Development of Synthetic Routes to Intermediates in the Biotin Biosynthetic Pathway

Mhairi Ishbel Brunton

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Department of Chemistry
University of Edinburgh
August 2003
Declaration

This thesis is submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Edinburgh. Unless otherwise stated, the work described is original and has not been submitted in whole or in part, for any degree at this or any other university.

Mhairi I. Brunton
August 2003
Abstract

The vitamin biotin plays an important role as a cofactor for carboxylation enzymes involved in a variety of metabolic processes. It is known that biotin biosynthesis in micro-organisms and plants occurs via a similar biosynthetic pathway, while mammalian biotin is produced by gut micro-flora. The initial steps in the biotin biosynthetic pathway are therefore important targets for the development of potential herbicides.

The requirement for this work was the development of a short and versatile route to α-aminoketones as potential substrates and inhibitors of the enzyme 7,8-diaminononanoate synthase (DANS). This is the second enzyme in biotin biosynthetic pathway, and uses the α-aminoketone 8-amino-7-oxononanoate (AON) as its substrate. α-Aminoketones are important intermediates in a number of other important biosynthetic pathways, and as a result a number of synthetic strategies have previously been developed. Here we describe the development of two routes to AON using the Dakin-West reaction and the nitroaldol reaction.

Condensation of an acid chloride with an oxazolone gives rise to the O-acyloxyoxazole which can be isomerised to its more thermodynamically stable C-acyl oxazolone isomer. Ring opening and deprotection of this species gives the required α-aminoketone via the dakin-West reaction. Initial nitroaldol condensation of a nitroalkane and an aldehyde affords the required nitroalcohol, which can be easily oxidised to the nitroketone. Hydrogenation affords the α-aminoketone as its hydrochloride salt.

The nitroaldol reaction is advantageous as it is not only short but allows variation in both chain length and substitution, thus providing an accessible route to a variety of α-aminoketone derivatives in good yield.
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Spencer, thank you for challenging me, and encouraging me to push myself harder in everything I do. Your continuing support and your belief in me when I was down have been invaluable. I only hope that I can give as much to you.
Abbreviations

μM micro molar
δC 13C NMR chemical shift
δH 1H NMR chemical shift
ACP Acyl carrier protein
ADP adenosine 5’-diphosphate
ALAS 5-aminolaevulinic acid synthase
AMP adenosine monophosphate
AON 8-amino-7-oxo nonanoate
AONS 8-amino-7-oxo nonanoate synthase
aqHCl Aqueous HCl
Ar aromatic
ATP adenosine 5’-triphosphate
B.sphaericus Bacillus sphaericus
BCCP biotin carboxyl carrier protein
bio biotin
bp Boiling point
bpxx Boiling point at xx millimetres mercury
br. broad
c.HCl Concentrated HCl
CCL Candida cylinracea
CoA Coenzyme A
d doublet
Da Dalton
DAN 7,8-diamino nonanoate
DANS diaminononanoate synthetase
DCM dichloromethane
DIPE diisopropyl ether
DMAP 4-N,N-dimethylamino pyridine
DNA deoxyribonucleic acid
DNP Dinitrophenyl hydrazine
DTB dethiobiotin
DTBS dethiobiotin synthetase
DTT dithiothreitol
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>ee</td>
<td>Enantiomeric excess</td>
</tr>
<tr>
<td>enz</td>
<td>enzyme</td>
</tr>
<tr>
<td>EPR</td>
<td>Electron pair resonance</td>
</tr>
<tr>
<td>Et₂O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>Et₃N</td>
<td>Triethyl amine</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>HSCoA</td>
<td>Coenzyme A (reduced form)</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>KBL</td>
<td>2-amino-3-ketobutyrate CoA ligase</td>
</tr>
<tr>
<td>k&lt;sub&gt;chem&lt;/sub&gt;</td>
<td>rate of non-enzymatic ring opening</td>
</tr>
<tr>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
<td>dissociation rate constant</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo Daltons</td>
</tr>
<tr>
<td>k&lt;sub&gt;enz&lt;/sub&gt;</td>
<td>the rate of enzyme reaction</td>
</tr>
<tr>
<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Inhibition rate constant</td>
</tr>
<tr>
<td>k&lt;sub&gt;rac&lt;/sub&gt;</td>
<td>rate of racemisation</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>mol</td>
<td>moles</td>
</tr>
<tr>
<td>mmol</td>
<td>milli molar</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>NADP</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate (reduced form)</td>
</tr>
<tr>
<td>nmr</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>°C</td>
<td>Degree celcius</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>PDC</td>
<td>Pyridinium dichromate</td>
</tr>
<tr>
<td>plc</td>
<td>Preparative layer chromatography</td>
</tr>
<tr>
<td>PLP</td>
<td>pyridoxal-5’-phosphate</td>
</tr>
<tr>
<td>PMP</td>
<td>pyridoxamine phosphate</td>
</tr>
<tr>
<td>PPL</td>
<td>porcine pancreatic lipase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PTC</td>
<td>phase transfer catalysis</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosyl methionine</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecylsulfate polyacrilamide gel electrophoresis</td>
</tr>
<tr>
<td>SPT</td>
<td>serine palmitoyl transferase</td>
</tr>
<tr>
<td>STBA</td>
<td>sodium tri-β-butoxyaluminoalumohydrate</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>tlc</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMG</td>
<td>1,1,3,3-tetramethylguanidine</td>
</tr>
<tr>
<td>v/v</td>
<td>volume /volume</td>
</tr>
</tbody>
</table>
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4.1 Summary

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Appendix 1: Courses Attended
1. Introduction
1.1 An Introduction to Biotin

The early twentieth century saw the discovery of a large number of enzyme cofactors. Amongst these was biotin, a water soluble cofactor discovered from three parallel fields of research. In 1901 Wildiers \(^1\) discovered that bios, a material derived from beer wort, stimulated the growth of yeasts. It was later discovered that his “bios” was a mixture of biotin, alanine and pantothenic acid \(^2\). In the 1930s Burk described coenzyme R \(^3\), a compound necessary for the growth of *Rhizobium*, a nitrogen fixing bacteria, which was later discovered to be identical to biotin \(^4,5\). At the same time three groups were conducting research into a substance protecting animals from the fatal effects of ingesting raw egg whites. Boas called this “protective factor x”, while Györky used “vitamin H” and Williams “the protective factor against egg white injury”, it has since been realised that all of these factors were also identical to biotin \(^6\). It is now known that biotin is an essential vitamin for all mammals, and is produced in minute quantities by plants, bacteria and yeasts. It is thought that concentrations in bacteria are never more than nanomolar \(^7\), whilst in plant mesophyll cells the biotin concentration can reach 11 \(\mu\)M \(^8\).

1.1.1 Structure of Biotin

Biotin (Figure 1.1) was first isolated by as its methyl ester by Kögl and Tönnis in 1935 \(^9\), its structure was determined by du Vigneaud in 1942 \(^10\) and was confirmed by the first total synthesis by Harris in 1944 \(^11\). X-ray studies have shown biotin to have an imidazolidone ring \(cis\)-fused to a tetrahydrothiophene ring with a valeric acid side chain at the C-2 position. Biotin contains three asymmetric carbons and therefore has eight stereochemical forms, although only one form, (+)-biotin (1, Figure 1.1), is biologically active.
1.1.2 Biotin as a Cofactor

It was suggested that biotin may play a role in carbon dioxide fixation as early as 1943\textsuperscript{12}, and experimental evidence was found to support this in 1953\textsuperscript{13}. A study by Wakil\textsuperscript{14} showed biotin to be an essential component of the acetyl CoA carboxylase enzyme and the central role of biotin in carboxylation reactions was revealed. To act as a mobile carrier of activated carbon dioxide biotin must be bound to the biotin carboxyl carrier protein (BCCP, Figure 1.2). BCCP is a small (22kDa) monomeric protein that covalently links biotin at the ε-amino group of a lysine residue. The flexible chain linking biotin to the BCCP enables the biotin group to swing between the two catalytic domains of the carboxylase enzyme, facilitating the transfer of the carboxyl group between the donor and acceptor sites. Biotin acts as a carboxyl carrier for three distinct categories of enzymes, as outlined below.

1.1.2.1 Class I: Carboxylase Enzymes

\[
\begin{align*}
\text{Enz-biotin + ATP + HCO}_3^- & \xrightleftharpoons{\text{Mg}^{2+}} \text{Enz-biotin-\text{CO}_2^- + ADP + PO}_4^{3-} + H^+ \\
\text{Enz-biotin-\text{CO}_2^- + R-H} & \xrightleftharpoons{} \text{Enz-biotin + R-\text{CO}_2^-}
\end{align*}
\]

Scheme 1.1: Partial reactions for Class I biotin dependant carboxylases

In class I carboxylase enzymes biotin mediates the transfer of a carboxylate group from hydrogen carbonate to acceptor molecules such as pyruvate, propionyl CoA, acetyl CoA, β-methylcrotonyl CoA, geranyl CoA and urea. The reaction takes place in two reversible steps as shown in Scheme 1.1. The initial step in the sequence is the adenosine 5'-triphosphate (ATP) dependant formation of the carboxy-biotin intermediate. Formation of the carboxy-biotin complex activates the carboxyl group allowing the transfer of CO\textsubscript{2} without additional energy input.
1.1.2.2 Class II: Decarboxylase Enzymes

Class II decarboxylase enzymes regulate the transport of sodium ions in anaerobes, and are linked to the decarboxylation of β-keto acids and their thioesters, such as oxaloacetate, methylmalonyl CoA and glutaconyl CoA. In this case ATP is not required and “active” CO₂ is transferred directly from the keto acid to the biotin containing enzyme (see Scheme 1.2).

1.1.2.3 Class III: Transcarboxylase Enzymes

The only known class III transcarboxylase enzyme is methylmalonyl CoA: pyruvate carboxytransferase, which mediates the formation of methylmalonyl CoA from oxaloacetate in Propionibacterium shermanii. As in the case of class II enzymes this reaction does not require ATP, and the CO₂ moiety is transferred directly from an acyl derivative to the biotin containing enzyme (see Scheme 1.3).

1.1.3 Mechanism of Biotin Carboxylation

The first indication of the mechanism of biotin carboxylation came from work by Lynen in 1959. He found that β-methylcrotonyl CoA carboxylase, in the presence of ATP and Mg²⁺, catalysed the carboxylation of free (+)-biotin. Diazomethane trapping of the enzymatic reaction intermediate recovered the dimethyl ester of 1′-N-carboxy-biotin (3, Figure 1.3), indicating carboxylation at the 1′-N position on biotin. This was confirmed by x-ray analysis of the bis-p-bromoanilide derivative of carboxybiotin by Bonnemere in 1965. It was suggested that since N-carboxybiotin is apparently chemically inert, the active enzymatic species could be O-carboxybiotin, which might rearrange to the more stable N-acyl compound during trapping and isolation. Although attractive, this theory was disproved when it was found that synthetic 1′-N-carboxybiotin was a chemically and kinetically competent substrate for acetyl CoA carboxylase.
Tracer experiments were used to investigate the carboxyl substrate for the carboxylation of biotin. Using $[^{18}\text{O}]$-bicarbonate as the substrate for the propionyl carboxylase reaction Kaziro found that two $^{18}\text{O}$ atoms were incorporated in the carboxyl group of methyl malonyl-CoA, and the third in the phosphate group released from ATP. Although this strongly suggested that the bicarbonate ion was the true substrate it did not rule out dehydration to carbon dioxide in the enzyme active site, however it was also shown that the bicarbonate ion was directly involved in the cleavage of the $\beta-\gamma$ Mg-ATP bond.

There have been a large number of mechanistic pathways proposed for the carboxylation of biotin. However only three are mechanistically attractive, these are shown in Figure 1.4.

**Figure 1.4: Three Proposed Mechanisms for the carboxylation of biotin**
Kaziro proposed that the bicarbonate ion was preactivated by ATP (Pathway A) forming the carboxyphosphate intermediate (4). This could then react directly with biotin to form 1'-N-carboxybiotin (2) as shown in (a), or decompose to enzyme bound CO₂ and carboxylate biotin as shown in (b).

In 1967 Lynen proposed biotin was pre-activated by phosphorylation of the urea oxygen. The resultant O-phosphobiotin (5) could then react with bicarbonate in a concerted six-electron pericyclic reaction to form (2), as shown in Pathway B.

Pathway C was proposed by Kluger in 1979 and also involves the pre-activation of biotin to form O-phosphobiotin (5). In this route (5) is attacked by a bicarbonate ion to form the carboxyphosphate (4) and the imidazolidone anion of biotin (6). Kluger suggested that (4) and (6) react, possibly with pre-formation of CO₂ to form carboxybiotin (2).

It has been demonstrated that enzymatic displacements at phosphorus centres take place with “in-line” geometry, and inversion of stereochemistry. This implies that Pathways A and B, Figure 1.4, would result in inversion of configuration, while Pathway C would have an overall retention of configuration. To find the true mechanism of biotin carboxylation Hansen and Knowles used the [γ-17O, γ-18O]-γ-phosphorothioate of ATP, a chiral ATP analogue, as the substrate for the pyruvate carboxylase enzyme. It was found that the reaction product, [16O, 17O, 18O]-thiophosphate had overall inversion of stereochemistry at the phosphorus centre, thus ruling out Pathway C.

Incubation studies showed that biotin carboxylase could produce ATP from Mg-ADP and carbamoyl phosphate, thus supporting the involvement of a carboxyphosphate (4) in the reaction mechanism, and indicating Pathway A as the most likely reaction mechanism. Despite the initial thought that the imidazolidone nitrogen would not act as a nucleophile, this is now considered to be the most probable pathway.

Despite much research on the mechanism of biotin carboxylation none of the proposed mechanisms have been completely experimentally verified. This is primarily due to problems performing reliable isotope exchange experiments on reversible processes and the failure to trap any of the putative intermediates.
1.1.4 Mechanism of Carboxyl Transfer

In their study on propionyl CoA carboxylase Retey and Lynen\textsuperscript{26} found that carboxylation proceeded with retention of configuration at the carbon accepting the carboxyl group. They also found that proton exchange between the substrate and solvent only occurred with enzymatic turnover. This lead them to propose a concerted pericyclic mechanism for carboxyl transfer, as shown in Pathway 1, Figure 1.5.

The unprecedented nature of the mechanism in pathway 1 prompted further study in the area. In their work on the same enzyme Stubbe and Abeles\textsuperscript{27} found that HF was eliminated when $\beta$-fluoropropionyl-CoA was used as a substrate. This led them to suggest a stepwise mechanism and the involvement of a carbanion intermediate, as shown in pathway 2, Figure 1.5. Knowles\textsuperscript{25} proposed a third mechanism, pathway 3, Figure 1.5, based on kinetic studies by Sauers\textsuperscript{20}. This is similar to pathway 2, but Knowles suggests that decarboxylation occurs prior to substrate carbanion attack.

Knowles and O'Keefe\textsuperscript{28} and Attwood\textsuperscript{29} confirmed a stepwise mechanism for the carboxyl transfer in their work identifying the rate determining steps in transcarboxylase and pyruvate carboxylase respectively. Knowles and O'Keefe compared the kinetic isotope effect of $[^{13}\text{CD}_3]$-
pyruvate and $[^{13}\text{CH}_3]$-pyruvate as substrates for the transcarboxylase enzyme. In a concerted reaction the rate would be slowed by deuterium substitution, but the $^{13}\text{C}$ effect would be unchanged. If the reaction were to proceed in a stepwise fashion it would be expected that deuterium substitution would slow deprotonation and carboxylation would become less rate limiting, therefore lowering the $^{13}\text{C}$ effect. The results of their studies showed a lowering of the $^{13}\text{C}$ effect, substantiating a stepwise mechanism. The investigations on pyruvate carboxylase by Attwood found a similar result.

1.1.5 Biotin Binding Proteins

There are several proteins known to bind biotin, with the most well known being the egg white glycoprotein avidin and streptavidin, isolated from *Streptomyces avidinii*. Avidin and streptavidin are homotetrameric 70kDa proteins. Each subunit is folded into an eight stranded anti-parallel β-barrel, binding biotin at one end between two tryptophan residues. The biotin-avidin interaction is one of the strongest protein-ligand interactions known ($K_d = 10^{-15}$ M), and the complex can withstand extremes of pH, buffer salts and chaotrophic agents such as guanidine. This has provided researchers with a valuable tool for use in a variety of biochemical and immunological studies.

1.1.6 Effects of Biotin Deficiency

Biotin deficiency in mammals occurs as the result of prolonged antibiotic treatment destroying gut microflora, the main producer of biotin, or from excessive ingestion of raw egg whites. A shortage of biotin reduces the activity of biotin dependant enzymes and indirectly results in impairment of lipid and protein synthesis and inhibition of RNA synthesis. The physiological effect of biotin deficiency in mammals can include dermatitis, alopecia, skin lesions, and nausea, however the symptoms are relieved by a dietary supplement of biotin.

1.2 Biotin Biosynthesis: an overview

1.2.1 The *E.coli* Biotin Operon

Biotin biosynthesis in *Escherichia coli* (*E.coli*) is controlled by the genes encoded in the 5.8 kilobase biotin (*bio*) operon, located at 17 minutes on the *E.coli* genome, between the attachment site of the lambda prophage (*att*λ) and the *uvrB* gene. Complementation analysis of biotin mutants showed the *bio* operon to consist of five genes: *bioA; bioB; bioC; bioD and...
bioF, coding for the principle enzymes in biotin biosynthesis\(^{32,33}\). Deletion mapping was used to establish the gene order of the operon\(^{34,35}\), and on locating the position of the operator and promoter it was concluded that the operon was divergently transcribed\(^{36}\) (see Figure 1.6). The promoter/operator is located between the bioA and bioB genes, and is transcribed to the left into bioA and to the right into bioFCD and B.

![Diagram of the E.coli biotin operon](image)

**Figure 1.6: The E.coli biotin operon**

In addition to the genes found in the bio operon, there are three genes located at separate locations on the E.coli genetic map. The gene bioH has been found at 74 minutes on the E.coli genome\(^{37}\), and although little is known about its function, current evidence suggests that bioH may be an acyl carrier protein\(^{38}\). Eisenberg identified two other genes: bioP, a permeability gene; and bioR, a regulatory gene\(^{39}\); it has since been discovered that this is identical to the birA gene discovered by Barker and Campbell\(^{40}\).

### 1.2.2 Regulation of the Biotin Operon in *E.coli*

Pai and Lichstein first showed evidence for the control of biotin biosynthesis in *E.coli* in 1965\(^{41}\). It was found that biotin production decreased with increasing biotin concentrations in the growth media, and it was later discovered that this was due to repression rather than to feedback inhibition\(^{42}\). It is now known that the protein BirA, product of the bioR gene, is the controlling factor in biotin biosynthesis.

BirA is a 321 amino acid (35.3 kDa) bifunctional protein, acting as a biotin activating enzyme and a transcriptional regulator\(^{43}\). Activation of biotin is necessary for ligation to the BCCP and is achieved by the formation of biotinyl-AMP using ATP. This proceeds via a BirA-biotinyl-AMP intermediate, which can act as a co-repressor, binding to the 40 base-pair bio operator and repressing transcription of the biotin biosynthetic genes. BirA therefore syntheses its own co-repressor, a unique property amongst DNA binding proteins.
1.2.3 Biotin Biosynthetic Pathway

It took nearly twenty years from the discovery of the role of biotin in cell biochemistry to elucidate the biotin biosynthetic pathway in *E. coli*\(^4^4\). The route (Figure 1.7) is now known to be ubiquitous using similar enzymes in bacteria, fungi, and higher plants \(^4^5\), as shown below.

![Biotin Biosynthetic Pathway diagram](image)

The first step in the pathway is the decarboxylative condensation of L-alanine and pimeloyl-CoA (7) to produce 8-amino-7-oxo nonanoate (AON, 8). This is catalysed by the pyridoxal-5'-phosphate (PLP) dependant *bioF* gene product, 8-amino-7-oxo nonanoate synthase (AONS). AON is converted to 7,8-diamino nonanoate (DAN, 9) in a transamination reaction mediated by the *bioA* gene product, diaminononanoate synthetase (DANS) another PLP dependant enzyme. Formation of dethiobiotin (DTB, 10) is the result of carbonyl insertion in DAN catalysed by the *bioD* gene product dethiobiotin synthetase (DTBS). The final step in the biosynthetic pathway is the insertion of a sulfur atom into DTB, catalysed by the *bioB* gene product biotin synthase, yielding biotin (1).
1.3 Enzymes involved in *E.coli* Biotin Biosynthesis

1.3.1 Pimeloyl-CoA Synthesis

The initial step in biotin biosynthesis in any organism is the formation of pimeloyl-CoA, however the synthesis varies between different bacterial and plant hosts. In *B.sphaericus* and some plants the *bioW* gene has been found to encode a pimeloyl-CoA synthetase enzyme\textsuperscript{46,47}. The *B.sphaericus* protein is a homodimer with subunit mass of 28kDa, catalysing the reaction of pimelic acid and HSCoA, in the presence of ATP and Mg\textsuperscript{2+} to form pimeloyl-CoA and pyrophosphate.

There is no gene equivalent to *bioW* in *E.coli*. However \textsuperscript{13}C labelling studies have prompted Sanyal and Ifuku to propose that pimeloyl-CoA in *E.coli* is produced in a pathway similar to that of fatty acid and polyketide synthesis\textsuperscript{48,49}. The suggestion that pimeloyl-CoA is produced from malonyl-CoA starter units supports the findings of Lynen\textsuperscript{50} who postulated that pimeloyl-CoA was synthesised from the condensation of three malonyl-CoA molecules. Although *E.coli* does not have the *bioW* gene it does have two other genes: *bioC* and *bioH*, which have been implicated in pimeloyl-CoA synthesis. Lemoine and co-workers have suggested that the *bioC* gene product may act as an acyl carrier protein, catalysing the stepwise condensation of malonyl-CoA starter units by the addition of acetate groups in a pathway similar to that used in chalcone synthase\textsuperscript{38}. The same group have also proposed that the *bioH* gene product transfers the pimeloyl moiety from an active cysteinyl residue on the *bioC* protein to HSCoA, preventing the accumulation of free pimelate in the cell. Studies on *bioH* in our laboratory\textsuperscript{51} have found that 99\% of the over-expressed and purified protein has the expected mass of 28,502 Da. *E.coli* *BioH* was incubated with CoA, MgCl\textsubscript{2} and DTT and analysed by LC-MS. The spectra showed a peak at 28,506 Da corresponding to unmodified *BioH*, but also two additional peaks were found at 28,849 Da and 29,272 Da. The peak at 29,272 Da corresponds to an addition of 765 Da, the mass of a HSCoA molecule, however the peak at 28,849 Da represents an addition of 343 Da, and is as yet unidentified. These results combined with genetic complementation analysis and enzymatic assays have lead Tomczyk *et al* to suggest that *BioH* may act as a CoA donor to a pimeloyl-ACP or a condensing enzyme.
1.3.2 8-Amino-7-oxononanoate Synthase

8-Amino-7-oxononanoate synthase (AONS) is the product of the bioF gene, and catalyses the decarboxylative condensation of pimeloyl-CoA (7) and L-alanine to produce AON (8) using a PLP co-factor. Eisenberg and co-workers purified AONS in the 1970's, but the sequence of the E.coli bioF gene was not established until 1988. The bioF gene has also been cloned from B.sphaericus and over-expressed in E.coli, and preliminary data for the crystallised enzyme published. Work in our laboratory has determined the structure and mechanism of the E.coli AONS.

The crystal structure of E.coli AONS (see Figure 1.9) has revealed that the enzyme functions as a homodimer, with each monomer having a mass of 41,464 Da, containing three separate domains. The small N-terminal domain (turquoise) acts as a linker, wrapping around the opposing monomer. The N-terminal domain is linked to the large central domain (purple),
which is composed of a seven stranded β-sheet, flanked by two α-helices, and curved around two more. This is in turn linked to the small (approximately 100 residues) C-terminal domain (yellow).

Each monomer of \textit{E.coli} AONS contains an active site, located within a deep cleft between the central and C-terminal domains, and using amino acid residues form both monomers. The PLP binding residue is lysine-236, inside the active site cleft, and therefore allows space for the pantothenoyl arm of pimeloyl-CoA to bind. Analysis of the holoenzyme structure has shown that PLP binding induces a conformational change. On formation of the internal aldimine a histidine (H133) residue to moves to lie in parallel with the pyridine ring of PLP, creating important π-interactions. A further conformational change occurs on product binding, in this instance the pyridine ring of PLP rotates approximately 15° with respect to the position of the internal aldimine. Product binding also causes the final turn in the C-terminal helix to unwind, causing the tip of the domain to move and imitate a lid closing on the external product aldimine.

