triethylamine) (Scheme 4.2.3) produced the aldehyde 119 as an off-white semisolid in moderate (40-60%) yield. However, attempts to increase the scale of the reaction did not improve the yield; and in all attempts, significant amounts of an unidentified side product were also isolated. Comparison of the $^1$H NMR spectra of aldehyde 119 and the side product (Figure 4.2) showed some similarities, notably the AB-quartet signals corresponding to the CH$_2$OTBS moiety of the desired product at $\delta = 4.2$ and 4.6 in aldehyde 119 and (possibly) at $\delta = 4.2$ and 4.8 in the unidentified side product; however, the absence of the distinctive doublet at $\delta = 5.3$ corresponding to 3-H and the absence of an aldehyde resonance appeared to suggest an unrelated structure for the side product.

Alternatively, small-scale oxidation of alcohol 116 in a two-phase mixture using sodium hypochlorite and catalytic TEMPO (see Scheme 2.2.5 for the mechanism of the TEMPO-catalysed oxidation) resulted in a 66% yield of aldehyde 119 and a slightly cleaner (to TLC) reaction mixture. Attempts to scale up this reaction were also somewhat unsatisfactory, however, mainly because the rate of reaction of 116 was rather low (addition of NaOCl was carried out over a 45-minute period, as necessary to maintain an intense yellow colour in the solution) and estimation of the end-point was difficult. More than once, solvent extraction and chromatography resulted in starting material being recovered, implying little or no reaction; and on one occasion, the product was apparently contaminated with hypohalous acid, as it decomposed rapidly when stored in a refrigerator. The best batch yield of pure 119 obtained via TEMPO oxidation was 61% (245 mg), comparable to yields obtained from the Swern oxidation of 116.
Figure 4.2. $^1$H NMR spectra of (top) aldehyde 119 and (bottom) the side product from the Swern oxidation of alcohol 116.
4.2.2.3. An unexpected cyclisation

It was considered that protection of the aldehyde moiety of compound 119 as the diethyl acetal would provide an alternative "link point" to the synthesis of nectrisine from triol 74, as the resulting silyl ether (111) had been synthesised from oxime 104 (see Section 4.1.2.2). Selective unmasking of the oxime silyl ether would then allow a direct link to the synthesis outlined in Scheme 4.1.4; alternatively, a protocol for the reduction of silyl ether 111 (presumably with N-O bond cleavage, and concomitant loss of the silyl group) would yield amines 105 and 106, and the synthesis of nectrisine would proceed as outlined in Section 4.1.2.3. Initial studies by Mark Smith of the protection of the aldehyde moiety of 119 via a p-toluenesulfonic (tosic) acid-catalysed transacetalisation with triethyl orthoformate however did not yield compound 111. Instead, a number of products were observed by TLC during the course of the reaction, from which, after quenching with triethylamine and removal of excess triethyl orthoformate followed by chromatography, a major product was isolated corresponding to a spot observed at $R_f = 0.24$ [petrol: EtOAc (9:1)].

$^1$H NMR analysis of the fraction isolated revealed the following unexpected results:

- Loss of a single TBS group, assumed to be that attached to the oxime oxygen;
Addition of a single ethyl group to the molecule (rather than two, as for the formation of the diethyl acetal of aldehyde 119);

- A surprising upfield shift of the proton attached to C-3 of the acyclic precursor, from $\delta = 5.3$ (a C-3 resonance at $\delta = 5.3$ was also observed in the expected diethyl acetal product 111 formed by silylation of oxime 104) to 4.6.

Further analysis of the compound isolated (see Section 4.3) appeared to suggest that an unexpected cyclisation had taken place, and that the product isolated from the reaction mixture was in fact the six-membered heterocycle 120, a 5,6-dihydro-4H-1,2-oxazine.

At first, the synthesis of compound 120 from aldehyde 119 proved difficult to repeat. The timing of the reaction was found to be critical; too long a reaction time resulted in the rapid disappearance of the spot observed at $R_f \approx 0.24$ and the appearance of a non-polar fraction which stained vivid red-orange with resorcinol; from such reaction mixtures the product assumed to be 1,2-oxazine 120 was not isolated. Reaction times of between 30 and 60 min were found to be optimal, depending on room temperature, with careful monitoring of the reaction by TLC essential. The nature and quantity of the base used to quench the reaction mixture also appeared critical, as addition of excess base also caused rapid decomposition of the material; on a 0.5 mmol scale, quenching of the reaction mixture with five drops of pyridine from a Pasteur pipette appeared optimal. Several attempts were made to vary the initial conditions of reaction in the hope of improving the isolated yield of 120: these included a change in the solvent from triethyl orthoformate to ethanol (a number of products were observed by TLC, none apparently corresponding to compound 120 and none proving to be characterisable by $^1$H NMR), use of alternative acid catalysts (acetic acid also produced an uncharacterisable mixture of products, evidently not containing compound 120, whereas Amberlyst 15® dry bead H⁺ resin did appear...

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1 Impure, non-polar fractions evidently containing a significant percentage of oxime ether 111 ($^1$H NMR evidence) were isolated from this mixture, but proved impossible to purify completely.
slowly to form 120, although the product decomposed on overnight stirring) and use of different temperatures (the reaction appeared to proceed at 0°C exactly as at RT). The most favourable conditions employed were eventually using tosic acid (= 5 mg per 100 mg aldehyde) in triethyl orthoformate (= 2 cm³ per 100 mg aldehyde), reaction times of 30-60 min, quenching with 5 drops of pyridine, and removal of triethyl orthoformate by azeotropic distillation with ethanol on a rotary evaporator. This procedure reliably produced ≈ 30% yields of 1,2-oxazine 120, which could be easily
purified from the other byproducts of reaction by chromatography on silica. Oxazine 120 was isolated as a clear oil, with a lifetime of the order of 1 week when refrigerated, and considerably less in chloroform solution in an NMR tube.

The synthesis of oxazine 120 from 5BX is summarised in Scheme 4.2.4. Evidence for the six-membered structure of 120, and possible mechanisms for its formation, are discussed in Section 4.3.

4.3. Novel Heterocycles derived from 5-0-Benzyl-D-Xylulose

4.3.1. 1,2-Oxazines: Some Literature Background

5,6-Dihydro-4H-1,2-oxazines (hereafter referred to more simply as 1,2-oxazines) are heterocycles most often formed by the inverse electron demand Diels-Alder cycloaddition of an α-nitrosoalkene to a nucleophilic dienophile. The α-nitrosoalkene, which acts as a heterodiene in the cycloaddition, is generated from the 1,4-elimination of an α-halogenated oxime, while a wide range of olefinic species have been employed as the nucleophilic component of the cycloaddition, including dienes and conjugated alkenes, aromatic heterocycles, enol ethers, enamines and silyl enol ethers. The resulting cycloadducts are formed with extremely high regioselectivity; with an enol ether, for instance, as the nucleophile, the resultant 1,2-oxazine is exclusively that which possesses the alkoxy (or silyloxy) substituent at C-6, rather than at C-5.

Some representative transformations of 1,2-oxazines include the following, which are summarised in Scheme 4.3.1:

- (a) ring opening of 1,2-oxazines which can stabilise a positive charge at C-6 (for instance, those derived from enol ethers) can be accomplished using HCl in methanol; cyclisation of the intermediates thus formed gives rise to five-membered nitrones or 6-membered pyridine N-oxides, depending on the nature of the C-3 substituent in the oxazine,
- (b) reduction of 6-alkoxy-1,2-oxazines (derived from enol ethers) with aluminium amalgam or triethyl borate and palladium, or of 1,2-oxazines with protected amino substituents at C-6 (derived from enamines) using hydrogenation over Raney® nickel produces highly substituted proline analogues which are
intermediates in the synthesis of a range of angiotensin-converting enzyme (ACE) inhibitors;

Scheme 4.3.1. Synthesis and transformation of some representative 1,2-oxazines

- (c) transition metal-induced conversion of 1,2-oxazines using molybdenum hexacarbonyl yields pyrroles in moderate to good yield;\(^{203}\)
- (d) mild acidic unmasking of 6-silyloxy-1,2-oxazines yields 6-hydroxy-1,2-oxazines which can be ring-opened on heating in aqueous formaldehyde to produce the corresponding 1,4-dicarbonyl compounds, though not always in high yield;\(^{203}\)
• (e) **reduction** of 6-silyloxy-1,2-oxazines **under protic conditions** using NaBH₄ in ethanol yields the ring-opened products, 4-hydroxyoximes, however using sodium cyanoborohydride in acetic acid no N-O bond cleavage is observed, and fully reduced 1,2-oxazines are isolated, with concomitant loss of the C-6 silyloxy substituent;²⁰⁵

• (f) **reduction** of 6-silyloxy-1,2-oxazines **under aprotic conditions** using DIBAL-H leads to the formation of five-membered N-hydroxypyrrolidines or nitrones.²⁰⁴

4.3.2. **SYNTHESIS AND TRANSFORMATION OF A NOVEL 1,2-OXAZINE**

4.3.2.1. **The characterisation of 1,2-oxazine 120**

The synthesis of an unexpected cyclic product from the attempted acid-catalysed acetalisation of aldehyde 119 in triethyl orthoformate has been described in **Section 4.2.2.3.** The proton NMR spectrum of the isolated product is reproduced in **Figure 4.3.** Assignment of the 1,2-oxazine structure 120 to the isolated product was made on the basis of the following evidence:

• ¹H NMR integrals were consistent with the presence of three TBS groups and a single ethoxy group per molecule; a molecular ion of m/z 534 [MH⁺] consistent with the molecular formula C₂₅H₅₅NO₅Si₃ was observed in the Chemical Ionisation mass spectrum; furthermore a C=N stretch at νₘₐₓ 1585 cm⁻¹ (exactly comparable to that reported by Gilchrist et al. for the C=N stretches of a range of unsaturated 1,2-oxazines¹⁹⁷,¹⁹⁸) was observed, implying that addition or other reaction across the oxime double bond had not taken place.

• A significant upfield shift in the ¹³C resonance of the C=N moiety, from δ = 162 in the acyclic precursors to δ = 146.

• The observation of a doublet with small coupling constant (J 2.0 Hz) at δ = 4.6 in the ¹H NMR spectrum of the product; decoupling experiments correlated this proton with 3-H of the precursor aldehyde, which was observed at δ ≈ 5.3 in the acyclic precursors and also in the silyl ether 111.

• The observation of a doublet with small coupling constant (J 3.0 Hz) at δ = 4.7 in the ¹H NMR spectrum of the product; decoupling experiments showed that this proton was coupled to 2-H of the precursor aldehyde (5-H in the 1,2-oxazine structure).
- A $^{13}$C signal at $\delta$ 104.5 corresponding to C-6 in the proposed structure, exactly as observed by Reissig and co-workers for related 6-ethoxy-1,2-oxazines.$^{201}$

This evidence is summarised in Figure 4.4.

![Figure 4.3. $^1$H NMR spectrum of 1,2-oxazine 120 (CDCl$_3$)](image)

**Figure 4.3. $^1$H NMR spectrum of 1,2-oxazine 120 (CDCl$_3$)**

![Figure 4.4. Spectroscopic evidence for the structure of 1,2-oxazine 120](image)

**Figure 4.4. Spectroscopic evidence for the structure of 1,2-oxazine 120**
4.3.2.2. Mechanistic proposals

It was notable that, in numerous failed attempts to synthesise 1,2-oxazine 120 from aldehyde 119, triethyl orthoformate and tosic acid, the non-polar material recovered from the reaction mixtures after chromatography appeared, by $^1$H NMR analysis, to contain (inter alia) the silyl ether 111. However, attempts to synthesise 1,2-oxazine 120 by stirring of a solution of silyl ether 111 in triethyl orthoformate or ethanol in the presence of tosic acid did not appear to yield the desired product. These considerations have led to the proposal of a putative mechanism for the acid-catalysed cyclisation of aldehyde 119 based on initial formation of a hemiacetal intermediate, followed by cyclisation and desilylation as the final step (Scheme 4.3.2). The operation of such a reaction scheme would imply that the diethyl acetal derivative 111 is considerably more stable than the proposed hemiacetal intermediate; thus steady accumulation of this product over time would result in a decrease in the proportion of 1,2-oxazine present in the reaction mixture. It is clear, however, that such a scheme does not explain the apparent rapid decomposition of the 1,2-oxazine if the reaction mixture is not quenched after a relatively short time.

A mechanistic scheme such as that proposed in Scheme 4.3.2 would, however, enable some explanation to be offered for the numerous side products observed by TLC under the reaction conditions. An alternative cyclisation, for instance, would be that which occurs when the nitrogen, rather than the oxygen, of the oxime silyl ether acts as a nucleophile, with the formation of a five-membered ring (a nitrone). The nitrone structure, 121, is in fact isomeric with 120, and it was considered that the two possible molecular structures would be difficult to differentiate on the basis of spectroscopic evidence, which raised the question of whether the structure of the major compound isolated from the reaction mixture might not, in fact, be the nitrone 121 rather than the 1,2-oxazine 120 (cyclisations forming five-membered rings being generally kinetically favoured over those which form six-membered rings). However, on the basis of consistency (vide supra) between the spectroscopic data observed for compound 120 and the data reported by Gilchrist$^{197,198}$ and Reissig$^{201}$ for related compounds - and particularly the high-field $^{13}$C resonance of C-3 in the proposed structure, a carbon atom which would be expected to be considerably deshielded by a neighbouring positive charge if the isomeric nitrone structure had been formed - we inclined to the opinion that the correct structure was, in fact, the 1,2-oxazine 120.
Scheme 4.3.2. Possible mechanism for formation of 1,2-oxazine 120
4.3.2.3. Conformation and stereochemistry of 1,2-oxazine 120

It was apparent from the $^1$H and $^{13}$C NMR spectra of 1,2-oxazine 120 that the heterocycle was isolated as a single diastereomer. Since the stereochemistry at C-4 and C-5 was fixed by the transketolase-catalysed condensation, only the stereochemistry at C-6 remained to be determined.

Gilchrist$^{198}$ and Reissig$^{201,205}$ both report that a “half-chair” conformation, similar to that adopted by cyclohexene, is the most favourable conformation for 6-substituted 1,2-oxazines. Assuming a “half-chair” conformation to be favourably adopted by 1,2-oxazine 120, the very small coupling constant $J_{4,5}$ (2.0 Hz) would imply that a trans-diaxial arrangement of the bulky silyloxy substituents at C-4 and C-5 existed in the most stable conformation of 1,2-oxazine 120 (Figure 4.5). In a “half-chair” model, 4-H and 5-H occupy (pseudo)-equatorial positions, and the Karplus equation predicts a small coupling constant between these two protons.

The small coupling constant $J_{5,6}$ (3.0 Hz) also appeared to imply a (pseudo)-equatorial disposition of the protons H-5 and H-6 in a “half-chair” model of 1,2-oxazine 120. This would imply an axial positioning of the ethoxy group, as observed by Gilchrist et al.$^{198}$ and by Reissig and co-workers$^{201,205}$ for 6-alkoxy-and 6-silyloxy-substituted 1,2-oxazines. The “anomeric effect” has been invoked to explain the stabilisation of the 6-(pseudo)-axial conformation in such compounds (since 6-silylalkyl and 6-stannylalkyl substituted 1,2-oxazines preferentially adopted a more sterically favourable conformation with a (pseudo)-equatorial 6-substituent),$^{201,202}$ and therefore this would be the predicted conformation for 1,2-oxazine 120.

It was hoped that $^1$H NMR nuclear Overhauser effect (nOe) studies would provide further evidence of an axially substituted ethoxy group at C-6. Unfortunately, nOe studies on 1,2-oxazine 120 were hampered by the instability of this compound in chloroform-$d$ solution and by the lack of an appropriate alternative NMR solvent.* A series of nOe difference spectra in CDCl$_3$ were obtained, unfortunately they were complicated by signals due to an increasing degree of decomposition of the material, and were inconclusive. It was later pointed out$^{206}$ that the distance between 5-H and 6-H, and hence the degree of nOe enhancement between them, was likely to be similar whether 6-H was axially or equatorially positioned. Molecular model building confirmed this possibility, and further nOe studies were not undertaken.

It is presently thought that the most likely conformation for 1,2-oxazine 120 is the “all-trans” conformation shown in Figure 4.5, and that the assignment of

* Relatively polar solvents tended not to dissolve 1,2-oxazine 120, and C$_6$D$_6$ did not allow a satisfactory separation of the critical proton resonances for nOe studies to be undertaken in this solvent.
absolute configuration at C-6 would therefore be (S). Unambiguous determination of the stereochemistry at C-6, however, could not be achieved.

Figure 4.5. Possible conformation and stereochemistry for 1,2-oxazine 120

4.3.3. SYNTHESIS AND CHARACTERISATION OF N-HYDROXYPYRROLIDINES

4.3.3.1. Reduction of 1,2-oxazine 120

Scheme 4.3.3. The formation of trisilylated 1,4-dideoxy-1,4-imino-D-arabinitol

It was envisaged that 1,2-oxazine 120 might prove to be a useful intermediate for the synthesis of a range of sugar analogues, including nectrisine. Reduction of 1,2-oxazines using hydrogenation over Raney® nickel had been shown to yield highly-substituted five-membered heterocycles (proline analogues), useful in the synthesis of ACE inhibitors. In preliminary studies by Mark Smith in this group, it was shown that 1,2-oxazine 120 was also readily reduced under analogous conditions, and that the intermediates thus formed readily cyclised
to form the trisilylated derivatives 122 and 123 of aza sugar 1,4-dideoxy-1,4-imino-D-arabinitol (42) and its C-2 epimer, in a 2:1 diastereomer ratio, in 59% overall yield (Scheme 4.3.3).

