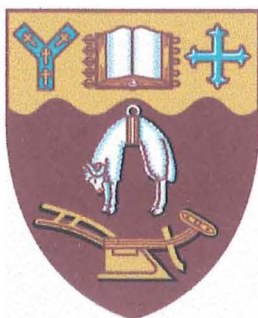


# Conformationally Restricted Amino Acid Analogues Based on Lactams

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A thesis  
submitted in partial fulfillment  
of  
Master of Science in Chemistry  
in the  
University of Canterbury  
by  
James Gardiner

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University of Canterbury

1998

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# Abstract

This thesis describes the synthesis of enamino esters 3(*S*)-(*E*)-benzoylamino-3-benzyl-5-ethoxycarbonylmethylidene-1-methylpyrrolidin-2-one **77**, and 3(*S*)-(*E*)-benzoylamino-3-benzyl-5-ethoxycarbonylmethylidene-1,2,4-dimethoxybenzylamino-pyrrolidin-2-one **79**. These were designed as a new class of conformationally restricted amino acid analogue.

Chapter one gives a general introduction to the importance of peptides in nature and the pivotal role peptides play in drug discovery and design. A number of examples are examined to provide an insight into key elements required in peptidomimetic design.

Chapter two introduces the process of peptidomimetic design, in particular that of *cis*-amide bond mimics. The synthesis of a new class of conformationally restricted peptidomimetic based on lactams is examined.

Chapter 3 discusses the synthesis of succinic acid-derived, and aspartic acid-derived,  $\beta$ -keto esters and amides **23**, **24**, **28**, via a Meldrum's acid procedure. This method provides the basis for the preparation of cyclic enamino esters. The preparation of the  $\beta$ -keto amide methyl-5-(N-ethoxycarbonylmethylcarbamoyl)-4-oxo-pentanoate **24** allowed chain extension in the *C*-direction in one easy step.

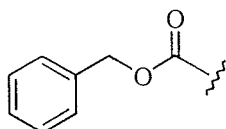
Chapter 4 examines the importance of ene-lactams in nature and the synthesis of enamino esters **55**, **56**, **59**, **61**, and enamino amides **63** and **64**, via the reaction of a  $\beta$ -keto ester, or amide, with an amine. Compounds **59** and **61** represent examples of 3-substituted *cis*-amide bond mimics that allow chain extension in both the *N* and *C* directions and subsequent incorporation into a peptide sequence.

Chapter 5 describes the synthesis of the target phenylalanine-derived 3,3-disubstituted enamino esters **77** and **79**, using the methodology established in chapter 4. Reaction of the enolate derived from (2*R*, 4*S*)-2-phenyl-3-benzoyl-4-benzyl-1,3-oxazolidin-5-one **65**, with *tert*-butylbromoacetate gave (2*R*, 4*S*)-2-phenyl-3-benzoyl-4-benzyl-4-(*tert*-butyloxycarbonylmethyl)-1,3-oxazolidin-5-one **67**. An x-ray crystal structure of **67** revealed that the reaction had proceeded with an inversion of configuration at C4, with the oxazolidinone ring being essentially planar in the solid state. This also revealed that

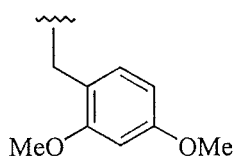
the stereochemistry at C3 could be determined by the stereochemistry of the amino acid from which it was derived. The *tert*-butyl ester was hydrolyzed and the resulting acid was converted to the  $\beta$ -keto ester **71** upon reaction with meldrum's acid and EtOH. The  $\beta$ -keto ester (2*R*, 4*S*)-2-phenyl-3-benzoyl-4-benzyl-4-(3-ethoxycarbonyl-2-oxopropyl)-1,3-oxazolidin-5-one **71**, was reacted with either methylamine, or 2,4-dimethoxybenzylamine (DMBNH<sub>2</sub>), and heated at 150° C, under reduced pressure, to give the target 3,3-disubstituted enamino esters **77** and **79**. The target enamino ester **77** and **79** allow chain extension in both the *N* and *C*-directions and are designed to be incorporated into a peptide sequence to mimic a *cis*-amide bond and allow investigation into the bio-active conformation of a specific peptide.