Kinetic and spectroscopic studies on \textit{E.coli} AONS have suggested a mechanistically attractive pathway for the formation of AON (see Figure 1.10). The initial step in the pathway is displacement of the AONS-PLP internal aldimine by the attack of L-alanine (Step 1). This causes displacement of the Lysine-236 from AONS and formation of the external aldimine by transimination of L-alanine. The entry of pimeloyl-CoA into the active site (Step 2) induces a conformational change, allowing abstraction of the α-proton from the external aldimine. This results in the formation of a quinonoid intermediate (Step 3), which attacks pimeloyl-CoA, releasing CoA and forming a new carbon-carbon bond (Step 4). Loss of the carboxyl group from L-alanine results in the formation of a second quinonoid intermediate (Step 5), which picks up a proton from the positively charged lysine-236 residue (Step 6). The final step in the mechanism (Step 7) is transimination by the basic lysine-236 residue reforming the original internal aldimine and releasing AON (8).
Structural and mechanistic studies have revealed that AONs belong to the α-oxoamine synthase family of enzymes. These PLP dependant enzymes catalyse the decarboxylative condensation of an amino acid and a CoA thioester to form an α-aminoketone. The other enzymes in this group are: 5-aminolaevulinic acid synthase (ALAS), catalysing the condensation of glycine and succinyl-CoA to form 5-aminolaevulinic acid, an intermediate used in the initial stages of haem biosynthesis; serine palmitoyl transferase (SPT), a membrane bound protein involved in sphingolipid biosynthesis, which catalyses the condensation of palmitoyl-CoA and serine to form 3-ketodihydrospingosine; and 2-amino-3-ketobutyrate CoA ligase (KBL), catalysing the reaction of acetyl-CoA and glycine to form 2-amino-3-ketobutyrate, used in threonine biosynthesis.
1.3.3 7,8-Diaminononanoate Synthetase

The formation of 7,8-diaminononanoate (DAN, 9) from AON is catalysed by the bioA gene product, 7,8-diaminononanoate synthetase (DANS). DANS has an absolute requirement for PLP, and is unique amongst amino-transferases, catalysing the stereospecific transfer of the amino group from S-adenosyl methionine (SAM) to the re face of AON. It was originally thought that methionine acted as the amino donor, however it was subsequently shown that SAM was the true amino donor.

Eisenberg and Stoner purified DANS to 86% homogeneity in 1975, and found that the protein was a dimer with a subunit mass of approximately 47 kDa, and an iso-electric point of 4.7. The dimeric structure of DANS (see Figure 1.12) was confirmed in 1999 with the publication of the crystal structure of the enzyme. It was revealed that each dimer consisted of two domains, a

Figure 1.11: Formation of DAN from AON using DANS

Figure 1.12: Crystal Structure of DANS
small domain, comprised of the N and C-terminal regions (turquoise and yellow respectively),
split by a larger (central) domain. This large domain is made up of a 7-stranded β-sheet,
surrounded by α-helices. Each monomer contains two structural sodium atoms, shown in blue.

The DANS active site is located in a cleft formed by both the large and small domains of one
monomer, but also uses amino acid residues from the other subunit. The PLP cofactor forms an
external aldimine with the lysine-274 residue, located at the bottom of the cleft. AON binds
with its polar head towards the cofactor and its acid tail close to the entrance of the cleft.65

The absolute requirement of PLP by DANS puts it in the family of vitamin-B_6-depenant
aminotransferases, whose mechanism of action is well established. In the case of DANS
the transfer of the SAM amino group to AON occurs via a ping-pong mechanism (see Figure
1.13). In first half of the reaction the internal PLP-DANS aldimine is displaced by the
formation of the SAM-PLP external aldimine, this is converted to the DANS-pyridoxamine
phosphate (DANS-PMP) form, and the putative product S-adenosyl-2-oxo-4-methylthiobutyric
acid leaves the active site. This speculative reaction product has never been isolated from
enzymatic incubations, and it is suggested that it may readily decompose to 5'-methylthioadenosine and 2-oxo-3-butenoic acid under non-enzymatic conditions. The second
half reaction begins with binding of AON to the active site, this receives the amino group from
DANS-PMP, to form DAN and regenerate the DANS-PLP internal aldimine.

It has been suggested that the bioA and bioF genes could be derived from a common ancestral
gene as the two gene products share a number of similar features including substrate
specificity and requirement of the same PLP cofactor. Otsuka showed that AONS and DANS
shared a 20% amino acid homology, and the binding sites for both PLP and adenosyl derivative
binding sites are found in similar positions. Despite this similarity it is noteworthy that DANS
shares homology with other α-aminotransferases and AONS with other α-oxoamine synthases.
1.3.4 Dethiobiotin Synthetase

\[
\begin{align*}
\text{DAN} & \rightarrow \text{DTBS} \\
\text{Mg-ATP} + \text{CO}_2 & \rightarrow \text{DTBS} \\
\end{align*}
\]

Figure 1.14: Formation of DTBS from DAN using DTBS

The bioD gene product, dethiobiotin synthetase (DTBS), catalyses the formation of the imidazolidone ring of biotin, converting DAN into dethiobiotin (DTB, see Figure 1.14). It is interesting to note that DTBS is one of the few CO\(_2\) utilising enzymes that does not involve a biotin prosthetic group.

Much of the early work on DTBS were carried out by Eisenberg and Krell who revealed the substrate-product relationship between DAN and DTB\(^6\), and established the requirement for CO\(_2\), Mg\(^{2+}\) and ATP\(^7\). Eisenberg and Krell subsequently purified DTBS to near homogeneity and showed the active form of the enzyme to be a homodimer with a molecular weight of approximately 42 kDa\(^7\). The genetic sequence was deduced by Otsuka\(^5\) and later corrected by Alexeev\(^7\) who found that DTBS was a 224 amino acid protein with the N-terminal methionine residue cleaved by a post translational process.

Figure 1.15: Structure of DTBS dimer
The homodimeric nature of *E. coli* DTBS was confirmed when the crystal structure of the enzyme was solved independently by two groups\(^{73,74}\). Each DTBS monomer (see Figure 1.15) consists of one subunit folded as an α/β-domain, containing a central seven-stranded parallel β-sheet shown in blue surrounded by α-helices shown in yellow. The active site of the enzyme is located within a concave space at the interface of the two monomers, with the substrate (DAN) binding to one surface and ATP binding to the other\(^{75}\).

\[
\begin{align*}
&\text{DAN [9]} \quad \text{CO}_2 \quad \text{8-Amino-carbamate [11]} \\
&\text{DTBS [10]} \quad \text{ATP} \quad \text{7-Amino-carbamate [12]}
\end{align*}
\]

Figure 1.16: Mechanism of amino-carbamate formation

Krell and Eisenberg\(^ {71}\) used \(^{14}\)C-ATP as a substrate for the DTBS reaction to determine the stoichiometric relationship between ATP and DTB. They found that adenosine diphosphate (ADP) and DTB were formed in equimolar quantities from DAN and DTBS. The overall stoichiometry of the reaction was established by Baxter *et al*\(^ {76}\) who found that one mole of ATP was required to form 1 mole of DTB, thus indicating a single activation step for the formation of two amide bonds. In the course of this work radiolabel pulse chase experiments revealed that the first step in the DTBS catalysed reaction was amino-carbamate formation. By trapping this intermediate using diazomethane Baxter and co-workers suggested that the initial amide bond was formed at N-8 on DAN resulting in the 8-amino-carbamate (11, Figure 1.16). Analysis of the enzyme-DAN-carbamate crystal structure by Huang *et al*\(^ {74}\) and Alexeev *et al*\(^ {75}\) subsequently found that the true intermediate was the 7-amino-carbamate (12, Figure 1.16). Further work by Gibson\(^ {77}\) isolated four times as much 7-amino carbamate as 8-amino carbamate from diazomethane trapping mixtures of DAN, \(^{14}\)CO\(_2\), and DTBS. The conclusion of this work found that DTBS conferred specificity on carbamate formation, activating DAN at the 7-position.
With the first step in the reaction mechanism established to proceed without ATP activation, Baxter and Baxter\textsuperscript{78} carried out incubation studies to establish the mechanism of the ring closure step in the DTBS mechanism. It was known that 1 mole of ATP was converted to ADP and inorganic phosphate, but there were two possible reaction intermediates, a phosphoric or an ADP mixed anhydride (13 & 14, Figure 1.17). They incubated DTBS with \textsuperscript{18}O labelled ATP, DAN, bicarbonate and Mg\textsuperscript{2+} and analysed the distribution of \textsuperscript{18}O in the products by \textsuperscript{31}P NMR. From this it was revealed that the reactive intermediate formed was the carbamate-phosphoric mixed anhydride (13) and ring-closure followed Pathway A (Figure 1.17).

### 1.3.5 Biotin Synthase

The final step in the biotin biosynthetic pathway is the insertion of sulfur into the carbon skeleton of DTB. This process is catalysed by the bioB gene product biotin synthase, an iron-
sulfur cluster containing enzyme 48 that requires an extensive number of co-factors.

*E. coli* Biotin synthase is a 346 amino acid protein with a predicted mass of approximately 38.5 kDa. Cloning, purification and over-expression of the *bioB* gene product from *E. coli* was first carried out by Sanyal. Native gel electrophoresis revealed two protein species with molecular weights of 82 kDa and 104 kDa; both of these species gave the expected monomeric molecular weight of approximately 39 kDa on SDS gels. The 82 kDa species was found to be a homodimer containing one [2Fe-2S] cluster per monomer, while the 104 kDa species was a homodimer with a single [2Fe-2S] cluster. It was found that the 104 kDa species could be converted to the 82 kDa species by incubation with FeCl₃ (Fe³⁺), Na₂S (S²⁻) and 2-mercaptoethanol. EPR studies on the Fe-S cluster from the 82 kDa species confirmed the presence of one [2Fe-2S] cluster per monomer. It was found that one iron ion is bonded to two sulfur ligands, while the other has a cysteine ligand and a terminal oxygenic ligand. Initial results suggested that anaerobic reduction of the dimer converts the two [2Fe-2S] clusters to one [4Fe-4S] cluster where each iron is bonded to a cysteine ligand, oxidation of this species results in partial formation of the original cluster. Recent work by Ollagnier-De Choudens et al confirms that although [2Fe-2S] clusters occur in the aerobic dimeric form, the anaerobic dimer contains two [4Fe-4S] clusters. The group has also found that in the absence of external iron and sulfur the anaerobic dimer contains only one [4Fe-4S] cluster. Iron-sulfur clusters can play an important role as a radical initiator, facilitating the reductive one electron cleavage of SAM to form methionine and a 5'-deoxyadenosyl radical.

In addition to the iron-sulfur cluster biotin synthase requires a number of co-factors for activity including: fructose-1,6-bisphosphate; SAM; Fe²⁺; reduced nicotinamide adenine dinucleotide phosphate (NADPH); *E. coli* flavodoxin; and *E. coli* flavodoxin NADP⁺ oxidoreductase. It has also been suggested that there is a requirement for a thiamine pyrophosphate dependant enzyme, and one of the following amino acids: asparagines; aspartate; glutamine or serine.

The investigation into the mechanism of the biotin synthase enzyme can be split into two main fields: the source of the sulfur donor and the mechanism of C-H to C-S bond formation. Early studies on the nature of the sulfur donor proved inconclusive. Recent studies undertaken by Shaw have found that that sulfur from [³⁵S]-cysteine can be incorporated into a reaction intermediate. The group of Marquet has found that regeneration of the *B. sphaericus* biotin synthase cluster with FeCl₃ and Na₂³⁴S results in the production of ³⁴S-biotin. Shaw has also shown that SAM is required to form an enzyme generated intermediate, which is then converted to biotin in another SAM dependant step. Assays using [¹⁴C-methyl]-SAM have shown that
[14C]-methionine is the product, supporting the hypothesis that SAM is cleaved to form methionine and a 5'-deoxadenosyl radical. The group proposed a mechanism where the conversion from DTB to the intermediate is dependant on a substrate or enzyme bound radical from the reductive cleavage of SAM, as is the conversion of the intermediate to biotin step.

![Proposed thiol intermediates](image)

Figure 1.19: Proposed thiol intermediates

Early studies on the nature of C-H to C-S bond formation by Parry\(^87,88\) found that sulfur introduction at C-6 and C-9 took place without loss of hydrogen at C-7, C-8 or C-5, tritium labelling studies revealed that sulfur incorporation involved loss of one hydrogen form both C-6 and C-9. The synthesis of chirally tritiated dethiobiotin showed that it was the 6-pro-S-hydrogen was lost from the C-6 position\(^89\) with overall retention of configuration at this centre. This suggests that functionalisation takes place in a single step, however both hydroxyl and thiol groups have been implicated as intermediates. Marquet\(^90\) and Emoto\(^91\) have found evidence against the formation of hydroxylated dethiobiotin intermediates, however this does not rule out the existence of hydroxylated dethiobiotin in a transient enzyme bound state. The presence of 6- and 9-mercaptopdethiobiotin (15 and 16, Figure 1.19) have been proposed as intermediates in the biotin synthase reaction by Marquet et al\(^92\) and Baxter et al\(^93\) respectively. Baxter found that 9-mercaptopdethiobiotin could support the growth of and E.coli bioA mutant (strain 6435), whilst Marquet found that 6-mercaptopdethiobiotin could replace DTB or biotin in a biotin auxotrophic strain of B.sphaericus. Biological evaluation of both compounds with resting cells of B.sphaericus has found that 9-mercaptopdethiobiotin is transformed to biotin with a 10% conversion rate compared to DTB. Incubation of\(^35\)S-(17) of the same cells in the presence of DTT has clearly shown the conversion of (17) into biotin, with no observed degradation. This has been substantiated by the identification of (17) as an intermediate in the biotin biosynthetic pathway in Lavendula vera\(^8\).
1.4 Pyridoxal Phosphate Enzymes involved in Biotin Biosynthesis

1.4.1 Pyridoxal-5’-Phosphate Dependant Enzymes

The vitamin B₆ (18, Figure 1.20) derived cofactor pyridoxal-5’-phosphate (PLP, 19) is used by a great many enzymes catalysing transformations at the α-, β-, or γ-carbons of amino acids or other important amino containing compounds. The biotin biosynthetic pathway contains two enzymes catalysing PLP dependant transformations at α-carbon centres (see sections 1.3.2 and 1.3.3 on AONS and DANS).

PLP has two basic roles in the reactions it mediates: through the aldehyde group it forms imine bonds with the amino groups of its substrates (20, Figure 1.20); and it acts as an electron sink, providing extensive charge delocalisation and stabilising the carbanion intermediates formed in catalysis.

The types of enzyme utilising a PLP cofactor can be split into three main categories, according to the carbon centre at which the transformation occurs (see Figure 1.21).

α-carbon reactions: catalysing transamination, racemisation, or decarboxylation at the α-carbon. In this case the nature of the enzyme dictates the substituent that will undergo reaction.
**β-carbon reactions**: catalysing elimination or replacement at the β-carbon. These reactions typically involve elimination of X if it is a good leaving group, or replacement of X with a nucleophile X'.

**γ-carbon reactions**: catalysing elimination or replacement at the γ-carbon. As with β-carbon reactions, these involve elimination of Y or its replacement by nucleophile Y'.

A cartoon showing the different types of PLP dependant reactions can be seen in Figure 1.22.

![Cartoon representing origins of PLP reaction specificities](image)

**1.4.2 The Mechanism of PLP catalysis**

PLP dependant enzymes are well studied and the catalytic action of these enzymes is therefore well documented. PLP exists as a Schiff base, with the aldehyde group forming an imine bond with the ε-amino group of a lysine residue on the enzyme, known as the internal aldimine. Binding of the substrate displaces the lysine residue and forms an external aldimine, where the PLP forms a Schiff base with the substrate. The general mechanism for a PLP mediated reaction was proposed by Metzler, Ikawa and Snell who performed a series of non-enzymatic model
reactions using metal chelating PLP-amino acid Schiff bases. It was found that there is the same basic reaction sequence for all categories of PLP dependant enzyme:

1. initial imine formation;
2. chemical modification via a carbanionic intermediate;
3. hydrolysis of the product imine.

Dunathan proposed an answer to the question of the specificity of a PLP mediated reaction. The Dunathan hypothesis\(^96\) suggested that the C\(^5\) bond perpendicular to the pyridine ring of PLP has the largest overlap of its HOMO with the LUMO of the extended \(\pi\)-system of the imine and the pyridinium ring, and is therefore broken most easily. The hypothesis was confirmed by x-ray crystallographic studies on aspartate aminotransferase\(^97\), tryptophan synthase\(^98\) and tyrosine phenol-lyase\(^99\), which found the C\(^5\)-H bond to be perpendicular to the pyridine ring. Similar studies on dialkyl glycine decarboxylase found the carboxylate group to be held perpendicular to the pyridine ring\(^100\).

On displacement of a substituent from the C\(^4\) of the external aldimine a quinonoid intermediate is formed (see Figure 1.22). This is generally an unstable intermediate as it is formed only transiently, however formation of this intermediate is the main catalytic role in the action of PLP dependant enzymes. The reactions following the formation of the quinonoid intermediate are complex, requiring the controlled migration of protons and leaving groups. It is thought that this is mediated by the active site residues of the individual enzymes, with the position of the acidic and basic residues at the active site dictating the substrate and reaction specificity of the enzyme\(^101\).

It can be seen in Figure 1.22 that each intermediate quinonoid can be transformed into a number of different products. This results in the enzyme having two major roles: accelerating the rate of the desired reaction; and preventing the occurrence of alternative reaction pathways. In doing this the enzyme can therefore utilise the versatile PLP cofactor effectively.

### 1.4.3 Inhibition of PLP Dependant Enzymes

PLP dependant enzymes are attractive targets for the design of enzyme activated irreversible inhibitors for two main reasons\(^102\). PLP dependant enzymes are involved in the metabolism of many physiologically important compounds. Specific inhibitors of these enzymes could therefore be of therapeutic interest, as the metabolism of these compounds may result in a pathological condition. PLP dependant enzymes are ideal for the design of inhibitors as the
mechanism of action is well understood, facilitating the selection of a suitable group for activation during catalysis. The covalent binding between substrate and PLP can be utilised, as any reactive intermediate generated cannot easily leave the active site, resulting in an advantageous situation for bond formation between the intermediate and enzyme. The nature of PLP as an electron sink, stabilising carbanionic intermediates can be used to generate an electrophilic intermediate that can react with suitable nucleophilic groups present in the side chain of the amino acid residues in the enzyme active site.

The most general approach to the design of inhibitors for PLP dependant enzymes has been to incorporate suitable groups into the substrate to alter the fate of the quinonoid intermediate and generate an electrophilic imine to react with the residues in the active site. The structure of these imines is normally closely related to that of the parent compound, with the most effective alterations being the addition of an alkene, alkyne or halogen functionality.

1.5 Inhibition of Biotin Biosynthesis

Biotin biosynthesis via the previously described pathway occurs only in plants and microorganisms, and as such is an ideal target for the development of inhibitors as non-toxic herbicides. It has been shown that actithiazic acid and aniclenomycin, compounds isolated from the culture filtrates of *Streptomyces* species, are inhibitors of biotin synthase\(^{103}\) and DANS\(^{104}\), and inhibit the growth of mycobacteria. Preliminary experiments on lavender cells (*Lavandula vera*) have shown that plant biotin synthase has also been inhibited by the anti-microbial actithiazic acid\(^45\), supporting the theory that inhibitors of microbial enzymes could also have herbicidal properties.

1.5.1 Inhibition of PLP Dependant Enzymes used in Biotin Biosynthesis

![Figure 1.23: Intermediate (18) used to design inhibitors of AONS](image-url)
The AONS enzyme in the biotin biosynthetic pathway has been extensively studied, and there is much known about its mechanism of action (see section 1.3.2). This has lead to the design of substrate analogues and inhibition studies by the group of Marquet\textsuperscript{105}. From their studies on the mechanism of the \textit{B.sphaericus} AONS enzyme\textsuperscript{54,106,107} the group designed six analogues based on the reaction intermediate (21, Figure 1.23) and transition state intermediate and tested them as inhibitors of AONS. The results of these tests can be seen in Table 1.1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Inhibition Type</th>
<th>( K_1 (\mu M) )</th>
<th>( K_1 / K_m ) L-Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Alanine</td>
<td><img src="image" alt="D-Alanine Structure" /></td>
<td>Competitive/L-Ala</td>
<td>590</td>
<td>0.24</td>
</tr>
<tr>
<td>22</td>
<td><img src="image" alt="Structure 22" /></td>
<td>Slow-binding, competitive/L-Ala</td>
<td>7</td>
<td>0.003</td>
</tr>
<tr>
<td>23</td>
<td><img src="image" alt="Structure 23" /></td>
<td>No inhibition</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td><img src="image" alt="Structure 24" /></td>
<td>No inhibition</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td><img src="image" alt="Structure 25" /></td>
<td>Competitive/L-Ala</td>
<td>68</td>
<td>0.027</td>
</tr>
<tr>
<td>26</td>
<td><img src="image" alt="Structure 26" /></td>
<td>Competitive/L-Ala</td>
<td>80</td>
<td>0.032</td>
</tr>
</tbody>
</table>

The first compound tested was D-alanine, which was found to be a competitive inhibitor of AONS. D-alanine is not a substrate for the enzyme as it does undergo C2-H proton exchange with the solvent, but binds more tightly to the active site than L-alanine. This occurs as the acylation step occurs with inversion of configuration, and D-alanine can mimic the reaction intermediate, binding tightly to the active site.

In their design of the other inhibitors tested the Marquet group based their design on work by Bartlett and Giangiordano\textsuperscript{108}. Their work found that the phosphonate group possessed the structural and electronic requirements to mimic the tetrahedral transition state generated from...
the hydrolysis of esters and related compounds. Marquet et al also used an alcohol group as another tetrahedral transition state analogue. These functionalities provided mixed results, with compounds 22, 25, and 26 exhibiting competitive inhibition while compounds 23 and 24 showed no inhibition. It was thought that the presence of three negative charges on 24 at pH 7.0 may have prevented interaction with the active site, while it was rationalised that the diastereomers of 23 may adopt different conformations preventing interaction with the active site.

1.5.2 Inhibition of Other Enzymes used in Biotin Biosynthesis

As the most extensively studied enzyme in the biotin biosynthetic pathway DTBS was the target for inhibition by the group of Baxter\textsuperscript{109}. This group targeted the nucleoside base binding site of the DTBS protein with a view to inhibiting its action.

The group of Sawyer used the detailed knowledge of the ATP binding site in DTBS\textsuperscript{73,74,75,110} to design a small molecule that would bind specifically to the purine binding elements in the active site. Three atoms of the adenine base form specific H-bonds to the DTBS protein: the 6-NH\textsubscript{2} forms H-bonds to the carbonyls of proline204 and asparagine175; N-7 is H-bonded to the amine of asparagine175, the optimal position for nucleotide binding is ensured by a second H-bond from the asparagine175 amine to valine113; the third H-bond is between N-1 and the main chain nitrogen of leucine206. Hydrophobic interactions sandwich the adenine ring between proline206, valine17 and the asparagine175-threonine 178 loop. These features confer specificity for ATP on the binding site, with CTP and GTP acting only as poor substrates. It was found that the simplest molecule to fit these binding specificities was 6-hydroxypirimidin-4(3H)-one (6-HP4, 28, Figure 1.1).

\[ \text{ATP} \quad \text{6-Hydroxypirimidin-4(3H)-one} \]

\[ \text{6-Hydroxypirimidin-4(3H)-one, (6-HP4) [28]} \]

\[ \text{A TP [27]} \]

Sawyer et al have shown that 6-HP4 is bound specifically in the adenine binding pocket of DTBS with many protein ligand interactions. 6-HP4 is H-bonded to the carbonyl and amine
groups of asparagine175, to the nitrogen of leucine206 and the carbonyl of alanine207; there are also similar hydrophobic interactions to those formed between the active site and adenine. The difference in binding arises around the carbonyl of proline204, with ATP as the substrate there is a H-bond present, however this does not occur when 6-HP4 is the substrate. 6-hp does however make a hydrophobic interaction with the side chain of isoleucine203. It was noted that 6-HP4 could bind to the active site in a number of different orientations and tautomeric forms, however 28 (Figure 1.1) gives the optimum number of interactions with the active site.

Kinetic measurements showed that 6-HP4 is a competitive inhibitor for DTBS, although it does have a relatively high $K_i$ (11.2 ± 1.0 mM). It was expected that 6-HP4 would bind more weakly than ATP as 6-HP4 lacks the charged sugar-phosphate tail of ATP. Comparing the 6-HP4 inhibition constant is to the ATP analogue AMP-PNP ($K_i$ ca 50μM) it can be seen that this is $10^3$ times better, however the 6-HP4 inhibition constant is 5 times greater than that of adenine ($K_i = 2.2 ± 0.5$ mM).

1.6 Aim of This Study

The biotin biosynthetic pathway is an ideal target for the development of pathway specific inhibitors that could act as non-toxic herbicides (see section 1.5). The main body of this work has been directed towards the identification and developments of synthetic routes to intermediates in the biotin biosynthetic pathway, with a view to the development of synthetic inhibitors of the enzymes involved in their formation.

The first objective was to develop a synthetic route to 8-amino-7-oxononanoate (AON, 8), based on previous work in our laboratory. The second objective was to identify and develop a new route towards the synthesis of AON, and extend this route to allow the synthesis of an array of AON analogues. The third objective was to test these analogues against the AONS and DANS enzymes, and identify any substrate activity with the DANS enzyme.
2. Dakin-West Chemistry
2.1 Dakin West Reaction

The base catalysed formation of an \( \alpha \)-acetylamino alkyl methyl ketone from an \( \alpha \)-amino acid and acetic anhydride (Figure 2.1) was first reported by Dakin and West in 1928\(^{112,113,114} \). Initial studies on the reaction of acetic anhydride and pyridine with several amino acids,

\[
\text{Amino Acid} \xrightarrow{\text{base}} \text{\( \alpha \)-Acetyl amino alkyl methyl ketone} + \text{CO}_2
\]

found that primary \( \alpha \)-amino acids were converted into the corresponding \( \alpha \)-amino ketones with the release of an equimolar amount of carbon dioxide. Further work has shown that the Dakin-West reaction works equally well with secondary and non amino acids. Changing the basic catalyst from pyridine to an alkyl pyridine\(^{115} \), a tertiary alkyl amine\(^{115} \), or to sodium acetate\(^{116} \), has improved reaction yields, although increasing the steric bulk of the amine does reduce the reaction rate and yield. The scope of the reaction has been further extended to include larger acid anhydrides\(^{117} \), but in general yields decrease with increasing anhydride chain length.