Of potentially greater interest, however, were reductive processes which did not cleave the N-O bond, and consequently left the oxazine ring structure intact. The reduction of the C=N bond of 6-silyloxy- and 6-alkoxy-1,2-oxazines with sodium cyanoborohydride in acetic acid has been reported to proceed without cleavage of the N-O bond; with the former compounds, loss of the C-6 silyloxy group was additionally observed.205 It was considered that reduction of 1,2-oxazine 120 with NaBH₃CN would generate a saturated oxazine (124); the functionality at C-6 being formally equivalent to an acetal moiety, cleavage of the saturated 1,2-oxazine 124 with iodonitrtrimethylsilane would in principle yield an aldehyde analogous to that involved in

Scheme 4.3.4. A postulated alternative synthesis of trisilylated nectrisine (107)
the iodonitromethan-mediated cyclisation of amine 105, in which case intramolecular cyclisation ought in principle to yield silylated nectrisine 107 (Scheme 4.3.4). This route to nectrisine would avoid the unreliable Raney® nickel-catalysed reduction which was previously employed (see Section 4.1.2.2); furthermore, we were also aware of the possibility of diastereoselective hydride addition in the synthesis of saturated 1,2-oxazine 124.

Reduction of 1,2-oxazine 120 (0.25 mmol) with sodium cyanoborohydride (≈ 25 mmol) in acetic acid did not, however, yield the expected 1,2-oxazine 124. TLC analysis of the reaction mixture revealed the rapid consumption of starting material and the formation of a compound which stained as a red-purple spot (Rf = 0.19 [petrol: EtOAc (9:1)]) when resorcinol was used to develop the TLC plate, along with a fainter, more polar fraction (Rf = 0.10). Neutralisation of the acetic acid, solvent extraction and careful chromatography on silica yielded the major product as a translucent crystalline solid (50 mg, 37%). 1H NMR analysis (see Figure 4.6) showed the loss of the C-6 ethoxy substituent, and at first it was assumed that the large excess of NaBH4CN employed in the reaction had resulted in cleavage of the C-6 ethoxy group, though Reissig and co-workers had not reported such a result with 6-ethoxy-1,2-oxazines.205 Thus the saturated 1,2-oxazine structure 125 was initially assigned to the product of reduction of 1,2-oxazine 120. The reduction product appeared to have been formed as a single diastereomer, as judged by 1H and 13C NMR analysis.

The minor product from the reduction was also isolated, and yielded milligramme quantities of a clear oil which could not be precisely identified by 1H NMR. It appeared that the oil contained more than one product, and that in at least one of the products the ethoxy group remained intact, but no better resolution of the components of the mixture could be obtained and it was not possible to characterise this minor fraction further.
4.3.3.2. Crystal structure determination

The 1,2-oxazine reduction product, assumed to be 125, was isolated as a crystalline solid. Since all the previous intermediates in the synthetic scheme employed from 5BX had been oils or waxes, it had not been possible to confirm the stereochemical integrity of the intermediates. Given that a single diastereomer had apparently been formed by the reduction of 120, an X-ray structure was of particular interest in confirming the stereochemistry of the reduction product. Crystals of the material were therefore grown by slow evaporation of a solution of the reduction product in diethyl ether; the crystals proved to be extremely thin, and diffracted rather poorly (the initial data set was refined to a crystallographic R-factor of 18%). However, the resulting X-ray structure\(^1\) of the oxazine reduction product (Figure 4.7) showed quite unambiguously that the molecule was not the expected saturated

\(^1\) The crystal structure determination was performed by Dr. Simon Parsons at Edinburgh University.
1,2-oxazine 125, but that a ring contraction had occurred, and the resulting structure was a five-membered ring (an N-hydroxypyrrolidine), 126. The stereochemistry at the new chiral centre in the product (redesignated the 2-position in the ring) was also clearly shown to be (R), with all substituents adopting a pseudoequatorial arrangement about the five-membered ring.

Figure 4.7. X-ray crystal structure of N-hydroxypyrrolidine 126 (structure courtesy of Dr. Simon Parsons)
Ring contractions of 1,2-oxazines had not been reported during reductions with NaBH₃CN; however similar processes had been observed under acidic conditions¹²⁰,¹²³ - yielding nitrones or pyridine N-oxides, depending on the nature of the substituent at C-6 of the oxazine - and in the reduction of 6-silyloxy-1,2-oxazines under aprotic conditions using DIBAL-H,¹²⁴ in which case the products isolated were N-hydroxypyrrolidines. Hippeli and Reissig explained this ring contraction by invoking an initial complexation of the aluminium to the oxygen atom of the 1,2-oxazine, followed by a rearrangement to form a nitrilium cation.¹²⁴ Reduction of the nitrilium cation and loss of the silyloxy group yields an aluminate complex which is hydrolysed on aqueous quenching to the N-hydroxypyrrolidine. An analogous scheme can be proposed for the ring contraction of 1,2-oxazine 120, involving initial activation of the oxazine oxygen through complexation with boron, or (more likely) through simple protonation (Scheme 4.3.5). Rearrangement to the nitrilium ion, reduction with hydride (apparently with high stereoselectivity), and subsequent elimination of the ethoxy group assisted by the ring nitrogen, would result in the formation of N-hydroxypyrrolidine derivative 126. The extremely high diastereoselectivity observed for the reaction is difficult to account for, although it is notable that the product formed is the least sterically hindered, “all-trans” pyrrolidine.

A precise melting point for the crystalline form of 126 was not obtained. It was observed that at temperatures of 86-88°C, a phase transition appeared to occur, the high-temperature form of 126 being an amorphous glassy material. This glassy form of 126 did not become completely fluid until a temperature of ≈ 103°C.

Further evidence for the novel N-hydroxypyrrolidine structure is reported in Section 4.3.3.4.

4.3.3.3. Deprotection studies

One of the major disadvantages of the synthetic schemes hitherto employed was the lack of an efficient protocol for the desilylation of materials such as trisilylated nectrisine (107). The difficulty of product purification when desilylation of these materials is attempted, as well as the apparent formation of product mixtures which are difficult to characterise when conventional silyl deprotection agents such as TBAF are employed, makes the development of alternative strategies for the deprotection of multiply silylated materials such as 107 and 126 highly desirable. The combination of a clean desilylation reaction, simple purification conditions, and ease of use on a scale of ≈ 50 mg does not yet appear to have been realised.
Scheme 4.3.5. Proposed mechanism for the formation of
*N*-hydroxyperrrolidine 126

*N*-Hydroxyperrrolidines are five-membered sugar analogues, and as such may have activity as glycosidase inhibitors. A recent report on the synthesis of six-membered *N*-alkoxy substituted aza sugars (*N*-alkoxypiperidines) remarks that the electron-withdrawing effect of the *N*-alkoxy group can be expected to attune the basic strength of the ring nitrogen functionality nearer to the physiological pH range than is the case for the related *N*-unsubstituted aza sugars (*e.g.* deoxynojirimycin 33); thus,
N-alkoxypiperidines may prove to be more powerful glycosidase inhibitors even than deoxynojirimycin and related aza sugars.\textsuperscript{207} The structure of the fully desilylated N-hydroxypyrrolidines 127 closely resembles that of the known glycosidase inhibitors, 1,4-dideoxy-1,4-imino-D-arabinitol (42) and nectrisine (72) (Figure 4.8); thus, N-hydroxypyrrolidines such as 127 may prove to be compounds with highly potent biological activity. To the best of our knowledge, the evaluation of enantiomerically pure N-hydroxypyrrolidine sugar analogues as glycosidase inhibitors has not been investigated.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.8.png}
\caption{Comparison of N-hydroxypyrrolidine 127 with known five-membered glycosidase inhibitors}
\end{figure}

Given the failure (vide supra) of conventional deprotection procedures (e.g. TBAF, acetic acid/water/THF, and TFA/water) to produce characterisable pure samples of nectrisine from the trisilylated compound 107, it was decided to use an alternative protocol for the desilylation of 126: namely, treatment of a solution of 126 (50 mg) in 1:1 acetonitrile/THF with aqueous HF solution.\textsuperscript{208} Such solutions have been observed to desilylate a range of primary and secondary silyloxy groups extremely rapidly at RT;\textsuperscript{209} in this case, TLC analysis of the reaction mixture showed a slow but steady consumption of starting material over a 6 h period (additional aliquots of aqueous HF were added at \(\approx 2\) h intervals). The mixture was quenched with solid NaHCO\textsubscript{3}, filtered, and the filtrate evaporated to dryness to yield a gummy solid which was assumed to contain the desired product 127, sodium fluoride and silyl fluoride byproducts. Repeated washing of this solid with ethanol to remove the NaF, and evaporation of the filtrate to dryness, yielded a white powder which was subjected to prolonged evaporation on a high-vacuum line (to remove silyl fluoride). Analysis of the final product by \(^1\)H NMR (D\textsubscript{2}O) (Figure 4.9) showed that, although traces of silylated impurities remained, the deprotection had yielded the fully desilylated product 127 in almost quantitative yield.
Although time did not permit an extensive study of this HF/acetonitrile/THF desilylation protocol, its efficacy in providing relatively pure unprotected sugar analogue 127 on a small scale appeared to recommend it strongly as an alternative to more conventional desilylation methods, as product purification was straightforward and no chromatography was necessary.

The synthesis of novel heterocyclic sugar analogues as single diastereomers from the aldehyde 119 is summarised in Scheme 4.3.6.

Scheme 4.3.6. The synthesis of novel 1,2-oxazine 120 and N-hydroxypyrrolidines 126 and 127

4.3.3.4. NMR studies on N-hydroxypyrrolidines 126 and 127

The precise assignment of the \textsuperscript{1}H NMR resonances in the spectra of the novel N-hydroxypyrrolidines 126 and 127 was achieved through a series of two-dimensional NMR experiments conducted at 600 MHz.\footnote{The two-dimensional NMR experiments were conducted by Dr. John Parkinson at Edinburgh University.} \textsuperscript{1}H-\textsuperscript{1}H (COSY) and \textsuperscript{1}H-\textsuperscript{13}C (HMQC) correlation spectra for the trisilylated heterocycle 126 are reproduced in the Appendix, and the assignment of the proton resonances in the NMR spectrum are reported in Chapter Five. The proton assignments of unprotected 127 (see Figure 4.9 for the \textsuperscript{1}H spectrum of 127) were inferred by direct comparison with the trisilylated derivative 126 (see Figure 4.6).
1H NMR nOe studies were undertaken to validate the assignment of stereochemistry at C-2 of the N-hydroxypyrrolidines 126 and 127. Some of the more significant interactions are shown in Figure 4.10 for trisilylated 126 and Figure 4.11 for the unprotected N-hydroxypyrrolidine 127 (owing to decomposition of 126 in CDCl₃, some nOe signals were somewhat unclear, notably those for irradiation of 5-H). The most significant results are the appreciable interactions between 2-H, 3-H and 4-H even when the protons are on opposite faces of the molecule (this is not uncommon for five-membered ring compounds, where the ring itself has a degree of flexibility), and the very large (7%) enhancement of 2-H in the unprotected N-hydroxypyrrolidine 127 on irradiation of 5-H, suggesting close proximity of 2-H to one of the protons at C-5, as might be expected for a five-membered ring structure.

2 The nOe studies were carried out by Drs. John Parkinson and Ian Sadler at Edinburgh University.
Figure 4.10. Nuclear Overhauser effect enhancements for silylated $N$-hydroxypyrrolidine 126

Figure 4.11. Nuclear Overhauser effect enhancements for $N$-hydroxypyrrolidine 127
4.4. Conclusions

The development of transketolase from *E. coli* as a catalyst in enzymatic carbon-carbon bond forming processes is dependent upon a number of factors. The initial factor - that of enzyme availability - has been addressed by the research groups of John Woodley, John Ward and Malcolm Lilly at UCL. With the availability of large amounts of the enzyme, the next target was to make available a range of novel substrates. Routes to both enantiomerically pure and racemic α-hydroxyaldehyde substrates have been investigated; the former routes have the advantage that they result in a cleaner biotransformation process, although their efficiency has not so far been demonstrated on a large scale. One α-hydroxyaldehyde with potential synthetic utility, 3-O-benzylglycereraldehyde (81), is available in multigramme quantities in the research laboratory, and is an excellent substrate for *E. coli* transketolase; biotransformations using this aldehyde have been performed with very high efficiency in unbuffered aqueous solution using a pH autotitrator to maintain the process optimum pH of 7.0. In separate studies this biotransformation has been shown to be highly amenable to scale-up and, subject to an appropriate reactor configuration, almost quantitative yields of the biotransformation product, 5-O-benzyl-D-xylulose, 82, can be obtained.

The utility of the chiral triols derived from TK-mediated biotransformations as synthetic intermediates has been demonstrated in the synthesis of a range of novel heterocyclic compounds, including the trisilylated derivative 107 of the unsaturated aza sugar nectrisine, the 5,6-dihydro-4H-1,2-oxazine 120 and the novel *N*-hydroxypyrrolidine, 127. To the best of our knowledge this is the first synthesis of enantiomerically pure, multiply substituted 1,2-oxazines and *N*-hydroxypyrrolidines, whose structural and electronic properties suggest that they may have considerable potency as glycosidase inhibitors. At present, these targets are available in relatively poor overall yield, and optimisation of the synthetic steps which yield the 1,2-oxazine 120 and the protected *N*-hydroxypyrrolidine 126 is highly desirable (though preceding synthetic steps can be accomplished with high efficiency). Further study of the desilylation protocol employed to produce the final product 127 is also necessary, since although highly successful on a small scale, its general efficacy in the synthesis of sensitive molecules of this type on a rather larger scale remains to be demonstrated.

In conclusion, therefore, this thesis presents a demonstration of the synthetic utility of *E. coli* transketolase as an effective catalyst in the synthesis of a range of
novel, enantiomerically pure products. Methodology to enable the subsequent use of transketolase as a process catalyst in the preparation of novel chiral intermediates and biologically active molecules has also been described in detail.
Chapter Five
Chapter Five. Experimental

5.1. General Experimental

Melting points (m.p.) were determined using a Gallenkamp melting point apparatus and are uncorrected.

Optical rotations ($[\alpha]_D$) were recorded using an Optical Activity AA-100 polarimeter and are reported in units of $10^1$ deg cm$^2$ g$^{-1}$. Optical rotations were not recorded for compounds which consisted of inseparable mixtures of diastereomers or double bond isomers.

Infra-red (IR) spectra were recorded as thin films or KBr discs on a Perkin-Elmer 881 grating spectrophotometer and a Bio-Rad FTS-7 Fourier Transform spectrophotometer. Absorption maxima are reported in reciprocal centimetres (cm$^{-1}$). The following abbreviations are used: vs, very strong; s, strong; m, medium; w, weak; and br, broad.

Ultra-violet (UV) absorptions were recorded using 1.5 cm solution cells on a Phillips PU 8720 UV-visible scanning spectrophotometer. Absorption maxima are reported in nanometres (nm).

Proton magnetic resonance spectra ($^1$H NMR) were recorded on Varian Gemini 2000 (200 MHz), Bruker AM200 (200 MHz), AM250 (250 MHz), AC250 (250 MHz), AM300 (300 MHz), AM400 (400 MHz) and Varian Inova (600 MHz) spectrometers. Chemical shifts ($\delta_H$) are reported in parts per million (ppm) downfield from tetramethylsilane as reference. Coupling constants ($J$) are reported in hertz (Hz) to the nearest 0.5 Hz. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; dt, double triplet; ddd, double doublet of doublets; ABq, AB-quartet; m, multiplet; br, broad. In some cases complex patterns were observed which closely resembled more straightforward coupling patterns; where the actual coupling constants could not be easily measured in these cases, the reported coupling constants are the apparent coupling constants $J_{app}$.

Carbon magnetic resonance spectra ($^{13}$C NMR) were recorded on Bruker AM250 (62.9 MHz), AC250 (62.9 MHz), AM300 (75.5 MHz) and AM400 (100.6 MHz) spectrometers. Chemical shifts ($\delta_C$) are reported in parts per million (ppm) downfield from tetramethylsilane as reference.

Electron impact mass spectra (EI) were recorded on a VG 12-253 spectrometer.
Chemical ionisation mass spectra (CI) were recorded on a VG Biotech Quattro II spectrometer (low resolution) and a VG ZAB-E spectrometer (high resolution) at the EPSRC Mass Spectrometry Service, University of Wales, Swansea.

Gas chromatography/mass spectra (GCMS) were recorded on a Hewlett Packard 5890 series II GC with 5972 series electron impact (EI) mass detector. GCMS analysis was performed on an HP5 column (15 m) at 15 p.s.i. maintaining a temperature of 60°C for 5 minutes with a temperature gradient of 10°C min⁻¹ thereafter to 250°C, and an injector temperature of 250°C and a detector temperature of 280°C.

Chiral gas chromatographic analysis (GC) was performed using a Perkin-Elmer Autosystem GC with a CP Chirasil-Dex-CB® column (25 m) at 20 p.s.i.

Biotransformations under pH controlled conditions were carried out using a Radiometer (Copenhagen) ABU80 autotitrator, a Metrohm 718 STAT Titriuto autotitrator and an Anglicon pH autotitrator at pH 7.0, with 1.0 molar hydrochloric acid as titrant except where noted in the text.

Anhydrous diethyl ether and tetrahydrofuran were obtained fresh by distillation from sodium benzophenone ketyl. Anhydrous methanol, dimethylsulfoxide and N,N-dimethylformamide were obtained direct from Aldrich. Anhydrous dichloromethane was distilled from CaH₂ before use, or obtained direct from Aldrich. All other solvents used in reactions were spectrograde and used as received. “Petrol” refers to the light fraction of petroleum ether distilling between 40 and 60°C.