# Abbreviations

ACE	angiotensin converting enzyme
AIDS	acquired immune deficiency syndrome
Cbz or (Z)	benzyloxycarbonyl



DMB	2,4-dimethoxybenzyl
-----	---------------------



DMF	N,N-dimethylformamide
GH	growth hormone
GSH	glutathione
HIV	human immunodeficiency virus
IC <sub>50</sub>	concentration required to inhibit cell growth by 50%
NOE	nuclear overhauser effect
PTH	parathyroid hormone
TFA	trifluoroacetic acid
THF	tetrahydrofuran

# Introduction



Peptides and proteins are the indispensable agents of biological function and as such they are essential components of all organisms. In addition to their bio-catalytic functions, in the form of enzymes, proteins are also important components of tissues. Peptides play a vital role within an organism acting as chemical regulators and messengers. Numerous examples of biologically active peptides have been identified and characterized in recent decades. Biologically active peptides have been found to act as neurotransmitters, neuromodulators and hormones. Peptides have further been identified as pivotal components in the regulation of physiological processes within an organism. By interaction with a specific receptor, these peptides control a series of vital functions such as metabolism, cell-cell communication, digestion, immune response, respiration, sensitivity to pain, reproduction, behaviour and electrolyte levels. It is therefore of no surprise that biologically active peptides are of enormous interest to medicine and medicinal chemists. Medical conditions including hypertension, emphysema, gastrointestinal diseases, diabetes, Acquired Immune Deficiency Syndrome (AIDS), neuropsychiatric disorders, fertility disorders, prostate cancer, pain and inflammation can be treated with therapeutic agents which imitate, or block, the function of biologically active peptides and their receptors or enzymes.

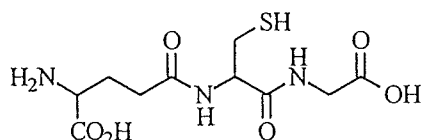
This introduction will give a brief overview of the structure and properties of biologically important peptides, along with a number of examples illustrating their importance in human biology. Finally, the role of peptides in the process of drug discovery and design will be addressed, with a particular emphasis placed on their use in the development of natural product-based systems and peptidomimetics.

## Biologically Active Peptides

Advances in chemistry, medicine and molecular biology have allowed the identification and characterization of many biologically active peptides and proteins found in nature. Peptides play an essential role in human biology in such processes as nerve stimulation and biochemical regulation (e.g. hormones). Due to their exceptional specificity, such peptides are not recognized by incompatible receptors, and the level needed to induce a response can remain relatively low. What follows is an account of a

number of important biologically active peptides that have been shown to play vital roles in human biological function.

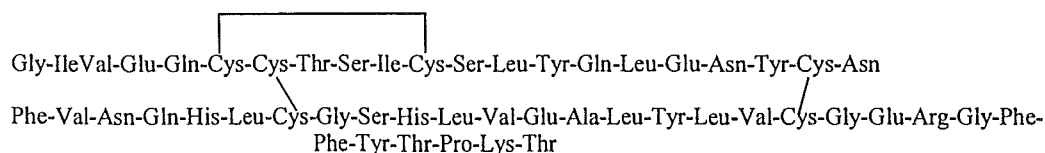
### Glutathione



Glutathione (L- $\gamma$ -glutamyl-L-cysteineglycine or GSH) is widely distributed in nature and occurs in virtually all animal cells, often in relatively high concentrations (0.1 – 10 mM).<sup>1</sup> This simple tripeptide is the most abundant intracellular thiol in almost all aerobic biological species due to the relative stability of its  $\gamma$ -peptide bond to degradation by protease enzymes. GSH has evolved as a molecule that provides protection against oxidation<sup>2</sup> and as such it has a number of important functions in metabolism, catalysis and transport. As an antioxidant its function is closely associated with the maintenance of a reductive environment in the interior of a cell. GSH maintains enzymes and other cellular components in a reduced state, while also protecting the cell against damaging radical and non-radical products of oxygen.