2.1.1 Reaction Mechanism.

\[
\begin{align*}
\text{L-amino acid} & \xrightarrow{\text{oxazolone intermediate (29)}} \text{resonance stabilised carbanion} \\
& \xrightarrow{\text{acylated oxazolone (30)}} \text{\( \alpha \)-acyl amino alkyl methyl-\( \beta \)-keto ester} \\
& \xrightarrow{\text{\( \alpha \)-acylamino alkyl methyl-\( \beta \)-keto acid (31)}} \text{\( \alpha \)-acylamino alkyl methyl ketone (32)}
\end{align*}
\]

A number of mechanisms were originally proposed for the Dakin-West reaction, but all met with objections. Dakin and West proposed the involvement of an oxazolone intermediate that gives rise to the same product as the parent amino acid\(^{113} \). Kinetic studies\(^{118} \) have shown that the
oxazolone reacts at the same apparent rate as the parent amino acid to form an α-acylamino ketone. The initial (fast) step in the reaction mechanism, Figure 2.2, is acylation and cyclisation of the amino acid to form the oxazolone (29). Reaction with the basic catalyst forms the resonance-stabilised carbanion, which is subsequently acylated by the acid anhydride to form 30. This then undergoes ring opening and deprotection to from the α-acylamino-β-keto acid (31). The driving force for the reaction is the decarboxylation of 31, and the subsequent tautomerisation to form the α-acylamino ketone (32).

2.1.2 Improvements on the Dakin-West Reaction

Over time the Dakin-West reaction has been improved to give higher yields and a more efficient use of starting materials. Attenburrow, Elliot and Penny \(^{116}\) found that reacting the sodium salt of an N-benzoyl amino acid with acetic anhydride at room temperature gave the corresponding acyl oxazolone, which formed the α-acylamino ketone in higher yields than previously encountered. Steglich and Höfle have made further improvements in developing a “stepwise” Dakin-West reaction, which are outlined in detail below.

2.2 Stepwise Dakin West Reaction

In the course of a detailed study on the mechanism of acylation of oxazol-5-ones Steglich and Höfle made two main improvements to the Dakin-West reaction \(^{119,120,121,122}\). The first of these was replacement of the acid anhydride acylating agent with an acid chloride \(^{119}\). Previous

![Figure 2.3: Stepwise Dakin-West reaction](image)
studies\textsuperscript{112,116} had shown that the reaction of an oxazolone with an acid chloride was difficult to control and resulted in poor yields, while the corresponding acid fluoride could be used more easily\textsuperscript{117}. Steglich and Höfle found that employing a stepwise process exemplified in Figure 2.3, where the pre-formed oxazolone was treated with Et\textsubscript{3}N and an acid chloride gave a more satisfactory result. The reaction proceeded at room temperature under kinetic control to form the O-acyloxy oxazole (34) in high yield. The second development made by Steglich and Höfle was to improve the yield and efficiency of the acyl migration step. By changing the catalyst used from pyridine to 4-N,N-dimethylamino pyridine (DMAP)\textsuperscript{120} the reaction time was decreased, the conditions were milder and the yield improved. Acyl migration was initially carried out by heating the O-acyloxy oxazole in pyridine at $95^\circ\text{C}$ for several hours, then distilling the mixture to give the thermodynamically stable C-acyl oxazolone (35). However it was found that addition of 0.05 molar equivalents of DMAP resulted in isomerisation at room temperature in 0.5-1h.

2.2.1 Changes in the Reaction Mechanism

In their early work on the mechanism of the Dakin–West reaction Steglich and Höfle studied the reaction of an oxazolone with an acetic anhydride-pyridine mixture using NMR\textsuperscript{121}. They originally proposed (Figure 2.2) that the resonance stabilised carbanion reacted directly with the acetic anhydride to form the C-acylated oxazolone, however the NMR study indicated the presence of a previously undiscovered reaction intermediate (37, Figure 2.4). It seems reasonable that the acid anhydride reacts with the pyridine to form 37 and that subsequent rearrangement allows formation of the C-acyl oxazolone.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2_4.png}
\caption{Steglich and Höfle acylation intermediate}
\end{figure}

The mechanism proposed by Steglich and Höfle for the transformation of O-acyloxy oxazole to C-acyl oxazolone (Figure 2.5) involves the initial formation of an ion-pair intermediate (38)\textsuperscript{122}. DMAP reacts with the O-acyloxy oxazole, abstracting the acyl group from the oxygen and forming an ion pair. This then forms a similar intermediate to that of (37, Figure 2.4) with rearrangement and elimination of DMAP giving the required C-acyl oxazolone.
The Dakin-West reaction is a good starting point for the synthesis of α-amino ketones and the work of Steglich and Höfle has improved the yield, conditions and versatility of the reaction. It was therefore decided to investigate the "stepwise" Dakin-West reaction as a possible method for the synthesis of 8-amino-7-oxo nonanoate (AON, 8).

2.3 Stepwise Dakin West Reaction in Synthesis of AON

2.3.1 Using 4-methyl-2-phenyl oxazolone

Previous work in our laboratory used N-benzoyl alanine as the starting point for the synthesis of AON, as shown in Figure 2.6. It was therefore decided to repeat this synthesis and develop the work in an attempt to improve reaction yields. N-benzoyl alanine (39) was prepared from benzoyl chloride and alanine under standard Schotten Bauman conditions at 4°C. Reaction of 39 with acetic anhydride formed 2-methyl-4-phenyl oxazolone (40) via cyclisation of the mixed anhydride (Figure 2.7). The melting point of 40 was found to be 36-38°C, corresponding to that of the to racemate (mp=37-39°C). Racemisation is expected in this instance as the C-4 proton has a pKₐ of 8.4, allowing facile interconversion of enantiomers, enhanced by heating in an acetic anhydride / acetic acid mixture.
Acylation of 40 with methyl-6-chlorcarbonyl hexanoate (41) and Et<sub>3</sub>N at room temperature for 14h formed the kinetically favoured O-acyloxy oxazole (42, Figure 2.6), which was found to decompose slowly with time. Conversion to the more thermodynamically stable C-acyl oxazolone (43) isomer occurred on treatment of 42 with DMAP in dry THF for 14h. It was found that increasing reaction time gave greater formation of 43, but this was accompanied by the slow decomposition of 42, and the optimum reaction time was found to be 14-16h.

On the basis of the earlier literature it was proposed that the most suitable strategy for the formation of AON (8) would be a one-pot sequence involving a ring opening and deprotection sequence. This was achieved in low yield by treatment of 43 with HCl (6M) at 80°C for 24h. TLC analysis revealed a number of reaction products including monomethyl heptanedioate, N-benzoyl alanine, and alanine in addition to AON. The products were separated and purified by ion exchange chromatography over DOWEX-50 (H<sup>+</sup> form, eluting with 0-1M HCl).

**Route A**

**Route B**

![Figure 2.7: Oxazol-5-one formation](image)

![Figure 2.8: Acidic ring opening of C-acyl oxazolone](image)
The 1,3-dicarbonyl centres present in the C-acyl oxazolone mean there are two possible protonation routes, as shown in Figure 2.8. Route A shows the expected ring opening sequence where initial protonation occurs at the carbonyl group on C-5. However the ring opened moiety can undergo further hydrolysis by one of two routes. If route (a) is followed decarboxylation and deprotection occur to form AON. If however route (b) is followed the side chain is cleaved to form N-benzoyl alanine and methyl heptanedioate.

Route B shows a much less desirable protonation course, where initial attack occurs at the carbonyl group on C-1’, giving rise to a retro-Claisen reaction where methyl heptanedioate and 4-methyl-2-phenyl-oxazol-5-ol are formed. Further hydrolysis of the oxazolol gives rise to N-benzoyl alanine.

In order to improve the yield of the final ring opening and deprotection of the C-acyl oxazolone it was decided to investigate the use of 2,4-dimethyl oxazolone and 2-methyl-4-trifluoromethyl oxazolone as starting materials for the synthesis of AON (8), and represents a new approach to the synthesis of AON. It was hoped that by choosing an oxazolone with an electron donating C-2 substituent and one with an electron withdrawing C-2 substituent that the effect of ring substituent on the retro-Claisen reaction could be determined. It was speculated that altering the C-2 substituent on the oxazolone would change the stability of the ring opened oxazolone to allow a more facile deprotection strategy to be employed, and promote hydrolysis via Route A (Figure 2.8).

2.3.2 Using 2,4-dimethyl oxazolone

Figure 2.9 shows the formation of AON via the Dakin-West reaction using N-acetyl alanine as the starting material. 2,4-Dimethyl oxazolone (44) was formed from N-acetyl alanine using an activated ester method. Dicyclohexyl carbodiimide was added to N-acetyl alanine in dry THF to form an activated ester, which underwent cyclisation forming 44 and dicyclohexyl urea. The DCU was precipitated with ether and filtered to give 44 as a waxy solid. Formation of the O-acyloxy oxazole (45) and the C-acyl oxazolone (46) were performed analogously to the formation of 42 and 43 (Figure 2.6). Ring opening and deprotection were carried out in a one pot sequence, heating in HCl (4M) at 80°C for 4h and subsequent stirring at room temperature for 12h. The product mixture was separated and purified by ion exchange chromatography using DOWEX-50 (H+ from, eluting with HCl 0-1M), to give a mixture of products, with AON in 10% yield.
Chapter 2

2.3.3 Using 2-methyl-4-trifluoromethyl oxazolone

Figure 2.9 shows the synthesis of AON using alanine as the starting material. 2-Trifluoro-4-methyl oxazolone (47) was formed from alanine and trifluoroacetic anhydride at 140°C via cyclisation of the mixed anhydride. Reaction with methyl-6-chlorocarbonyl hexanoate (41) and Et₃N formed the O-acyloxy oxazole (48), which was isomerised to the C-acyl oxazolone (49) using DMAP. Ring opening and deprotection with HCl (4M) at 80°C for 12h gave AON (8) in 17%, in addition to the other products as seen previously.

2.3.4 Comparison of the Three Methods for the Synthesis of AON

It can be seen above that using benzoyl alanine as the starting material for the Dakin-West reaction affords 2-methyl-4-phenyl oxazolone (40) in 62% yields, with the acylation affording the O-acyloxy oxazole (42) in 74% yield and isomerisation to the C-acyl oxazolone occurring in 68% yield. Final ring opening and deprotection affords AON (8) in 14%, giving an overall yield of 4%. When N-acetyl alanine is used as the starting material 2,4-dimethyl oxazolone is formed in 58% yield. Subsequent acylation gives the O-acyloxy oxazole (45) in 64% yield, with isomerisation giving the C-acyl oxazolone in 48% yield. In this instance ring opening and deprotection affords AON (8) in 10% yield, an overall yield of 2%. The oxazolone 2-methyl-4-trifluoromethyl oxazolone (47) is formed in 95% yield from alanine and trifluoroacetic anhydride, with acylation giving the O-acyloxy oxazole (48) in 68% yield. Subsequent isomerisation affords the C-acyl oxazolone (49) in 65% yield, with deprotection and ring opening giving AON (8) in 17%, an overall yield of 7%. A summary of these results is shown in Graph 2.1.

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The differences in yield observed in the formation of oxazolones 40, 44 and 47 can be attributed to due to an easier separation of 47 from its by products. In this instance distillation under reduced pressure afforded pure 47, whilst 40 and 44 required repeated crystallisation to afford a waxy solid. The yields of O-acyloxy oxazoles (42, 45 and 48) are comparable for each of the oxazolone starting materials used. The yields experienced for the formation of the C-acyl oxazolones 43 and 49 are comparable, however the yield of the C-acyl oxazolone 46 is much lower. This can be rationalised by looking at the ion-pair intermediate (38, Figure 2.5) formed on treatment of the parent O-acyloxy oxazole with DMAP. The intermediate 38 has a delocalised negative charge which can be readily stabilised by both the phenyl and trifluormethyl substituents, however, this does not occur with the methyl substituent, thus lowering the yield of this reaction step. Low yields are experienced when all three C-acyl oxazolones undergo ring opening and deprotection, however Graph 2.1 shows that the 49 gives the highest yield of AON (8), due to a more facile hydrolysis as a result of the electron withdrawing effect of the CF$_3$ substituent. In the case of 46 the inductive effect of the methyl group causes a more difficult hydrolysis thus reducing the reaction yield.

As this strategy had not been as successful as anticipated, a different approach to the final ring opening and deprotection step was adopted. It was decided to investigate an enzymatic ring opening of the C-acyl oxazolone followed by a separate deprotection step to give the final product.
2.4 An Enzymatic Approach to the Synthesis of AON

By using an enzymatic ring opening method it was hoped that the problem faced by competing hydrolysis routes (Figure 2.8) would be avoided. The advantage of taking an enzymatic approach would be production of AON as its biologically active enantiomer (R,S AON) where as the stepwise Dakin-West reaction produced AON as a mixture of enantiomers. To this end it was decided to investigate the use of the enzyme Lipozyme®, which could be employed in the enantioselective oxazolone ring opening.

2.4.1 The Use of Enzymes in Organic Chemistry

In the last twenty years extensive studies on the use of enzymes in synthetic organic chemistry have shown that there is a large degree of practical application for enzymes in chemical synthesis. Despite this knowledge chemists have been slow to employ enzymes in the course of their day to day reactions. There are many reasons for this lack of enthusiasm with the most important reason being the perceived necessity to work in aqueous solution. Research has now shown that this need not be the case, as many enzymes can function in organic solvents with little or no added water. This realisation has opened up the use of enzymes in synthetic chemistry as one no longer needs to worry about the insolubility of organic compounds in water, the promotion of side reactions by water, or unfavourable thermodynamic equilibria leading to low yields.

2.4.2 Lipase Catalysed Oxazolone Ring Opening

![Partial in situ racemisation](image)

Figure 2.10: Enantioselective enzymatic ring opening of oxazolones

In the early nineties there was much research carried out on the enantioselective synthesis of L-α-amino acids from oxazol-5-ones. This work focused on the enantioselective methanolysis, or butanolysis, of 4-substituted-2-phenyl oxazolones (Figure 2.10) using a lipase,
such as porcine pancreatic lipase (PPL) or *Candida cylinracea* (CCL)\(^{131}\) in an organic solvent. It had been found that enol esters underwent transesterification much faster than simple alkyl esters\(^ {136}\); it was therefore proposed that oxazolones, as cyclic aza enol esters, should behave in the same way, as the reactions had similar mechanisms. Solvent studies showed that the reaction medium had an effect on the optical purity of the amino acid, and that changing the solvent used could improve the enantioselectivity of the lipase\(^ {132}\). It was suggested that it was possible to prepare any \(\alpha\)-amino acid in high optical purity and in the desired stereochemical form via the lipase ring opening of the oxazolone derivative.

![Figure 2.11: Racemisation and enzymatic ring opening of oxazolones](image)

An important finding of these studies was that the rate of racemisation (\(k_{\text{rac}}\)) of the two isomers of the oxazolone must be faster than the rate of enzyme catalysed ring opening (\(k_{\text{enz}}\)). This in turn must be faster than the rate of non-enzymatic ring opening (\(k_{\text{chem}}\)). It was therefore important to find conditions which to promote \(k_{\text{rac}}\) and \(k_{\text{enz}}\) whilst reducing \(k_{\text{chem}}\)\(^ {133,134}\).

When the lipase Lipozyme\(^ \text{®} \) (*Mucor miehei*) was used in solvent studies by Bevinakatti in 1992\(^ {132}\) it was found that the S-isomer of the protected amino acid was formed. These studies showed dichloromethane to have the greatest enantioselectivity (69% ee), while diisopropyl ether (DIPE) the solvent used in previous studies showed the least selectivity (33% ee). Further studies by Turner in 1995\(^ {135}\) showed that using Lipozyme\(^ \text{®} \) in toluene with 2 equivalents of \(n\)-butanol and 0.25 equivalents of Et\(_3\)N added gave the required product in 69% yield, and 73% ee. Turner *et al* found that Lipozyme\(^ \text{®} \) degraded slowly over time to form an isozyme which competed with the action of Lipozyme\(^ \text{®} \). The addition of Et\(_3\)N reduced the activity of this isozyme leading to overall higher yields.
2.4.3 The Use of Lipozyme® in the Synthesis of AON

The method of Turner et al\textsuperscript{135} was employed for the ring opening of the C-acyl oxazolones and the synthesis of AON. The C-acyl oxazolones were treated with Lipozyme® in a solution of toluene containing n-butanol (2 equivalents) and Et\textsubscript{3}N (0.25 equivalents). The reaction mixture was shaken on an orbital shaker for 72h and purified by preparative layer chromatograph (plc).

Analysis of the purified products revealed the major product to be dibutyl heptanedioate, with the other bands present containing the monoester. This could be the result of an acid type catalysis occurring in the enzymatic reaction. Acid catalysis would cause the same protonation pathway as seen in Figure 2.8, where there is competing hydrolysis at both the carbonyl at C-5 and C-1'. Work by the group of Sih in 1993\textsuperscript{133} found that the reaction rate and enantioselectivity depended greatly on the nature of the C-4 substituent. Previous studies on the enzymatic ring opening of oxazolones has focused on small alkyl groups as substituents at the C-4 position, with Sih finding that the reaction rate decreased markedly for bulky C-4 substituted oxazolones. In the case of 43, 46 and 49 the C-4 substituent is a bulky acyl group, which may not be recognised by the enzyme active site, or may not fit into the enzyme active site. This, in addition to the carbonyl group on the side chain probably account for the formation of dibutyl heptanedioate over AON.

2.5 Conclusions

Having investigated the use of three different oxazolones in the stepwise Dakin-West synthesis of AON it was found that using 4-methyl-2-phenyl oxazolone as the starting material gave AON in 4% overall yield, whilst 4-methyl-2-trifluoromethyl oxazolone gave AON in 7% overall yield and 2,4-dimethyl- oxazolone gave AON in 2% overall yield. The low yield can be attributed to competing hydrolysis routes caused by the presence of a 1,3-dicarbonyl centre, whereby the C-acyl oxazolone can be protonated on either the ring or the side chain carbonyl, resulting in a variety of products. To try to overcome this problem an enzymatic approach was taken to the oxazolone ring opening. This resulted in the formation of dibutyl heptanedioate, and could be the result of the bulky acyl side chain blocking the active site of the enzyme.

To overcome these problems several different approaches could be adopted. It has been reported in the literature that the use of acetic anhydride and pyridine, or anhydrous oxalic acid can prevent the competing hydrolysis. In this instance the most suitable method to employ would be oxalic acid, as amino ketones form stable pyrazine salts in basic conditions. A second approach trialed in the course of this work was to reduce the side chain carbonyl group to the alcohol with
sodium cyanoborohydride, achieving the alcohol in 85% yield. It was thought that the alcohol would be less susceptible to side reactions during hydrolysis and the resulting amino alcohol could be easily oxidised to the amino ketone. Due to time constraints this approach was not taken any further and other route for the synthesis of AON (8) and related compounds were investigated.
3. Nitroaldol Chemistry
3.1 Nitroaldol Reaction in the Synthesis of AON (8)

The nitroaldol reaction provides a versatile approach to a range of nitroalcohols, as the aldehyde and nitroalkane starting materials can be varied depending on the nitroalcohol required. This intermediate can be reduced to form an aminoalcohol, or oxidised to give a nitroketone, which can subsequently be reduced to an aminoketone. It was therefore decided to adopt this approach for the synthesis of 8-amino-7-oxononanoate (AON, 8).

On completion of the synthesis of AON it was anticipated that the compounds would be assayed with the 8-amino-7-oxononanoate (AONS) and 7,8-diaminononanoate (DANS) enzymes. It was hoped that assaying with both AONS and DANS would show product and substrate binding respectively and allow an in depth study on the binding of AON in the substrate binding site of DANS. To create a successful probe for this enzyme it was decided to synthesise a series of compounds related to AON. These compounds were designed to be varying in chain length and either contain a methyl group between the ketone and amino groups, or contain only a methylene group between the two functional groups. It was also decided to test the DANS and AONS enzymes with the aminoalcohols of identical structure.

3.1.1 Henry (Nitroaldol) Reaction

![Diagram of nitroaldol reaction](image)

**Figure 3.1: Nitroaldol (Henry) Reaction**

The nitroaldol or Henry reaction\textsuperscript{137,138} is a classical chain-lengthening reaction using a nitroalkane and a carbonyl compound to form a nitroalcohol (50, Figure 3.1). The versatility of the nitroaldol intermediate (50) makes this reaction a suitable starting point for a number of different transformations, such as nitroketone, aminoalcohol and nitroalkene formation (see Figure 3.2)\textsuperscript{139,140}.

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The Henry reaction is an aldol type reaction and as such relies upon a basic catalyst for generation of the stabilised carbanion intermediate (51, Figure 3.3). Stabilisation of the negative charge results in the formation of the ambident nucleophile (51a, 51b), related to both the nitroalkane and the nitronic acid (aci-nitro species, 52) through nitro-aci nitro tautomerism. It might be expected that carbanion 51 would form an O-alkylated product due to the more electronegative nature of the oxygen end of the molecule, however this is not the case as the sole reaction product is the C-alkylated nitroalcohol 50.

Figure 3.3: Equilibria involved in formation of nitroalcohol (50)

The nitroaldol reaction has been traditionally used to perform syntheses involving simple functional group interconversions (see Figure 3.2) and its progression to utilisation as an efficient and reliable enantioselective transformation has been aided by the availability of new chiral catalysts. These compounds have been used in the synthesis of adrenalin related...
mediators of the sympathetic nervous system\textsuperscript{141,142,143}; the carbohydrate components of anthracycline antibiotics\textsuperscript{144}, HIV protease inhibitors\textsuperscript{145,146} and azole antifungal agents\textsuperscript{147}.

3.1.2 General Catalysts

The success of the nitroaldol reaction depends on the choice of reaction conditions and catalyst employed. The choice of these is dependant on several factors such as the functional groups present; the solubility of the reactants and the ease of generation of the nitronate salt.

Early studies on the nitroaldol reaction used strongly basic conditions, using alkoxides and hydroxides in alcohol or aqueous media as catalysts. More recently organic bases such as 1,1,3,3-tetramethylguanidine (TMG) in ether and THF; and amines such triethylamine and diisopropylamine in alcohol have been used as catalysts. Current work has concentrated on the development of new catalysts to reduce side reactions (e.g. dehydration of the nitroalcohol); to prevent the formation of normal aldol by-products; to avoid epimerisation of centres remote from the nitro group and to reduce by-products from Nef-type reactions.

![Figure 3.4: Proazaphosphatran catalysts developed by Kisanga and Verkade](image)

To reduce self-condensation reactions where ketones are used as the carbonyl substrate Kisanga and Verkade have developed a series of proazaphosphatranes catalysts\textsuperscript{148} (53-55, see Figure 3.4). The undesirable self-condensation is reduced as the proazaphosphatranes exist as the protonated complex 56, forming a complex with the desired nitroalkane. To optimise the reaction conditions Kisanga and Verkade included magnesium sulfate in the reagent mixture to act as a Lewis acid and activate the carbonyl group.

Recent work has focussed on the development of solvent-free and aqueous phase reactions. Ballini has established conditions for both of these types of reactions using the phase transfer catalyst cetyltrimethylammonium chloride to promote the reaction in aqueous conditions\textsuperscript{149} and Amberlyst A-21\textsuperscript{150} as the catalyst for the solvent free reaction of a series of aldehydes with 4-nitro-2-butanol. Other solvent free reactions have used microwave irradiation with ammonium acetate\textsuperscript{151} or powdered KOH\textsuperscript{152} as the chemical catalysts for the reaction.
3.1.3 Asymmetric catalysts

The driving force for the development of asymmetric catalysts for the nitroaldol reaction has been the requirement for new routes to chiral aminoalcohols. These can be readily formed by the reduction of the nitroalcohol from the nitroaldol reaction, and various syntheses of complex natural products have been based on this strategy (see section 3.1).

Shibasaki has developed Binol catalysts (57, 58, Figure 3.5) that have been used in the synthesis of several compounds of pharmaceutical interest. In their synthesis of the β-adrenoreceptor antagonist propanolol the Shibasaki group successfully used a lanthanum-(R)-binaphthol complex derived from 57 to catalyse the formation of 60. Their procedure was further modified in their synthesis of the β-adrenergic agent R-(−)-arbutamine, where the binol derivative 58 was used to form the intermediate 61. Corey has used the chiral quaternary salt 59 (Figure 3.5) to catalyse the formation of the nitroaldol intermediate (62) required for the synthesis of the HIV protease inhibitor Amprenavir. Menzel et al found that the catalyst giving the most favourable ratio of the desired L-arabino isomer of the nitroaldol 63: an intermediate in the synthesis of L-acosamine, the carbohydrate unit of anthracycline antibiotics; was a mixture of tetra-N-butyl ammonium fluoride (TBAF) and methyl-tert-butyl ether (MTBE). A summary of conditions employed for the use of these catalysts is shown in Table 3.1.
Chapter 3

Nitroaldol Chemistry

Table 3.1: Examples of asymmetric nitroaldol catalysts

<table>
<thead>
<tr>
<th>Nitroaldol</th>
<th>Target Compound</th>
<th>Catalyst Mix</th>
<th>Yield (%)</th>
<th>e.e. (%)</th>
<th>Diastereoselectivity</th>
<th>Ref.</th>
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<tr>
<td></td>
<td></td>
<td>Dilithium-52</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>oYNO₂S-(-)-Propanolol</td>
<td>LaCl₃.7H₂O, NaOtBu, H₂O/THF</td>
<td>80</td>
<td>92</td>
<td>141</td>
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<td></td>
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<td>R-(-)-Arbutamine</td>
<td>53</td>
<td>93</td>
<td>142</td>
<td></td>
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<tr>
<td></td>
<td>Amprenavir</td>
<td>Sm(O'Pr)₃, H₂O, n-BuLi</td>
<td>93</td>
<td>92</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L-Acosamine</td>
<td>Oxidation/Reduction</td>
<td>95</td>
<td></td>
<td>27:62:3:8</td>
<td></td>
</tr>
</tbody>
</table>

3.1.4 The Synthesis of AON (8)

The nitroaldol reaction requires an aldehyde and a nitroalkane as its starting material. The desired nitroalkanes: nitroethane and nitromethane are readily available from a variety of commercial sources, however the desired aldehydes were not commercially available. To this end a series of aldehydes were synthesised and reacted with nitroethane and nitromethane to form the desired nitroalcohols. These were subsequently oxidised to form nitroketones and the nitro group reduced to the aminoketone, or the nitro group of the nitroalcohol reduced to form the desired aminoalcohol.
3.2 Aldehyde Synthesis

The aldehyde ethyl 7-oxoheptanoate (64) is pivotal for the formation of AON via the nitroaldol reaction. As such its synthesis was carefully investigated and five approaches evaluated: Baeyer-Villiger oxidation of cycloheptanone; Rosenmund reduction of ethyl-6-chlorocarbonylhexanoate; aluminium hydride reduction of ethyl-6-chlorocarbonylhexanoate, using sodium tri-t-butoxyaluminohydride; and tin hydride reduction of ethyl-6-chlorocarbonylhexanoate. These are outlined in Figure 3.6, and discussed in detail below.