Aqueous solution concentrations are expressed in moles of solute per 1000 cm³ of solvent and reported as molarities (M), whereby a 1.0 M solution contains 1.0 mol dm⁻³ solute. “Brine” refers to a saturated aqueous solution of sodium chloride.

All reagents were used as obtained from commercial sources unless otherwise stated.

_E. coli_ transketolase was supplied by the Department of Biochemical Engineering at University College, London. Preparation of the enzyme is described in Section 5.2 below. All other enzymes and cofactors were obtained from Sigma and used as received.

Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F₂₅₄ 0.25 mm glass-backed plates. The plates were visualised using alkaline potassium permanganate (KMnO₄), resorcinol, 2,4-dinitrophenylhydrazine (2,4-DNP), ammonium molybdate (molybdate) or by irradiation under a low-frequency ultraviolet lamp (UV). Flash column chromatography was performed using Merck Kieselgel 60, 230-400 mesh.
5.2. Transketolase Production

Transketolase employed in biotransformations reported in this chapter was extracted from *E. coli* strain JM107 transformed with p'asmid pQR700 (see Section 1.4.3.1). Large-scale fermentations were performed at University College, London. Cell harvest and sonication followed by single-step ammonium sulfate fractionation produced a clarified extract which was approximately 70% pure transketolase and which was suitable for storage by freezing in 1.0 cm$^3$ aliquots. Transketolase solution was thawed and centrifuged before addition to biotransformation mixtures.

5.3. Experimental Procedures

5.3.1. Experimental Details to Chapter Two

5.3.1.1. (R)-2-Hydroxy-3-phenylpropanoic acid ((R)-55)

\[
\text{Ph} \quad \text{OH} \quad \text{CO}_2\text{H}
\]

D-(R)-Phenylalanine (9.96 g, 60.4 mmol) was dissolved in 1.2 M sulfuric acid solution (120 cm$^3$). Sodium nitrite was added in four equal (1.69 g, 24.5 mmol) portions at half-hour intervals. Stirring was maintained at RT for 16.5 h after the last addition of NaNO$_2$; thereafter the mixture was diluted with H$_2$O to a volume of 200 cm$^3$, extracted with Et$_2$O (4 x 100 cm$^3$) and evaporated *in vacuo* to yield the crude $\alpha$-hydroxyacid (R)-55 as a yellow solid, which was not purified further: 7.30 g (73%); $\delta_h$ (250 MHz; CDCl$_3$) 2.99 (1 H, dd, J 8.0, 12.0, PhCHH), 3.20 (1 H, dd, J 5.0, 12.0, PhCHH), 4.55 (1 H, m, CH), 7.30 (5 H, m, Ph).

(±)-2-Hydroxy-3-phenylpropanoic acid ((±)-55). An analogous procedure yielded crude acid (±)-55 from DL-phenylalanine: 4.39 g (54%); $^1$H NMR analysis was in agreement with that reported for (R)-55 above.
5.3.1.2. Methyl (R)-2-hydroxy-3-phenylpropanoate ((R)-56)

\[
\text{Ph} \quad \begin{array}{c}
\text{OH} \\
\text{CO}_2\text{Me}
\end{array}
\]

a) by refluxing in methanol. α-Hydroxyacid (R)-55 (4.39 g, 26.4 mmol) was dissolved in MeOH (100 cm\(^3\)) containing catalytic conc. \(\text{H}_2\text{SO}_4\) and heated at reflux for 18 h. After cooling to RT the mixture was evaporated \textit{in vacuo} and purified by chromatography [petrol: EtOAc (2:1)] to yield the ester as a pale yellow solid: 3.65 g (77%); \(\nu_{\text{max}}\) (KBr disc)/cm\(^{-1}\) \(3289\)br (OH), \(3023\)s (CH), \(2950\)s (CH), \(1749\)vs (C=O); \(\delta_{\text{H}}\) (250 MHz; CDCl\(_3\)) 2.71 (1 H, d, \(J\ 7.0\), OH), 2.97 (1 H, dd, \(J\ 8.0, 12.0\), PhCH\(_2\)), 3.13 (1 H, dd, \(J\ 5.0, 12.0\), PhCH\(_2\)), 3.78 (3 H, s, OCH\(_3\)), 4.45 (1 H, m, CH), 7.28 (5 H, m, Ph); \(\delta_{\text{C}}\) (62.9 MHz; CDCl\(_3\)) 40.6 (CH\(_2\)), 52.4 (OCH\(_3\)), 71.4 (CH), 127.0, 128.5, 129.6 (aromatic CH), 137.0 (aromatic quarternary), 175.0 (C=O); \(m/z\) (EI) 180 [M\(^+\)], 162, 121, 103, 91, 77; (Found: \(m/z\) (EI) [M\(^+\)], 180.07937. \(\text{C}_{10}\text{H}_{20}\text{O}_3\) requires \(m/z\) (EI) [M\(^+\)] 180.07864). A single recrystallisation from petrol yielded 2.77 g (58%) (R)-56 as fine white needles: m.p. 48.1°C; \([\alpha]_{D}^{26}\) -5.0° (c 1.08, MeOH).

Methyl (±)-2-hydroxy-3-phenylpropanoate ((±)-56). An analogous procedure starting from (±)-55 yielded the racemic α-hydroxyester as a yellow oil: 1.58 g (77%); \(\nu_{\text{max}}\) (thin film)/cm\(^{-1}\) \(3480\)vs (OH), 3032s (CH), 2956s (CH), 1739vs (C=O); other spectroscopic data in agreement with that reported above.

b) via acid chloride formation \textit{in situ}. α-Hydroxyacid (R)-55 (7.30 g, 44.0 mmol) was dissolved in MeOH (100 cm\(^3\)) and cooled in an ice-water bath. Thionyl chloride (4.3 cm\(^3\), 7.0 g, 59 mmol) was added dropwise, cautiously, with stirring over 20 min. Stirring was maintained at 0°C for 5 min and the mixture warmed to RT; a further portion of SOCl\(_2\) (1.0 cm\(^3\), 1.6 g, 14 mmol) was added. The mixture was stirred for a further 15 min, then evaporated \textit{in vacuo} to yield a viscous dark yellow oil which was purified by chromatography [heptane: EtOAc (2:1)]. The product was obtained as a yellow solid; TLC showed a trace contaminant, but no further purification was attempted: 6.46 g (82%); \(^1\text{H NMR}\) in agreement with that reported for (R)-56 above.

5.3.1.2. Methyl (±)-3-phenyl-2-tert-butyldimethylsilyloxypropanoate((±)-57)

\[
\text{Ph} \quad \begin{array}{c}
\text{OTBS} \\
\text{CO}_2\text{Me}
\end{array}
\]
Methyl ester (±)-56 (652 mg, 3.62 mmol) and imidazole (377 mg, 5.54 mmol) were dissolved in anhydrous \(N,N\)-dimethylformamide (DMF) (30 cm\(^3\)) under nitrogen. \textit{Tert-Butyldimethylsilyl trifluoromethanesulfonate} (TBS triflate) (0.80 cm\(^3\), 920 mg, 3.5 mmol) was added dropwise, and the solution stirred for 90 min. Saturated \(\text{NH}_4\text{Cl}\) solution (25 cm\(^3\)) was added to the solution, followed by water (20 cm\(^3\)), and the mixture extracted with Et\(_2\)O (3 x 25 cm\(^3\)). The combined organic extracts were washed with saturated \(\text{NaHCO}_3\) solution (50 cm\(^3\)) and brine (50 cm\(^3\)), dried (\(\text{MgSO}_4\)) and evaporated \textit{in vacuo}. The residue was purified by chromatography on silica [petrol: EtOAc (2:1)] and the product obtained as a pale yellow oil: 585 mg (55%); \(R_f\) 0.73 [petrol: EtOAc (2:1), UV]; \(\nu_{\text{max}}\) (thin film)/cm\(^{-1}\) 2955s (CH), 2932s (CH), 2860s (CH), 1759vs (C=O), 1258s (Si-C); \(\delta_H\) (250 MHz; CDCl\(_3\)) -0.17 (6 H, 2 x s, (CH\(_3\))\(_2\)Si), 0.80 (9 H, s, 'Bu), 2.88 (1 H, dd, \(J\) 9.0, 13.5, PhCHH), 3.07 (1 H, dd, \(J\) 4.0, 13.5, PhCHH), 3.72 (3 H, s, OCH\(_3\)), 4.34 (1 H, dd, \(J\) 4.0, 9.0, CH), 7.25 (5 H, m, Ph); \(\delta_C\) (62.9 MHz; CDCl\(_3\)) -5.9, -5.5 ((CH\(_3\))\(_2\)Si), 18.2 ((CH\(_3\))\(_3\)C-Si), 25.6 ((CH\(_3\))\(_3\)C-Si), 41.7 (CH\(_2\)), 51.7 (OCH\(_3\)), 73.9 (CH), 126.7, 128.3, 129.9 (aromatic CH), 137.6 (aromatic quarternary), 173.6 (C=O); \(m/z\) (El) 294 (M\(^+\), very small), 279, 237; (Found: \(m/z\) (El) [M\(^+\)] 294.16489. \(\text{C}_{16}\text{H}_{26}\text{O}_3\text{Si}\) requires \(m/z\) (El) [M\(^+\)] 294.15512).

5.3.1.4. \((\pm)-3\)-Phenyl-2-\textit{tert}-butyldimethylsilyloxypropanal ((±)-58)

Ester (±)-57 (585 mg, 1.99 mmol) was dissolved in toluene (35 cm\(^3\)), flushed with nitrogen, cooled to -78°C (acetone-dry ice bath), and treated with a 1.5 M solution of DIBAL-H in toluene (3.3 cm\(^3\); 5.0 mmol). The mixture was stirred for 2 h, quenched at -78°C by dropwise addition of 2 M HCl solution (35 cm\(^3\)), allowed to warm to 0°C, and extracted with EtOAc (3 x 40 cm\(^3\)). The organic layers were dried (\(\text{MgSO}_4\)) and evaporated \textit{in vacuo}, and the residue purified by chromatography on silica [\(\text{CH}_2\text{Cl}_2\) (neat)], yielding the aldehyde (±)-58 as a clear oil: 363 mg (69%); \(R_f\) 0.67 [\(\text{CH}_2\text{Cl}_2\) (neat); 2,4-DNP]; \(\nu_{\text{max}}\) (thin film)/cm\(^{-1}\) 2957s (CH), 2932s (CH), 2860s (CH), 1740vs (C=O), 1259s (Si-C); \(\delta_H\) (250 MHz; CDCl\(_3\)) -0.17 (6 H, 2 x s, (CH\(_3\))\(_2\)Si), 0.85 (9 H, s, 'Bu), 2.78 (1 H, dd, \(J\) 9.0, 13.5, PhCHH), 2.99 (1 H, dd, \(J\) 4.0, 13.5, PhCHH), 4.13 (1 H, dd, \(J\) 4.0, 9.0, CH), 7.28 (5 H, m, Ph), 9.60 (1 H, s, CHO); \(\delta_C\) (62.9 MHz; CDCl\(_3\)) -5.4, -5.1 ((CH\(_3\))\(_2\)Si), 18.1 ((CH\(_3\))\(_3\)C-Si), 25.7
(\(\text{CH}_3\)\(_3\)C-Si), 39.2 (\(\text{CH}_2\)), 79.0 (\(\text{CH}\)), 126.7, 128.3, 129.9 (aromatic \(\text{CH}\)), 136.8 (aromatic quarternary), 203.3 (CHO); \(m/z\) (El) 264 (M\(^+\), very small), 263, 235, 207.

5.3.1.5. Methyl (R)-3-phenyl-2-trimethylsilyloxypropanoate ((R)-59)

To a solution of \(\alpha\)-hydroxyester (R)-56 (1.00 g, 5.56 mmol) and imidazole (567 mg, 8.34 mmol) in dry tetrahydrofuran (20 cm\(^3\)) under nitrogen was added TMSCl (0.76 cm\(^3\), 650 mg, 5.9 mmol). The mixture was stirred for 30 min then treated with diethyl ether (20 cm\(^3\)), the resulting white precipitate removed by filtration, and the filtrate evaporated in vacuo and purified by chromatography on neutralised silica [petrol: ethyl acetate (2:1) as eluant; silica was neutralised prior to use by washing with a solution of 2% triethylamine in petrol]. The purified material was obtained as a clear oil: 1.15 g (82%); \(R_f\) 0.60 [petrol: EtOAc (2:1); KMnO\(_4\)]; \([\alpha]_D^{20} +32.0^\circ\) (c 0.92, MeOH); \(v_{\text{max}}\) (thin film)/cm\(^{-1}\) 3032m (CH), 2957m (CH), 2902m (CH), 1757vs (C=O), 1251vs (Si-C); \(\delta_h\) (250 MHz; CDCl\(_3\)) -0.06 (9 H, s, SiMe\(_3\)), 2.88 (1 H, dd, \(J\) 9.0, 13.5, PhCHH), 3.08 (1 H, dd, \(J\) 4.0, 13.5, PhCHH), 3.73 (3 H, s, OCH\(_3\)), 4.30 (1 H, dd, \(J\) 4.0, 9.0, CH), 7.24 (5 H, m, Ph); \(\delta_c\) (62.9 MHz; CDCl\(_3\)) -0.59 (SiMe\(_3\)), 41.4 (CH\(_2\)), 51.9 (OCH\(_3\)), 73.5 (CH), 126.7, 128.3, 129.7 (aromatic CH), 137.6 (aromatic quarternary), 183.0 (C=O); \(m/z\)(El) 252 (M\(^+\), very small), 237, 193, 89, 73; (Found: \(m/z\)(El) [M\(^+\)], 252.11885. C\(_{13}\)H\(_{20}\)O\(_3\)Si requires \(m/z\) (El) [M\(^+\)] 252.11817).

**Methyl (±)-3-phenyl-2-trimethylsilyloxypropanoate ((±)-59).** An analogous procedure yielded the racemate (±)-59 as a clear oil: 737 mg (58%); all spectroscopic properties in agreement with those of the enantiomerically pure material.

5.3.1.6. (R)-2-Hydroxy-3-phenylpropanal ((R)-54)

\[\text{Ph} \quad \text{OH} \quad \text{O} \]

Ester (R)-59 (1.09 g, 4.31 mmol) was dissolved in toluene (25 cm\(^3\)), cooled to -78°C, and treated with DIBAL-H (1.5 M solution in toluene; 5.5 cm\(^3\), 8.3 mmol) under N\(_2\). After stirring for 1 h the mixture was quenched with 2 M HCl (25 cm\(^3\)),
warmed to RT, extracted with EtOAc (3 x 20 cm³) and the organic extracts concentrated in vacuo. The residue remaining after solvent evaporation in vacuo was redissolved in a 3:1:1 mixture of acetic acid/water/THF (10 cm³), and stirred for 18 h at RT. Lyophilisation of the solution on a rotary evaporator, followed by chromatography on silica [petrol: EtOAc (2:1)] gave the title compound as a clear viscous oil: 612 mg (95%); δH (250 MHz; D₂O) 2.81 (1 H, dd, J 9.5, 14.0, PhCHH), 3.11 (1 H, dd, J 3.0 & 14.0, PhCHH), 3.88 (1 H, m, CH), 5.02 (1 H, d, J 5.0, CHO as gem-diol), 7.47 (5 H, m, Ph); δC (100.6 MHz; D₂O) 37.8 (CH₃), 75.2 (CH), 91.8 (CHO as gem-diol), 126.9, 129.0, 129.9 (aromatic CH), 138.9 (aromatic quarternary).

(±)-2-Hydroxy-3-phenylpropanal ((±)-54). An analogous procedure yielded the racemic α-hydroxyaldehyde from ester (±)-59: 136 mg (69%); ¹H NMR spectrum in agreement with that of the enantiomerically pure material.

5.3.1.7. (R)-1,1-Diethoxy-3-phenylpropan-2-ol ((R)-60)

Aldehyde (R)-54 (110 mg, 0.73 mmol) was dissolved in ethanol (10 cm³) and stirred at RT for 20 h in the presence of catalytic conc. H₂SO₄. The mixture was neutralised by addition of Amberlite® basic anion exchange resin which had been pre-washed in NaOH (2 M), filtered, concentrated in vacuo, and purified by chromatography on silica [petrol: EtOAc (2:1)] to yield acetal (R)-60 as a yellow oil: 102 mg (63%); [α]D +210° (c 1.56, CHC₂Cl₂); Rf 0.50 [petrol: EtOAc (2:1); 2,4-DNP]; νmax (thin film)/cm⁻¹ 3480br (OH), 3029w (CH (aryl)), 2977s (CH), 2930m (OCH₂), 2898m (OCH₃), 1496m (C-C (aryl)); δH (250 MHz; CDCl₃) 1.24 (3 H, t, J 7.0, CH₃), 1.25 (3 H, t, J 7.0, CH₃), 2.18 (1 H, d, J 4.0, OH), 2.73 (1 H, dd, J 8.5, 14.0, PhCHH), 2.99 (1 H, dd, J 4.0, 14.0, PhCHH), 3.55-3.86 (4 H, m, OCH₂CH₃), 3.75 (1 H, m, CH(OH)), 4.31 (1 H, d, J 6.0, CH(OEt)₂), 7.28 (5 H, m, Ph); δC (62.9 MHz; CDCl₃) 15.4 (CH₃), 36.3 (PhCH₃), 63.3, 63.4 ((CH₂CH₂)₂), 72.8 (CH(OH)), 104.4 (CH(OEt)₂), 126.3, 128.4, 129.6 (CH (aromatic)), 138.7 (aromatic quarternary); m/z (EI) 103, 91; (Found: m/z (EI) [M⁺] 224.14056. C₁₃H₂₀O₃ requires m/z (EI) [M⁺] 224.14125).
(±)-1,1-Diethoxy-3-phenylpropan-2-ol ((±)-60). An analogous procedure yielded the racemic acetal (±)-60 as a clear oil from (±)-54: 169 mg (81%); spectroscopic properties in agreement with those of the enantiomerically pure material.