Glutathione was first observed as a reducing agent in yeast as early as 1888, but it was not until 1921 that F. G. Hopkin carried out a successful isolation. Its formulation as an  $\gamma$ -peptide of glutamic acid, cysteine and glycine was made by Pirie and Pinhey in 1929.

### Insulin

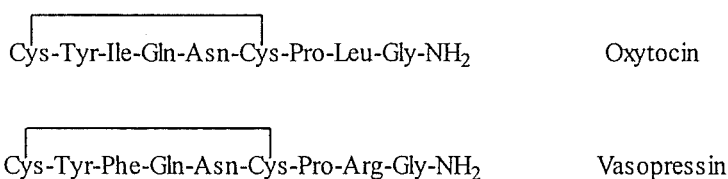


Insulin<sup>3</sup> is the primary peptide responsible for the regulation of glucose metabolism. It is produced by the so-called Langerhans islet cells in the pancreas, where it is released to act on nearby liver cells to regulate the breakdown of glycogen to glucose. A deficiency

of insulin leads to increased blood glucose levels and the common deficiency disease *diabetes mellitus*.

While diabetes still remains the third leading cause of death in the US today (a serious health risk) treatment of patients is possible through the industrial synthesis of human insulin via microbial fermentation.<sup>4</sup> This process involves the incorporation of the appropriate DNA sequence into a cell's nucleus with the resulting expressed peptide being isolated for clinical use. This powerful technique utilizes the cells own protein synthesizing ability to synthesize a peptide surplus to its needs. Insulin was first isolated in 1922 by Frederick G. Banting and Charles H. Best and consists of two peptide strands linked by disulphide bridges to give a complex three-dimensional structure.

### Oxytocin and Vasopressin



The peptide hormones<sup>5</sup> oxytocin and vasopressin originate in the pituitary gland, or neurohypophysis, an important regulatory gland at the base of the brain. Both are relatively small polypeptides with strikingly similar structures (where oxytocin has leucine and isoleucine, vasopressin has arginine and phenylalanine). Despite the similarity of the two amino acid sequences, these two polypeptides have quite different physiological roles.

Oxytocin is found only in females and stimulates uterine contractions during childbirth and the release of milk from the mammary gland during child rearing.

Vasopressin occurs in both males and females and causes contractions of peripheral blood vessels, and hence an increase in blood pressure. Its major function, however, is as an antidiuretic (induces water retention in the kidneys) and is often referred to as an antidiuretic hormone.

The structure of oxytocin and vasopressin also illustrates the importance of disulphide linkages between cysteine residues in maintaining the overall structure of a polypeptide. In these two molecules the disulphide linkages lead to a cyclic structure.

Although the physiological effects of these peptides were first observed, by H. H. Dale, as early as 1906, it was not until 1953 that the structure of oxytocin was determined by du Vigneaud, Ressler and Trippit. The structure of vasopressin soon followed with efforts in these areas leading to great advances in peptide synthesis and elucidation. Vincent du Vigneaud was later awarded the Nobel prize for his role in this work.<sup>6</sup>

### Renin-Angiotensin System

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

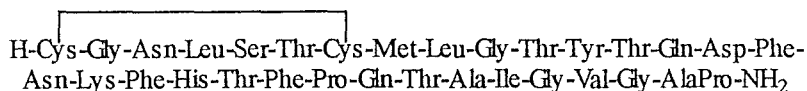
1

The Renin-Angiotensin system plays an important role in the regulation of blood pressure.<sup>7</sup> Increases in instances of cardiovascular diseases, such as hypertension and heart failure, have been attributed to an imbalance in this system. The system involves two enzymes, Renin and Angiotensin Converting Enzyme (ACE), which catalyze the release of angiotensin (1), one of the most potent known vasoconstrictors. This process begins in the liver with the synthesis of the large 60kD protein angiotensinogen. This peptide is then passed into the blood stream and is transported to the kidneys where it is cleaved by an aspartic acid protease (Renin) to give Angiotensin I. Angiotensin I is then further cleaved by ACE, primarily in the lungs, to give the biologically active octapeptide Angiotensin II (1). Upon binding to specific receptors on the surface of cells, 1 affects blood pressure directly by constriction of blood vessels, and indirectly by the release of the hormone aldosterone, from the adrenal gland, to induce sodium ion and water retention. Inactivation of 1 occurs by enzymatic cleavage of the C-terminal residue to give Angiotensin III.