3.2.1 Baeyer-Villiger Oxidation of Cycloketones

The Baeyer-Villiger reaction is the oxidation of ketones with peroxy acids to form esters or lactones. The two-step mechanism (see Figure 3.7) proceeds via initial addition of the peroxy acid to the carbonyl of the ketone, forming the intermediate 65. The second step is
usually rate determining and involves the migration of the R’ group from carbon to oxygen and the breaking of the oxygen-oxygen bond in a concerted process.

\[ \text{K}_2\text{S}_2\text{O}_8, \text{H}_2\text{SO}_4, \text{EtV} \rightarrow \text{POC, DCM} \]  

Figure 3.8: Baeyer-Villiger oxidation of cycloheptanone to form ethyl 7-oxoheptanoate (64)

The Baeyer-Villiger reaction has wide ranging use\textsuperscript{153}, and was adopted for the synthesis of ethyl 7-hydroxyheptanoate (66) by Robinson and Smith\textsuperscript{154} as early as 1937, and later used for the synthesis of ethyl 7-oxoheptanoate (64) by Ballini\textsuperscript{155} in 1991 (see Figure 3.8).

Oxidation of cycloheptanone with potassium persulfate in ethanol and sulfuric acid affords a lactone intermediate, which undergoes ring opening to give ethyl 7-hydroxyheptanoate (66). It could be conjectured that the alcohol can then be oxidised using PCC or PDC in DCM to give ethyl 7-oxoheptanoate (64). In their work Robinson and Smith also reported the synthesis of ethyl-6-hydroxyhexanoate and ethyl-5-hydroxypentanoate in acceptable yields.

### 3.2.1.1 Baeyer-Villiger synthesis of ethyl 7-hydroxyheptanoate (66) and related compounds

The successful use of the Baeyer-Villiger reaction in the synthesis of aldehydes by Ballini et al and Robinson and Smith gave an indication that this might be a useful route to the aldehydes required. It was therefore decided to adopt this method for the synthesis of a range of aldehydes to be used in the nitroaldol reaction.

The oxidation of four cycloketones: cycloheptanone, cyclooctanone, cyclohexanone and cyclopentanone was investigated. Typically the cycloketone in ethanol were added drop-wise to a solution of potassium persulfate, sulfuric acid, water and ethanol at 15°C, following the procedure of Ballini\textsuperscript{155}. Ballini et al suggest purification by distillation, however in our hands it was found that distillation was unsuccessful, but that purification of the products could be achieved by silica gel chromatography. The yields of ethyl 7-hydroxyheptanoate (66), ethyl 8-hydroxyoctanoate (67), ethyl 6-hydroxyhexanoate (68) and ethyl 5-hydroxypentanoate (69) obtained are shown in Table 3.2.
Table 3.2: Yields of the Baeyer-Villiger synthesis of 66 and related compounds\textsuperscript{155}.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Starting Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td><img src="image" alt="Structure" /></td>
<td>22</td>
<td>Cycloheptanone</td>
</tr>
<tr>
<td>67</td>
<td><img src="image" alt="Structure" /></td>
<td>37</td>
<td>Cyclooctanone</td>
</tr>
<tr>
<td>68</td>
<td><img src="image" alt="Structure" /></td>
<td>33</td>
<td>Cyclohexanone</td>
</tr>
<tr>
<td>69</td>
<td><img src="image" alt="Structure" /></td>
<td>28</td>
<td>Cyclopentanone</td>
</tr>
</tbody>
</table>

In their original study on the formation of 66 from cycloheptanone Robinson and Smith found that the yields obtained are sensitive to reaction conditions, especially temperature\textsuperscript{154}. The reactions were therefore repeated taking care to ensure a maximum temperature of 15°C throughout, however this did not greatly improve the yields. In their original study Robinson and Smith achieved a maximum yield of ca. 50%, although this was improved by Ballini \textit{et al} who reported yields of 85%. A possible explanation for the low reaction yield in our hands could be the production of a side product giving off a strong odour of pineapple which has been isolated in the past\textsuperscript{154}. Robinson and Smith suggested that this compound was a dilactone with a 16-membered ring, related to 7-hydroxheptoic acid; based on the work of Natta \textit{et al}\textsuperscript{156} on large dilactones and Ruzicka \textit{et al}\textsuperscript{157} on the relation between odour and large ring dilactones. Despite the attraction of characterising this elusive compound I avoided the temptation to pursue this further.

### 3.2.1.2 Synthesis of ethyl 7-oxoheptanoate (64) and related compounds

After numerous trials ethyl 7-oxoheptanoate (64), ethyl 8-oxooctanoate (70), ethyl 6-oxohexanoate (71) and ethyl 5-oxopentanoate (72) were synthesised by oxidation of their respective hydroxy esters. The best procedure proved to involve adding a solution of hydroxy ester in DCM drop-wise to a stirring solution of pyridinium dichromate (PDC) in DCM, cooled to 5°C. The products were routinely purified by silica gel chromatography. While this approach appeared to be straightforward, considerably difficulty was encountered.
Table 3.3: Oxidation of hydroxy esters to form Ethyl 7-oxoheptanoate (64) and related compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)</th>
<th>Starting Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td><img src="image" alt="Structure" /></td>
<td>51</td>
<td>11</td>
<td>66</td>
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<tr>
<td>70</td>
<td><img src="image" alt="Structure" /></td>
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<td>15</td>
<td>67</td>
</tr>
<tr>
<td>71</td>
<td><img src="image" alt="Structure" /></td>
<td>46</td>
<td>15</td>
<td>68</td>
</tr>
<tr>
<td>72</td>
<td><img src="image" alt="Structure" /></td>
<td>42</td>
<td>12</td>
<td>69</td>
</tr>
</tbody>
</table>

In achieving satisfactory yields, as shown in Table 3.3. $^1$H NMR confirmed the structures of compounds 64, 70, 71 & 72. Each $^1$H nmr spectrum exhibited an: ethyl ester methyl triplet and methylene quartet at 1.2ppm and 4.1ppm; -CH$_2$CHO methylene triplet at 2.2-2.5ppm; and the aldehyde proton (-CHO) singlet at 9.8ppm.

In an effort to improve this procedure PDC was substituted by pyridinium chlorochromate (PCC), however this was unsuccessful. Incomplete oxidation of starting material was responsible for the low yields of 64, 70, 71 and 72, and efforts to increase the yields by increasing reaction temperature proved futile, resulting in the formation of the corresponding acids.

In light of the low yield experienced from the Baeyer-Villiger oxidation to form the hydroxy ester starting material it was decided not to optimise this reaction, and methods of aldehyde formation from acid chlorides$^{158}$ were investigated.
3.2.2 Rosenmund Reduction of Acid Chlorides

The Rosenmund reaction, first described in 1918\textsuperscript{159}, is one of the most direct methods for the conversion of acid chlorides to aldehydes. Typically the Rosenmund reaction is carried out by bubbling hydrogen through a hot suspension of the acid chloride and supported palladium catalyst. A major limitation of this procedure is over-reduction of the acid chloride, forming the corresponding alcohol. This can be avoided by the addition of a suitable catalyst poison, to inactivate the catalyst\textsuperscript{160}. Temperature plays an important role in the Rosenmund reaction, with optimal yields achieved if the temperature is kept near the lowest temperature required for hydrogen chloride evolution\textsuperscript{160}.

The Rosenmund reaction was developed by Sakurai and Tanabe\textsuperscript{161} who found that the reaction could be carried out at room temperature using acetone as a solvent if N,N-dimethylaniline was used as a hydrogen chloride acceptor. It was later found that N,N-dimethylaniline was itself reduced under the reaction conditions and Peters and van Bekkum refined the process using ethylidisopropylamine as the hydrogen chloride acceptor\textsuperscript{162}.

3.2.2.1 Rosenmund Reduction of Ethyl 6-chlorocarbonylhexanoate (74)

As a test case, to evaluate the Rosenmund procedure ethyl 7-oxoheptanoate (64) was synthesised from ethyl 6-chlorocarbonylhexanoate (74). Ethyl 6-chlorocarbonylhexanoate (74) was prepared in 93% yield from ethyl heptanedioate (73) and oxalyl chloride. Freshly distilled 74 was added to a pre-hydrogenated suspension of palladium on charcoal in acetone and diisopropylethylamine, and stirred in a hydrogen atmosphere for 1h according to the procedure of Peters and van Bekkum\textsuperscript{162}. Aqueous work up and distillation of the product (bp\textsubscript{0.02} = 79°C) afforded ethyl 7-oxoheptanoate (64) in a disappointing 26% yield. Repetition of this procedure gave erratic results, with yields varying greatly, but never greater than 26%. It has been noted by others that over reduction is a common problem with the
Rosenmund reaction\textsuperscript{160}, however this problem was found to be minimal (always less than 1\%) using the method of Peters and van Bekkum\textsuperscript{163}. A possible explanation for the low yields experienced here could be product polymerisation during distillation, resulting in some of the dark residues recovered.

### 3.2.3 Sodium tri-\textit{t}-butoxyaluminohydride reduction of Ethyl 6-chlorocarbonylhexanoate (74)

In the course of a study on the reducing characteristics of sodium aluminium hydride derivatives Brown and Cha\textsuperscript{164} found that sodium tri-\textit{t}-butoxyaluminohydride (STBA) gave facile and efficient reduction of acid chlorides to aldehydes as shown in Figure 3.11.

![Figure 3.11: STBA reduction of ethyl-6-chlorocarbonylhexanoate (74) to form (64)](image)

In the initial trails STBA was prepared by reacting sodium aluminium hydride in diglyme with 3 equivalents of \textit{t}-butanol at 0\(^\circ\)C for 6h. The reagent was then used to effect the reduction of ethyl-6-chlorocarbonylhexanoate (74) to ethyl 7-oxoheptanoate (64). The procedure involved drop-wise addition of STBA in diglyme to a cooled (-78\(^\circ\)C) solution of 74 in dry THF. Purification by silica gel chromatography and tlc analysis of the crude reaction mixture from this procedure revealed the sole product to be ethyl 7-oxoheptanoate (64), however purified yields of 64 were never more than 27\%. This was due to difficulties in separating the product form the diglyme. Several approaches to this problem were attempted: repeated extraction with water did remove the diglyme, but also caused some decomposition of 64; purification by silica gel chromatography was partially successful; and distillation proved unsuccessful resulting only in a reduced volume of diglyme present with the product. Although this concept was successful in concept, showing the only aldehyde present by tlc (DNP staining), the difficulty in removal of the solvent without adversely affecting the yield of aldehyde proved impossible to overcome and other more practical approaches had to be studied.
3.2.4 Organotin Hydride Reduction of Acid Chlorides

The next logical step for the synthesis of ethyl 7-oxoheptanoate (64) was to employ tin hydride chemistry. Organotin hydrides have been known to reduce acid chlorides since the early 1970s, however the original reaction conditions were not suitable for the formation of aldehydes due to the competing formation of esters (see Figure 3.12). The reaction was modified in the early 1980s by the addition of a tetrakis(triphenylphosphine)palladium catalyst, and this was found to be completely selective towards the formation of aldehydes. Detailed studies on this modified procedure found that it could be applied to a wide range of acid chlorides - both aliphatic and aromatic, and that the reaction tolerated the presence of a wide range of reducible functionalities.

3.2.4.1 Mechanism of Action

It was originally thought that the tin hydride mediated reduction of acid chlorides had a free radical mechanism. In the case of the palladium mediated reduction this route has been eliminated and Four and Guibe have proposed two plausible mechanisms both of which involve the acylhydridopalladium (II) complex (75, Figure 3.13). The first pathway (AB, Figure 3.13) involves the oxidative addition of the acid chloride to (PPh₃)₄Pd, forming the acylchloropalladium (II) complex (76), this is followed by a metathesis reaction between 76 and Bu₃SnCl giving Bu₃SnCl and 75. The first step in the second pathway (CD, Figure 3.13)
is the oxidative addition of Bu$_3$SnH to (PPh$_3$)$_4$Pd giving 77, a subsequent metathesis reaction of this with the acid chloride gives rise to 75. In both pathways the aldehyde is formed by reductive elimination from 75.

### 3.2.4.2 Synthesis of Ethyl 7-oxoheptanoate (64) and related compounds

![Figure 3.14: Tin hydride reduction of ethyl-6-chlorocarbonylhexanoate (79) to form (64)](image)

Ethyl 7-oxoheptanoate (64) was prepared from ethyl 6-chlorocarbonylhexanoate (74) using the procedure of Four and Guibe$^{167}$ (see Figure 3.14). Other aldehydes were prepared from their corresponding acid chlorides. Ethyl 6-chlorocarbonylhexanoate (74); methyl 5-chlorocarbonylpentanoate (78); and ethyl 7-chlorocarbonylheptanoate (79) were routinely prepared in good yield by heating the parent acid at reflux with oxalyl chloride in DCM. The acid chlorides were purified by Kugelrohr distillation (for yields see Table 3.4) and the structures were confirmed by $^1$H NMR evidence. The ethyl ester groups of 74 and 79 gave a triplet at 1.2ppm (CH$_3$CH$_2$) and a quartet at 4.1ppm (-CH$_2$); the methyl ester group of 75 gave a singlet at 3.7ppm; the CH$_2$COCl methylene protons gave a triplet at 2.9ppm. It was found that all the acid chlorides could be stored at -20°C for up to 4 weeks without significant decomposition.

### Table 3.4: Yields of formation of ethyl 6-chlorocarbonylhexanoate (74), and related acid chlorides (78 & 79).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>bp (°C, mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
<td><img src="image" alt="Structure 74" /></td>
<td>93</td>
<td>90 (0.04)</td>
</tr>
<tr>
<td>78</td>
<td><img src="image" alt="Structure 78" /></td>
<td>96</td>
<td>50 (0.02)</td>
</tr>
<tr>
<td>79</td>
<td><img src="image" alt="Structure 79" /></td>
<td>91</td>
<td>100 (0.02)</td>
</tr>
</tbody>
</table>

Mhairi I. Brunton
August 2003
Ethyl 7-oxoheptanoate (64) was prepared by adding Bu₃SnH (1.1 equivalents) to a suspension of ethyl 6-chlorocarbonylhexanoate (74) and (PPh₃)₄Pd (0.01 equivalents) under N₂. The reaction proceeded rapidly and was found to be complete within 1h. The catalyst was removed by initial evaporation of the bulk of the solvent followed by precipitation with hexane and filtration. The aldehyde was purified by Kugelrohr distillation (bp₀.₀₂ = 79°C) and could be stored at -20°C for up to 2 weeks.

Ethyl 4-oxobutanoate (80); methyl 6-oxohexanoate (81); and ethyl 8-oxooctanoate (70) were prepared in a similar manner starting from ethyl succinyl chloride, methyl 5-chlorocarbonylpentanoate (78); and ethyl 7-chlorocarbonylheptanoate (79) respectively. The yields and boiling points of these compounds are shown in Table 3.5. It was found that distillation afforded pure aldehydes, and NMR characterisation showed the presence of the aldehyde proton (⁻CHO) at 9.8ppm (singlet), and the (⁻CH₂CHO) methylene protons at 2.4ppm (triplet). The presence of the ethyl ester groups was shown by signals at ca. 1.2ppm (triplet) and ca. 4.1ppm (quartet) corresponding to the methyl and methylene protons, and the methyl ester group was shown by a signal at 3.7ppm (singlet). No tributyltin chloride residues were present in the purified compounds.

Table 3.5: Yields of formation of the ethyl 7-oxoheptanoate (64) and related aldehydes (80, 81 & 70)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)*</th>
<th>bp (°C, mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td><img src="image" alt="Structure" /></td>
<td>74</td>
<td>69</td>
<td>79 (0.02)</td>
</tr>
<tr>
<td>80</td>
<td><img src="image" alt="Structure" /></td>
<td>80</td>
<td>80#</td>
<td>60 (0.02)</td>
</tr>
<tr>
<td>81</td>
<td><img src="image" alt="Structure" /></td>
<td>83</td>
<td>80</td>
<td>65 (0.02)</td>
</tr>
<tr>
<td>70</td>
<td><img src="image" alt="Structure" /></td>
<td>87</td>
<td>79</td>
<td>95 (0.02)</td>
</tr>
</tbody>
</table>

* From starting acid, # from ethyl succinyl chloride
3.3 Nitroalcohol Synthesis

The nitroaldol reaction can be catalysed by a number of different reagents as shown in sections 3.1.2 and 3.1.3, (pp.45). Trial reactions using aldehyde and nitroalkane supported on silica\(^\text{168}\) or alumina\(^\text{169,170}\) yielded nitroalcohol, however on scale up it was found that incomplete use of starting materials and dehydration of the product gave low yields of nitroalcohol. This coupled with problems experienced isolating the product from the solid support meant that other methods for the synthesis of nitroalcohols were investigated.

3.3.1 Synthesis of ethyl 7-hydroxy-8-nitrononanoate (82) and related compounds

Wollenberg and Miller studied the nitroaldol reaction as a method of synthesising nitroalkanes in 1978\(^\text{171}\). Their work found that bases previously reported as catalysts failed to initiate any reaction between octanal and nitromethane. They found however, that potassium fluoride and potassium hydroxide in iso-propanol could be used successfully to synthesise nitroalcohols, which were subsequently acylated and reduced to give the desired nitroalkane. It was therefore decided to use powdered KOH in iso-propanol as the catalyst for the synthesis of aliphatic nitroalcohols as intermediates in the synthesis of a series of aminoketones including 8-amino-7-oxononanoate (8).

Ethyl 7-hydroxy-8-nitrononanoate (82, Figure 3.15) was synthesised using the method of Wollenberg and Miller: ethyl 7-oxoheptanoate (64) was added solution of nitroethane and powdered KOH (0.05 equivalents) in iso-propanol, and the reaction mixture stirred for 6h. Precipitation of the catalyst and filtration of the suspension gave ethyl 7-hydroxy-8-nitrononanoate (82) which was purified by silica gel chromatography in a yield of 95%. The related compounds: ethyl 4-hydroxy-5-nitrohexanoate (83), methyl 6-hydroxy-7-nitrooctanoate (84) and ethyl 8-hydroxy-9-nitrodecanoate (85) were synthesised in an analogous manner, with the yields shown in Table 3.6. Characterisation of compounds 82-85
by $^1$H NMR confirmed the anticipated structures: the compounds typically showed an ethyl ester triplet at 1.2ppm and quartet at 4.1ppm (82, 83 & 85); a methyl ester singlet at 3.7ppm (84); a multiplet at 3.7-4.5ppm [-CH(OH)-], a multiplet at 4.5ppm [-CH(NO$_2$)CH$_3$] and a doublet at 1.6ppm (-CHCH$_3$).

Table 3.6: Yields of nitroaldols 82, 83, 84 & 85 from the reaction of aldehydes 64, 80, 81 & 70 with nitroethane

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>82</td>
<td><img src="image" alt="Structure" /></td>
<td>95</td>
<td>66</td>
</tr>
<tr>
<td>83</td>
<td><img src="image" alt="Structure" /></td>
<td>88</td>
<td>70*</td>
</tr>
<tr>
<td>84</td>
<td><img src="image" alt="Structure" /></td>
<td>89</td>
<td>71</td>
</tr>
<tr>
<td>85</td>
<td><img src="image" alt="Structure" /></td>
<td>70</td>
<td>55</td>
</tr>
</tbody>
</table>

* From starting acid, # from ethyl succinyl chloride

Figure 3.16: Nitroaldol reaction of ethyl 7-oxoheptanoate (64) and nitromethane to form ethyl 7-hydroxy-8-nitrooctanoate (86)

The nitromethane analogues of compounds 82-85: ethyl 7-hydroxy-8-nitrooctanoate (86, see Figure 3.16); ethyl 4-hydroxy-5-nitro pentanoate (87); methyl 6-hydroxy-7-nitro heptanoate (88); and ethyl 8-hydroxy-9-nitro nonanoate (89) were subsequently synthesised in the same manner as above, and the yields are shown in Table 3.7. The structures of compounds 86-89.
Table 3.7: Yields of nitroaldols 86, 87, 88 & 89 from the reaction of aldehydes 64, 80, 81 & 70 with nitromethane

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>86</td>
<td>![Structure 86]</td>
<td>92</td>
<td>63</td>
</tr>
<tr>
<td>87</td>
<td>![Structure 87]</td>
<td>90</td>
<td>72</td>
</tr>
<tr>
<td>88</td>
<td>![Structure 88]</td>
<td>86</td>
<td>69</td>
</tr>
<tr>
<td>89</td>
<td>![Structure 89]</td>
<td>82</td>
<td>65</td>
</tr>
</tbody>
</table>

* From starting acid, \(^9\) from ethyl succinyl chloride.

were also confirmed by \(^1\)H NMR and each spectrum showed: an ethyl ester methyl triplet at ca. 1.2ppm (-CH\(_3\)) and methylene quartet at ca. 4.1ppm (-CH\(_2\)CH\(_3\)) (86, 89 & 88) or a methyl ester singlet at 3.7ppm in the case of 87; and a multiplet at 4.6ppm (for the -CHOH & -CH\(_2\)NO\(_2\) groups).

By employing the method of Wollenberg and Miller the nitroalcohols 82-85 were formed in 70-95% yield, giving acceptable overall yields in the range 55-71% from the starting acids (see Table 3.6). The yield of ethyl 8-hydroxy-9-nitrodecanoate (85) was low in comparison to the other compounds in the series: analysis of the reaction mixture by tlc showed that this was due to partial decomposition of 85 by dehydration to the nitroalkene ethyl 9-\(^-\)nitrodecenoate, a widely reported by-product of the nitroaldol reaction\(^{139}\). The nitromethane analogue ethyl 8-hydroxy-9-nitro nonanoate (89) was synthesised with a reaction time of 1h, and it can be seen in Table 3.7 that although this condensation has a lower yield than the other compounds in the series, the yield is higher than that experienced for the formation of 85. The series of nitroketones 86-89 were synthesised in yields of 82-92%, giving overall yields of 63-72% fro the starting acids (as shown in Table 3.7).
3.4 Nitroketone Synthesis

The next step required for the synthesis of AON, and related aminoketones was oxidation of nitro alcohols 82-89 to the corresponding nitroketones. The majority of studies on the oxidation of nitroalcohols to nitroketones have used chromium (VI) reagents. The earliest research by Levy and Scaife 172 and Hurd and Nilson 173 used sodium dichromate in sulfuric acid as the oxidant for the synthesis of simple α-nitroketones. Later research has used milder conditions such as PCC in dichloromethane at room temperature 174. It was found that this system gave good yields of α-nitroketones, typically 65%-87%, and that the conditions employed were mild enough to leave other functional or protecting groups unaltered. A drawback of this system are the long reaction times: typically 1.5 equivalents of PCC are added to the nitroalcohol in DCM and the mixture stirred for 24h at room temperature before addition of another 0.75 equivalents of oxidant and the mixture stirred for a further 12h. Oxidation of nitroalcohols has been carried out using phase transfer catalysis (PTC) 175. Rosini et al oxidised a series of nitroalcohols by the addition of potassium dichromate or potassium chromate in 30% sulfuric acid to a solution of the nitroalcohol and tetra-n-butylammonium hydrogen sulfate (0.1mol per mol nitroalcohol) in DCM. It was found that these reactions proceeded smoothly, giving good yields after 2h; the study also found that acid labile protecting groups were left intact. In subsequent studies Santaniello 176 found that chromic acid (H₂CrO₄) supported on silica gel could be used for the oxidation of hydroxy compounds, however this catalyst has low stability and has not been used for the oxidation of nitroalcohols. In their study on the synthesis of nitroalcohols and nitroketones on solid support Mélot et al 169 found that although alumina supported CrO₃ gave low yields of nitroketones from nitroalcohols, while montmorillonite K10 supported CrO₃ gave simple nitroketones from nitroalcohols in good yields. The addition of the supported reagent to the nitroalcohol can cause spontaneous inflammation, but by adding the nitroalcohol to a suspension of montmorillonite reagent in DCM at -15°C they found that the exothermic reaction could be controlled to give yields of 76%-93% after 3.5h. A subsequent study by Ballini et al 168 found that wet alumina supported CrO₃ could be used for the in situ solvent free oxidation of supported nitroalcohols. Their procedure used neutral alumina to catalyse the formation of the desired nitroalcohol.