5.3.1.8. Methyl (R)-2-hydroxy-2-phenylethanoate (methyl (R)-mandelate) ((R)-63)

(R)-Mandelic acid (4.19 g, 27.6 mmol) was dissolved in MeOH (100 cm³), catalytic conc. H₂SO₄ added, and the mixture heated at reflux for 22 h. The mixture was cooled to RT, concentrated in vacuo, and purified by chromatography [petrol: EtOAc (2:1)] to yield the methyl ester (R)-63 as a yellow solid: 4.45 g (97%); m.p. 54.4°C (lit: 54-56°C); [α]°D⁺ -146° (c 1.08, MeOH) (lit: [α]°D⁻ -144°); Rf 0.32 [petrol: EtOAc (2:1), KMnO₄]; νmax (KBr disc)/cm⁻¹ 3446br (OH), 3036m (CH), 2954m (CH), 5.18 (1 H, s, CH(OH)), 7.39 (5 H, m, Ph); δc (62.9 MHz; CDCl₃) 52.9 (CH₃), 73.0 (CH(OH)), 126.7, 128.6, 128.7 (aromatic CH), 138.4 (aromatic quarternary), 174.2 (C=O); m/z(EI) 166, 107, 77; (Found: m/z(EI) [M⁺] 166.06323. C₉H₁₀O₃ requires m/z(EI) [M⁺] 166.06299).

Methyl (S)-2-hydroxy-2-phenylethanoate (methyl (S)-mandelate) (S)-63. The same procedure yielded (S)-63 from (S)-mandelic acid: 4.19 g (95%); [α]°D⁻ +139° (c 1.3, MeOH) (lit: [α]°D⁻ +144°).

Methyl (±)-2-hydroxy-3-phenylethanoate (methyl mandelate). Similarly racemic 63 was prepared from DL-mandelic acid: 5.16 g (94%).

5.3.1.9. Methyl (R)-2-phenyl-2-trimethylsilyloxyethanoate ((R)-64)

Methyl (R)-mandelate (R)-63 (819 mg, 4.93 mmol) and imidazole (500 mg, 7.35 mmol) were dissolved in dry THF (20 cm³). TMSCl (0.63 cm³, 4.95 mmol) was added dropwise and the mixture stirred at RT for 20 min under N₂. Et₂O (20 cm³) was then added, the mixture filtered to remove the precipitate, and the filtrate concentrated.
in vacuo. Purification of the TMS protected material was accomplished by chromatography on silica neutralised by flushing with a solution of 2% triethylamine in petrol [petrol: EtOAc (2:1)] to give the target compound (R)-64 as a clear oil: 866 mg (74%); $\nu_{\text{max}}$ (thin film)/cm$^{-1}$ 3043s (CH), 2956s (CH), 2899s (CH), 1757vs (C=O), 1734vs (C=O), 1252vs (Si-C); $\delta_{\text{H}}$ (250 MHz; CDCl$_3$) 0.14 (9 H, s, SiMe$_3$), 3.68 (3 H, s, OCH$_3$), 5.24 (1 H, s, CH(OSiMe$_3$)), 7.29-7.40 (5 H, m, Ph); $\delta_{\text{C}}$ (62.9 MHz; CDCl$_3$) -0.16 (SiMe$_3$), 52.1 (OCH$_3$), 74.3 (CH(OSiMe$_3$), 126.7, 128.3, 128.5 (aromatic CH), 139.1 (aromatic quarternary), 172.5 (C=O); m/z (EI) 238 [M$^+$], 223, 195, 179.

Methyl (S)-2-phenyl-2-trimethylsilyloxyethanoate (S)-64. An analogous procedure yielded the ester (S)-64 from (S)-63: 893 mg (75%); spectroscopic properties in agreement with those of the (R)-ester.

Methyl (±)-2-phenyl-2-trimethylsilyloxyethanoate (±)-64. Similarly, racemic 64 was prepared from the $\alpha$-hydroxyester (±)-63: 942 mg (77%).

5.3.1.10. (R)-2-Hydroxy-2-phenylethanal (α-Hydroxyphenylacetaldehyde or mandelaldehyde) ((R)-61)

α-Silyloxyester (R)-64 (352 mg, 1.48 mmol) was dissolved in toluene (12 cm$^3$), cooled to -78°C, and treated with DIBAL-H (1.5 M solution in toluene; 1.8 cm$^3$, 2.7 mmol). The mixture was stirred under N$_2$ at -78°C for 60 min, quenched with 2 M HCl (12 cm$^3$), extracted with ethyl acetate (3 x 12 cm$^3$), dried (MgSO$_4$) and concentrated in vacuo. The residue was redissolved in AcOH/H$_2$O/THF (3:1:1; 10 cm$^3$) for 64 h, lyophilised on a rotary evaporator, and purified by chromatography on silica [petrol: EtOAc (2:1)] to give the title compound as a clear oil: 128 mg (64%); R$_f$ 0.11-0.26 [petrol: EtOAc (2:1), 2,4-DNP]; $\delta_{\text{H}}$ (250 MHz; D$_2$O) 4.62 (1 H, d, J 7.0, $\alpha$-CH), 5.23 (1 H, d, J 7.0, CH(OD)$_2$), 7.53 (5 H, m, Ph).

(S)-2-Hydroxy-2-phenylethanal (S)-61. By an analogous procedure the (S)-enantiomer was obtained from (S)-64: 138 mg (52%); $^1$H NMR data in agreement with that of the (R)-aldehyde.

(±)-2-Hydroxy-2-phenylethanal (±)-61. Similarly the racemic material was prepared from (±)-64: 150 mg (56%).
5.3.1.11. (R)-2,2-Diethoxy-1-phenylethanol ((R)-65)

Aldehyde (R)-61 (128 mg, 0.94 mmol) was dissolved in ethanol (10 cm³), Amberlyst® H+ exchange resin beads were added, and the mixture stirred at RT for 48 h. The resin beads were removed by filtration, the filtrate neutralised (using Amberlite® basic gel resin pre-washed in 2 M NaOH) and concentrated in vacuo, and the acetal purified by chromatography [petrol: EtOAc (2:1)]. Acetal (R)-65 was isolated as a clear oil: 152 mg (77%); [α] D 27 -21° (c 0.98, CHCl₃); Rf 0.40 [petrol: EtOAc (2:1), 2,4-DNP]; δ H (300 MHz; CDCl₃) 1.05 (3 H, t, J 7.5, CH₃), 1.25 (3 H, t, J 7.5, CH₃), 2.78 (1 H, d, J 2.5, OH), 3.21-3.84 (4 H, m, (OCH₂CH₃)₂), 4.38 (1 H, d, J 7.5, CH(OEt)₂), 4.59 (1 H, dd, J 2.5, 7.5, CH(OH)), 7.25-7.50 (5 H, m, Ph); δ c (75.5 MHz; CDCl₃) 15.1, 15.3 (CH₃), 63.6, 64.6 (OCH₂CH₃), 74.7 (CH(OH)), 106.0 (CH(OEt)₂), 127.2, 127.8, 128.1 (aromatic CH), 139.6 (aromatic quaternary); m/z(EI) 210 [M⁺], 209, 165, 119, 103, 91, 75; (Found: m/z(EI) [M⁺] 210.12545. C₁₂H₁₈O₃ requires m/z(EI) [M⁺] 210.12560).

(S)-2,2-Diethoxy-1-phenylethanol (S)-65. A slightly impure sample of aldehyde (S)-61 (74 mg, 0.54 mmol) was converted to the diethyl acetal (S)-65 as described above: 22 mg (19%); [α] D 27 +17° (c 0.2, CHCl₃); spectroscopic properties in agreement with those of the (R)-acetal.

(±)-1,1-Diethoxy-2-phenylethanol (±)-65.-Similarly the racemic acetal was prepared from aldehyde (±)-61: 63 mg (65%).

5.3.1.12. (±)-2-Hydroxy-3-methylbutanoic acid (68)

DL-valine (5.98 g, 51.1 mmol) was dissolved in 0.5 M sulfuric acid solution (100 cm³) and treated with NaNO₂ in four equal (1.38 g, 20.0 mmol) portions at half-hour intervals. Stirring was continued at RT for a further 24 h, the mixture extracted with Et₂O (4 x 100 cm³) and the organic extracts dried (MgSO₄) and evaporated in vacuo to yield the crude α-hydroxyacid as a yellow waxy solid which
was not purified further: 4.13 g (68%); $\delta$ (250 MHz; D$_2$O) 1.04 (3 H, d, J 6.5, CH$_3$), 1.12 (3 H, d, J 6.5, CH$_3$), 2.57 (1 H, m, CHMe$_2$), 4.29 (1 H, br s, CH(OH)).

5.3.1.13. Methyl (±)-2-hydroxy-3-methylbutanoate (69)

\[
\text{CH}_3
\]

$\alpha$-Hydroxyacid 68 (4.12 g, 34.9 mmol) was dissolved in methanol (100 cm$^3$), catalytic conc. H$_2$SO$_4$ was added, and the mixture was heated at reflux for 20 h. After cooling to RT the mixture was concentrated in vacuo and purified by chromatography [petrol: EtOAc (2:1)] to yield the methyl ester 69 as a yellow oil: 2.54 g (55%); $R_f$ 0.39 [petrol: EtOAc (2:1); KMnO$_4$]; $\nu_{\text{max}}$ (thin film)/cm$^{-1}$ 3511br (OH), 2965s (CH), 1736vs (C=O); $\delta$ (250 MHz; CDCl$_3$) 0.85 (3 H, d, J 7.0, CH$_3$), 0.99 (3 H, d, J 7.0, CH$_3$), 2.02 (1 H, m, CHMe$_2$), 2.73 (1 H, d, J 6.0, OH), 3.76 (3 H, s, OCH$_3$), 4.01 (1 H, m, CH(OH)); $\delta$ (75.5 MHz; CDCl$_3$) 16.0, 18.7 (CH$_3$), 32.1 (CHMe$_2$), 52.2 (OCH$_3$), 75.1 (CH(OH)), 175.3 (C=O); $m/z$(El) 117, 101, 90.

5.3.1.14. Methyl (±)-3-methyl-2-tert-butyldimethylsilyloxybutanoate (70)

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\text{OTBS}
\]

$\alpha$-Hydroxyester (±)-69 (325 mg, 2.46 mmol) and imidazole (254 mg, 3.74 mmol) were dissolved in anhydrous DMF (12 cm$^3$). The mixture was stirred at RT under nitrogen, and TBS triflate (740 mg, 0.65 cm$^3$, 2.8 mmol) was added dropwise. The mixture was stirred for a further 1 h, then quenched with saturated aqueous ammonium chloride solution (10 cm$^3$) and diluted with water (10 cm$^3$). Extraction with Et$_2$O (3 x 15 cm$^3$), washing of the ether extracts with saturated aqueous NaHCO$_3$ (25 cm$^3$) and brine (25 cm$^3$), drying of the organic layer (MgSO$_4$) and evaporation in vacuo produced a yellow liquid which was purified by chromatography [petrol: EtOAc (2:1)] to yield the desired product as a pale yellow oil: 440 mg (73%); $\nu_{\text{max}}$ (thin film)/cm$^{-1}$ 2958vs (CH), 2932vs (CH), 2859vs (CH), 1757vs (C=O), 1251s (Si-C); $\delta$ (250 MHz; CDCl$_3$) 0.04 (3 H, s, Si(CH$_3$)(CH$_3$)(Bu)), 0.06 (3 H, s, Si(CH$_3$)(CH$_3$)(Bu)), 0.88 (3 H, d, J 7.0, (CH$_3$)$_2$CH), 0.90 (9 H, s,
Si(CH₃)(CH₃)(tBu)), 0.93 (3 H, d, J 7.0, (CH₃)₂CH), 2.03 (1 H, septet, J 5.0, (CH₃)₂CH), 3.71 (3 H, s, OCH₃), 3.97 (1 H, d, J 5.0, α-CH); δ_c (62.9 MHz; CDCl₃) -5.43, -5.08 (Si(CH₃)₂Bu), 17.0 ((CH₃)₂CH), 18.3 (C(CH₃)₃), 18.9 ((CH₃)₂CH), 25.7 (C(CH₃)₃), 32.8 ((CH₃)₂CH), 51.4 (OCH₃), 77.2 (α-CH), 173.9 (C=O); m/z (EI) 247 [MH⁺], 189, 161, 129, 89.

5.3.1.15. (±)-2-Hydroxy-3-methylbutanal (66)

α-Silyloxyester 70 (499 mg, 2.03 mmol) was dissolved in toluene (20 cm³), cooled to -78°C, and treated with DIBAL-H (1.5 M solution in toluene; 2.0 cm³, 3.0 mmol) under N₂. After stirring for 1 h, the mixture was quenched with 2 M HCl (20 cm³), warmed to RT, and extracted with EtOAc (3 x 20 cm³). The organic extracts were dried (MgSO₄) and concentrated in vacuo to yield a clear viscous oil which was redissolved in THF (3 cm³) and cooled to 0°C. Tetra-n-butylammonium fluoride (TBAF) (1.0 M solution in THF; 2.0 cm³, 2.0 mmol) was added, and stirring maintained for 1 h under N₂. The mixture was concentrated in vacuo and purified by chromatography: [petrol: EtOAc (2:1)]; TLC showed a mixture of products, from which a single fraction (15 mg, 7%) was isolated as a clear oil. Addition of D₂O caused a white amorphous solid to settle out, which redissolved on standing for 2 days at RT. NMR analysis of the solution after this time showed a mixture of the free aldehyde and hydrate in a 1:2 ratio.

**Hydrate:** δ_h (250 MHz; D₂O) 1.01 (3 H, d, J 7.0, (CH₃)₂CH), 1.05 (3 H, d, J 7.0, (CH₃)₂CH), 1.96 (1 H, septet, J 7.0, (CH₃)₂CH), 3.36 (1 H, m, α-CH), 5.06 (1 H, d, J 5.5, CH(OD)₂); δ_c (75.5 MHz; D₂O) 15.4, 18.2 ((CH₃)₂CH), 28.6 ((CH₃)₂CH), 77.9 (CH(OD)), 89.8 (CH(OD)₂).

**Free aldehyde:** δ_h (250 MHz; D₂O) 1.07 (3 H, d, J 7.0, (CH₃)₂CH), 1.14 (3 H, d, J 7.0, (CH₃)₂CH), 2.37 (1 H, septet, J 7.0, (CH₃)₂CH), 4.30 (1 H, m, α-CH), 9.75 (1 H, s, CHO); δ_c (75.5 MHz; D₂O) 15.5, 17.6 ((CH₃)₂CH), 29.0 ((CH₃)₂CH), 80.6 (CH(OD)), 205.6 (CHO).
5.3.1.16. \((\pm)-3,3\text{-Diethoxypropane-1,2-diol (Glyceraldehyde diethyl acetal)} \((\pm)-76\)

\[
\begin{align*}
\text{EtO} & \quad \text{OH} \\
\text{OH} & \quad \text{OEt}
\end{align*}
\]

\(\text{a) from acrolein diethyl acetal.} \) Acrolein diethyl acetal (5.29 g, 40.7 mmol) and N-methylmorpholine-N-oxide (NMO) (5.18 g, 44.3 mmol) were dissolved in acetone (120 cm\(^3\)). Osmium tetroxide (100 mg, 0.39 mmol) was dissolved in THF (1 cm\(^3\)) and added to the reaction mixture, and stirring maintained for 18 h at RT. After this time the osmium (VIII) ions in the reaction mixture were reduced by addition of a slurry of Na\(_2\)S\(_2\)O\(_4\) (3.0 g) and talc (7.0 g) in H\(_2\)O (200 cm\(^3\)) and stirring for 30 min until a grey metallic colouration was observed in the mixture. The suspension was filtered under reduced pressure through a Celite\textsuperscript{\textregistered} pad, and the resulting black filtrate extracted with EtOAc (4 x 150 cm\(^3\)). The organic extracts were dried (MgSO\(_4\)), concentrated \textit{in vacuo}, and purified by chromatography [EtOAc (neat)]. The product \((\pm)-76\) was obtained as a pale yellow oil: 1.39 g (21%); \(\delta_H\) (400 MHz; CDCl\(_3\)) 1.23 (3 H, t, \(J\) 6.0, CH\(_3\)), 1.25 (3 H, t, \(J\) 6.0, CH\(_3\)), 2.22 (1 H, t, \(J\) 7.0, CH\(_2\)OH), 2.49 (1 H, d, CHOH), 3.56-3.67, 3.72-3.83 (7 H, multiplets, (CH\(_3\)CH\(_2\)),CH, CHOH, CH\(_2\)OH), 4.51 (1 H, d, \(J\) 8.5, CH(OEt)\(_2\)); \(\delta_C\) (100.6 MHz; CDCl\(_3\)) 15.3, 15.4 (CH\(_3\)), 62.5 (CH\(_2\)OH), 63.7, 64.5 ((OCH\(_3\)Me)\(_2\)), 71.5 (CH(OH)), 103.6 (CH(OEt)\(_2\)); \(m/z\) (GCMS) 119, 103, 91.