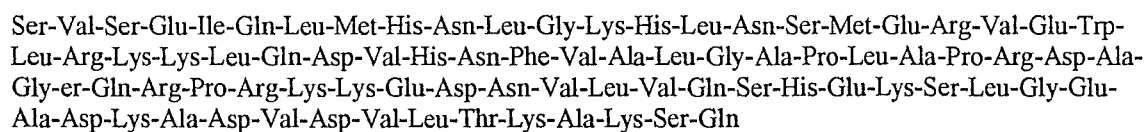
Successful antihypertensive drugs such as Captopril<sup>8</sup> are used in medicine to mediate such cardiovascular disorders. Captopril, like other hypertensive agents, inhibits the

action of ACE on its substrate angiotensin I. Determination of the amino acid sequence of the biologically active peptide Angiotensin II occurred in 1956.

### Calcitonin and Parathyroid Hormone



calcitonin

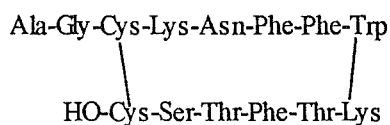


human parathyroid hormone (PTHh)

The vital regulation of the level of calcium ions in the blood is under the control of two peptide hormones. The parathyroid hormone (PTH) raises the concentration of blood calcium by mobilizing calcium uptake from the bones, while the thyroid hormone calcitonin directs the flow of calcium ions in the opposite direction. Thus a constant balance of calcium ion level is maintained in the blood.

The discovery of calcitonin<sup>9</sup> in 1962 by D. H. Copp led to it becoming a major focus of investigation in medicine. It was hoped that it would be of value in the treatment of osteoporosis and other diseases associated with the loss of calcium from the bones. Of the calcitonin isolated from various species, the hormone from salmon shows exceptionally high activity and is therefore used for therapy. However, the human hormone, despite its lower activity, is often preferable as it avoids the concerns of antibody formation. The complete sequence of the human parathyroid hormone<sup>10</sup> was obtained in 1978 and found to be a chain consisting of 84 amino acid residues.

### Somatistatin



Somatistatin, first isolated and characterized in 1973,<sup>11</sup> is a 14-residue cyclic peptide synthesized in the hypothalamus. Its biological function is as a release-inhibiting hormone, preventing the release of growth hormone (GH) from the pituitary gland. It also inhibits the secretion of several other hormones including those acting on the pancreas to prevent the release of insulin and glucagon. A lowered level of glucagon is beneficial in certain types of diabetes mellitus, therefore somatistatin became an important target in medical research.

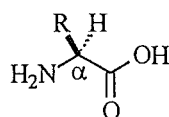
Somatistatin has been implicated in a vast array of actions in the central and peripheral nervous systems as well as in autocrine and paracrine regulation. As such it has been shown to have multiple receptor sites with five human somatistatin receptor sites having been identified.<sup>12</sup> Identification of these receptor sites has led to therapeutic uses of somatistatin, and its analogues, in the treatment of gastric ulcers, acromegalia, carcinoid tumours and intestinal scleroderma.

## Properties of Amino Acids and Peptides

Chemically, peptides and proteins (there is no clear distinction between the two) consist of linear sequences of amino acid building blocks joined by an amide linkage called a peptide bond. Two or more linked amino acids are called a peptide, while a long polypeptide chain is usually referred to as a protein. Although there are many hundreds of amino acids found in nature, there remains an exclusive group of only twenty primary amino acids of which nearly all peptides and proteins are made. Even with this seemingly small group of building blocks, the scope for diversity within any one peptide sequence is still quite staggering. For example, a simple tetrapeptide sequence offers over 200,000 possible combinations. Mammals e.g. humans, can only synthesize ten of the twenty primary amino acids. Those that can be synthesized in the body are classified as **non-essential**; that is, they are not required to be part of the diet. In contrast, those amino acids that cannot be synthesized are classified as **essential** and must be obtained from dietary sources, in particular the digestion of plant material. Plants synthesize amino acids by way of nitrogen fixation and the reduction of nitrates