On the basis of work by Ballini et al and Mélot et al it was decided to carry out a series of trial reactions using both supported and solution phase chromium reagents for the oxidation of compounds 82-89. The first trial used wet alumina supported CrO₃ 168 added to 82, and...
although this method gave a reasonable yield of nitroketone it was found that the results were difficult to reproduce, and product isolation proved difficult. The second trial used montmorillonite supported chromium trioxide which was added to 82 at -15°C. This method gave an acceptable yield of nitroketone (ca. 60%), but again problems were experienced in extracting the product from solid oxidant. Trials using PCC and PDC in DCM gave good yields of nitroketone, however the most successful reagent proved to be Jones’ reagent. It was therefore decided to use this as the method for the oxidation of compounds 82-89.

3.4.1 Synthesis of ethyl 8-nitro-7-oxononanoate (90) and related compounds

![Figure 3.17: Jones' Oxidation of ethyl 7-hydroxy-8-nitrononanoate (82) to form ethyl 8-nitro-7-oxononanoate (90)](image)

Ethyl 8-nitro-7-oxononanoate (90) was synthesised by the Jones’ oxidation of ethyl 7-hydroxy-8-nitrononanoate (82, see Figure 3.17), affording after purification ethyl 8-nitro-7-oxononanoate (90) in 91% yield. The related nitroketones: ethyl 5-nitro-4-oxohexanoate (91); methyl 7-nitro-6-oxooctanoate (92); and ethyl 9-nitro-8-oxodecanoate (93) were synthesised in an identical manner and the yields are shown in Table 3.8. Characterisation of the nitroketones 90, 91, 92 & 93 by 1H NMR showed the presence of all the desired functional groups: an ethyl ester triplet at ca. 1.2ppm (-CH₃) and quartet at ca. 4.0ppm (-CH₂-) (for 90, 91 & 93); methyl ester singlet at 3.7ppm (for 92); all four compounds showed a doublet at 1.6ppm (-CHCH₃) and a quartet at 5.2ppm (-CH(CH₃)NO₂).
Table 3.8: Yields of the nitroketones 90, 91, 92 & 93 from the Jones’ oxidation of the corresponding nitroalcohols (82, 83, 84 & 85).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)*</th>
</tr>
</thead>
<tbody>
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<td>60</td>
</tr>
<tr>
<td>91</td>
<td><img src="image" alt="Structure 91" /></td>
<td>89</td>
<td>62#</td>
</tr>
<tr>
<td>92</td>
<td><img src="image" alt="Structure 92" /></td>
<td>88</td>
<td>62</td>
</tr>
<tr>
<td>93</td>
<td><img src="image" alt="Structure 93" /></td>
<td>87</td>
<td>48</td>
</tr>
</tbody>
</table>

* From starting acid, # from ethyl succinyl chloride

Figure 3.18: Jones’ Oxidation of ethyl 7-hydroxy-8-nitrooctanoate (86) to form ethyl 8-nitro-7-oxooctanoate (94)

The nitromethane analogues of compounds 90-93: ethyl 8-nitro-7-oxooctanoate (94, Figure 3.18); ethyl 5-nitro-4-oxopentanoate (95); methyl 7-nitro-6-oxohexanoate (96); and ethyl 9-nitro-8-oxononanoate (97) were synthesised in the same manner using the nitroalcohols 86, 87, 88 & 89 as starting materials. $^1$H nmr characterisation of these compounds showed: the expected ethyl ester methyl triplet at ca. 1.2ppm (-CH$_3$) and methylene quartet at ca. 4.1ppm (-CH$_2$CH$_3$) in compounds 94, 95 & 97; the methyl ester singlet at 3.7ppm in 96; and the (-CH$_2$NO$_2$) singlet at ca. 5.3ppm in all four nitroketones.
Table 3.9: Yields of the nitroketones 94, 95, 96 & 97 from the Jones' oxidation of the corresponding nitroalcohols (86, 87, 88 & 89).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)*</th>
</tr>
</thead>
<tbody>
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<td><img src="image" alt="" /></td>
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<td>95</td>
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<td>63</td>
</tr>
<tr>
<td>97</td>
<td><img src="image" alt="" /></td>
<td>90</td>
<td>59</td>
</tr>
</tbody>
</table>

* From starting acid, * from ethyl succinyl chloride

It has been shown above that although previous studies have oxidised nitroalcohols to nitroketones in good yield (61% or greater), the methods used have involved long reaction times, intricate procedures or supported reagents. By changing the oxidant for the reaction to Jones reagent the reaction procedure can be simplified and the yields improved. The reaction using Jones’ reagent requires a simple titration of reagent onto a solution of the nitroalcohol in acetone, thus avoiding complex procedures, or extraction of the nitroketone product from a solid support. A further advantage in using Jones’ reagent is the shortening of reaction times: with the substrates used the reaction time never exceeded 1h. It can be seen in Table 3.8 and Table 3.9 the yields for the Jones reagent mediated oxidation are not only higher, but the range of yields are narrower than previous studies (see above). Jones’ reagent therefore provides a simple and high yielding method for the oxidation of nitroalcohols to nitroketones.
3.5 Aminoketone & Aminoalcohol Synthesis

The final step in the synthesis of AON and its related aminoketones and aminoalcohols was reduction of the nitro group of the parent nitroketones and nitroalcohols. In principle the reduction of aromatic nitro compounds to amines can be effected under mild reaction conditions and without complication using a number of different reducing agents. Although the mechanism has not been greatly studied it is presumed that that nitroso and hydroxylamine intermediates are involved (see Figure 3.19) as treatment of these compounds with reducing agents gives amines. It has been suggested that the mechanism of metal and acid mediated reduction follows the pathway shown in Figure 3.19.

![Figure 3.19: Reduction of aromatic nitro compounds to amines](image)

A different problem lies in the reduction of aliphatic nitro compounds, and a number of studies on their reduction have been undertaken. In preliminary studies it was found that the rate of reduction of aliphatic nitro compounds is considerably slower than that of aromatic nitro compounds, and that the reaction products can poison the reduction catalyst. It was found that the successful reduction of aliphatic nitro compounds required high catalyst loading, vigorous conditions or long reaction times.

The main obstacle in the reduction of nitro compounds comes not from the conditions required for the nitro reduction, but from the presence of other functional groups, which must be retained or reduced to afford the desired compound. Most hydrogenation catalysts were found to be unsuitable for the selective reduction of the nitro group of nitroketones, as they first reduce the carbonyl group, and often promote the cyclisation of the aminoketones formed. It was found that the Mannich bases derived from \( p \)-nitroacetophenone could be successfully reduced to the corresponding aminoketones using 5% palladium on charcoal in methanol as the catalyst. The selective reduction of the nitro functional group in aromatic nitroketones is thought to be the result of preferential adsorption of the nitro group to the
catalyst\textsuperscript{181}, with cyclisation only occurring of the carbonyl and nitro groups are spatially proximate\textsuperscript{183}.

In 1986 Tamura \textit{et al} found that hydrogenating $\alpha$-nitroketones over 5\% platinum sulfide on carbon (Pt-S-C) effected the chemoselective reduction of the nitro group, forming an $\alpha$-aminoketone hydrochloride\textsuperscript{184}. In a study of the scope of this reaction the group isolated $\alpha$-aminoketone hydrochlorides in good yields from a number of different $\alpha$-nitroketone starting materials. It was therefore decided to use this method for the synthesis of 8-amino-7-oxononanoate (8) and its related compounds.

### 3.5.1 Synthesis of 8-amino-7-oxononanoate (8) and related aminoketones

![Chemical Structure](image)

8-Amino-7-oxononanoate (8) was synthesised from ethyl 8-nitro-7-oxononanoate (90) using the method of Tamura \textit{et al}\textsuperscript{184} (see Figure 3.20). Ethyl 8-nitro-7-oxononanoate (90) in ethanol-\textit{c}.HCl (20:1, \textit{v}/\textit{v}) was added to a suspension of 5\% Pt-S-C in ethanol-\textit{c}.HCl (20:1, \textit{v}/\textit{v}) and the reaction mixture flushed with hydrogen three times before hydrogenation at 50°C for 16h. After removal of the catalyst by filtration the crude aminoketone was taken up in aqHCl (5M) and stirred at 50°C for a further 4h to remove any residual ester protecting group. Purification of the crude product by ion exchange chromatography (DOWEX-50, H\textsuperscript{+} form) gave the desired product: 8-amino-7-oxononanoate (8) in 68\% yield. The related compounds: 5-amino-4-oxohexanoate (98), 7-amino-6-oxooctanoate (99), and 9-amino-8-oxodecanoate (100); were synthesised in the same manner using the corresponding $\alpha$-nitroketones. The yields of these are shown in Table 3.10. Characterisation of this series of compounds by $^1$H nmr confirmed the anticipated structures, with the presence of the methyl (-CH$_3$) protons as a doublet at \textit{ca}. 1.5ppm; the methine protons of the -COCH(CH$_3$)NH$_2$ group as a quartet at \textit{ca}. 4.1ppm; and the CH$_2$CO$_2$R and the CH$_2$COR methylene protons as triplets at \textit{ca}. 2.2 and \textit{ca}. 2.5 respectively.
Table 3.10: Yields of the aminoketones 8, 98, 99 & 100 from the reduction of the corresponding nitroketones (90, 91, 92 & 93).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)*</th>
</tr>
</thead>
<tbody>
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<tr>
<td>98</td>
<td><img src="image2" alt="Structure 98" /></td>
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<td>99</td>
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<td>40</td>
</tr>
<tr>
<td>100</td>
<td><img src="image4" alt="Structure 100" /></td>
<td>57</td>
<td>27</td>
</tr>
</tbody>
</table>

* From starting acid, * from ethyl succinyl chloride

Figure 3.21: Synthesis of 8-amino-7-oxooctanoate (101) from the reductive hydrogenation of ethyl 8-nitro-7-oxooctanoate (94)

The nitromethane analogues of compounds 8, and 98-100: 8-amino-7-oxooctanoate (101, see Figure 3.21), 5-amino-4-oxopentanoate (102), 7-amino-6-oxoheptanoate (103), and 9-amino-8-oxo nonanoate (104) were also synthesised according to the method of Tamura et al\textsuperscript{184} using compounds 94-97 as starting materials. The yields of compounds 101-104 are shown in Table 3.11. Characterisation by \textsuperscript{1}H NMR confirmed the structures showing the expected splitting patterns: the methylene (-COCH\textsubscript{2}NH\textsubscript{3}) singlet at \textit{ca.} 3.8-4.0ppm; and the methylene triplets (-CH\textsubscript{2}CO\textsubscript{2}R and - CH\textsubscript{2}COR) at \textit{ca.} 2.2 and 2.5ppm respectively.
Table 3.11: Yields of the aminoketones 101, 102, 103 & 104 from the reduction of the corresponding nitroketones (94, 95, 96 & 97)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)</th>
</tr>
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<tr>
<td>103</td>
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<td>39</td>
</tr>
<tr>
<td>104</td>
<td><img src="image" alt="Structure" /></td>
<td>59</td>
<td>35</td>
</tr>
</tbody>
</table>

* From starting acid, * from ethyl succinyl chloride

Table 3.10 and Table 3.11 above show that the reductive hydrogenation of compounds 90-97 using the method of Tamura et al gives the desired α-aminoketone hydrochlorides (8, and 98 - 104) in yields of 57 - 70%, giving an overall yield of 27 - 47%.

3.5.2 Synthesis of 8-amino-7-hydroxynonanoate (106) and related aminoalcohols

Figure 3.22: Synthesis of 8-amino-7-hydroxynonanoate (109) from the reductive hydrogenation of ethyl 7-hydroxy-8-nitrononanoate (82)

With the successful development of a synthetic approach to α-aminoketones it was decided to extend this to the synthesis of the related α-aminoalcohols. To this end the aminoalcohol analogues of compounds 8, and 98-104 were synthesised from the α-nitroalcohols 82-89. 8-amino-7-hydroxy nonanoate (105, Figure 3.22) was synthesised from ethyl 7-hydroxy-8-
nitrononanoate (82) using the method of Tamura et al\textsuperscript{144} as described above. Typically ethyl 7-hydroxy-8-nitrononanoate (82) in ethanol-c. HCl (20:1, v/v) was added to a suspension of 5\% Pt-S-C in ethanol-c. HCl (20:1, v/v) and the reaction mixture flushed with hydrogen three times before hydrogenation at 50\textdegree C for 16h. The residual ester protecting group was removed after filtration of the catalyst by stirring in aq HCl (5M, 5cm\textsuperscript{3}) at 50\textdegree C for 4h. Purification of the crude product by ion exchange chromatography (DOWEX-50, H\textsuperscript{+} form) gave 8-amino-7-hydroxynonanoate in 69\% yield. The related compounds: 5-amino-4-hydroxyhexanoate (106); 7-amino-6-hydroxyoctanoate (107); and 9-amino-8-hydroxydecanoate (108) were synthesised in the same way from compounds 83-89, and the yields are shown in Table 3.12. Characterisation of these compounds by \textsuperscript{1}H NMR showed the presence of the expected groups: the -CH\textsubscript{2}CO\textsubscript{2}R methylene protons as a triplet at 2.2ppm; the -CH\textsubscript{2}CH(OH)- methylene protons as a multiplet at ca. 1.9ppm; the -CHOH and the -CH(CH\textsubscript{3})NH\textsubscript{2} methine protons as a multiplet at ca. 4.3ppm; and the -CH(CH\textsubscript{3})NH\textsubscript{2} methane protons as a doublet at ca. 1.4ppm.

Table 3.12: Yields of the amine/alcohols 105, 106, 107 & 108 from the reduction of the corresponding nitroalcohols (82, 83, 84 & 85).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)*</th>
</tr>
</thead>
<tbody>
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<td>46</td>
</tr>
<tr>
<td>106</td>
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<td>107</td>
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</tr>
<tr>
<td>108</td>
<td><img src="image4" alt="Structure" /></td>
<td>59</td>
<td>32</td>
</tr>
</tbody>
</table>

* From starting acid, # from ethyl succinyl chloride
The nitromethane analogues of compounds 105-108: 8-amino-7-hydroxyoctanoate (109, Figure 3.23); 5-amino-4-hydroxypentanoate (110); 7-amino-6-hydroxyheptanoate (111); and 9-amino-8-hydroxynonanoate (112) were synthesised in the same manner as above, using compounds 86-89 as starting materials. The yields of compounds 109-112 can be seen in Table 3.13. Characterisation of these compounds confirmed the expected structures showing the presence of: the -CH₂CO₂H methylene protons as a triplet at ca. 2.3ppm; the -CH₂CH(OH)- methylene protons as a multiplet at ca. 1.8ppm; and the -CHOH- methine protons and the -CH₂NH₂ methylene protons as a multiplet at ca. 4.5ppm.

Table 3.13: Yields of the aminoalcohols 109, 110, 111 & 112 from the reduction of the corresponding nitroalcohols (86, 87, 88 & 89).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Overall Yield (%)*</th>
</tr>
</thead>
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<tr>
<td>112</td>
<td><img src="image4" alt="Structure" /></td>
<td>61</td>
<td>40</td>
</tr>
</tbody>
</table>

* From starting acid, # from ethyl succinyl chloride

The reduction of nitroalcohols to aminoalcohols is the most studied reduction of the aliphatic nitro compounds, due to the importance of aminoalcohols as biologically active compounds, and the resistance of the hydroxy group to reduction by the reagents used. A variety of
approaches to the synthesis of aminoalcohols have been adopted: hydrogenation with Raney nickel, platinum oxide or palladium on barium sulfate to give aminoalcohols in yields of 80-95%; chemical reduction using a combination of metal and acid giving aminoalcohols in 92-98% yield; or electrochemical reduction using a lead cathode and dilute $\text{H}_2\text{SO}_4$ or $\text{HCl}$ as the electrolyte forming aminoalcohols in yields of 75-94%. Table 3.12 and Table 3.13 show that the method of Tamura et al. can be applied to the reduction of nitroalcohols, giving the desired aminoalcohols in yields of 58-69%.

3.6 Conclusions

The nitroaldol reaction, followed by oxidation of the nitroaldol product and reduction of the nitro group to an amine was investigated as a general approach to long chain aminoketone and aminoalcohol synthesis.

Synthesis of the precursor aldehydes required for the nitroaldol reaction was achieved in good yield using the method of Four and Gibe (see section 3.2.4 pp.54). The desired aldehydes: ethyl 7-oxoheptanoate (64); ethyl 4-oxobutanoate (80); methyl 6-oxohexanoate (71); and ethyl 8-oxooctanoate (70) were synthesised in 74%, 80%, 83%, and 87% yield respectively. Reaction of these aldehydes with nitroethane and mtromethane in the presence of powdered potassium hydroxide according to the method of Wollenberg and Miller gave the desired series of nitroalcohols via the nitroaldol reaction. Ethyl 7-hydroxy-8-nitrononanoate (82); ethyl 4-hydroxy-5-nitro hexanoate (83); methyl 6-hydroxy-7-nitro octanoate (84); ethyl 8-hydroxy-9-nitrodecanoate (85); ethyl 7-hydroxy-8-nitrooctanoate (86); ethyl 4-hydroxy-5-nitropentanoate (87); methyl 6-hydroxy-7-nitroheptanoate (88); and ethyl 8-hydroxy-9-nitrononanoate (89) were synthesised in 95%, 88%, 89%, 70%, 92%, 90%, 86% and 82% yields respectively. Oxidation of these nitroalcohols to the desired nitroketones was achieved using Jones’ reagent. The nitroketones: ethyl 8-nitro-7-oxo nonanoate (90); ethyl 5-nitro-4-oxohexanoate (91); methyl 7-nitro-6-oxooctanoate (92); ethyl 9-nitro-8-oxodecanoate (93); ethyl 8-nitro-7-oxooctanoate (94); ethyl 5-nitro-4-oxo pentanoate (95); methyl 7-nitro-6-oxoheptanoate (96); and ethyl 9-nitro-8-oxononanoate (97) were synthesised in yields of 91%, 89%, 88%, 87%, 94%, 93%, 91%, and 90%.

The hydrogenation method developed by Tamura et al. was designed for the reduction of mostly aromatic $\alpha$-nitroketones to the corresponding $\alpha$-aminoketone hydrochlorides. It has been shown above that this method can now be extended to non-aromatic nitroketones containing other functional groups. Table 3.10 and Table 3.11 show that the reductive
hydrogenation of compounds 90-97 in the presence of 5% Pt-S-C and ethanolic HCl gives the desired α-aminoketone hydrochloride in yields of 57% or greater.

The desired series of α-aminoketones: 8-amino-7-oxononanoate (8); 5-amino-4-oxo hexanoate (98); 7-amino-6-oxooctanoate (99); and 9-amino-8-oxodecanoate (100); 8-amino-7-oxooctanoate (101); 5-amino-4-oxopentanoate (102); 7-amino-6-oxoheptanoate (103); and 9-amino-8-oxononanoate (104) were prepared from their parent α-nitroketones using the method of Tamura et al.\textsuperscript{184}. Hydrogenation of the parent α-nitroketone in the presence of a partially poisoned platinum catalyst (5% Pt-S-C) and ethanolic HCl afforded the desired α-aminoketones (8, and 98-104) in yields of 58%, 60%, 64%, 57%, 67%, 70%, 62%, and 59%.

The related α-aminoalcohols were synthesised in the same manner as above to extend the study of the interaction of these compounds with the 8-amino-7-oxononanoate synthase (AONS) and the 7,8-diaminononanoate synthetase (DANS) enzymes. 8-amino-7-hydroxy nonanoate (105); 5-amino-4-hydroxyhexanoate (106); 7-amino-6-hydroxyoctanoate (107); 9-amino-8-hydroxydecanoate (108); 8-amino-7-hydroxyoctanoate (109); 5-amino-4-hydroxypentanoate (110); 7-amino-6-hydroxyheptanoate (111); and 9-amino-8-hydroxynonanoate (112) were synthesised in 69%, 63%, 65%, 59%, 68%, 64%, 58%, and 61% yields respectively from the hydrogenation of their parent α-nitroalcohols.

It has been shown above that the nitroaldol reaction provides a excellent starting point for the synthesis of both α-aminoketones and α-aminoalcohols. This method is advantageous as it allows variation in both the length of the desired amino compound, and the substitution between the amino and carbonyl or hydroxyl groups, thus providing a useful tool for binding studies involving the AONS and DANS enzymes. By using a synthetic pathway with a good deal of variability the range of compounds synthesised can be extended to include compounds suitable for use with other enzymes in the α-oxoamine synthase family: 5-aminolaevulinic acid synthase (ALAS); serine palmitoyl transferase (SPT); and 2-amino-3-ketobutyrate CoA ligase (KBL).
4. Summary and Future Work
4.1 Summary

This work has been directed towards the development of new synthetic routes to 8-amino-7-oxononanoate (8), an important intermediate in the biotin biosynthetic pathway. The routes developed had to be versatile to allow extension to analogues of AON which could be used as potential substrates or inhibitors of the 7,8-diaminononanoate synthetase enzyme.

The first approach was the development of a synthetic route to 8 using Dakin-West chemistry, based on previous work in our laboratory. To this end the oxazolone 2-methyl-4-phenyl oxazolone (40) was used as the starting point for the stepwise Dakin-West reaction, affording AON (8) as the final product in 4% overall yield. The low yield of 8 can be attributed to competing hydrolysis pathways (Figure 2.8, pp.34), since it might be anticipated that by changing the C-2 substituent on the oxazolone the hydrolysis could be directed along the desired pathway. Two oxazolones: 2,4-dimethyl oxazolone (43) and 2-methyl-4-trifluoromethyl oxazolone (46); were synthesised as intermediates. 2,4-Dimethyl oxazolone (43) afforded 8 in 2% overall yield whilst 2-methyl-4-trifluoromethyl oxazolone (46) afforded 8 in 7% overall yield. The higher yield experienced using 46 as the initial oxazolone can be ascribed to the electron withdrawing effect of the trifluoromethyl C-2 substituent stabilising hydrolysis intermediates and promoting hydrolysis via Route A (Figure 2.8). In a further attempt to limit the competing hydrolysis routes experienced in the one pot ring opening and deprotection step an enzymatic ring opening of the C-acyl oxazolone using Lipozyme® followed by a separate deprotection step to give AON (8) was investigated. Analysis of the purified products showed the major products to be dibutyl and monobutyl heptanediol. This can be attributed to a number of factors: acid catalysis causing competing hydrolysis pathways; the nature of the C-acyl C-4 substituent altering the rate of the enzymatic reaction; or the C-4 acyl group not being recognised or fitting into the active site of the enzyme.

The nitroaldol reaction, followed by oxidation of the nitroaldol product and reduction of the nitro group to an amine was investigated as a general approach to a series of \( \alpha \)-aminoketones and \( \alpha \)-aminoalcohols.

A series of aldehydes: ethyl 7-oxoheptanoate (64); ethyl 4-oxobutanoate (80); methyl 6-oxohexanoate (81); and ethyl 8-oxooctanoate (70) were synthesised in good yield and used as the starting material for the nitroaldol reaction. This allowed the synthesis of eight \( \alpha \)-aminoketones: 8-amino-7-oxononanoate (8); 5-amino-4-oxo hexanoate (98); 7-amino-6-oxooctanoate (99); and 9-amino-8-oxodecanoate (100); 8-amino-7-oxooctanoate (101); 5-
amino-4-oxopentanoate (102); 7-amino-6-oxoheptanoate (103); and 9-amino-8-oxononanoate (104) in overall yields of 35%, 37%, 40%, 27%, 40%, 47%, 39%, and 35%. The related α-aminoalcohols: 8-amino-7-hydroxy nonanoate (105); 5-amino-4-hydroxyhexanoate (106); 7-amino-6-hydroxyoctanoate (107); 9-amino-8-hydroxydecanoate (108); 8-amino-7-hydroxyoctanoate (109); 5-amino-4-hydroxypentanoate (110); 7-amino-6-hydroxyheptanoate (111); and 9-amino-8-hydroxynonanoate (112) were synthesised in overall yields of 46%, 44%, 46%, 32%, 43%, 46%, 40%, and 40%. It has been shown that the hydrogenation method developed by Tamura et al\textsuperscript{184} for the reduction of aromatic nitroketones can be applied to aliphatic nitroketones and nitroalcohols, although the yields experienced are reduced. Tamura found that using a platinum catalyst over a palladium catalyst avoided the formation of aminoalcohols in aromatic systems as platinum has a lower affinity for the \( \pi \)-electrons of the aryl group, thus reducing the occurrence of carbonyl reduction. Although this factor is not as crucial in alkyl \( \alpha \)-nitroalkyl ketones Tamura et al found that 5\% Pt-S-C was a more suitable catalyst than 5\% Pd-C in all systems. A further study could use a palladium catalyst for the reduction of compounds 90-97 and compare the yields of \( \alpha \)-aminoketone hydrochlorides synthesised.

In summary the stepwise Dakin-West reaction and the nitroaldol reaction both allow the introduction of a high degree of variation in the \( \alpha \)-aminoketones synthesised. However it has been shown that the Dakin-West reaction poses some problems in the final stages of the reaction sequence with the 1,3-dicarbonyl centres present causing competing hydrolysis routes. The nitroaldol reaction can be readily used to synthesise both \( \alpha \)-aminoketones and \( \alpha \)-aminoalcohols, and it is anticipated that these compounds can be used to probe the enzymes in the biotin biosynthetic pathway.

4.2 Future Work

Future work in this area can be divided into two main areas: development of the synthetic methods used and enzymatic studies to probe the AON analogues as potential substrates or inhibitors of the 7,8-diaminononanoate enzyme.