\(\text{b) from DL-glyceraldehyde. }\) DL-Glyceraldehyde (12.5 g, 0.139 mol) was suspended in ethanol (500 cm\(^3\)) containing Amberlyst\textsuperscript{\textregistered} H\textsuperscript{+} resin beads, and stirred at RT. After 96 h a further 150 cm\(^3\) EtOH and fresh Amberlyst\textsuperscript{\textregistered} resin was added. After 112 h the mixture had become clear; the solution was filtered, treated with Amberlite\textsuperscript{\textregistered} basic gel resin (pre-washed in 2 M NaOH), filtered, and concentrated \textit{in vacuo} to yield crude acetal \((\pm)-76\): 19.5 g (86%); spectroscopic properties in agreement with those reported above. A small quantity \((\approx 5\text{%})\) unreacted glyceraldehyde was also observed in the \(^1\text{H}\) NMR spectrum, but no further purification was attempted.

\(\text{c) from 3-O-benzylglyceraldehyde diethyl acetal. }\) 3BG diethyl acetal \((\pm)-78\) (820 mg, 3.23 mmol) was dissolved in THF (50 cm\(^3\)). Palladium on activated charcoal (BDH, 10\text{%} grade; 500 mg) was added. The mixture was repeatedly evacuated using a water pump and flushed with hydrogen, and allowed to stand for 18.5 h under an atmosphere of hydrogen. The mixture was filtered through Celite\textsuperscript{\textregistered}, and the filtrate concentrated \textit{in vacuo} and purified by chromatography [EtOAc (neat)].
The diol (±)-76 was obtained as a clear oil: 249 mg (47%); spectroscopic properties in agreement with those reported above.

5.3.1.17. 2,2-Diethoxyacetaldehyde (31)

(±)-GDA 76 (1.37 g, 8.34 mmol) was dissolved in water (50 cm$^3$) and cooled to 0°C. Sodium periodate (1.97 g, 9.21 mmol) was added, and the mixture stirred at 0°C for 5 min then allowed to warm to RT. The solution was extracted with CH$_2$Cl$_2$ (5 x 100 cm$^3$), dried (MgSO$_4$), concentrated in vacuo and purified by chromatography [EtOAc (neat)]. Aldehyde 31 was isolated as a clear viscous oil: 289 mg (26%); $\delta$$_H$ (300 MHz; D$_2$O) 1.34 (6 H, t, J 7.0, CH$_3$), 3.78-3.99 (4 H, in, CH$_2$CH$_3$), 4.51 (1 H, d, J 5.0, CH(OD)$_2$), 5.00 (1 H, d, J 5.0, CH(OEt)$_2$); $\delta$$_C$ (75.5 MHz; D$_2$O) 13.9 (CH$_3$), 64.1 (CH$_2$CH$_3$), 88.6 (CH(OD)$_2$), 102.5 (CH(OEt)$_2$); m/z (GCMS) 103, 75, 73, 61.

5.3.1.18. (±)-3,3-Diethoxy-2-trimethylsilyloxypropanenitrile (79)

Diethoxyacetaldehyde 31 (225 mg, 1.70 mmol) was dissolved in CH$_2$Cl$_2$ (15 cm$^3$). A catalytic amount of zinc iodide was added, and the mixture stirred and cooled to 0°C under N$_2$. Trimethylsilyl cyanide (0.25 cm$^3$, 190 mg, 1.9 mmol) was added dropwise, and the mixture stirred for 4 h. The mixture was quenched by addition of CH$_2$Cl$_2$ (20 cm$^3$) and water (20 cm$^3$), and the organic layer separated and washed with a further 20 cm$^3$ water. The organic layer was dried (MgSO$_4$), concentrated in vacuo, and the remaining solvent evaporated under a stream of N$_2$, and the α-silyloxy nitrile 79 isolated as a yellow-brown oil which was not purified further: 231 mg (59%); $\delta$$_H$ (200 MHz; CDCl$_3$) 0.20 (9 H, s, SiMe$_3$), 1.22 (3 H, t, J 6.0, CH$_3$CH$_2$), 1.26 (3 H, t, J 6.0, CH$_3$CH$_2$), 3.71 (4 H, m, CH$_3$CH$_2$), 4.30 (1 H, d, J 6.0, α-CH), 4.50 (1 H, d, J 6.0, (EtO)$_2$CH); m/z (GCMS) 232 [MH$^+$], 170, 142, 103, 75.
5.3.1.19. (±)-3,3-Diethoxy-2-hydroxypropanal ((±)-75)

Glyceraldehyde diethyl acetal (±)-76 (2.41 g, 14.7 mmol) and 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO) radical (50 mg, 0.33 mmol) were dissolved in 1:1 ethyl acetate/toluene (160 cm³) and cooled to 0°C with stirring. Benzyl trimethyl ammonium chloride (523 mg, 2.81 mmol), NaBr (335 mg, 3.25 mmol) and saturated aqueous NaHCO₃ (35 cm³) were added. The resulting two-phase mixture was treated dropwise with a solution made up of 1.1 M NaOCl solution (15 cm³), saturated aq. NaHCO₃ (15 cm³) and brine (20 cm³) (solution pH 8.6) over a 1 h period, at a rate just sufficient to maintain a yellow colour in the solution. After addition of the last drop of buffered NaOCl, the mixture was subjected to continuous extraction with EtOAc for 15.5 h. The organic layer was separated, washed with saturated aq. NaHCO₃ (20 cm³) and brine (20 cm³), dried (MgSO₄), concentrated in vacuo, and purified by chromatography on silica [EtOAc (neat)]. The aldehyde (±)-75 was isolated as a yellow-orange oil: 409 mg (17%); δₜ (300 MHz; D₂O) 1.26 (6 H, t, J 6.0, CH₃), 3.57 (1 H, dd, J 4.0 & 5.5, CH(OD)), 3.59-3.73, 3.74-3.90 (4 H, in, CH₂CH₂), 4.64 (1 H, d, J 5.5, CH(OEt)₂), 5.07 (1 H, d, J 4.0, CH(OD)₂); δₖ (75.5 MHz; D₂O) 13.8, 13.9 (CH₃), 64.0, 64.1 (CH₂CH₂), 72.9 (CH(OD)₂), 88.5 (CH(OD)₂), 101.7 (CH(OEt)₂); m/z (GCMS) 161 [M-H+] 117, 103.

5.3.1.20. (±)-E-1,2-Epoxy-4-phenylbut-3-ene ((±)-89)

Sodium hydride (60% dispersion in mineral oil) (2.48 g, 62.8 mmol) was washed with petrol (20 cm³) and to it was added, under nitrogen, anhydrous THF (30 cm³) and dimethylsulfoxide (DMSO) (30 cm³). The mixture was cooled to 0°C in an ice-salt bath. A solution of trimethylsulfonium iodide (12.1 g, 60.0 mmol) in DMSO (50 cm³) was added with stirring, followed by cinnamaldehyde (2.64 g, 20.0 mmol). The mixture was stirred at 0°C for 30 min, then at RT for 1 h, quenched cautiously with ice-water (100 cm³) and extracted with CH₂Cl₂ (3 x 100 cm³). The combined organic extracts were washed with brine (2 x 100 cm³), dried (MgSO₄), and
the solvent evaporated in vacuo. The product was purified by chromatography on silica [petrol : EtOAc (3:1)] followed by heating for 1 h at 80°C on a Kugelrohr apparatus to remove the last traces of DMSO. The epoxide (±)-89 was obtained as a yellow oil: 1.94 g, 66%; δ H (250 MHz; CDCl₃) 2.75 (1 H, dd, J 2.5, 5.0, CH₂), 3.05 (1 H, dd, J 4.0, 5.0, CH₂), 3.55 (1 H, m, CH), 5.90 (1 H, dd, J 8.0, 16.0, CH=CHPh), 6.80 (1 H, d, J 16.0, CH=CHPh), 7.30 (5 H, m, Ph).

5.3.1.21. (±)-E-2-Azido-4-phenyl-3-buten-1-ol ((±)-88)

Epoxide 89 (247 mg, 1.69 mmol) and sodium azide (210 mg, 3.23 mmol) were dissolved in a 2:1 mixture of acetone/water (3.8 cm³), heated to 65°C and stirred for 2 h. Ammonium chloride (80 mg) was added, and the mixture cooled to RT over 20 min. The acetone was removed in vacuo and the remaining liquid diluted with water (to 10 cm³ total volume) and extracted with CH₂Cl₂ (4 x 10 cm³). On drying (MgSO₄) and evaporation in vacuo, a yellow-brown oil was obtained which was purified by chromatography on silica [petrol: EtOAc (5:1)] to yield the azido-alcohol (±)-88 as a yellow oil: 180 mg (56%); Rf 0.11 [petrol: EtOAc (5:1)]; ν max (thin film)/cm⁻¹ 3388br (OH), 2119vs (N₃); δ H (250 MHz; CDCl₃) 2.11 (1 H, m, OH), 3.69 (2 H, m, CH₂OH), 4.24 (1 H, m, CH), 6.15 (1 H, dd, J 8.0, 16.0, CH=CHPh), 6.74 (1 H, d, J 16.0, CH=CHPh), 7.36 (5 H, m, Ph); δ C (62.9 MHz; CDCl₃) 65.0 (CH₂), 66.3 (CH(N₃)), 128.4 (CH=CHPh), 135.3 (CH=CHPh), 123.0, 126.7, 128.7 (aromatic CH), 135.7 (aromatic quarternary).

5.3.1.22. (±)-2-Bromo-3-hydroxypropanoic acid ((±)-84)

To a solution of DL-serine (5.36 g, 51.0 mmol) and potassium bromide (20.9 g, 175 mmol) in 1.25 M sulphuric acid (100 cm³) were added, with stirring, four equal portions of sodium nitrite (1.38 g, 20.0 mmol) at half-hour intervals. The mixture was stirred for 2 h after the last addition of NaNO₂, extracted with diethyl ether (3 x 125 cm³), dried (MgSO₄) and evaporated in vacuo. The last traces of Et₂O were removed from the resulting oil by evacuation on a high-vacuum pump, and the
acid (±)-84 was recovered as a viscous yellow oil which was not purified further: 5.81 g (67%); ν<sub>max</sub> (thin film)/cm<sup>-1</sup> 3100-3500 br (OH), 2952 br (OH), 1726 vs (C=O), 1411 m (OH), 662 m (C-Br); δ<sub>H</sub> (250 MHz; D<sub>2</sub>O) 4.07 (2 H, d, J 6.5, CH<sub>2</sub>), 4.60 (1 H, t, J 6.5, CH).

5.3.1.23. Methyl (±)-2-bromo-3-hydroxypropanoate ((±)-91)

![Chemical structure of methyl (±)-2-bromo-3-hydroxypropanoate](image)

To a stirred solution of α-bromoacid 84 (5.69 g, 33.7 mmol) in methanol (150 cm<sup>3</sup>) were added 10 drops of concentrated H<sub>2</sub>SO<sub>4</sub>. The mixture was heated to reflux on an oil bath and stirred for 20 h. After allowing the mixture to cool, the methanol was removed in vacuo and the residue purified by chromatography on silica [petrol: ethyl acetate (4:1), followed by petrol: ethyl acetate (1:1)] yielding the methyl ester (±)-91 as a pale yellow oil: 4.878 g (79%); R<sub>f</sub> 0.13 [petrol: EtOAc (4:1), UV]; ν<sub>max</sub> (thin film)/cm<sup>-1</sup> 3466 br (OH), 2957 s (CH), 1743 vs (C=O); δ<sub>H</sub> (300 MHz; CDCl<sub>3</sub>) 2.65 (1 H, br s, OH), 3.81 (3 H, s, OCH<sub>3</sub>), 3.92 (1 H, m, J 5.5, 12.0, CHH), 4.04 (1 H, m, J 7.5, 12.0, CHH), 4.33 (1 H, m, CH); δ<sub>C</sub> (75.5 MHz; CDCl<sub>3</sub>) 44.3 (CH), 53.2 (OCH<sub>3</sub>), 63.8 (CH<sub>2</sub>), 169.4 (C=O).

5.3.1.24. Methyl (±)-2-azido-3-hydroxypropanoate ((±)-92)

![Chemical structure of methyl (±)-2-azido-3-hydroxypropanoate](image)

To a solution of the methyl ester 91 (1.01 g, 5.49 mmol) in distilled water (20 cm<sup>3</sup>) was added, with stirring, sodium azide (715 mg, 11 mmol) and "Aliquat 336" phase transfer catalyst (methyl trioctyl ammonium chloride) (202 mg, 0.5 mmol). The mixture was warmed to 55°C and stirred for 16 h, cooled to RT over 1 h, and extracted with Et<sub>2</sub>O (4 x 20 cm<sup>3</sup>). After drying (MgSO<sub>4</sub>) and evaporation in vacuo, the resulting yellow oil was purified by silica chromatography [petrol: EtOAc (5:3)] to yield the ester (±)-92 as a pale yellow oil: 423 mg (53%); R<sub>f</sub> 0.20 [petrol: EtOAc (5:3), KMnO<sub>4</sub>]; ν<sub>max</sub> (thin film)/cm<sup>-1</sup> 3453 br (OH), 2960 m (CH), 2115 vs (N<sub>3</sub>), 1742 vs (C=O); δ<sub>H</sub> (250 MHz; CDCl<sub>3</sub>) 3.01 (1 H, br s, OH), 3.78 (3 H, s, OCH<sub>3</sub>), 3.88 (2 H, m, CH<sub>2</sub>), 4.04 (1 H, m, CH).
5.3.1.25. *Methyl (±)-2-azido-3-tert-butyldimethylsilyloxypropanoate* ((±)-93)

\[
\begin{align*}
\text{TBSO} & \quad \text{N}_3 \\
& \quad \text{CO}_2\text{Me}
\end{align*}
\]

α-Azidoester 92 (806 mg, 5.56 mmol) was added to a solution of imidazole (570 mg, 8.38 mmol) in anhydrous DMF (35 cm³) under nitrogen. TBS triflate (1.4 cm³, 1.6 g, 6.1 mmol) was added dropwise. The mixture was stirred at RT for 90 min under nitrogen, quenched by careful addition of saturated aqueous ammonium chloride solution (50 cm³) and extracted with Et₂O (3 x 50 cm³). The combined ether extracts were further washed with saturated NaHCO₃ solution (100 cm³) and brine (100 cm³), dried (MgSO₄), and evaporated *in vacuo*. The residue was purified by silica chromatography [petrol: EtOAc (3:1)] and the protected compound 93 was obtained as a clear oil (1.07 g, 74%): Rₚ 0.58 [petrol: EtOAc (3:1), molybdate]; vₚₓ (cm⁻¹) 2956m (CH), 2933m (CH), 2107vs (N₃), 1754vs (C=O), 1257s (Si-C); δₜ (250 MHz; CDCl₃) -0.07 (6 H, 2 x s, (CH₃)₂Si), 0.88 (9 H, s, 'Bu), 3.79 (3 H, s, OCH₃), 3.80 (1 H, br s, CH), 4.03 (2 H, m, CH₂); δₜ (75.5 MHz; CDCl₃) -5.8, -5.7 ((CH₃)₂Si), 18.1 ((CH₃)₃C-Si), 25.6 ((CH₃)₃C-Si), 52.5 (OCH₃), 63.2 (CH), 64.5 (CH₂), 169.2 (C=O); m/z (El) 259 (M⁺, very weak), 202, 115; (Found: m/z (El) [M⁺] 259.13429. C₁₀H₂₁N₃O₃Si requires m/z (El) [M⁺] 259.13522).

5.3.1.26. *α-Azido-3-tert-butyldimethylsilyloxypropanal* ((±)-94)

\[
\begin{align*}
\text{TBSO} & \quad \text{N}_3 \\
& \quad \circ
\end{align*}
\]

Ester 93 (549 mg, 2.12 mmol) was dissolved in toluene (40 cm³), and cooled to -78°C under nitrogen using an acetone/dry ice bath. DIBAL-H solution (1.5 M in toluene; 2.8 cm³, 4.2 mmol) was added dropwise to the mixture, after which stirring at -78°C was maintained for 90 min. The mixture was quenched by slow addition of 2 M aqueous HCl (40 cm³) at -78°C, allowed to warm to = 0°C, and extracted with EtOAc (75 cm³, then 2 x 60 cm³). The residue was dried (MgSO₄) and purified by chromatography on silica [CH₂Cl₂ (neat)] yielding the aldehyde 94 as a pale yellow oil: 245 mg (51%); vₚₓ (thin film)/cm⁻¹ 2957s (CH), 2932s (CH), 2887s (CH), 2861s (CH), 2105vs (N₃), 1740s (C=O), 1255s (Si-C); δₜ (250 MHz; CDCl₃) 0.09, 0.10 (6 H, 2 x s, (CH₃)₂Si), 0.89 (9 H, s, 'Bu), 3.83 (1 H, m, CH), 4.10 (2 H, m, CH₂),
9.15 (1 H, s, CHO); δ_C (62.9 MHz; CDCl_3) -5.74, -5.67 ((CH_3)_2Si), 18.1 ((CH_3)_2C-Si), 25.7 ((CH_3)_3C-Si), 62.6 (CH_2), 68.7 (CH), 197.0 (CHO); m/z (EI) 230 (MH^+, very weak), 229 (M^+, very weak); (Found: m/z (EI) [MH^+] 230.13161. C_9H_20N_3O_2Si requires m/z (EI) [MH^+] 230.12465).