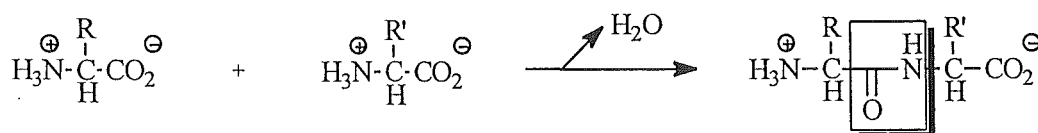
from the soil to give ammonia. This is then incorporated into a number of biosynthetic pathways to produce the various amino acids required for metabolism.

The general structure of a primary amino acid consists of an amino group ( $\text{NH}_2$ ), a carboxylic acid ( $\text{COOH}$ ) and a side chain group  $\text{R}$  attached to a central carbon atom ( $\text{C}_\alpha$ ). The primary amino acids differ only in the nature of the  $\text{R}$  group and it is the interactions between these groups within a peptide sequence that gives rise to the unique chemical and biological characteristics of that sequence. Of the two possible enantiomers, only that designated as the L-enantiomer is found in most peptides and proteins.



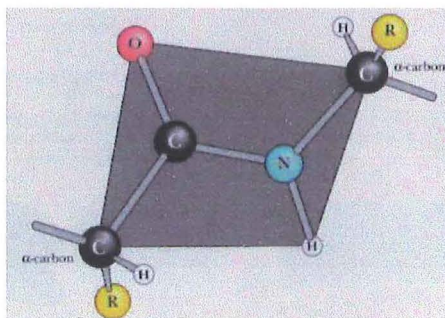
A primary L-amino acid

The formation of a peptide bond between two amino acids involves the formation of a covalent bond between the carbonyl group of one amino acid and the amino group of another.



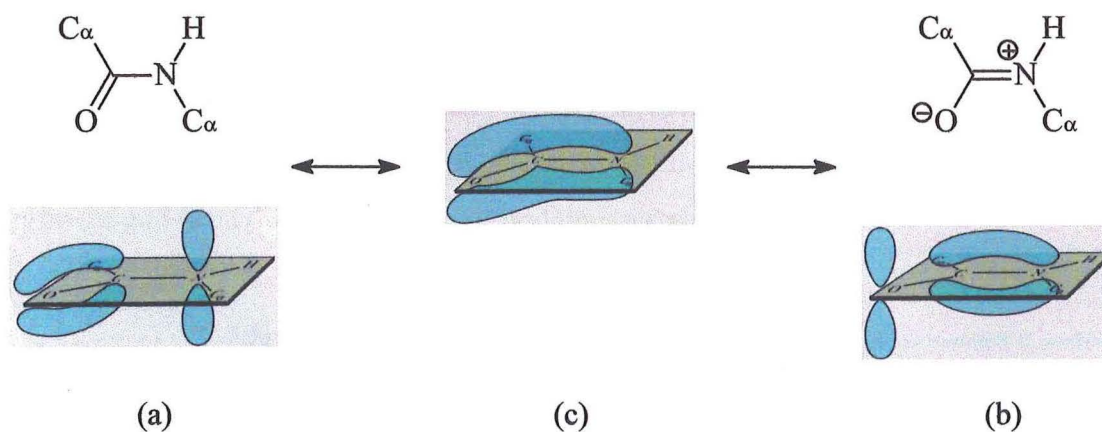
Formation of a peptide bond

The peptide backbone of a peptide or protein consists of the repeated sequence  $-\text{N}-\text{C}_\alpha-\text{CO}-$ , where the  $\text{N}$  is the amide nitrogen,  $\text{C}_\alpha$  the  $\alpha$ -carbon of the amino acid in the polymer, and the final  $\text{C}$  is the carbonyl carbon of the amino acid, which is in turn linked to the next amino acid in the chain. The geometry of the peptide bond is shown below. Note that the carbonyl oxygen and the amide hydrogen have been drawn *trans*. This conformation is favoured thermodynamically as it results in less steric hindrance between the  $\text{R}$  groups of adjacent amino acids. However as we shall see in later examples, it is the manipulation of this conformation (and in many cases manipulation towards the *cis* configuration) that gives rise to biological activity.