The Dakin-West chemistry could be further developed to overcome the problems experienced due to the competing hydrolysis routes resulting from the presence of the 1,3-dicarbonyl centre of the C-acyl oxazolone in compounds 42, 45 and 58. It has been reported in the literature that the use of acetic anhydride and pyridine, or anhydrous oxalic acid can prevent the competing
hydrolysis. In this instance the most suitable method to employ would be oxalic acid, as amino ketones form stable pyrazine salts in basic conditions. A second approach could be to reduce the side chain carbonyl group to the alcohol, ring opening and deprotection would then give the α-aminoalcohol which could be readily oxidised to the α-aminoketone. Trial reductions were carried out reducing 42 with sodium cyanoborohydride, achieving the alcohol in 85% yield. It is anticipated that this compound would be less susceptible to side reactions during hydrolysis, thus giving the desired α-aminoalcohol in good yield. Selective oxidation of the secondary hydroxyl group could then be undertaken using Jones' reagent, giving the desired α-aminoketones. Although this sequence would involve the addition of a reaction step, it is thought that this would be advantageous as hydrolysis would proceed more smoothly.

With a robust synthetic route to AON established we can now undertake enzymatic studies on the 7,8-diaminononanoate synthetase (DANS) enzyme using the compounds synthesised. Enzymatic studies using AON would give greater insight into the reaction mechanism of the enzyme. By repeating these studies using the other α-aminoketones and the α-aminoalcohols synthesised we could examine their potential as substrates or inhibitors of the reaction. Here the α-aminoalcohols may prove useful as they cannot decarboxylate via the β-keto acid route favoured by PLP dependant transaminases.
5. Experimental
General Procedure

All starting materials were purchased from commercial sources (Acros, Aldrich, Biorad, Fisher, Fluka, Nova Biochem, Pharmacia) and were used without further purification unless otherwise stated.

Thin Layer Chromatography was carried out on aluminium sheets pre-coated with Merck silica gel 60 F254. Compounds were visualised by UV absorption, iodine staining, potassium permanganate (2% KMnO4 in 2% NaHCO3) or ninhydrin dip (0.3% ninhydrin in EtOH-collidine, 19:1). Silica gel chromatography was carried out using Matrex Silica 60 (particle size 35-70μm).

Melting points were determined on an electrically heated Griffin melting point apparatus and are uncorrected.

NMR spectra were recorded on a Varian Gemini 2000 (200MHz) or a Brucker AC250 (250MHz) instrument. The chemical shifts (δ) are given in ppm relative to tetramethylsilane, and the coupling constants (J) are in Hz.

Fast Atom Bombardment mass spectra were recorded on a Kratos MS 50 TC instrument using glycerol, thioglycerol or NOBA as the matrix. Electrospray Ionisation mass spectra were recorded on a MicroMass platform II spectrometer with acetonitrile/water (50:50) as the solvent.

Tetrahydrofuran was dried by reflux over sodium/benzophenone, distilled and stored over 4Å molecular sieves, or sodium wire. Dichloromethane was heated at reflux over calcium hydride, distilled and stored in the same manner. Triethylamine and diisopropylethylamine were dried by reflux over potassium hydroxide, distillation and were stored over calcium hydride. Toluene was distilled and stored over 4Å molecular sieves. Jones Reagent was prepared by dissolving CrO3 (26g) in conc. H2SO4 (23cm³).

Organic extracts were routinely dried over anhydrous MgSO4 and filtered prior to evaporation.
N-benzoyl-L-alanine (39)

Alanine (5.02g, 57mmol) was dissolved in 0.6M aqNaOH (20cm³) and enough benzoyl chloride added to adjust the pH to 8. The mixture was cooled (4°C) and benzoyl chloride (8.35g, 59mmol) and aqNaOH (2M, ~30cm³) added in tandem over 30 minutes to ensure a constant pH of 8-8.5. The cloudy solution was stirred at 4°C for 16h, conc.aqNaOH added (20cm³, pH10) and the solution extracted with EtOAc (3×50cm³). aqHCl (10M) was added to adjust the pH of the solution to 2 and the solution extracted with EtOAc (3×50cm³). Evaporation gave a colourless solid that was crystallised (aqEtOH) to yield 39 (4.27g, 39%). mp = 150°C (lit153 mp = 150-151°C), M⁺ 193.17352 (C₁₀H₁₁NO₃ requires 193.19924); δH (200MHz, CDCl₃): 1.59 (3H, d, J=7.0 3-CH₃); 4.78-4.85 (1H, q, J=7.0, 2-CH); 6.74 (1H, br. d, J=8.6, -NH); 7.42-7.82 (5H, m, 5 Ar-CH), δC (200MHz, CDCl₃): 16.7 (C-3), 50.7 (C-2), 126.4, 128.5, 131.8 & 132.5 (6 x Ar-C), 169.1 (C-4), 180.3 (C-1). All data compared favourably to samples synthesised previously in this laboratory 111.

4-methyl-2-phenyl-4H-oxazol-5-one (40)

36, (3.62g, 19mmol) was dissolved in acetic anhydride/dioxan (1:1, 28cm³) and stirred with the exclusion of atmospheric water vapour (CaCl₂ drying tube) for 1h at 50°C. The solution was cooled to room temperature and stirred for a further 1.5h. Removal of solvent, filtration through silica (ca.2g, eluting with DCM, 20cm³) and evaporation gave a pale yellow oil that solidified on standing. The resultant solid was crystallised from ether to yield 40 (2.02g, 62%). mp = 37°C (lit124 37-39°C); νmax (oil, cm⁻¹) 1825 (CO₂), 1649 (C=N); M⁺ 175. 18546 (C₁₀H₉NO₂ requires 175.18396) δH(200MHz, CDCl₃): 1.55 (3H, d, J=7.3, 4'-CH₃); 4.42 (1H, q, J=7.5, 4-CH); 7.29-8.19 (5H, m, Ar-H), δC(200MHz, CDCl₃): 16.4 (C-4'), 125.6 (C-ipso), 127.3 & 128.4 (4 x o-, m-C), 132.1 (C-p), 160.9 (C-2), 179.2 (C-5). All data compared favourably to samples synthesised previously in this laboratory 111.
Methyl 6-chlorocarbonyl hexanoate (41)

![Methyl 6-chlorocarbonyl hexanoate](image)

Oxalyl chloride (7.615g, 60mmol) was added drop-wise to monomethyl heptanedioic acid (5.225g, 30mmol) in dry DCM (20 cm³). The resulting solution was stirred under N₂ for 1h, heated to 50°C and stirred for a further 1.5h. Excess solvent was removed in vacuo and the oil distilled to afford 41 (5.493g, 95%). bP0.4mm = 87°C, δH (200MHz, CDCl₃): 1.32-1.44 (2H, m, 4-CH₂); 1.54-1.78 (4H, m, 3,5-CH₂); 2.28 (2H, t, J=7.3, 2-CH₂); 2.86 (2H, t, J=7.3, 6-CH₂); 3.48 (3H, s, 1'-CH₃); δC (200MHz, CDCl₃): 24.5 (C-3), 24.7 (C-4), 27.3 (C-5), 33.4 (C-6), 46.4 (C-2), 60.5 (C-1'), 173.2 (C-1), 173.5 (C-7). All data compared favourably to samples synthesised previously in this laboratory.

Methyl, 4-methyl-2-phenyl-oxazol-5-yl heptanedioate (42)

![Methyl, 4-methyl-2-phenyl-oxazol-5-yl heptanedioate](image)

38 (293mg, 1.52mmol) was added drop-wise to a cooled (4°C) solution of 37(273mg, 1.57mmol) and Et₃N (147mg, 1.45mmol) in dry THF (5cm³). The reaction mixture was stirred under N₂ for 16h at RT. The excess solvent was removed in vacuo and ether (15cm³) added. The organic layer was washed with aqHCl (0.3M, 2×15cm³), and water (15cm³), dried and evaporated to a pale yellow oil. Silica gel chromatography (20g, hexane/ethyl acetate, 4:1) afforded 41 (566mg, 74%) as a colourless oil. M⁺ = 331.14182 (C₁₈H₂₁NΟ₅ requires 331.14197); δH (200MHz, CDCl₃): 1.46 (2H, m, 4-CH₂); 1.71 (4H, m, 3,5-CH₂); 2.08 (3H, s, 4''-CH₃); 2.36 (2H, t, J=7.3, 6-CH₂); 2.64 (2H, t, J=7.5, 2-CH₂); 3.68 (3H, s, 1'-CH₃); 7.40-7.95 (5H, m Ar-H); δC (200MHz, CDCl₃): 9.9 (C-4'''), 23.9, 24.2, 28.5 (C-7'', -8'', -9''), 33.3, 33.6 (C-6'', -10''), 50.9 (C-12''), 120.5 (C-ipso), 125.4, 128.3 (4 x C-o, -m), 127.2 (C-4), 129.9 (C-p), 145.1 (C-5), 154.4 (C-2), 170.0 (C-5''), 173.9 (C-11''). All data compared favourably to samples synthesised previously in this laboratory.
7-(4-methyl-5-oxo-2-phenyl-oxazol-4-yl)-methyl 7-oxo-heptanoate (43)

DMAP (7.0mg, 64.7μl) was added to a solution of 42 (389mg, 1.08 mmol) in dry THF (7cm³). The solution was stirred under N₂ at room temperature for 16h, solvent removed in vacuo, and the product dissolved in ether (15cm³). The organic phase was washed with aqHCl (0.1M, 2×15cm³), water (15cm³) and brine (15cm³), dried and evaporated to a yellow oil. Silica gel chromatography (20g, hexane/ethyl acetate, 4:1) afforded 43 as a colourless oil (263mg, 68%). M⁺ 331.14175 (C₁₈H₂₁N₂O₅ requires 331.14197); δH (200MHz, CDCl₃): 1.24 (2H, m, 8'-CH₂); 1.58 (4H, m, 7',9'-CH₂); 1.70 (3H, s, 4'-CH₃); 2.27 (2H, t, J=7.5, 5'-CH₂); 2.55 (2H, t, J=5.8, 10'-CH₂); 3.56 (3H, s, 12'-CH₃); 7.52-8.07 (5H, m, Ar-H); 13C (200MHz, CDCl₃): 9.9 (C-4'), 23.9, 24.2, 28.5 (C-1', -5', -9'), 33.3, 33.6 (C-2', -10'), 50.9 (C-1'), 84.6 (C-4), 120.5 (C-ipso), 125.4, 128.3 (4 x C-a, -m), 127.2 (C-4), 129.9 (C-p), 145.1 (C-5”), 154.4 (C-2), 170.0 (C-11’), 173.9 (C-5’). All data compared favourably to samples synthesised previously in this laboratory ¹¹¹.

8-Amino-7-oxo-nonanoate hydrochloride (8)

43 (117mg, 324.7μmol) was dissolved in aqHCl (4M, 10cm³), and the solution stirred at 80°C for 24h. The reaction mixture was cooled to RT, washed with DCM (3x3cm³) and the aqueous phase evaporated to dryness. Ion exchange chromatography (DOWEX-50X, H⁺ form, eluting from 0-1M HCl), and crystallisation of the resultant solid from ethanol/ether afforded 8 (10mg, 13.8%). Rf = 0.45 (butanol/acetic acid/water, 3:1:1); νmax (nujol, cm⁻¹) 3134 & 3040 (NH₃ & COOH), 1754 (COOH); M⁺: 188.12882 (C₉H₁₈NO₃ requires 188.12867); δH (200MHz, D₂O): 1.19 (2H, m, 4-CH₂); 1.39 (3H, d, J=7.4, 9-CH₃); 1.45 (4H, m, 3,5-CH₂); 2.22 (2H, t, J=7.3, 2-CH₂); 2.52 (2H, t, J=7.3, 6-CH₂); 4.10 (1H, q, J=7.5, 8-CH); δC (250MHz, D₂O): 14.6 (C-9), 22.2, 23.9, 27.5 (C-3, -4, -5) 33.7 (C-2), 37.8 (C-6), 54.8 (C-8), 179.0 (C-1), 209.3 (C-7).
2,4-dimethyl oxazolone (44)

N-acetyl alanine (1.007g, 7.6mmol) was dissolved in dry THF (15cm³) and DCC (1.691g, 8.2mmol) added portion-wise. The solution was stirred with the exclusion of water (CaCl₂ drying tube) for 2h at RT. Solvent was removed in vacuo, DCM (15cm³) added and the suspension was filtered to remove DCU. Evaporation of the filtrate gave an oil that solidified on standing. Crystallisation from ether afforded 44 (497mg, 57.8%) as a colourless solid. mp = 35°C, δ_H (200MHz, CDCl₃): 1.46 (3H, d, J=7.7, 4'-CH₃); 2.21 (3H, s, 2-CH₃); 4.18 (1H, m, 4-CH); δ_C (200MHz, CDCl₃): 12.2 (C-4'), 15.3 (C-2'), 28.3 (C-4), 130.4 (C-2), 172.3 (C-5).

Methyl, 2,4-dimethyloxazol-5-yl heptanedioate (45)

41 (293mg, 1.52mmol) was added drop-wise to a cooled (4°C) solution of 44 (184mg, 1.63mmol) and Et₃N (147mg, 1.45mmol) in dry THF (5cm³). The reaction mixture was stirred under N₂ for 16h at room temperature before removal of excess solvent in vacuo and addition of ether (15cm³). Washing with aqHCl (0.3M, 2x15cm³), and water (15cm³), drying and evaporation gave a pale yellow oil. Silica gel chromatography (20g, hexane/ethyl acetate, 4:1) afforded 45 (293mg, 64%) as a colourless oil. M⁺ 269.12669 (C₁₃H₁₉NO₅ requires 269.12632); δ_H (200MHz, CDCl₃): 1.45 (2H, m, 4-CH₂); 1.67 (4H, m, 3,5-CH₂); 1.97 (3H, s, 2''-CH₃); 2.07 (3H, s, 4''-CH₃); 2.32 (2H, t, J=7.3, 6-CH₂); 2.61 (2H, t, J=7.3, 2-CH₂); 3.64 (3H, s, 1'-CH₃); δ_C (200MHz, CDCl₃): 9.9 (C-4'), 15.3 (C-2'), 23.9, 24.2, 28.5 (C-7', -8', -9'), 33.3, 33.6 (C-6', -10'), 50.9 (C-12'), 127.2 (C-4), 145.1 (C-5), 154.4 (C-2), 170.0 (C-5'), 173.9 (C-11').
7-(2,4-dimethyl-5-oxo-oxazol-4-yl)-methyl 7-oxo-heptanoate (46)

DMAP (10mg, 81.9µmol) was added to a solution of 45(508mg, 1.79mmol) in dry THF (10cm³) and the solution stirred under N₂ at room temperature for 16h. Excess solvent was removed in vacuo and the product taken up in ether (15cm³). The organic layer was washed with aqHCl (0.1M, 2x15cm³), water (15cm³) and brine (15cm³), dried and evaporated to a pale yellow oil. Silica gel chromatography (hexane/ethyl acetate, 4:1) afforded 46(244.9mg, 48.2%) as a colourless oil. ¹H (200MHz, CDCl₃): 1.45 (2H, m, 4'-CH₂); 1.67 (4H, m, 3,5-CH₂); 1.76 (3H, s, 4''-CH₃); 1.97 (3H, s, 2''-CH₃); 2.29 (2H, t, J=7.3, 6-CH₂); 2.59 (2H, t, J=7.3, 2-CH₂); 3.64 (3H, s, 1'-CH₃); 8 C (200MHz, CDCl₃): 9.9 (C-4'), 15.3 (C-2'), 23.9, 24.2, 28.5 (C-7', -8', -9'), 33.3, 33.6 (C-6', -10'), 50.9 (C-12'), 82.3 (C-4), 145.1 (C-5), 154.4 (C-2), 170.0 (C-5'), 173.9 (C-11').

8-amino-7-oxononanoate hydrochloride (8) from 46

46(247.9mg, 875.0µmol) was dissolved in 2M aqHCl (5cm³), and the solution stirred at 80°C for 8h, before being cooled to RT and stirred for a further 12h. The mixture was extracted with DCM (3x4cm³) and the aqueous phase evaporated to dryness. The solid was dissolved in water (0.5cm³), purified by ion-exchange chromatography (DOWEX-50X, H⁺ form, eluting with HCl, 0-1M) and crystallised (ethanol/ether) to give 8 as its HCl salt (20mg, 10%). For data see 8-Amino-7-oxo-nonanoate hydrochloride (8), pp.79

Mhairi I. Brunton  
October 2002

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4-methyl-2-trifluoromethyl-2H-oxaol-5-one (47)\textsuperscript{127}

\[
\begin{array}{c}
\text{F}_3\text{C}-\text{N} \\
\text{O} \quad \text{O}
\end{array}
\]

L-alanine (996mg, 11mmol) was added to trifluoroacetic anhydride (5.35g, 26mmol) and the resulting solution was stirred at 140°C for 2h. The mixture was cooled to RT, Bu\textsubscript{3}N (11mg, 56μmol) added and distilled to yield 47(1.79g, 96%) as a pale yellow oil. bp\textsubscript{20mm} =34°C\textsuperscript{127}, δ\textsubscript{H} (200MHz, CDCl\textsubscript{3}) 2.41 (3H, s, 4'-CH\textsubscript{3}), 6.08 (1H, br s, 2-CH). Proton nmr compared favourably to literature\textsuperscript{127}.

Methyl, 4-methyl-2-trifluormethyloxazol-5-yl heptanedioate (48)

\[
\begin{array}{c}
\text{F}_3\text{C}-\text{N} \\
\text{O} \quad \text{O}
\end{array}
\]

41 (293mg, 1.52mmol) was added drop-wise to a cooled (4°C) solution of 47 (242mg, 1.45mmol) and Et\textsubscript{3}N (147mg, 1.45mmol) in dry THF (5cm\textsuperscript{3}). The reaction mixture was stirred under N\textsubscript{2} for 16h at room temperature before removal of excess solvent in vacuo and addition of ether (15cm\textsuperscript{3}). The crude solution was washed with aqHCl (0.3M, 2×15cm\textsuperscript{3}), and water (15cm\textsuperscript{3}), dried and evaporated to a pale yellow oil. Silica gel chromatography (20g, hexane/ethyl acetate, 4:1) afforded 48 (262mg, 65%) as a colourless oil. δ\textsubscript{H} (200MHz, CDCl\textsubscript{3}): 1.35 (2H, m, 4-CH\textsubscript{2}); 1.60 (4H, m, 3,5-CH\textsubscript{2}); 2.10 (3H, s, 4''-CH\textsubscript{3}); 2.25-2.38 (4H, t, J=7.2, 2,6-CH\textsubscript{2}); 3.68 (3H, s, 1'-CH\textsubscript{3}); δ\textsubscript{C} (200MHz, CDCl\textsubscript{3}): 10.4 (C-4''), 23.6, 24.5, 28.6 (C-7'', -8'', -9''), 33.2, 33.4 (C-6'', -10''), 50.9 (C-12''), 121.1 (C-2''), 127.2 (C-4), 145.1 (C-5), 154.4 (C-2), 170.0 (C-5''), 173.9 (C-11'').
Chapter 5

Experimental

7-(4-methyl-5-oxo-2-trifluoromethyl-oxazol-4-yl)-methyl-7-oxo-heptanoate (49)

![Chemical Structure]

DMAP (9mg, 70μmol) was added to a solution of methyl, 48 (406mg, 1.25mmol) in dry THF (7cm³) and the solution stirred under N₂ at room temperature for 16h. After removal of solvent in vacuo the product was dissolved in ether (15cm³) and washed with aqHCl (0.1M, 2×15cm³), water (15cm³) and brine (15cm³), before drying and evaporation to a pale yellow oil. Silica gel chromatography (hexane/ethyl acetate, 4:1) afforded 49 (262mg, 65%) as a colourless oil. δH (200MHz, CDCl₃): 1.33 (2H, m, 4'-CH₂), 1.63 (4H, m, 3,5-CH₂), 1.74 (3H, s, 4''-CH₃), 2.35 (4H, t, J=7.1, 2,6-CH₂), 3.66 (3H, s, 12''-CH₃); δC (200MHz, CDCl₃): 9.9 (C-4''), 23.9, 24.2, 28.5 (C-7'', -8'', -9''), 33.4, 33.8 (C-6'', -10''), 60.4 (C-12''), 82.7 (C-4), 124.3 (C-2''), 145.1 (C-5), 154.7 (C-2), 171.5 (C-5''), 173.3 (C-11'').

8-amino-7-oxononanoate (8) from 49

![Chemical Structure]

49 (130mg, 402μmol) was dissolved in 3M aqHCl (5cm³), and stirred at 80°C for 24h. The solution was cooled, extracted with DCM (3×4cm³) and the aqueous phase evaporated to dryness. Purification by ion-exchange chromatography (DOWEX-50, H⁺ form, eluting with HCl, 0-1M) and crystallisation (ethanol/ether) afforded 8 as its HCl salt (15mg 17%). For data see 8-Amino-7-oxo-nonanoate hydrochloride (8), pp. 79
Lipase Ring opening of 43

43 (20mg, 60.4μmol) was added to a suspension of n-butanol (8.2mg, 0.12mmol), Et₃N (1.87mg, 18.5μmol) and Lipozyme® (20mg) in dry toluene. The reaction mixture was shaken on an orbital shaker at 200rpm, 37°C for 72h. Lipozyme® was removed by filtration and solvent evaporated under reduced pressure to give a pale yellow oil. The crude product was purified by preparative layer chromatography (plc), eluting with hexane/ether (1:1) to yield dibutyl heptanedioate (8mg, 48.5%) as a colourless oil. δH (200MHz, CDCl₃): 0.92 (6H, t, J=7.3, 4',4''-CH₃); 1.38 (6H, m, 4,3',3''-CH₂); 1.60 (8H, m, 3,5,2',2''-CH₂); 2.29 (4H, t, J=7.4, 2,6-CH₂); 4.06 (4H, t J=6.6, 1',1''-CH₂)

Lipase ring opening of 46

46 (20mg, 74μmol) was dissolved in dry toluene (3cm³) and n-butanol (11mg, 140μmol), Et₃N (2mg, 19μmol) and Lipozyme® (20mg) added. The solution was shaken on an orbital shaker at 200rpm, 37°C for 72h. Lipozyme® was removed by filtration and solvent evaporated under reduced pressure to give a pale yellow oil. The crude product was purified by plc, eluting with hexane/ether (1:1) to yield dibutylpimelate (8mg, 40%) as a colourless oil. For data see Lipase ring opening of 43 (above).

Lipase ring opening of 49

49 (20mg, 62μmol) was added to a suspension of n-butanol (9mg, 110μmol), Et₃N (2mg, 19μmol) and Lipozyme® (20mg) in dry toluene (3cm³). The suspension was shaken on an orbital shaker at 200rpm, 37°C for 72h. Lipozyme® was removed by filtration and solvent evaporated under reduced pressure to give a pale yellow oil. The crude product was purified by plc, eluting with hexane/ether (1:1) to yield dibutylpimelate (10mg, 53%) as a colourless oil. For data see Lipase ring opening of 43 (above).
Ethyl 7-hydroxyheptanoate (66)\(^{155}\)

Potassium persulfate (32.5g, 120mmol) was added gradually to a cooled (15°C) solution of H\(_2\)SO\(_4\) (96%, 0.55mol, 31cm\(^3\)), water (0.55mol, 10cm\(^3\)) and ethanol (24cm\(^3\)) and stirred for 0.5h. Cycloheptanone (4.487g, 40mmol) in ethanol (16cm\(^3\)) was added drop-wise at 15°C, and reaction mixture stirred at room temperature for 16h. The solution was diluted with water (240cm\(^3\)), extracted with Et\(_2\)O (3×80cm\(^3\)) dried and evaporated to give a pale yellow oil. Purification by silica gel chromatography (20g, hexane/Et\(_2\)O, 1:1) afforded ethyl 7-hydroxyheptanoate (66, 1.702g, 24%) as a colourless oil. M\(^+\) 174.22543 (C\(_9\)H\(_{18}\)O\(_3\) requires 174.22567); \(\delta\)\(_H\) (200MHz, CDCl\(_3\)): 1.21 (3H, t, \(J=7.2, 2'-\text{CH}_3\)); 1.34 (4H, m, 4,6-\text{CH}_2); 1.59 (4H, m, 3,5-\text{CH}_2); 2.26 (2H, t, \(J=7.5, 2'-\text{CH}_2\)); 3.59 (2H, t, \(J=6.5, 7-\text{CH}_2\)); 4.10 (2H, q, \(J=7.0, 1'-\text{CH}_2\)); \(\delta\)\(_C\) (200MHz, CDCl\(_3\)): 14.1 (C-2'), 24.5 (C-4), 28.3, 28.6 (C-3, -5), 33.9 (C-2), 38.9 (C-6), 60.2, 63.2 (C-1', -7), 173.5 (C-1). Data compared favourably to literature\(^{155}\).