5.3.1.27. (±)-2-Azido-3-hydroxypropanal ((±)-36)

a) by deprotection of the aldehyde (±)-94: Aldehyde 94 (150 mg, 0.66 mmol) was dissolved in a 3:1:1 mixture of acetic acid/water/THF (5 cm³) and stirred at RT for 24 h under nitrogen. The solvents were removed in vacuo, and the residue was purified by silica chromatography [Et_2O (neat)] to yield the azido-aldehyde (±)-36 as a clear oil, TLC of which [Et_2O (neat), 2,4-DNP] showed a streak at R_f = 0.3-0.5. The product was stored as a 50 mM solution in D_2O in a refrigerator: 50 mg (66%); δ_H (250 MHz; D_2O) 3.68 (1 H, ddd, J 3.5, 5.0, 7.5, CH), 3.77 (1 H, dd, J 7.5, 11.5, CHH), 3.96 (1 H, dd, J 3.5, 11.5, CHH), 5.19 (1 H, d, J 5.0, CH(OH)); δ_C (75.5 MHz; D_2O) 60.2 (CH_2), 66.8 (CH), 88.7 (CH(OH)).

b) by ozonolysis of the olefinic azido-alcohol 88: Alcohol 88 (118 mg, 0.62 mmol) was dissolved in a 1:1 mixture of CH_2Cl_2/methanol (10 cm³), cooled to -78°C using an acetone/dry ice bath, and treated with an ozone-oxygen stream from a laboratory ozonator for 10 min. A blue colour was observed in the solution after this time. The solution was purged with oxygen to remove excess dissolved ozone, then with nitrogen; dimethyl sulfide (1 cm³) was added; the mixture was allowed to warm to RT, water (0.5 cm³) was added, and stirring was maintained for 20 h. The solvents were removed in vacuo, residual water and DMSO were removed by heating on a Kugelrohr apparatus at 75°C for 30 min, and the resulting yellow oil was purified by chromatography on silica [petrol: Et_2O (3:1), petrol: Et_2O (1:1), Et_2O]. A fraction with R_f = 0.3-0.5 [Et_2O (neat)] was isolated; solvent evaporation yielded 28 mg of a clear oil. Initial ^1H NMR studies (D_2O) were hampered by the apparent decomposition of the product (with effervescence) in concentrated D_2O solution in the NMR tube, and after standing at RT for 2 days only decomposition products were observed by ^1H NMR.
5.3.2. Experimental Details to Chapter Three

5.3.2.1. (3S, 4R)-1,3,4-Trihydroxy-5-phenylpentan-2-one (95)

\[
\begin{align*}
\text{Ph} & \quad \text{OH} & \quad \text{O} & \quad \text{OH} \\
& & \text{OH} & & \\
\end{align*}
\]

a) in glycyglycine buffer (1 mmol). Aldehyde \((R)-54\) (277 mg, 1.85 mmol), lithium hydroxypropyruvate (LiHPA) (103 mg, 0.94 mmol), magnesium chloride (2.2 mg, 0.023 mmol), and thiamine pyrophosphate chloride (cocarboxylase) (12 mg, 26 \(\mu\)mol) were suspended in glycyglycine buffer (0.1 M, pH adjusted to 7.0 using 0.1 M NaOH). Transketolase solution (1.0 cm\(^3\), \(\approx\) 200 U) was added, and the mixture incubated for 16 h at 37°C. After cooling to RT, silica was added, and the mixture lyophilised on a rotary evaporator and dry loaded onto a silica column. Chromatography [EtOAc: MeOH (99:1)] yielded triol (3S, 4R)-95 as a viscous pale yellow oil: 87 mg (44%); \([\alpha]_D^{23} +26.7^\circ\) (c 1.07, CHCl\(_3\)); \(\nu_{\max}\) (thin film)/cm\(^{-1}\) 3413vs (OH), 1726vs (C=O); \(\delta\) H (300 MHz; CDCl\(_3\)) 2.91 (2 H, m, PhCH\(_2\)), 2.94 (1 H, br s, OH), 3.45 (1 H, br s, OH), 3.82 (1 H, br s, OH), 4.13 (2 H, m, CH(OH)CH(OH)), 4.32 (1 H, 0.5ABq, \(\text{J} 19.5\), CHHOH), 4.49 (1 H, 0.5ABq, \(\text{J} 19.5\), CHHOH), 7.27 (5 H, in, Ph); \(\delta\) C (62.9 MHz; CDCl\(_3\)) 39.7 (PhCH\(_2\)), 66.5 (CH\(_2\)OH), 73.3 (CH), 76.8 (CH), 127.0, 128.8, 129.5 (aromatic CH), 137.3 (aromatic quarternary), 211.6 (C=O).

b) in unbuffered solution in the pH stat (4 mmol). The following mixture was prepared in deionised water (60 cm\(^3\)) in the pH stat reaction vessel: aldehyde \((R)-54\) (601 mg, 4.00 mmol), LiHPA (443 mg, 4.03 mmol); magnesium chloride hexahydrate (41 mg, 0.20 mmol); TPP chloride (25 mg, 0.054 mmol); bovine serum albumin (BSA) (30 mg). The solution pH was adjusted manually to \(\approx\) 7 using aqueous NaOH (1 M solution), the pH stat control set to 7.0 (0.5 M aqueous HCl as titrant), and transketolase solution (\(\approx\) 200 U) added. The mixture was stirred at 25°C for 46 h (a further \(\approx\) 200 U transketolase was added after 22 h), treated with silica, lyophilised by dry loading of the residue onto a silica column followed by chromatography [EtOAc (neat)]. The product was obtained as a pale yellow viscous oil: 450 mg (54%); all spectroscopic data in agreement with that reported above.
5.3.2.2. (3S, 4R)-1,3,4-Trihydroxy-4-phenylbutan-2-one (97)

The following mixture was prepared in water (18 cm³) in the pH stat vessel: aldehyde (R)-61 (93 mg, 0.68 mmol); LiHPA (75 mg, 0.68 mmol); anhydrous MgCl₂ (8 mg, 85 μmol); TPP chloride (14 mg, 30 μmol); and BSA (9 mg). The solution pH was adjusted to ≈ 7 using 1 M NaOH, the pH stat control set to 7.0 (1 M HCl as titrant), and transketolase solution (0.50 cm³, ≈ 100 U) added. Stirring was maintained at RT for 17 h; thereafter silica was added, and the mixture lyophilised and purified by dry loading onto a silica column followed by chromatography [EtOAc: petrol (3:1)]. The product was obtained as a white amorphous solid: 58 mg (44%); R<sub>f</sub> 0.28 [EtOAc: petrol (3:1), 2,4-DNP]; δ<sub>δ</sub> (300 MHz; D<sub>2</sub>O) 4.53 (1 H, 0.5ABq, J 19.0, CHHOH), 4.62 (1 H, 0.5ABq, J 19.0, CHHOH), 4.66 (1 H, d, J 4.0, PhCH(OH)), 5.24 (1 H, d, J 4.0, PhCH(OH)CH(OH)), 7.55 (5 H, m, Ph); δ<sub>C</sub> (75.5 MHz; D<sub>2</sub>O) 65.9 (CH₂), 73.5 (PhCH(OH)), 78.3 (PhCH(OH)CH(OH)), 125.9, 127.7, 128.2 (aromatic CH), 139.1 (aromatic quarternary), 211.7 (C=O).

5.3.2.3. (3S, 4S)-5,5-Diethoxy-1,3,4-trihydroxypentan-2-one (74)

The following mixture was prepared in water (20 cm³) in the pH stat vessel: aldehyde (±)-75 (409 mg, 2.52 mmol); lithium hydroxypyruvate monohydrate (173 mg, 1.35 mmol); magnesium chloride hexahydrate (35 mg, 0.172 mmol); TPP chloride (24 mg, 52.1 μmol); and BSA (20 mg). The solution pH was adjusted to ≈ 7 using 0.1 M NaOH, the pH stat control set to 7.0 (1.0 M HCl as titrant) and transketolase solution (1.0 cm³, ≈ 200 U) added. Stirring was maintained at RT for 40 h, with a further addition of transketolase (1.0 cm³, ≈ 200 U) after which time silica was added and the mixture lyophilised and dry loaded onto a silica column. Chromatography [EtOAc (neat)] yielded the desired product 74 as a clear oil: 157 mg (56%, yield based on reactive (S)-enantiomer); [α]<sup>D</sup><sup>30</sup> -12.0° (c 0.8, CHCl₃) (lit: -18°); δ<sub>δ</sub> (400 MHz; CDCl₃) 1.26 (6H, t, J 7.0, CH₃), 3.69-3.84 (4H, m, MeCH₂), 3.96
(1H, dd, J 3.0, 5.0, (EtO)₂CHCH(OH), 4.50 (2H, s, CH₂OH), 4.58 (1H, d, J 3.0, (EtO)₂CHCH(OH)CH(OH), 4.63 (1H, d, J 5.0, (EtO)₂CH); δc (100.6 MHz; CDCl₃) 15.2, 15.3 (CH₃), 64.3, 65.2 (CH₂CH₃), 66.9 (CH₂OH), 72.1 (CH(OH)), 75.1 (CH(OH)), 103.0 ((EtO)₂CH), 211.2 (C=O).

5.3.2.4. (3S, 4R)-5-Benzyl oxy-1,3,4-trihydroxypentan-2-one
(5-α-Benzyl-D-xylulose) (82)

(±)-3-O-Benzylglyceraldehyde diethyl acetal (±)-78 (3.51 g, 13.8 mmol) was suspended in water (100 cm³), the solution pH adjusted to 1.0 by addition of 1.0 M HCl via an autotitrator, and the mixture stirred at RT for 68 h to release 3-O-benzylglyceraldehyde 81. The resulting solution/suspension was diluted to 500 cm³ volume by addition of water, the pH adjusted to = 7 by careful addition of 5 M NaOH followed by 0.1 M NaOH, and to the mixture was added TPP chloride (153 mg, 0.332 mmol), magnesium chloride hexahydrate (59 mg, 0.291 mmol), and BSA (67 mg). The pH was readjusted to = 7 using 0.1 M NaOH, and transketolase solution (3.0 cm³; = 600 U) added. After stirring for a further 5 min to allow the enzyme and cofactors to incubate, reaction was initiated by addition of lithium hydroxypyruvate monohydrate (886 mg, 6.92 mmol). The pH was maintained at 7.0 by autotitration of 1.0 M HCl solution. After 22.5 h, THF (20 cm³) was added to solubilise the remaining unreacted aldehyde. Stirring was continued for a further 2.5 h; thereafter the mixture was treated with silica, lyophilised on a rotary evaporator, dry loaded onto a silica column, and purified by chromatography [petrol: EtOAc (3:2), EtOAc: MeOH (9:1)]. The product was isolated as a pale yellow, viscous oil: 1.29 g (78%, yield based on reactive (R)-aldehyde); δh (300 MHz; D₂O) 3.75 (1H, dd, J 10.0, 8.0, PhCH₂OCHH), 3.79 (1H, dd, J 10.0, 6.0, PhCH₂OCHH), 4.32 (1H, m, 4-H), 4.53 (1H, d, J 2.0, 3-H), 4.61 (1H, 0.5ABq, J 19.0, 1-H), 4.73 (1H, 0.5ABq, J 19.0, 1-H), 4.74 (2H, s, PhCH₂), 7.47-7.61 (5H, m, Ph); δc (100.6 MHz; D₂O) 66.5 (CH₂), 70.5 (CH₂), 70.6 (CH), 73.6 (CH₂), 75.9 (CH), 128.8, 128.9, 129.2 (aromatic CH), 137.3 (aromatic quaternary), 211.3 (C=O).

(S)-3-α-Benzylglyceraldehyde ((S)-81). In a separate biotransformation, resolved aldehyde (S)-81 was recovered as a clear oil: 534 mg (26% of total); δh (200 MHz; D₂O) 3.51-3.75 (3 H, multiplets, CH₃CH(OH) and CH₂CH(OH)),

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4.56 (2 H, s, PhCH₂), 4.89 (1 H, d, J 5.5, CH(OD)₂), 7.40 (5 H, s, Ph); m/z (GCMS) 107, 91, 89. ¹H NMR also showed a small amount of contamination with racemic acetal (±)-78.

5.3.2.5. (S)-3-Benzylxy-1,1-diethoxypropan-2-ol (3-O-Benzylglyceraldehyde diethyl acetal) ((S)-78)

\[
\text{Ph} \longrightarrow \text{O} \longrightarrow \text{OH} \longrightarrow \text{OEt} \longrightarrow \text{OEt}
\]

Aldehyde (S)-81 recovered from biotransformation (351 mg, 1.95 mmol) was dissolved in ethanol (20 cm³), Amberlyst® H⁺ exchange resin beads were added, and the mixture stirred at RT for 24 h. The mixture was filtered, neutralised with Amberlite® basic anion exchange resin (pre-washed in 1 M LiOH), filtered, and concentrated in vacuo. The residue was purified by chromatography [EtOAc (neat)] to yield the enantiomerically enriched (S)-acetal as a clear oil: 357 mg (72%);

\[
\begin{align*}
\delta_{\text{H}} (200 \text{ MHz; CDCl₃}) & : 1.20 (3 \text{ H, t, } J 7.0, \text{ CH₃}), \ 1.26 (3 \text{ H, t, } J 7.0, \text{ CH₃}), \ 3.44 (1 \text{ H, d, } J 8.0, \text{ OH}), \ 3.56-3.84 (7 \text{ H, multiplets, PhCH₂, } (\text{CH₃CH₂O})₂\text{CH}, \text{ CH(OH)}), \\
\delta_{\text{C}} (100.6 \text{ MHz; CDCl₃}) & : 15.3 (\text{CH₃}), \ 63.3, 63.7 (\text{CH₂CH₃}), \ 69.3 (\text{CH₂}), \ 70.9 (\text{CH}), \ 73.5 (\text{CH₂}), 102.4 ((\text{EtO})₂\text{CH}), 127.7, 127.8, 128.4 (\text{aromatic CH}), \ 138.0 (\text{aromatic quarternary}).
\end{align*}
\]

5.3.3. EXPERIMENTAL DETAILS TO CHAPTER FOUR

5.3.3.1. (3S, 4S)-5,5-Diethoxy-1,3,4-tri(tert-butyldimethylsilyloxy)pentan-2-one (102)

\[
\begin{align*}
\text{EtO} & \longrightarrow \text{O} \longrightarrow \text{TBSO} \longrightarrow \text{O} \longrightarrow \text{TBS}
\end{align*}
\]

Triol 74 (127 mg, 0.572 mmol) was dissolved in anhydrous CH₂Cl₂ (5 cm³), flushed with N₂, cooled to 0°C with stirring, and treated with triethylamine (0.42 cm³,
305 mg, 3.02 mmol) and TBS triflate (0.67 cm$^3$, 770 mg, 2.9 mmol). After stirring for 1 h the mixture was quenched with saturated aqueous NH$_4$Cl (5 cm$^3$), and extracted with CH$_2$Cl$_2$ (3 x 10 cm$^3$). The combined organic extracts were dried (MgSO$_4$), concentrated in vacuo, and purified by chromatography [CH$_2$Cl$_2$ (neat)], yielding the trisilylated material 102 as a pale yellow oil: 223 mg (69%); $[\alpha]_D^{20} +8.7^\circ$ (c 1.13, CHCl$_3$) (lit: +9$^\circ$ (c 0.72, CHCl$_3$)); R$_f$ 0.45 [CH$_2$Cl$_2$ (neat), resorcinol]; $\delta_H$ (200 MHz; CDCl$_3$) 0.08, 0.10 (18 H, 2 x s, Si(CH$_3$)$_2$Bu), 0.89-0.96 (27 H, 3 x s, Si(CH$_2$)$_2$Bu), 1.20 (6 H, t, J 7.0, (CH$_3$CH$_2$O)$_2$CH), 3.46-3.70 (4 H, m, (CH$_3$CH$_2$O)$_2$CH), 4.01 (1 H, dd, J 2.5, 6.0, 4-H), 4.39 (1 H, d, J 2.5, 5-H), 4.42 (1 H, d, J 6.0, 3-H), 4.54 (2 H, apparent doublet, $J_{app}$ 2.5, CH$_2$OTBS); m/z (GCMS) 259, 189, 133, 117, 103.

5.3.3.2. E- and Z-(3S, 4S)-5,5-Diethoxy-2-hydroxyimino-1,3,4-tri(tert-butyldimethylsilyloxy)pentane (104)

![Diagram of compound 104](image)

Compound 102 (174 mg, 0.31 mmol) was dissolved in anhydrous methanol (5 cm$^3$). Hydroxylamine hydrochloride (107 mg, 1.54 mmol) and KHCO$_3$ (134 mg, 1.34 mmol) were added, and the mixture heated at reflux under an atmosphere of nitrogen. A further 4 cm$^3$ methanol was added after 1.5 h. After stirring for a total of 5 h, the mixture was cooled to RT, treated with silica, evaporated to dryness in vacuo, dry loaded onto a silica column, and purified by chromatography [petrol: EtOAc (9:1)] yielding the oximes 104 as a mixture of geometric isomers (A:B) as a viscous clear oil: 131 mg (73%); $\delta_H$ (250 MHz; CDCl$_3$) 0.04-0.10 (18 H, 6 x s, Si(CH$_3$)$_2$), 0.87-0.90 (27 H, 3 x s, SiC(CH$_3$)$_3$), 1.11-1.27 (6 H, m, (CH$_3$CH$_2$O)$_2$CH), 3.45-3.69 (4 H, m, (CH$_3$CH$_2$O)$_2$CH), 3.90 (B, 0.33 H, m, 4-H), 4.13 (A, 0.67 H, m, 4-H), 4.38 (1 H, 0.5ABq, J 16.0, 1-H), 4.49 (1 H, 0.5ABq, J 16.0, 1-H), 4.76 (A, 0.67 H, d, J 7.0, 5-H), 4.79 (B, 0.33 H, d, J 7.0, 5-H), 5.11 (1 H, d, J 2.0, 3-H), 10.22 (B, 0.33 H, br s, NOH), 10.48 (A, 0.67 H, br s, NOH); $\delta_C$ (62.9 MHz; CDCl$_3$) -5.6, -5.5, -5.3, -5.2, -4.9, -4.4, -4.3, -4.2, -4.1 (Si(CH$_3$)$_3$), 14.9, 15.1, 15.2, 15.4 (CH$_3$CH$_2$O), 18.1, 18.2, 18.3 (Si-C(CH$_3$)$_3$)), 25.7, 25.8, 25.9 (Si-C(CH$_3$)$_3$)), 57.1 (B, 1-C), 57.2 (A, 1-C), 61.3, 62.5, 63.3, 63.7 (CH$_3$CH$_2$O), 69.0 (A, CH(OH)), 167.
71.3 \((B, CH(\text{OH}))\), 71.9 \((A, CH(\text{OH}))\), 74.3 \((B, CH(\text{OH}))\), 100.4 \((A, 5-\text{C})\), 102.1 \((B, 5-\text{C})\), 157.2 \((B, C=\text{N})\), 158.0 \((A, C=\text{N})\).