Ethyl 8-hydroxyoctanoate (67)

Using the procedure described above ethyl 8-hydroxyoctanoate (67) was synthesised from potassium persulfate (44g, 150mmol), H\(_2\)SO\(_4\) (96%, 0.7mol, 39cm\(^3\)), water (0.7mol, 13cm\(^3\)), ethanol (56cm\(^3\)) and cyclooctanone (6.3g, 50mmol) in 37% yield (3.5g). Rf\(^=\) 0.46 (1:1, hexane/ether); M\(^+\) 188.14129 (C\(_{10}\)H\(_{20}\)O\(_3\) requires 188.14133); \(\delta\)\(_H\) (200MHz, CDCl\(_3\)): 1.21 (3H, t, \(J=7.1, 2'-\text{CH}_3\)); 1.30 (6H, m, 4,5,6-\text{CH}_2); 1.58 (4H, m, 3,7-\text{CH}_2); 2.25 (2H, t, \(J=7.5, 2'-\text{CH}_2\)); 3.59 (2H, t, \(J=6.6, 8-\text{CH}_2\)); 4.09 (2H, q, \(J=7.1, 1'-\text{CH}_2\)); \(\delta\)\(_C\) (200MHz, CDCl\(_3\)): 14.6 (C-2'), 24.5, 25.2 (C-4, -5), 28.6, 28.9 (C-3, -6), 32.8 (C-2), 39.5 (C-7), 60.9, 63.4 (C-1', -8), 174.1 (C-1)
Ethyl 6-hydroxyhexanoate (68)

Using the procedure described above ethyl 6-hydroxyhexanoate (68) was synthesised from potassium persulfate (32.4g, 0.12mol), H₂SO₄ (96%, 0.56mol, 31cm³), water (0.56mol, 10cm³), ethanol (45cm³) and cyclohexanone (3.93g, 40mmol) in 44% yield (2.84g). Rf = 0.45 (1:1 hexane/EtOAc); M⁺ 160.11014 (C₈H₁₆O₃ requires 160.11001); δH (200MHz, CDCl₃): 1.16 (3H, t, J=7.1, 2'-CH₃); 1.31 (4H, q, 4'-CH₂); 1.53 (4H, m, 3,5-CH₂); 2.22 (2H, t, J=7.3, 2-CH₂); 3.53 (2H, t, J=6.4, 6-CH₂); 4.03 (2H, q, J=6.6, 1'-CH₂); δC (200MHz, CDCl₃): 13.7 (C-2'), 27.9, 28.4 (C-3, -4), 32.8 (C-2), 39.2 (C-5), 61.0, 64.5 (C-1', -6), 174.0 (C-1).

Ethyl 5-hydroxypentanoate (69)

Using the procedure described above ethyl 5-hydroxypentanoate (69) was synthesised from potassium persulfate (32.5g, 0.12mol), H₂SO₄ (96%, 0.56mol, 31cm³), water (0.56mol, 10cm³), ethanol (45cm³) and cyclooctanone (3.37g, 40mmol) in 28% yield (1.64g). Rf= 0.52 (1:1, hexane/ether); M⁺ 146.09461 (C₇H₁₄O₃ requires 146.09435); δH (200MHz, CDCl₃): 1.19 (3H, t, J=7.1, 2'-CH₃); 1.62 (2H, m, 3,4-CH₂); 2.28 (2H, t, J=7.1, 2-CH₂); 3.57 (2H, t, J=5.9, 5-CH₂); 4.06 (2H, q, J=7.4, 1'-CH₂); δC (200MHz, CDCl₃): 14.4 (C-2'), 28.9 (C-3), 32.2 (C-2), 38.5 (C-4), 61.5, 64.6 (C-1', -5), 173.6 (C-1). 13-C mnr data compares favourably to literature ¹⁸⁶.
Ethyl 7-oxoheptanoate (64) via Oxidation of 66\textsuperscript{155}

![Chemical Structure]

Ethyl 7-hydroxyheptanoate (66, 523mg, 3mmol) in DCM (2cm\textsuperscript{3}) was added drop-wise to PCC (1.94g, 9mmol) in DCM (7cm\textsuperscript{3}). The suspension was stirred at room temperature for 3h before dilution with ether (20cm\textsuperscript{3}) and filtration through Celite. Evaporation of the solution gave an orange oil which was purified by silica gel chromatography (20g, hexane/EtOAc, 2:1) to yield ethyl 7-oxoheptanoate (64, 215mg, 42%). M\textsuperscript{*} 172.11057 (C\textsubscript{9}H\textsubscript{16}O\textsubscript{3} requires 172.11001); \(\delta\text{H} (200MHz, CDCl\textsubscript{3}): 1.20 (3H, t, J=7.1, 2'-CH\textsubscript{3}); 1.32 (2H, m, 4-CH\textsubscript{2}); 1.60 (4H, m, 3,5-CH\textsubscript{2}); 2.27 (2H, t, J=7.5, 2 -CH\textsubscript{2}); 2.28 (2H, t, J=7.3, 6-CH\textsubscript{2}) 4.06 (2H, q, J=7.2, 1'-CH\textsubscript{2}); 9.82 (1H, s, 8-CH); \(\delta\text{C} (200MHz, CDCl\textsubscript{3}): 14.0 (C-2'), 21.5 (C-3), 24.1 (C-4), 24.4 (C-5), 28.3 (C-6), 43.5 (C-2), 60.1 (C-1'), 173.5 (C-1) 202.5 (C-7). Data compared favourably to literature\textsuperscript{155}.

Ethyl 8-oxooctanoate (70) via Oxidation of 67

![Chemical Structure]

Ethyl 8-oxooctanoate (70) was synthesised in the same manner as above using ethyl 8-hydroxyoctanoate (67, 0.57g, 3mmol) and PCC (1.94g, 9mmol) in DCM (5cm\textsuperscript{3}) in a yield of 61\% (0.34g). Rf =0.61 (2:1, hexane/EtOAc); M\textsuperscript{*} 186.12579 (C\textsubscript{10}H\textsubscript{18}O\textsubscript{3} requires 186.12567); \(\delta\text{H} (200MHz, CDCl\textsubscript{3}): 1.31 (3H, t, J=7.2, 1'-CH\textsubscript{2}); 1.40 (4H, m, 4,5-CH\textsubscript{2}); 1.69 (4H, m, 3,6-CH\textsubscript{2}); 2.35 (2H, t, J=7.4, 2-CH\textsubscript{2}); 2.48 (2H, t, J=7.2, 7-CH\textsubscript{2}) 4.18 (2H, q, J=7.2, 1'-CH\textsubscript{2}); 9.82 (1H, s, 8-CH); \(\delta\text{C} (200MHz, CDCl\textsubscript{3}): 13.7 (C-2'), 21.2 (C-4), 22.3 (C-5), 23.9 (C-3), 24.4 (C-6), 28.3 (C-2), 42.9 (C-7), 60.4 (C-1'), 174.3 (C-1) 200.9 (C-8)
Ethyl 6-oxohexanoate (71) via Oxidation of 68

Ethyl 6-oxohexanoate (71) was synthesised in the same manner as above using ethyl 6-hydroxyhexanoate (68, 1.06g, 6.6mmol) and PCC (4.27g, 20mmol) in DCM (11cm$^3$) in a yield of 40% (415mg). Rf = 0.53 (3:2, hexane/EtOAc); M$^+$ 158.09429 (C$_8$H$_{14}$O$_3$ requires 158.09435); $\delta_H$ (200MHz, CDCl$_3$): 1.23 (3H, t, J=7.1, 2'-CH$_3$); 1.62 (4H, m, 3,4-CH$_2$); 2.27 (2H, t, J=7.3, 2-CH$_2$); 2.41 (2H, t, J=7.3, 5-CH$_2$) 4.10 (2H, q, J=7.1, 1'-CH$_2$); 9.74 (1H, s, 6-CHO); $\delta_C$ (200MHz, CDCl$_3$): 14.5 (C-2'), 24.5 (C-3), 24.9 (C-4), 28.7 (C-2), 43.0 (C-5), 60.7 (C-1'), 172.9 (C-1) 201.5 (C-6)

Ethyl 5-oxopentanoate (72) via Oxidation of 69

Ethyl 5-oxopentanoate (72) was synthesised in the same manner as above using ethyl 5-hydroxypentanoate (69, 281mg, 1.9mmol) and PCC (1.23g, 5.7mmol) in DCM (3cm$^3$) in a yield of 21% (57mg). Rf = 0.38 (2:1 hexane/EtOAc); M$^+$ 144.07892 (C$_7$H$_{12}$O$_3$ requires 144.07869); $\delta_H$ (200MHz, CDCl$_3$): 1.12 (3H, t, J=7.1, 2'-CH$_3$); 1.54 (2H, m, 3-CH$_2$); 2.33 (2H, t, J=6.5, 2-CH$_2$); 2.50 (2H, t, J=7.1, 4-CH$_2$); 3.99 (2H, q, J=7.3, 1'-CH$_2$); 9.64 (1H, s, 5-CHO); $\delta_C$ (200MHz, CDCl$_3$): 14.5 (C-2'), 24.6 (C-3), 28.1 (C-2), 42.4 (C-4), 61.4 (C-1'), 173.0 (C-1) 200.6 (C-5)
**Ethyl 7-oxoheptanoate (64) via hydrogenation of 71**

Ethyl 6-chlorocarbonylhexanoate was added to a pre-hydrogenated suspension of disopropylethylamine (300mg, 2.4mmol) and Pd-C (10%, 200mg) in acetone (10cm³). The reaction mixture was hydrogenated for 3h before filtration of the catalyst and dilution of the filtrate with aqHCl (2M, 10cm³). Extraction with hexane, drying (MgSO₄) and evaporation afforded a pale yellow oil that was distilled to yield ethyl 7-oxoheptanoate (61, 88mg, 21%). For data see Ethyl 7-oxoheptanoate (64) via Oxidation of 66 pp.87.

**64 via sodium tri-t-butoxyaluminohydride reduction of 71**

Sodium tri-t-butoxyaluminohydride was prepared by stirring sodium aluminium hydride (5.4g, 100mmol) in diglyme (120cm³, dried over NaOH) for 24h. The resulting suspension was filtered under N₂ and the filtrate added to 'BuOH and stirred under N₂ for 6h at 0°C.

Sodium tri-t-butoxyaluminohydride (40cm³, 16mmol, 0°C) was added drop-wise to a solution of ethyl 6-chlorocarbonylhexanoate (3.00g, 14.5mmol) in dry THF (14.5cm³) at -78°C. The reaction mixture stirred for 1.5h at -78°C and warmed to room temperature. aqHCl (1M, 40cm³) added and extracted with EtOAc (3×30ml) evaporation gave an oil that was washed with water (3×30cm³) to remove diglyme. Drying (MgSO₄) and evaporation gave a colourless oil, purified by column chromatography (30% EtOAc in hexane) to give ethyl 7-oxoheptanoate (64, 667mg, 27%). For data see Ethyl 7-oxoheptanoate (64) via Oxidation of 66 pp.87.

**Acid Chloride Synthesis - General Procedure**

Oxalyl chloride (2 equivalents) was added dropwise to a solution of a diacid monoester in dry DCM. The resulting solution was stirred under N₂ for 1h, heated to 50°C and stirred for a further 1.5h. Excess solvent was removed in vacuo and the oil distilled to afford the desired acid chloride.
Ethyl 6-chlorocarbonyl hexanoate (74)

71 was prepared as for the general procedure (pp.89) using oxalyl chloride (2.539g, 20mmol), monoethyl heptanedioic acid (1.882g, 10mmol) and dry DCM (20 cm³) in a yield of 1.922g (93%). bp₀.₀⁴₅ₐ₄ = 90°C; M⁺ 206.55501 (C₉H₁₅O₂Cl requires 206.55518); δ̃H (200MHz, CDCl₃): 1.20 (3H, t, J=7.5, 2'-CH₃); 1.32-1.44 (2H, m, 4-CH₂); 1.54-1.78 (4H, m, 3,5-CH₂); 2.28 (2H, t, J=7.3, 2-CH₂); 2.86 (2H, t, J=7.3, 6-CH₂); 4.10 (2H, q, J=7.2, 1'-CH₂); δC (200MHz, CDCl₃): 14.0 (C-2'), 24.2 (C-3), 24.5 (C-4), 27.7 (C-5), 33.7 (C-6), 46.7 (C-2), 60.2 (C-1'), 173.2 (C-1), 173.5 (C-7).

Methyl 5-chlorocarbonyl pentanoate (78)

78 was prepared analogously to the general procedure (pp.89) using oxalyl chloride (2.539g, 20mmol), monomethyl hexanedioic acid (1.602mg, 10mmol) and dry DCM (10cm³) in a yield of 1.715g (96%). bp₀.₀⁵₅₅ = 50°C (Lit¹⁸⁷ bp₀.₀₁₅₅ = 61°C); M⁺: 178.52367 (C₇H₁₁O₃Cl requires 178.52386); δ̃H (200MHz, CDCl₃): 1.77 (2H, m, 3-CH₂); 1.80 (2H, m, 4-CH₂); 2.41 (2H, t, J=6.8, 2-CH₂); 2.99 (2H, t, J=7.0, 5-CH₂); 3.74 (3H, s, 1'-CH₃); δC (200MHz, CDCl₃): 24.5 (C-3), 27.7 (C-4), 33.7 (C-6), 46.7 (C-2), 61.4 (C-1'), 172.8 (C-1), 173.3 (C-6). 13-C mnr data compares favourably to literature¹⁸⁷.
Ethyl 7-chlorocarbonyl heptanoate (79)

![Image of ethyl 7-chlorocarbonyl heptanoate](image)

79 was prepared via the general procedure (pp.89) using oxalyl chloride (1.269g, 10mmol), monoethyl octanedioic acid (1.011g, 5mmol) and dry DCM (10cm³) in a yield of 925mg (91%). bp 0.4mm = 100°C (Lit bp 0.1mm = 78-80°C), M+ 220.57075 (C₉H₁₅O₂Cl requires 220.57084); δH (200MHz, CDCl₃): 1.34 (3H, t, J=7.0, 2'-CH₃); 1.45 (4H, m, 4,5-CH₂); 1.78 (4H, 3,6-CH₂); 2.38 (2H, t, J=7.2, 2-CH₂); 2.98 (2H, t, J=7.2, 7-CH₂); 4.21 (2H, q, J=7.2, 1'-CH₂) δC (200MHz, CDCl₃): 14.6 (C-2'), 24.0 (C-4), 24.3 (C-5), 27.2 (C-3), 27.7 (C-6), 33.7 (C-7), 46.7 (C-2), 59.9 (C-1'), 173.5 (C-1), 173.9 (C-8). All 13-C nmr data compares favourably to literature.

Aldehyde Synthesis – General Procedure

Bu₃SnH (1.1 equivalents) was added drop-wise to a stirred solution of acid chloride and (Ph₃P)₄Pd (0.001 equivalents) in dry THF. The reaction mixture was stirred for 1h before addition of hexane (10cm³) and filtration of the precipitate. Evaporation and distillation afforded the required aldehyde.

Ethyl 7-oxoheptanoate (64)

![Image of ethyl 7-oxoheptanoate](image)

64 was prepared using the general procedure (pp.89) with Bu₃SnH (1.164g, 4.0mmol), ethyl 6-chlorocarbonylhexanoate (744mg, 3.6mmol) and (Ph₃P)₄Pd (42mg, 36μmol) in dry THF (7cm³) in a yield of 459mg (74%). For data see Ethyl 7-oxoheptanoate (64) via Oxidation of 66 pp.87 bp 0.02 = 79°C.
**Ethyl 4-oxo butanoate (80)**

![Chemical Structure](image)

80 was prepared by means of the general procedure (pp.89) using Bu₃SnH (2.241g, 7.7mmol) ethyl succinyl chloride (1.152mg, 7mmol) and (Ph₃P)₄Pd (81mg, 70μmol) in dry THF (10cm³) in a yield of 725mg (80%). bp 0.02mm = 60°C; M⁺ 130.06310 (C₆H₁₀O₃ requires 130.06303); δH (200MHz, CDCl₃): 1.32 (3H, t, J=7.2, 2'-CH₂); 2.67 (2H, t, J=7.2, 2-CH₂); 2.85 (2H, t, J=6.1, 3-CH₂); 4.20 (2H, q, J=7.2, 1'-CF₂); 9.87 (1H, s, 4-CH); δC (200MHz, CDCl₃): 14.6 (C-2'), 28.3 (C-2), 43.5 (C-3), 60.9 (C-1'), 173.5 (C-1) 202.5 (C-4)

**Methyl 6-oxo hexanoate (81)**

![Chemical Structure](image)

81 was prepared according to the general procedure (pp.89) with Bu₃SnH (2.241g, 7.7mmol) methyl 5-chlorocarbonyl pentanoate (78, 1.250g, 7mmol) and (Ph₃P)₄Pd (81mg, 70μmol) in dry THF (7cm³) in a yield of 853mg (83%). bp 0.02mm = 65°C; M⁺ 144.07875 (C₇H₁₄O₃ requires 144.07869); δH (200MHz, CDCl₃): 1.39 (2H, m, 3-CH₂); 1.48 (2H, m, 4-CH₂); 1.71 (2H, t, J=7.6, 2-CH₂); 2.38 (2H, t, J=7.4, 5-CH₂); 3.72 (3H, s, 1'-CH₃); 9.83 (1H, s, 6-CH); δC (200MHz, CDCl₃): 24.1 (C-3), 24.4 (C-4), 28.3 (C-2), 43.5 (C-5), 60.1 (C-1'), 173.5 (C-1) 202.5 (C-6)

**Ethyl 8-oxo octanoate (70)**

![Chemical Structure](image)

70 was prepared in the manner of the general procedure (pp.89) using Bu₃SnH (1.339g, 4.6mmol), ethyl 7-chlorocarbonyl heptanoate (79, 929mg, 4.2mmol) and (Ph₃P)₄Pd (49mg, 42μmol) and dry THF (8cm³) in a yield of 679mg (87%). For data see Ethyl 8-oxooctanoate (70) via Oxidation of 67 pp.87; bp 0.02mm = 95°C.
Nitroalcohol Synthesis – General Procedure

Nitromethane or nitroethane (2 equivalents) and powdered KOH (0.1 equivalents) were stirred in 'PrOH (10 cm³) for 0.5 h and aldehyde in 'PrOH (3 cm³) added. The reaction mixture was stirred for 6 h, Et₂O (10 cm³) added and the resulting precipitate filtered through a pad of Celite (1 g). Evaporation and purification by silica gel chromatography (20 g, hexane/EtOAc, 3:2) gave the desired nitroalcohol.

Ethyl 7-hydroxy-8-nitro nonanoate (82)

82 was prepared in line with the general procedure (pp. 93) using nitroethane (210 mg, 2.8 mmol), powdered KOH (8 mg, 0.14 mmol) and ethyl 7-oxohexanoate (64, 245 mg, 1.4 mmol) in a yield of 330 mg (95%). M⁺ 247.14201 (C₁₁H₂₁NO₅ requires 247.14197); δH (200 MHz, CDCl₃): 1.23 (3H, t, J=7.0, 2'-CH₃); 1.37 (6H, m, 3,4,5-CH₂); 1.52 (3H, d, J=6.6, 9-CH₃); 1.62 (2H, m, 6-CH₂); 2.28 (2H, t, J=7.3, 2-CH₂); 3.87 (1H, m, 7-CH); 4.09 (2H, q, J=7.1, 1'-CH₂); 4.51 (1H, m, 8-CH₂); δC (200 MHz, CDCl₃): 12.3 (C-9), 14.1 (C-2'), 24.1 (C-4), 28.6, 28.7 (C-3, -5), 33.6, 33.9 (C-2, -6), 60.2 (C-1'), 71.8 (C-8), 86.2 (C-7), 173.6 (C-1)

Ethyl 4-hydroxy-5-nitro hexanoate (83)

83 was synthesised by means of the general procedure (pp. 93) using nitroethane (600 mg, 8.0 mmol), powdered KOH (22 mg, 0.4 mmol) and ethyl 4-oxo butanoate (80, 520 mg, 4.0 mmol) in a yield of 722 mg (88%). M⁺ 205.09510 (C₈H₁₅NO₅ requires 205.09502); δH (200 MHz, CDCl₃): 1.33 (3H, t, J=7.0, 2'-CH₃); 1.63 (3H, d, J=6.6, 6-CH₃); 1.80 (2H, m, 3-CH₂); 2.59 (2H, t, J=7.0, 2-CH₂); 4.05 (1H, m, 4-CHOH); 4.21 (2H, q, J=7.0, 1'-CH₂); 4.60 (2H, m, 5-CH); δC (200 MHz, CDCl₃): 12.6 (C-6), 14.4 (C-2'), 33.2, 34.0 (C-2, -3), 60.0 (C-1'), 71.5 (C-5), 86.7 (C-4), 173.2 (C-1)
Chapter 5

Experimental

Methyl 6-hydroxy-7-nitro octanoate (84)

![Chemical structure of methyl 6-hydroxy-7-nitro octanoate (84)]

84 was prepared in line with the general procedure (pp.93) using nitroethane (375mg, 5.0mmol), powdered KOH (14mg, 0.3mmol) and methyl 6-oxo hexanoate (81, 359mg, 2.5mmol) in a yield of 489mg (89%). M⁺ 219.11059 (C₉H₁₇NO₅ requires 219.11067); δH (200MHz, CDCl₃): 1.49 (4H, m, 3,4-CH₂); 1.61 (3H, d, J=7.0, 8-CH₃); 1.67 (2H, m, 5-CH₂); 2.38 (2H, t, J=7.2, 2-CH₂); 3.73 (3H, s, 1'-CH₃); 4.24 (1H, m, 6-CH); 4.59 (1H, m, 7-CH); δC (200MHz, CDCl₃): 12.0 (C-8), 28.5, 28.7 (C-3, -4), 33.5, 33.8 (C-2, -5), 58.7 (C-1’), 71.8 (C-7), 86.2 (C-6), 173.9 (C-1)

Ethyl 8-hydroxy-9-nitro decanoate (85)

![Chemical structure of ethyl 8-hydroxy-9-nitro decanoate (85)]

85 was prepared in accordance with the general procedure (pp.93) using nitroethane (135mg, 1.8mmol), powdered KOH (5mg, 0.09mmol) and ethyl 8-oxo octanoate (70, 169mg, 0.9mmol), in a yield of 164mg (70%). M⁺ 261.15753 (C₁₂H₂₃NO₅ requires 261.15762); δH (200MHz, CDCl₃): 1.32 (3H, t, J=7.0, 2'-CH₃); 1.41 (4H, m, 4,5-CH₂); 1.62 (3H, d, J=6.6, 10-CH₃); 1.67 (4H, m, 3,6-CH₂); 1.81 (2H, m, 7-CH₂); 2.36 (2H, t, J=7.0, 2-CH₂); 4.18 (2H, q, J=7.2, 1'-CH₂); 4.54 (2H, m, 8,9-CH); δC (200MHz, CDCl₃): 12.0 (C-10), 14.4 (C-2'), 24.3 (C-4, -5), 28.4, 28.8 (C-3, -6), 33.4, 33.7 (C-2, -7), 60.2 (C-1’), 71.8 (C-9), 86.2 (C-8), 173.2 (C-1)
Ethyl 7-hydroxy-8-nitro octanoate (86)

86 was prepared according to the general procedure (pp.93) using nitromethane (171mg, 2.8mmol), powdered KOH (8mg, 0.14mmol) and ethyl 7-oxoheptanoate (64, 245mg, 1.4mmol) in a yield of 301mg (92%). M⁺ 247.14201 (C₁₀H₁₉NΟ₅ requires 233.12632); δₜ (200MHz, CDCl₃): 1.13 (3H, t, J=7.0, 2'-CH₃); 1.34 (2H, m, 4-CH₂); 1.57 (4H, m, 3,5-CH₂); 1.84 (2H, m, 6-CH₂); 2.52 (2H, t, J=7.4, 2-CH₂); 4.32 (2H, q, J=7.2, 1’-CH₂); 4.55 (3H, m, 8-CH₂, 7-CH); δC (200MHz, CDCl₃): 14.4 (C-2’), 24.5 (C-4), 28.2, 28.4 (C-3, -5), 33.5, 33.6 (C-2, -6), 60.9 (C-1’), 71.1 (C-8), 85.4 (C-7), 174.0 (C-1)

Ethyl 4-hydroxy-5-nitro pentanoate (87)

87 was prepared in a similar manner to the general procedure (pp.93) using nitromethane (244mg, 4.0mmol), powdered KOH (11mg, 0.2mmol) and ethyl 4-oxo butanoate (80, 245mg, 1.4mmol) in a yield of 345mg (90%). M⁺ 191.07942 (C₇H₁₃NΟ₅ requires 191.07937); δₜ (200MHz, CDCl₃): 1.33 (3H, t, J=7.0, 2'-CH₃); 1.90 (2H, m, 3-CH₂); 2.60 (2H, t, J=7.0, 2-CH₂); 3.34 (1H, m, 4-CH); 4.21 (2H, q, J=7.2, 1’-CH₂); 4.48 (2H, m, 5-CH₂); δC (200MHz, CDCl₃): 14.3 (C-2’), 33.1, 33.6 (C-2, -3), 61.4 (C-1’), 70.9 (C-5), 85.8 (C-4), 173.8 (C-1)
Methyl 6-hydroxy-7-nitro heptanoate (88)

![Methyl 6-hydroxy-7-nitro heptanoate](image)

88 was synthesised according to the general procedure (pp.93) using nitromethane (305mg, 5.0mmol), powdered KOH (14mg, 0.3mmol) and methyl 6-oxo hexanoate (81, 305mg, 2.5mmol) in a yield of 414mg (86%). M° 205.09513 (C₈H₁₅NO₅ requires 205.09502); δH (200MHz, CDCl₃): 1.58 (4H, m, 3,4-CH₂); 1.71 (2H, m, 5-CH₂); 2.41 (2H, t, J=7.0, 2-CH₂); 3.73 (3H, s, 1'-CH₃); 4.78 (3H, m, 7-CH₂, 6-CH); δC (200MHz, CDCl₃): 28.3, 28.6 (C-3, -4), 33.2, 33.5 (C-2, -5), 59.3 (C-1'), 71.6 (C-7), 86.5 (C-6), 173.8 (C-1)

Ethyl 8-hydroxy-9-nitro nonanoate (89)

![Ethyl 8-hydroxy-9-nitro nonanoate](image)

89 was prepared in a similar manner to the general procedure (pp.93) using nitromethane (110mg, 1.8mmol), powdered KOH (5mg, 0.09mmol) and ethyl 8-oxo octanoate (70, 245mg, 1.4mmol) in a yield of 183mg (82%). M° 247.14978 (C₁₁H₂₁NO₅ requires 247.14969); δH (200MHz, CDCl₃): 1.32 (3H, t, J=7.0, 2'-CH₃); 1.39 (4H, m, 4,5-CH₂); 1.67 (6H, m, 3,6,7-CH₂); 2.35 (2H, t, J=7.4, 2-CH₂); 4.18 (2H, q, J=7.0, 1'-CH₂); 4.55 (3H, m, 9-CH₂, 8-CH); δC (200MHz, CDCl₃): 14.1 (C-2'), 24.5, 24.8 (C-4, -5), 28.5, 28.7 (C-3, -6), 33.4, 33.7 (C-2, -7), 60.9 (C-1'), 70.8 (C-9), 85.1 (C-8), 173.8 (C-1)

Nitroketone Synthesis – General Procedure

Jones Reagent (1 volume equivalent) was added to solution of nitroalcohol in acetone (3cm³). The reaction mixture was stirred at 15°C for 1h, warmed to room temperature and the chromium salts filtered through Celite. Et₂O (5cm³) was added to the filtrate and the solution washed with HCl (0.1M, 3×5cm³) and brine (5cm³) before evaporation. The resultant oil was purified by silica gel chromatography to yield the desired nitroketone.
Ethyl 8-nitro-7-oxo nonanoate (90)

90 was prepared in accordance with the general procedure (pp.96) using ethyl 7-hydroxy-8-nitro nonanoate (82, 173mg, 0.7mmol) and Jones’ Reagent (0.7cm³) in acetone (4cm³) in a yield of 155mg (91%). M⁺ 245.12645 (C₁₁H₁₉NΟ₅ requires 245.12632); δH (200MHz, CDCl₃): 1.19 (3H, t, J=7.1, 2'-CH₃); 1.30 (2H, m, 4-CH₂); 1.54 (4H, m, 3,5-CH₂); 1.65 (3H, d, J=7.0, 9-CH₃); 2.27 (2H, t, J=7.1, 6-CH₂); 2.53 (2H, t, J=6.8, 2-CH₂); 4.06 (2H, q, J=7.2, 1'-CH₂); 5.18 (1H, q, J=7.1, 8-CH); δC (200MHz, CDCl₃): 11.9 (C-9), 14.3 (C-2'), 24.6 (C-4), 28.4, 28.7 (C-3, -5), 33.2, 33.4 (C-2, -6), 60.7 (C-1'), 71.7 (C-8), 173.4 (C-1), 179.6 (C-7)

Ethyl 5-nitro-4-oxo hexanoate (91)

91 was prepared in accordance with the general procedure (pp.96) using ethyl 4-hydroxy-5-nitro hexanoate (83, 308mg, 1.5mmol) and Jones Reagent (1.5cm³) in acetone (10cm³) in a yield of 271mg (89%). M⁺ 203.07929 (C₈H₁₃NΟ₅ requires 203.07937); δH (200MHz, CDCl₃): 1.14 (3H, t, J=7.2, 2'-CH₃); 1.66 (3H, d, J=6.6, 6-CH₃); 2.25 (2H, t, J=7.2, 3-CH₂); 2.52 (2H, t, J=7.4, 2-CH₂); 5.21 (1H, q, J=7.2, 5-CH); δC (200MHz, CDCl₃): 12.7 (C-6), 14.3 (C-2'), 33.4, 33.9 (C-2, -3), 60.2 (C-1'), 71.6 (C-5), 173.1 (C-1), 179.4 (C-4)
**Methyl 7-nitro-6-oxo octanoate (92)**

![Structure of Methyl 7-nitro-6-oxo octanoate](image)

92 was prepared via the general procedure (pp.96) using methyl 6-hydroxy-7-nitro octanoate (84, 263mg, 1.2mmol) and Jones Reagent (1.2cm³) in acetone (10cm³) in a yield of 228mg (88%). M⁺ 217.09511 (C₉H₁₅NO₅ requires 217.09502); δ_H (200MHz, CDCl₃): 1.76 (4H, m, 3,4-CH₂); 1.79 (3H, d, J=7.4, 8-CH₃); 2.46 (2H, t, J=6.6, 2-CH₂); 2.70 (2H, t, J=6.4, 5-CH₂); 3.73 (3H, s, 1'-CH₃); 5.31 (1H, q, J=7.0, 7-CH); δ_C (200MHz, CDCl₃): 12.2 (C-8), 28.4, 28.8 (C-3, -4), 33.6, 33.9 (C-2, -5), 58.9 (C-1'), 71.9 (C-7), 173.8 (C-1), 179.9 (C-7)

**Ethyl 9-nitro-8-oxo decanoate (93)**

![Structure of Ethyl 9-nitro-8-oxo decanoate](image)

93 was prepared in an analogous manner to the general procedure (pp.96) using ethyl 8-hydroxy-9-nitro decanoate (85, 183mg, 0.7mmol) and Jones Reagent (0.7cm³) in acetone (4cm³) in a yield of 158mg (87%). M⁺ 259.14205 (C₁₂H₂₁NO₅ requires 259.14197); δ_H (200MHz, CDCl₃): 1.32(3H, t, J=7.0, 2'-CH₃); 1.41 (4H, m, 4,5-CH₂); 1.70 (4H, m, 3,6-CH₂); 1.78 (3H, d, J=7.4, 10-CH₃); 2.42 (2H, t, J=7.4, 2-CH₂); 2.64 (2H, t, J=7.4, 7-CH₂); 4.18 (2H, q, J=7.0, 9-CH); δ_C (200MHz, CDCl₃): 12.2 (C-10), 14.5 (C-2'), 24.2, 24.6 (C-4, -5), 28.3, 28.8 (C-3, -6), 33.3, 33.5 (C-2, -7), 60.4 (C-1'), 71.4 (C-9), 173.4 (C-1), 179.8 (C-8)
**Ethyl 8-nitro-7-oxo octanoate (94)**

![Chemical Structure](attachment:structure1.png)

94 was prepared in line with the general procedure (pp.96) using Jones Reagent (0.6cm³) and ethyl 7-hydroxy-8-nitro octanoate (86, 150mg, 0.6mmol) in acetone (3cm³) in a yield of 130mg (94%). M⁺ 247.14201 (C₁₀H₁₇NO₅ requires 231.11067); δₜ (200MHz, CDCl₃): 1.13 (3H, t, J=7.0, 2'-CH₃); 1.45 (2H, m, 4-CH₂); 1.68 (4H, m, 3,5-CH₂); 2.37 (2H, t, J=7.2, 2-CH₂); 2.61 (2H, t, J=7.0, 6-CH₂); 4.32 (2H, q, J=7.2, 1'-CH₂); 5.35 (2H, s, 8-CH₂); δₜ (200MHz, CDCl₃): 14.4 (C-2'), 24.5 (C-4), 28.2, 28.4 (C-3, -5), 33.5, 33.6 (C-2, -6), 60.9 (C-1'), 71.1 (C-8), 174.0 (C-1), 179.9 (C-7)

**Ethyl 5-nitro-4-oxo pentanoate (95)**

![Chemical Structure](attachment:structure2.png)

95 was prepared in a similar manner the general procedure (pp.96) using Jones Reagent (0.9cm³) and ethyl 4-hydroxy-5-nitro pentanoate (87, 172mg, 0.9mmol) in acetone (5cm³) in a yield of 158mg (93%). M⁺ 189.06380 (C₇H₁₁NO₅ requires 189.06372); δₜ (200MHz, CDCl₃): 1.11 (3H, t, J=7.2, 2'-CH₃); 2.45 (4H, m, 2-CH₂); 4.29 (2H, q, J=7.0, 1'-CH₂); 4.49 (3H, m, 5-CH₂); δₜ (200MHz, CDCl₃): 14.1 (C-2'), 33.2, 33.5 (C-2, -3), 61.6 (C-1'), 71.3 (C-5), 174.3 (C-1), 180.2 (C-7)
Methyl 7-nitro-6-oxo heptanoate (96)

96 was prepared by means of the general procedure (pp.96) using Jones Reagent (1.2 cm³) and ethyl 6-hydroxy-7-nitro heptanoate (88, 246 mg, 1.2 mmol) in acetone (15 cm³) in a yield of 222 mg (91%). M⁺ 203.07945 (C₈H₁₃NO₅ requires 203.07937); δH (200 MHz, CDCl₃): 1.15 (3H, s, J=7.0, 2'-CH₃); 1.34 (4H, m, 3,4-CH₂); 2.19 (2H, t, J=7.3, 2-CH₂); 2.51 (2H, t, J=7.2, 5-CH₂); 3.69 (3H, s, 1'-CH₃); 4.59 (2H, m, 7-CH₂); δC (200 MHz, CDCl₃): 28.4, 28.7 (C-3, -4), 33.1, 33.5 (C-2, -5), 58.9 (C-1'), 70.2 (C-7), 174.0 (C-1), 179.7 (C-6)

Ethyl 9-nitro-8-oxo nonanoate (97)

97 was prepared in accordance to the general procedure (pp.96) using Jones Reagent (CrO₃/H₂SO₄, 0.6 cm³) and ethyl 8-hydroxy-9-nitro nonanoate (89, 148 mg, 0.6 mmol) in acetone (10 cm³) in a yield of 132 mg (90%). M⁺ 245.12622 (C₁₁H₁₉NO₅ requires 245.12632); δH (200 MHz, CDCl₃): 1.29 (3H, t, J=7.2, 2'-CH₃); 1.39 (4H, m, 4,5-CH₂); 1.68 (4H, m, 3,6-CH₂); 2.34 (2H, t, J=7.2, 2-CH₂); 2.59 (2H, t, J=7.2, 7-CH₂); 4.16 (2H, q, J=7.0, 1'-CH₂); 5.34 (2H, s, 9-CH₂); δC (200 MHz, CDCl₃): 13.9 (C-2'), 24.2, 24.6 (C-4, -5), 28.6, 28.9 (C-3, -6), 33.4, 33.6 (C-2, -7), 61.4 (C-1'), 70.9 (C-9), 174.0 (C-1), 179.6 (C-8)
Aminoalcohol and Aminoketone Synthesis – General Procedure

Nitroalcohol or nitroketone (0.5mmol) in EtOH/c.HCl (20:1, 1cm³) was added drop-wise to a suspension of Pt-S-C (10%, 10mg) in EtOH/c.HCl (20:1, 2cm³) and hydrogenated at 50°C for 16h. The solution was filtered, washed with MeOH (3cm³), and the filtrate evaporated under reduced pressure. The residue was dissolved in aqHCl (5M, 5cm³), stirred at 50°C for 4h and evaporated to dryness. Purification by ion exchange chromatography (DOWEX-50, H⁺ form, eluting with HCl, 0-1M) gave the desired aminoalcohol or aminoketone as its HCl salt.

8-Amino-7-oxo-nonanoic acid (8)

\[
\begin{align*}
\text{O} & \quad \text{O} \\
& \quad \text{NH}_3\text{Cl}^-
\end{align*}
\]

8 was prepared in accordance with the general procedure (pp.101) using ethyl 8-nitro-7-oxo octanoate (123mg, 0.5mmol) in a yield of 55mg (58%). Rf = 0.46 (butanol/acetic acid/water, 3:1:1), M+1(ESI +ve): 188.3 (C₉H₁₇NO₃ requires 187.2); for other data see 8-Amino-7-oxo-nonanoate hydrochloride (8), pp.79

5-Amino-4-oxo hexanoate (98)

\[
\begin{align*}
\text{O} & \quad \text{O} \\
& \quad \text{NH}_3\text{Cl}^-
\end{align*}
\]

98 was prepared in keeping with the general procedure (pp.101) using ethyl 5-nitro-4-oxo octanoate (90, 102mg, 0.5mmol) in a yield of 55mg (60%). Rf = 0.48 (butanol/acetic acid/water, 3:1:1), M⁺: 146.08164 (C₆H₁₂NO₃ requires 146.08172); δH (200MHz,D₂O): 1.47 (3H, d, J=7.2, 6-CH₃); 2.59 (4H, m, 2,3-CH₂); 4.21 (1H, q, J=6.9, 5-CH); δC (250MHz, D₂O): 14.2 (C-6), 33.6 (C-2), 37.4 (C-3), 54.9 (C-5), 179.5 (C-1), 209.8 (C-4)
**7-Amino-6-oxo octanoate (99)**

![Chemical structure of 7-Amino-6-oxo octanoate (99)](image)

99 was prepared in the same way as the general procedure (pp.101) using ethyl 7-nitro-6-oxooctanoate (91, 109mg, 0.5mmol) in a yield of 67mg (64%). Rf = 0.45 (butanol/acetic acid/water, 3:1:1), MH+: 174.11313 (C8H16NO3 requires 174.11302); δH (200MHz,D2O): 1.43 (3H, d, J=7.4, 8-CH3); 1.51 (4H, m, 3,4-CH2); 2.29 (4H, t, J=7.2, 2,5-CH2); 4.15 (1H, q, J=7.3, 7-CH); δC (250MHz, D2O): 14.9 (C-8), 23.7, 27.6 (C-3, -4) 33.3 (C-2), 37.9 (C-5), 54.6 (C-7), 179.0 (C-1), 209.4 (C-6)

**9-Amino-8-oxodecanoate (100)**

![Chemical structure of 9-Amino-8-oxodecanoate (100)](image)

100 was prepared in an analogous manner to the general procedure (pp.101) using ethyl 9-nitro-8-oxodecanoate (92, 105mg, 0.4mmol) in a yield of 54mg (57%). Rf = 0.41 (butanol/acetic acid/water, 3:1:1), MH+: 202.14441 (C10H20NO3 requires 202.14432); δH (200MHz,D2O): 1.41 (3H, d, J=7.4, 10-CH3); 1.49 (4H, m, 3,6-CH2); 1.77 (4H, m, 4,5-CH2); 2.55 (4H, m, 2,7-CH2); 4.19 (1H, q, J=7.1, 9-CH); δC (250MHz, D2O): 14.5 (C-b), 22.6, 23.5, 27.8, 28.1 (C-3, -4, -5, -6) 33.6 (C-2), 38.0 (C-7), 54.8 (C-9), 178.7 (C-1), 208.9 (C-8)

**8-Amino-7-oxo octanoic acid (101)**

![Chemical structure of 8-Amino-7-oxo octanoic acid (101)](image)

101 was prepared in keeping with the general procedure (pp.101) using ethyl 8-nitro-7-oxo octanoate (93, 116mg, 0.5mmol) in a yield of 67mg (67%). Rf = 0.44 (butanol/acetic acid/water, 3:1:1), MH+: 174.11310 (C8H16NO3 requires 174.11302); δH (200MHz,D2O): 1.00 (2H, m, 4-CH2); 1.29 (4H, m, 3,5-CH2); 2.07 (2H, t, J=7.1, 2-CH2); 2.29 (2H, t, J=73, 6-CH2); 3.83 (2H, s, 8-CH2); δC (250MHz, D2O): 22.5, 23.7, 27.2 (C-3, -4, -5) 33.3 (C-2), 37.1 (C-6), 54.2 (C-8), 178.7 (C-1), 209.5 (C-7)
5-Amino-4-oxo pentanoate (102)

![Chemical Structure]

102 was prepared in a analogous manner to the general procedure (pp.101) using ethyl 5-nitro-4-oxo pentanoate (94, 95mg, 0.5mmol) in a yield of 59mg (70%). Rf = 0.47 (butanol/acetic acid/water, 3:1:1); MH⁺: 132.06615 (C₅H₁₀NO₃ requires 132.06607); δₜ (200MHz,D₂O): 2.58 (2H, t, J=5.6, 2-CH₂); 2.75 (2H, t, J=7.4, 3-CH₂); 3.99 (2H, s, 5-CH₂); δC (250MHz, D₂O): 33.2 (C-2), 37.1 (C-3), 54.7 (C-5), 179.4 (C-1), 209.2 (C-4)

7-Amino-6-oxo heptanoate (103)

![Chemical Structure]

103 was prepared in keeping with the general procedure (pp.101) using ethyl 7-nitro-6-oxoheptanoate (95, 102mg, 0.5mmol) in a yield of 61mg (62%). Rf = 0.44 (butanol/acetic acid/water, 3:1:1); MH⁺: 160.09746 (C₇H₁₄NO₃ requires 160.09737); δₜ (200MHz,D₂O): 1.17 (4H, m, 3,4-CH₂); 2.28 (4H, t, J=6.4, 2,5-CH₂); 4.04 (2H, s, 7-CH₂); δC (250MHz, D₂O): 23.8, 27.8 (C-3, -4) 33.3 (C-2), 37.3 (C-5), 54.2 (C-7), 179.7 (C-1), 209.3 (C-6)

9-Amino-8-oxononanoate (104)

![Chemical Structure]

104 was prepared in an identical method to the general procedure (pp.101) using ethyl 9-nitro-8-oxononanoate (96, 123mg, 0.5mmol) in a yield of 66mg (59%). Rf = 0.40 (butanol/acetic acid/water, 3:1:1); MH⁺: 188.12875 (C₉H₁₈NO₃ requires 188.12867); δₜ (200MHz,D₂O): 1.22 (2H, m, 4,5-CH₂); 1.49 (4H, m, 3,6-CH₂); 2.27 (2H, t, J=7.3, 2-CH₂); 2.50 (2H, t, J=7.3, 7-CH₂); 3.96 (2H, s, 9-CH₂); δC (250MHz, D₂O): 22.5, 23.4, 27.8, 28.3 (C-3,-4,-5,-6) 33.9 (C-2), 37.8 (C-7), 54.8 (C-9), 179.0 (C-1), 209.3 (C-8)
8-Amino-7-hydroxy nonanoic acid (105)

8-Amino-7-hydroxy nonanoic acid (105) was prepared in line with the general procedure (pp. 101) using ethyl 7-hydroxy-8-nitro nonanoate (82, 124mg, 0.5mmol) in a yield of 78mg (69%). Rf = 0.31 (butanol/acetic acid/water, 3:1:1), MH⁺: 190.14443 (C₉H₂₀NO₃ requires 190.14432); δH (200MHz,D₂O): 1.33 (2H, m, 4-CH₂); 1.44 (3H, d, J=7.4, 9-CH₃); 1.61 (4H, m, 3,5-CH₂); 1.96 (2H, m, 6-CH₂); 2.32 (2H, t, J=7.4, 2-CH₂); 4.29 (2H, m, 7,8-CH); δC (250MHz, D₂O): 14.6 (C-9), 22.2, 23.9, 27.5 (C-3, -4, -5) 33.7 (C-2), 37.8 (C-6), 54.8 (C-8), 86.4 (C-7), 179.0 (C-1).

5-Amino-4-hydroxy hexanoate (106)

5-Amino-4-hydroxy hexanoate (106) was prepared in an equivalent method to the general procedure (pp. 101) using ethyl 4-hydroxy-5-nitro hexanoate (83, 103mg, 0.5mmol) in a yield of 58mg (63%). Rf = 0.33 (butanol/acetic acid/water, 3:1:1), MH⁺: 148.09742 (C₆H₁₄NO₃ requires 148.09737); δH (200MHz,D₂O): 1.50 (3H, d, J=6.9, 6-CH₃); 2.61 (4H, m, 2,3-CH₂); 4.25 (2H, m, 4,5-CH); δC (250MHz, D₂O): 14.2 (C-6), 33.6 (C-2), 37.4 (C-3), 54.9 (C-5), 86.3 (C-4), 179.5 (C-1).

7-Amino-6-hydroxy octanoate (107)

7-Amino-6-hydroxy octanoate (107) was prepared in line with the general procedure (pp. 101) using ethyl 6-hydroxy-7-nitrooctanoate (84, 110mg, 0.5mmol) in a yield of 69mg (65%). Rf = 0.32 (butanol/acetic acid/water, 3:1:1), MH⁺: 176.12877 (C₈H₁₈NO₃ requires 176.12867); δH (200MHz,D₂O): 1.41 (3H, d, J=7.0, 8-CH₃); 1.55 (4H, m, 3,4-CH₂); 1.79 (2H, m, 6-CH₂); 2.55 (2H, t, J=7.4, 2-CH₂); 4.33 (2H, m, 6,7-CH); δC (250MHz, D₂O): 14.9 (C-8), 23.7, 27.6 (C-3, -4) 33.3 (C-2), 37.9 (C-5), 54.6 (C-7), 85.9 (C-6), 179.0 (C-1).
9-Amino-8-hydroxydecanoate (108)

108 was prepared in a comparable manner to the general procedure (pp.101) using ethyl 8-hydroxy-9-nitrodecanoate (85, 105mg, 0.4mmol) in a yield of 57mg (59%). Rf = 0.29 (butanol/acetic acid/water, 3:1:1), MH⁺: 204.15989 (C_{10}H_{22}NO₃ requires 204.15997); δ<sub>H</sub> (200MHz,D₂O): 1.32 (4H, m, 4,5-CH₂); 1.45 (3H, d, J=6.8, 10-CH₃); 1.55 (4H, m, 3,6-CH₂); 1.77 (2H, m, 7-CH₂); 2.56 (2H, t, J=7.3, 2-CH₂); 4.33 (2H, m, 8,9-CH); δ<sub>C</sub> (250MHz, D₂O): 14.5 (C-10), 22.6, 23.5, 27.8, 28.1 (C-3, -4, -5, -6) 33.6 (C-2), 38.0 (C-7), 54.8 (C-9), 86.8 (C-8), 178.7 (C-1)

8-Amino-7-hydroxy octanoate (109)

109 was prepared according to the general procedure (pp.101) using ethyl 7-hydroxy-8-nitro octanoate (86, 117mg, 0.5mmol) in a yield of 72mg (68%). Rf = 0.25 (butanol/acetic acid/water, 3:1:1), MH⁺: 176.12854 (C₈H₁₈NO₃ requires 176.12867); δ<sub>H</sub> (200MHz,D₂O): 1.32 (2H, m, 4-CH₂); 1.54 (4H, m, 3,5-CH₂); 1.89 (2H, m, 6-CH₂); 2.25 (2H, t, J=7.3, 2-CH₂); 4.49 (3H, m, 8-CH₂, 7-CH); δ<sub>C</sub> (250MHz, D₂O): 22.5, 23.7, 27.2 (C-3, -4, -5) 33.3 (C-2), 37.1 (C-6), 54.2 (C-8), 86.3 (C-7), 178.7 (C-1).
5-Amino-4-hydroxy pentanoate (110)

110 was prepared in a similar manner to the general procedure (pp.101) using ethyl 4-hydroxy-5-nitro pentanoate (87, 100mg, 0.5mmol) in a yield of 54mg (64%). Rf = 0.28 (butanol/acetic acid/water, 3:1:1), MH⁺: 134.08165 (C₅H₁₀NO₃ requires 134.08172); δH (200MHz,D₂O): 1.92 (2H, m, 3-CH₂); 2.29 (2H, t, J=7.0, 2-CH₂); 4.29 (2H, m, 4-CH, 5-CH₂); δC (250MHz, D₂O): 33.2 (C-2), 37.1 (C-3), 54.7 (C-5), 86.7 (C-4), 179.4 (C-1).

7-Amino-6-hydroxy heptanoate (111)

111 was prepared in accordance with the general procedure (pp.101) using ethyl 6-hydroxy-7-nitro heptanoate (88, 103mg, 0.5mmol) in a yield of 57mg (58%). Rf = 0.24 (butanol/acetic acid/water, 3:1:1), MH⁺: 162.11295 (C₇H₁₄NO₃ requires 162.11302); δH (200MHz,D₂O): 1.54 (4H, m, 3,4-CH₂); 1.76 (2H, m, 5-CH₂); 2.27 (2H, t, J=7.1, 2-CH₂); 4.78 (3H, m, 6-CH, 7-CH₂); δC (250MHz, D₂O): 23.8, 27.8 (C-3, -4) 33.3 (C-2), 37.3 (C-5), 54.2 (C-7), 85.7 (C-6), 179.7 (C-1).
9-Amino-8-hydroxynonanoate (112)

112 was prepared in a similar manner as the general procedure (pp. 101) using ethyl 8-hydroxy-9-nitrooctanoate (89, 124mg, 0.5mmol) in a yield of 69mg (61%). Rf = 0.29 (butanol/acetic acid/water, 3:1:1), MH⁺: 190.14439 (C₉H₂₀NO₃ requires 190.14432); δH (200MHz,D₂O): 1.35 (4H, m, 4,5-CH₂); 1.70 (6H, m, 3,6,7-CH₂); 2.31 (2H, t, J=7.1, 2-CH₂); 4.41 (3H, m, 8-CH, 9-CH₂); δC (250MHz, D₂O): 22.5, 23.4, 27.8, 28.3 (C-3, -4, -5, -6) 33.9 (C-2), 37.8 (C-7), 54.8 (C-9), 86.2 (C-8), 179.0 (C-1), 209.3 (C-8)
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Appendix 1
Appendix 1

Courses Attended

Basic Course On Radiation Protection, Radiation Protection Service, University of Edinburgh, October 1998

Royal Society of Chemistry, Perkin Division, Scottish Regional Meeting, University of St Andrews, December 1998

Current Awareness in Organic Chemistry, Zeneca Postgraduate Lecture Course, University of Edinburgh, May 1999

Royal Society of Chemistry, Bio-Organic Group Meeting, Firbush, 9th-11th September 1999

Royal Society of Chemistry, Bio-Organic Group Pre Doctoral Symposium, Oxford University, December 1999, Post presentation given

Royal Society of Chemistry, Bio-Organic Group Pre Doctoral Symposium, Warwick University, December 2000, Oral presentation given

Royal Society of Chemistry, Perkin Division, Scottish Regional Meeting, Heriot Watt University, December 2000

Royal Society of Chemistry, Medicinal Chemistry Summer School, Nottingham University, 9th-13th July 2001

Royal Society of Chemistry, Annual Conference, ICC Birmingham, 1st-3rd August 2001, Poster presentation given

Royal Society of Chemistry, Bio-Organic Group Meeting, Firbush, 14th-16th September 2001

Organic Research Seminars, Department of Chemistry, University of Edinburgh, 1998-2001, 2 Oral presentations given

Mhairi I. Brunton

October 2002