5.3.3.3. \((2R, 3R, 4S)-\) and \((2S, 3R, 4S)-2\)-Amino-5,5-diethoxy-1,3,4-tri(tert-butyldimethylsilyloxy)pentane \((105 \text{ and } 106)\)

The mixture of oximes \(104\) \((148 \text{ mg}, 0.260 \text{ mmol})\) was dissolved in ethanol \((6 \text{ cm}^3)\). Raney nickel \((50\% \text{ slurry in water}; 1.0 \text{ cm}^3)\) was added, and the reaction vessel repeatedly evacuated and flushed with hydrogen. The mixture was stirred under \(H_2\) for 21 h, filtered through Celite\(^{\circledR}\), concentrated \(\textit{in vacuo}\) and purified by chromatography on silica [petrol: EtOAc \((17:3)\)] to yield amines \(105\) and \(106\) as a 3:2 diastereomeric \((A:B)\) mixture, as judged by \(^1\text{H NMR integrals}: 93 \text{ mg (64%); \(R_f\) 0.03-0.10 [petrol: EtOAc \((9:1), \text{ resorcinol}\) ]; \(\delta_{\text{H}}\) \((250 \text{ MHz; } CDCl_3)\) 0.02-0.08 \((18 \text{ H, } 6 \times \text{s, Si(CH}_3)_3), 0.86-0.88 \((27 \text{ H, } 3 \times \text{s, Si-C(CH}_3)_3), 1.17 \((A, 3.6 \text{ H, t, J 7.0, CH}_3\text{CH}_2\text{O}), 1.20 \((B, 2.4 \text{ H, t, J 7.0, CH}_3\text{CH}_2\text{O}), 1.67 \((2 \text{ H, br s, NH}_2), 2.82 \((A, 0.6 \text{ H, dt, J 5.0, 9.0, 2-H}), 3.05 \((B, 0.4 \text{ H, dt, J 5.0, 4.5, 2-H}), 3.40-3.83 \((8 \text{ H, multiplets, CH}_3\text{CH}_2\text{O), 1-H, 3-H, 4-H}), 4.50 \((A, 0.6 \text{ H, d, J 3.5, 5-H}), 4.57 \((B, 0.4 \text{ H, d, J 4.0, 5-H}); \delta_{\text{C}}\) \((62.9 \text{ MHz; } CDCl_3)\) \(-5.4, -4.7 \((\text{Si(CH}_3)_3), 15.2, 15.3 \((\text{CH}_3\text{CH}_2\text{O}), 18.0, 18.1, 18.2 \((\text{Si-C(CH}_3)_3), 25.8 \((\text{Si-C(CH}_3)_3), 52.4 \((B, 2-C), 55.1 \((A, 2-C), 62.3, 62.4, 62.6, 63.1 \((\text{CH}_2\text{CH}_2\text{O}), 64.5 \((A, 1-C), 64.9 \((B, 1-C), 72.7 \((A, CH(OH)), 72.9 \((B, CH(OH)), 75.3 \((B, CH(OH)), 75.6 \((A, CH(OH)), 100.9 \((B, 5-C), 101.3 \((A, 5-C).\)

5.3.3.4. \(E\)-and \(Z\)-(3S, 4S)-5,5-Diethoxy-2-(tert-butyldimethylsilyloxy)iminoo-1,3,4-tri(tert-butyldimethylsilyloxy)pentane \((111)\)
The mixture of oximes 104 (71 mg, 0.12 mmol) was dissolved in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (4 cm\textsuperscript{3}) and cooled to 0°C under N\textsubscript{2}. Triethylamine (0.04 cm\textsuperscript{3}, 29 mg, 0.29 mmol) and TBS triflate (0.05 cm\textsuperscript{3}, 58 mg, 0.22 mmol) was added with stirring. After 50 min the mixture was quenched with water (4 cm\textsuperscript{3}), the organic layer separated, and the aqueous layer extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 10 cm\textsuperscript{3}). The combined organic extracts were dried (MgSO\textsubscript{4}), concentrated in vacuo, and purified by chromatography on silica [petrol: EtOAc (9:1)], yielding the product as a clear oil: 64 mg (76%); \textsuperscript{1}H (200 MHz; CDCl\textsubscript{3}) 0.01-0.15 (24 H, 12 x s, Si(CH\textsubscript{3})\textsubscript{2}), 0.87-0.94 (36 H, 8 x s, Si-C(CH\textsubscript{3})\textsubscript{3}), 1.16-1.24 (6 H, m, CH\textsubscript{3}CH\textsubscript{2}O), 3.42-3.68 (4 H, m, CH\textsubscript{3}CH\textsubscript{2}O), 4.02 (1 H, m, 4-H), 4.39 (2 H, m, 1-H), 4.41 (1 H, m, 5-H), 5.30 (1 H, d, J 2.0, 5-H). The double bond isomers were not clearly resolved by \textsuperscript{1}H NMR.

5.3.3.5. (2R, 3R, 4R)-and (2S, 3R, 4R)-3,4-Di(tert-butyldimethylsilyloxy)-2-tert-butyldimethylsilyloxymethyl-5-pyrroline (107 and 108)

A solution of amines 105 and 106 (36 mg, 63 \textmu mol) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (4 cm\textsuperscript{3}) under N\textsubscript{2} was treated with iodotrimethylsilane (0.10 cm\textsuperscript{3}, 140 mg, 0.70 mmol). After stirring for 1 h at RT the mixture was quenched by addition of saturated aq. NaHCO\textsubscript{3} (4 cm\textsuperscript{3}). Once fuming had ceased, the organic layer was separated and the aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 10 cm\textsuperscript{3}). The combined organic extracts were dried (MgSO\textsubscript{4}), concentrated in vacuo, and purified by chromatography on silica [petrol: EtOAc (17:3)], enabling separation of the diastereomers.

Silylated nectrisine (107): 18 mg (60%); R\textsubscript{f} 0.44 [petrol: EtOAc (17:3), resorcinol]; \textsuperscript{1}H (250 MHz; CDCl\textsubscript{3}) 0.04-0.14 (18 H, 6 x s, Si(CH\textsubscript{3})\textsubscript{2}), 0.88 (27 H, br s, Si-C(CH\textsubscript{3})\textsubscript{2}), 3.61 (1 H, dd, J 7.0, 11.0, CH\textsubscript{3}); 3.79 (1 H, dd, J 4.0, 7.0, CH\textsubscript{3}), 3.83 (1 H, m, 2-H), 4.12 (1 H, dd, J 3.0, 6.0, 3-H), 4.50 (1 H, apparent doublet, J\textsubscript{app} 3.0, 4-H), 7.49 (1 H, d, J 2.0, 5-H).
108: 11 mg (37%); Rf 0.35 [petrol: EtOAc (17:3), resorcinol]; δH (250 MHz; CDCl3) 0.01-0.09 (18 H, 6 x s, Si(CH3)2), 0.87 (27 H, br s, Si-C(CH3)2), 3.78 (1 H, dd, J 3.0, 10.0, CH2), 3.89 (1 H, J 3.0, 13.0, CH2), 3.95 (1 H, m, 2-H), 4.13 (1 H, m, 3-H), 4.76 (1 H, d, J 6.0, 4-H), 7.57 (1 H, br s, 5-H).

5.3.3.6. (2R, 3R, 4R)-3,4-Dihydroxy-2-hydroxymethyl-5-pyrroline (Nectrisine) (72)

a) using AcOH/H2O/THF. Pyrroline 107 (18 mg, 38 μmol) was dissolved in a 3:1:1 mixture of AcOH/H2O/THF (5 cm3), and the mixture stirred at RT for 170 h. After 48 h all starting material was consumed; TLC analysis [EtOAc (neat), resorcinol] showed a new product at Rf = 0.59. Thereafter the course of reaction was more difficult to follow. After 7 days an intense spot close to baseline was observed [EtOAc (neat)], so the mixture was treated with silica, evaporated to dryness in vacuo, and the resulting powder dry loaded onto a silica column and purified by chromatography [EtOAc (neat)]. A small quantity (2 mg) of a sticky, off-white solid was isolated, but insufficient material was available for characterisation.

b) using TFA. Pyrroline 107 (12 mg, 25 μmol) was dissolved in a 9:1 mixture of trifluoroacetic acid/water (5 cm3), and stirred at RT for 18 h. TLC analysis of the mixture after this time [EtOAc (neat), resorcinol] showed material close to the solvent front and an intense baseline spot. The mixture was duly evaporated to dryness in vacuo to yield a red-brown, waxy solid (5 mg). Treatment of this solid with D2O caused a white, amorphous solid to settle out; 1H NMR analysis of the supernatant (see Section 4.1.2.3) revealed signals not inconsistent with the presence of nectrisine (72) but insufficient material was available for full characterisation.
5.3.3.7. (3S, 4R)-5-Benzyloxy-1,3,4-tri(tert-butyldimethylsilyloxy)pentan-2-one (112)

5-O-benzyl-D-xylulose 82 (1.29 g, 5.39 mmol) was dissolved in anhydrous CH₂Cl₂ (50 cm³) under argon, and cooled to 0°C. To the stirred solution was added triethylamine (3.4 cm³, 2.5 g, 25 mmol) and TBS triflate (5.6 cm³, 6.5 g, 24 mmol). After 30 minutes the reaction was quenched by addition of saturated aq. NH₄Cl (50 cm³). The organic layer was separated, and the aqueous layer extracted with CH₂Cl₂ (2 x 40 cm³). The combined organic extracts were dried (MgSO₄), concentrated in vacuo, and purified by chromatography on silica [CHCl₃ (neat)] to give the title compound as a colourless oil: 2.62 g (83%); [α]²³ -5.6° (c 0.98, CHCl₃); Rf 0.61 [CH₂Cl₂ (neat), KMnO₄]; νmax (thin film)/cm⁻¹ 2951s, 2889s, 2855s (CH stretch), 1737s (C=O), 1470s, 1255vs (Si-C); δH (250 MHz; CDCl₃) 0.01-0.08 (18 H, 6 x s, CH₃Si), 0.87-0.94 (27 H, 3 x s, (CH₃)₃C-Si), 3.30 (1 H, dd, J 9.0, 6.0, 5-H), 3.55 (1 H, dd, J 9.0, 6.0, 5-H), 4.10 (1 H, m, 4-H), 4.30 (1 H, d, J 3.0, 3-H), 4.43 (1 H, 0.5ABq, J 12.0, 1-H), 4.50 (1 H, 0.5ABq, J 12.0, 1-H), 4.56 (2H, m, PhCH₂), 7.32 (5 H, m, Ph); δC (69.2 MHz; CDCl₃) -5.6, -5.4, -5.3, -5.1, -4.9, -4.7 (Si(CH₃)$_3$), 17.8, 17.9, 18.3 (Si-C(CH₃)$_3$), 25.6, 25.7 (Si-C(CH₃)$_3$), 68.9 (C-1), 70.0 (CH₂), 72.9 (CH₂), 73.4 (CH), 77.5 (CH), 127.4, 127.5, 128.1 (aromatic CH), 137.9 (aromatic quaternary), 209.1 (C=O); m/z (CI) 600 [MNH₄⁺], 583 [MH⁺], 492, 468, 451, 393, 343, 319; (Found: m/z (CI) [MH⁺] 583.367. C₃₀H₅₈O₅Si₃ requires m/z (CI) [MH⁺] 583.367).

5.3.3.8. E-and Z-(3S, 4R)-5-Benzyloxy-2-hydroxyimino-1,3,4-tri(tert-butyldimethylsilyloxy)pentane (113)

To a solution of compound 112 (2.97 g, 5.10 mmol) in dry methanol (60 cm³) was added hydroxylamine hydrochloride (1.63 g, 23.5 mmol) and KHCO₃ (2.51 g,
2.51 mmol). The mixture was heated at reflux for 1 h, and cooled; silica was added and the mixture evaporated to dryness in vacuo. The resulting powder was dry loaded onto a silica column and purified by chromatography [petrol: EtOAc (9:1)] to yield compound 113 as a mixture of (E)- and (Z)-oximes in a 2:1 (A:B) ratio: 2.16 g (71%); $\nu_{\text{max}}$ (thin film)/cm$^{-1}$ 3267br (OH), 2955vs, 2929vs, 2858vs (CH), 1470s, 1254vs (Si-C); $\delta_{\text{H}}$ (250 MHz; CDCl$_3$) 0.01-0.07 (18 H, 6 x s, CH$_3$Si), 0.86-0.92 (27 H, 3 x s, (CH$_3$)C-Si), 3.29-3.41 (1 H, m, 5-H), 3.54-3.63 (1 H, m, 5-H), 4.27 (1 H, m, 5-H), 4.38-4.77 (4 H, m, 1-H and PhCH$_2$), 5.12 (1 H, d, $J$ 3.0, 3-H), 7.32 (5 H, m, Ph), 10.00 (B, 0.33 H, br s, OH), 10.06 (A, 0.67 H, br s, OH); $\delta_{\text{C}}$ (62.9 MHz; CDCl$_3$) -5.7, -5.6, -5.5, -5.3, -5.2, -5.1, -4.8, -4.7, -4.6, -4.5 ((CH$_3$)$_3$Si), 17.9, 18.0, 18.2 ((CH$_3$)$_3$C-Si), 25.7, 25.8, 25.9 ((CH$_3$)$_3$C-Si), 56.7 (B, C-1), 62.2 (A, C-1), 68.7 (A, CH), 70.5 (A, CH$_2$), 71.6 (A, CH), 71.8 (B, CH$_2$), 72.6 (B, CH), 72.8 (A, CH$_2$), 73.1 (B, CH$_2$), 73.9 (B, CH), 127.2, 127.5, 128.1 (aromatic CH), 138.2 (aromatic quarternary), 157.7 (B, C=N), 158.2 (A, C=N); m/z (CI) 598 [MH$^+$], 582, 453, 411, 132, 108, 91; (Found: m/z (CI) [MH$^+$] 598.378; C$_{30}$H$_{59}$NO$_5$Si$_3$ requires m/z (CI) [MH$^+$] 598.378).

5.3.3.9. E- and Z-(3S, 4R)-5-Benzylxy-1,3,4-tri(tert-butyldimethylsilyloxy)-2-(tert-butyldimethylsilyloxy)iminopentane (115)

The mixture of oximes 113 (2.09 g, 3.50 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (50 cm$^3$) and cooled to 0°C under Ar. Triethylamine (0.88 cm$^3$, 640 mg, 6.3 mmol) and TBS triflate (1.20 cm$^3$, 1.38 g, 5.23 mmol) were added with stirring. After 25 min the mixture was quenched with saturated aq. NH$_4$Cl (50 cm$^3$), the organic layer separated, and the aqueous layer extracted with CH$_2$Cl$_2$ (2 x 50 cm$^3$). The combined organic extracts were dried (MgSO$_4$), concentrated in vacuo, and purified by chromatography on silica [petrol: EtOAc (9:1)], yielding oxime ethers 115 as a mixture of (E)-and (Z)-double bond isomers in a 2:1 (A:B) ratio as a clear oil: 2.44 g (98%); $\nu_{\text{max}}$ (thin film)/cm$^{-1}$ 2955s, 2930s, 2854s (CH), 1469s, 1253vs (Si-C); $\delta_{\text{H}}$ (250 MHz; CDCl$_3$) 0.01-0.11 (24 H, 8 x s, CH$_3$Si), 0.86-0.91 (36 H, 8 x s, (CH$_3$)$_3$C-Si), 3.27 (A, 0.67 H, dd, $J$ 6.0, 9.5, 5-H), 3.35 (B, 0.33 H, dd, $J$ 6.0, 9.5, 9.5, 172
5-H), 3.49 (A, 0.67 H, dd, J 5.5, 9.5, 5-H), 3.56 (0.33 H, dd, J 4.0, 9.5, 5-H), 4.18 (1 H, m, 4-H ), 4.43-4.72 (4 H, multiplets, 1-H and PhCH₃), 5.16 (1 H, d, J 5.0, 3-H), 7.30 (5 H, m, Ph); δₓ (69.2 MHz; CDCl₃) -5.7, -5.5, -5.4, -5.3, -5.2, -5.1, -5.0, -4.7, -4.6, -4.4 ((CH₃)₂Si), 17.8, 17.9, 18.1, 18.2 ((CH₃)₃C-Si), 25.5, 25.7, 25.9, 26.0 ((CH₃)₂C-Si), 55.6 (B, 1-C), 61.2 (A, 1-C), 67.9 (A, CH), 71.3 (A, CH₂), 72.4 (B, CH₂), 72.6 (B, CH), 72.7 (A, CH₂), 72.8 (A, CH), 73.1 (B, CH₂), 74.2 (B, CH), 127.0, 127.5, 127.9 (aromatic CH), 138.3, 138.4 (aromatic quarternary), 162.2 (B, C=N), 163.5 (A, C=N); m/z (Cl) 712 [MH⁺], 453, 132, 108, 106, 91; (Found: m/z (Cl) [MH⁺] 712.464. C₃₆H₇₁NO₅Si₄ requires m/z (Cl) [MH⁺] 712.464).

5.3.3.10. E-and Z-(2R, 3S)-2,3,5-Tri(tert-butyldimethylsilyloxy)-
4-(tert-butyldimethylsilyloxy)iminopentanol (116)

Compound 115 (2.44 g, 3.43 mmol) was dissolved in THF (50 cm³). Palladium on activated charcoal (BDH, 10% grade; 1.02 g) was added, and the reaction vessel repeatedly evacuated and flushed with H₂. The mixture was stirred at RT for 16 h under H₂, filtered through Celite® (using copious EtOAc to wash the residue), and the filtrate evaporated in vacuo to yield the crude alcohol 116 as a pale yellow waxy solid, as an approximately 1:1 (A:B) mixture of double bond isomers: 1.98 g (93%); δₓ (200 MHz; CDCl₃) 0.02-0.38 (24 H, 11 x s, CH₃Si), 0.87-0.96 (36 H, 9 x s, (CH₃)₃C-Si), 3.57 (2 H, m, 1-H), 4.05 (1 H, m, 2-H), 4.09 (A, 0.25 H, 0.5ABq, J 10.0, 5-H), 4.13 (B, 0.25 H, 0.5ABq, J 8.0, 5-H), 4.55 (B, 0.25 H, 0.5ABq, J 8.0, 5-H), 4.62 (A, 0.25 H, 0.5ABq, J10.0, 5-H), 4.83 (B, 0.5 H, d, J 12.0, 3-H), 5.22 (A, 0.5 H, d, J 8.0, 3-H); δₓ (62.9 MHz; CDCl₃) -5.7, -5.6, -5.5, -5.4, -4.9, -4.8, -4.7, -4.3, -4.0, -3.7, -3.1 ((CH₃)₂Si), 18.0, 18.2 ((CH₃)₃C-Si), 25.2, 25.5, 25.6, 25.7, 25.8, 26.0 ((CH₃)₂C-Si), 54.3 (B, 5-C), 61.3 (A, 5-C), 63.6 (B, 1-C), 63.9 (A, 1-C), 68.5 (CH), 74.4 (CH), 74.5 (CH), 74.7 (CH), 161.9 (B, C=N), 162.7 (A, C=N); m/z (EI) 621 [M⁺], 564, 546, 506, 476, 447, 432, 402; (Found: m/z (EI) [M⁺] 621.40972. C₂₉H₆₇NO₅Si₄ requires m/z (EI) [M⁺] 621.40964).
5.3.3.11. \textit{E-} and \textit{Z-}(2S, 3S)-2, 3,5-
\textit{Tri(tert-butylimethylsilyloxy)-4-(tert-butylimethylsilyloxy)}iminopentanal (119)

\[
\begin{align*}
\text{OTBS} & \quad \text{TBSO} \\
1 & \quad 2 \\
3 & \quad N \quad 4 \\
5 & \quad \text{OTBS} \\
O & \quad \text{OTBS}
\end{align*}
\]

\textbf{a) by Swern oxidation.} A solution of dry dimethylsulfoxide (0.25 cm\(^3\), 280 mg, 3.5 mmol) in anhydrous CH\(_2\)Cl\(_2\) (20 cm\(^3\)) was cooled to -60\(^\circ\)C under N\(_2\) in an acetone-Dry Ice Bath. Oxalyl chloride (2.0 M solution in CH\(_2\)Cl\(_2\); 0.90 cm\(^3\), 1.8 mmol) was added, and the mixture stirred for 10 min before addition of a solution of alcohol 116 (1.02 g, 1.64 mmol) in anhydrous CH\(_2\)Cl\(_2\) (10 cm\(^3\)). Stirring was maintained at -60\(^\circ\)C for 15 min, then triethylamine (1.10 cm\(^3\), 800 mg, 7.9 mmol) was added. The mixture was stirred for a further 15 min at -60\(^\circ\)C, then quenched with water (10 cm\(^3\)), allowed to warm to RT, and the organic layer separated. The aqueous layer was extracted with CH\(_2\)Cl\(_2\) (3 x 30 cm\(^3\)), and the combined organic extracts dried (MgSO\(_4\)), concentrated \textit{in vacuo}, and purified by chromatography on silica [petrol: EtOAc (19:1)] yielding aldehyde 119 as an off-white, semicrystalline solid, and an as yet unidentified byproduct as a clear oil:

\textit{Aldehyde 119}: 609 mg (60%); \(\delta_h\) (200 MHz; CDC\(_3\)) 0.02-0.13 (24 H, 6 x s, CH\(_3\)Si), 0.86-0.90 (36 H, 4 x s, (CH\(_3\))\(_3\)C-Si), 4.20 (1 H, 0.5ABq, \(J\ 12.5\), 5-H), 4.37 (1 H, d, \(J\ 6.0\), 2-H), 4.58 (1 H, 0.5ABq, \(J\ 12.5\), 5-H), 5.30 (1 H, d, \(J\ 6.0\), 3-H), 9.47 (1 H, s, CHO); \(m/z\) (El) 619 [M\(^+\)], 604, 591, 562, 533, 502, 446; (Found: \(m/z\) (El) [M\(^+\)] 619.39625. C\(_{29}\)H\(_{65}\)NO\(_5\)Si\(_4\) requires 619.39399).

\textbf{b) by oxidation using NaOCl and TEMPO.} Alcohol 116 (401 mg, 0.646 mmol) and TEMPO (catalytic) was dissolved in a 1:1 mixture of EtOAc/toluene (12 cm\(^3\)). Benzyl trimethyl ammonium chloride (44 mg, 0.237 mmol), NaBr (37 mg, 0.359 mmol) and saturated aq. NaHCO\(_3\) (6 cm\(^3\)) were added, and the mixture cooled to 0\(^\circ\)C. Oxidation was effected by slow dropwise addition over 45 min of a mixture of 1.1 M NaOCl/saturated aq. NaHCO\(_3\)/brine (1:1:2) at a rate just sufficient to maintain an intense yellow colour in the mixture. The mixture was allowed to stir for a further 30 min; thereafter the organic layer was separated, the aqueous layer extracted with EtOAc (3 x 10 cm\(^3\)), and the combined organic extracts washed with saturated aq. NaHCO\(_3\) (20 cm\(^3\)) and brine (20 cm\(^3\)), dried (MgSO\(_4\)), and purified by chromatography [petrol: EtOAc (19:1)]. The aldehyde 119 was isolated as a yellow semi-solid: 245 mg (61%); spectroscopic data in agreement with that reported above.
5.3.3.12. (4S, 5S)-6-Ethoxy-4,5-di(tert-butyldimethylsilyloxy)-3-(tert-butyldimethylsilyloxy)methyl-5,6-dihydro-4H-1,2-oxazine (120)

To a solution of aldehyde 119 (436 mg, 0.704 mmol) in triethyl orthoformate (8 cm³) was added p-toluenesulfonic acid (22 mg, 0.13 mmol). The mixture was stirred for 40 min, and quenched with pyridine (5 drops from a Pasteur pipette). Silica was added, and the mixture evaporated to dryness in vacuo, using repeated additions of ethanol to form an azeotropic mixture with (EtO)₂CH; the resulting powder was dry loaded onto a silica column and purified by chromatography [petrol: EtOAc (17:3)]. The oxazine 120 was isolated as a clear oil: 125 mg (33%); [α]²⁰ D -26.6° (c 1.1, CHCl₃); Rₚ 0.24 [petrol: EtOAc (9:1), resorcinol]; ν max (thin film)/cm⁻¹ 2955vs, 2932vs, 2859vs (CH), 1585s (C=N); δH (300 MHz; CDCl₃) 0.02-0.09 (18 H, 6 x s, CH₃Si), 0.77-0.88 (27 H, 3 x s, (CH₃)$_₃$C-Si), 1.17 (3 H, t, J 5.5, CH₂), 3.80 (1 H, m, MeCHH), 3.95 (1 H, dd, J 2.0, 3.0, 5-H), 4.18 (1 H, 0.5ABq, J 14.0, CHHOTBS), 4.42 (1 H, m, MeCHH), 4.62 (1 H, d, J 2.0, 4-H), 4.70 (1 H, d, J 3.0, 6-H), 4.82 (1 H, 0.5ABq, J 14.0, CHHOTBS); δC (69.2 MHz; CDCl₃) -5.8, -5.7, -5.3, -5.2, -4.9, -4.8 (CH₃Si), 14.9 (CH₂CH₂), 17.5, 17.6, 17.9 ((CH₃)$_₃$C-Si), 25.4, 25.5 ((CH₂)$_₂$C-Si), 53.8 (CH₂), 68.3 (CH₂), 76.1 (CH), 79.5 (CH), 104.5 (6-C), 146.2 (C=N); m/z (CI) 534 [MH⁺], 518, 492, 474, 388, 343, 333; (Found: m/z (CI) [MH⁺] 534.347. C$_{25}$H$_{55}$NO$_5$Si$_3$ requires m/z (CI) [MH⁺] 534.347).

5.3.3.13. (2R, 3R, 4R)-1-Hydroxy-3,4-di(tert-butyldimethylsilyloxy)-2-tert-butyldimethylsilyloxyethylpyrrolidine (126)

Oxazine 120 (145 mg, 0.272 mmol) was dissolved in glacial acetic acid (4 cm³). Sodium cyanoborohydride was added in five equal (34 mg, 0.576) portions over a 10 min period, allowing effervescence to cease before addition of each
subsequent portion. The mixture was stirred at RT for a further 1 h, quenched by cautious addition of saturated aq. NaHCO₃ (30 cm³) and extracted with EtOAc (3 x 25 cm³). The combined organic layers were dried (MgSO₄), treated with silica, and evaporated to dryness in vacuo. The resulting powder was dry loaded onto a silica column, and the product purified by chromatography [petrol: EtOAc (19:1)].

N-hydroxypyrrolidine 126 was isolated as a translucent crystalline solid: 50 mg (37%); [α]D²⁰ -17.6° (c 0.96, CHCl₃); Rₚ 0.19 [petrol: EtOAc (9:1), resorcinol]; δH (360 MHz; CDCl₃) 0.04-0.13 (18 H, 6 x s, CH₃Si), 0.81-0.96 (27 H, 3 x s, (CH₃)₃Si), 1.76 (1 H, s, 1-H), 2.94 (1 H, dt, J 5.5, 6.0, 2-H), 3.19 (2 H, m, 5-H), 3.76 (2 H, d, J 6.0, CH₂OTBS), 3.88 (1 H, dd, J 2.0, 5.5, 3-H), 4.07 (1 H, m, 4-H); δC (69.2 MHz; CDCl₃) -5.50, -5.48, -4.9, -4.7, -4.6 ((CH₃)₂Si), 17.7, 17.8, 18.2 ((CH₃)₃Si), 25.6, 25.7, 25.9 ((CH₃)₃C-Si), 63.1 (CH₂OTBS), 64.0 (5-C), 76.7 (2-C), 77.5 (4-C), 79.3 (3-C); m/z (CI) 492 [MH⁺], 476, 371, 344, 330; (Found: m/z (CI) [MH⁺] 492.3361. C₂₃H₅₃NO₄Si₃ requires m/z (CI) [MH⁺] 492.3361).

5.3.3.14. (2R, 3R, 4R)-1,3,4-Trihydroxy-2-hydroxymethylpyrrolidine (127)

N-Hydroxypyrrolidine 126 (51 mg, 0.104 mmol) was dissolved in a 1:1 mixture of acetonitrile/THF (2 cm³). Hydrofluoric acid solution (40% in H₂O; 0.300 cm³) was added, and the mixture stirred at RT for 5.5 h, with further additions of HF (0.300 cm³) after 2 h and 4.25 h. Thereafter the mixture was basified by cautious addition of solid NaHCO₃ (2.00 g), filtered, and the filtrate evaporated in vacuo to yield a white gum. This gum was washed with EtOH (40 cm³), and the filtrate evaporated to dryness to yield a white solid. Treatment with water, followed by filtration through a cotton wool plug, yielded a solution of N-hydroxyprpyrrolidine 127 in quantitative yield: δH (200 MHz; D₂O) 2.69 (1 H, dt, J 6.5, 11.0, 2-H), 3.08 (2 H, d, J 7.5, 5-H), 3.66 (2 H, d, J 6.5, CH₂OH), 3.75 (1 H, dd, J 5.5, 11.0, 3-H), 4.05 (1 H, m, 4-H); δC (150.8 MHz; D₂O) 59.6, 63.0, 74.7, 75.1, 77.4; m/z (CI) 150 [MH⁺], 98, 82; (Found: m/z (CI) [MH⁺] 150.0766. C₇H₁₁NO₄ requires m/z (CI) [MH⁺] 150.0766).
5.4. Analytical Procedures

5.4.1. Enzyme Assays

5.4.1.1. Assay for hydroxypyruvate

The following reagents were added to a 1.5 cm$^3$ spectroscopic cuvette: sample containing hydroxypyruvate (0.03 cm$^3$), 1 M HCl solution (to denature any enzymes present) (0.02 cm$^3$), water (0.62 cm$^3$), 1 M NaOH solution (to neutralise) (0.02 cm$^3$), Tris buffer (0.1 M, pH 7.6) (0.75 cm$^3$), NADH disodium salt (to final concentration of 0.3 mM), and glycerate dehydrogenase (Gly-DH) (to final concentration 0.03 U cm$^{-3}$). UV absorbance at 340 nm was measured immediately before, and 15-20 min after, addition of Gly-DH, and the change in absorbance ($\Delta A$) recorded. A series of standard hydroxypyruvate solutions (final cuvette concentrations 0 to 0.15 mM) were used to deduce calibration equations relating $\Delta A$ and hydroxypyruvate concentration.

5.4.1.2. Substrate specificity determinations using the hydroxypyruvate assay

In a 1 cm$^3$ Eppendorf tube, reaction mixtures were prepared containing the following components (expressed in their final concentrations): TPP chloride (0.2 mM), MgCl$_2$ (0.9 mM), lithium hydroxypyruvate (7.5 mM), glycylglycine buffer (70 mM, pH 7.6), the substrate aldehyde, and transketolase (= 3 U cm$^{-3}$), addition of which initiated the reaction. Controls were also set up without enzyme. At regular time intervals after the addition of TK, a 0.03 cm$^3$ aliquot was taken out of the reaction mixture and of each control, and each was assayed for hydroxypyruvate as in Section 5.3.1 (hydroxypyruvate concentrations being deduced from $\Delta A$ values using the calibration equations). Substrate specificities for each novel aldehyde were assessed using a rate parameter $v_{rel}$, expressing the initial rate of hydroxypyruvate depletion as a percentage of the initial depletion rate in a reaction mixture containing the same concentration of glycolaldehyde under the same conditions.

5.4.1.3. Transketolase activity assays

The following reagents, expressed in their final concentrations, were added to a 1.5 cm$^3$ spectroscopic cuvette: TPP chloride (0.25 mM), NADH disodium salt (0.154 mM), magnesium chloride (9 mM), $\alpha$-glycerophosphate dehydrogenase/triosephosphate isomerase (0.2 U cm$^{-3}$), phosphoriboisomerase (0.2 U cm$^{-3}$),
D-ribulose-5-phosphate-3-epimerase (0.2 U cm\(^{-3}\)), glycylglycine buffer (70 mM, pH 7.6), transketolase (0.01 - 0.05 U), and D-ribose-5-phosphate (3 mM). After allowing 1 min for mixing of all components after addition of ribose-5-phosphate, the decrease in UV absorbance at 340 nm was monitored over a 10 min period. The slope of the linear portion of the resulting graph was used to determine transketolase activity at the temperature of measurement.

5.4.2. **Kinetic Resolution of (±)-Glyceraldehyde Diethyl Acetal (76)**

The following mixtures were prepared in nine separate vials: toluene (10 cm\(^3\)); diol (±)-76 (100 mg, 0.61 mmol); vinyl butyrate (100 mg, 0.88 mmol); decane (50 mg, 0.35 mmol). To each vial was added a catalytic quantity of enzyme as follows:

1. Novozyme®
2. Lipozyme® (immobilised *Mucor miehei* lipase)
3. Porcine pancreatic lipase (PPL)
4. *Candida rugosa* lipase (CRL)
5. Lipase OF®
6. \(\alpha\)-Chymotrypsin
7. *Pseudomonas fluorescens* lipase (PFL)
8. Alcalase®
9. Control: no enzyme added.

The mixtures were incubated at 30°C for 21 h, and analysed at regular intervals by TLC and GCMS (to determine the percentage conversion of starting material \(c\), by reference to the standard signal (decane) in the GCMS trace). For those samples which showed a significant consumption of (±)-76, analysis by chiral GC was undertaken to determine the enantiomeric excesses of 76 and hence the enantiomeric ratio (E) values for the reaction using the expression:

\[
E = \frac{\ln\{(1 - \text{e.e.})(1 - c)\}}{\ln\{(1 + \text{e.e.})(1 - c)\}}
\]  
(ref. 186)

Results were as follows:

1. Substrate entirely consumed (no resolution);
2. Substrate entirely consumed (no resolution);
3. No reaction;
4. \(E = 1.73\);
5. No reaction;
6. Slow reaction, no e.e. observed;
7) $E = 1.34$;
8) No e.e. observed.
Thus a successful kinetic resolution of (±)-glyceraldehyde diethyl acetal $76$ was not found.
Appendices and References
Appendix I

\[^1\text{H} - ^1\text{H} \text{ correlation (COSY) spectrum of } N\text{-hydroxypyrrolidine} \] 126
Appendix II

$^1$H-$^{13}$C correlation (HMOC) spectrum of N-hydroxypyrrolidine 126
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