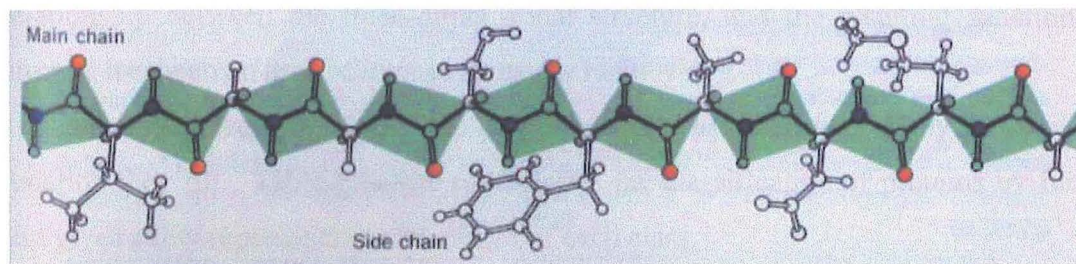
### Peptide bond geometry

One important property of a peptide bond is its ability to display partial double bond character. Two resonance structures can be shown to exist for a peptide bond. A pure double bond (a) between C and O would permit free rotation about the C-N bond, while the other resonance structure (b) would prohibit C-N bond rotation, but would place too great a charge on O and N. The true electron density (c) lies somewhere in between. The barrier to C-N bond rotation of, about 88kJ/mol, is enough to keep the amide group planar.



An essentially planar amide bond has a number of consequences, including placing all six atoms in a peptide bond group in the same plane. This creates a type of link effect with the principle point of rotation being about the  $\alpha$ -carbon.



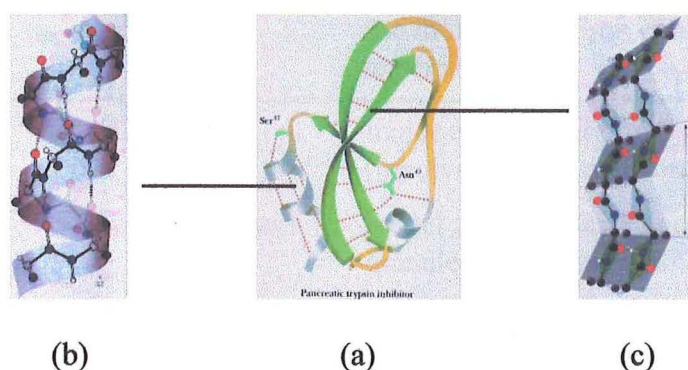


### Peptide 'link' effect.

The typical charge separation in an amide bonds means that the peptide backbone is quite polar, and hence is suitable for hydrogen bonding between the positively charged hydrogen on the nitrogen and the negatively charged carbonyl oxygen.



This type of interaction leads to a folding and coiling of the peptide chains, in an ordered way, to give secondary and tertiary structure. Three-dimensional structures such as the  $\alpha$ -helix and  $\beta$ -pleated sheet result and these occur regularly throughout proteins and form the basis of overall protein structure.<sup>13</sup>



(Diagram of pancreatic trypsin inhibitor showing (a) secondary and tertiary structure:  
(b)  $\alpha$ -helix, (c)  $\beta$ -pleated sheet.)

As virtually every cellular activity is dependent on one or more particular proteins, the relationship between the three-dimensional structure, and the resulting function of a protein, has been an area of intense study for many years.<sup>14</sup>

A convenient way to classify the enormous number of proteins is by the biological function they fill. Table 1 below summarizes the classification of proteins by function and gives a few representative examples of each class.

**Table 1** Biological Functions of Proteins and Some Representative Examples

Class	Example	Function
Enzymes	-Chymotrypsin	-catalyzes hydrolysis of dietary proteins
	-Catalase	-catalyzes hydrolysis of RNA
	-HIV	-catalyzes processing of AIDS virus proteins
Regulatory	-Insulin	-regulates glucose metabolism
	- <i>lac</i> repressor	-inhibits enzyme system responsible for lactose metabolism
	-CAP	-increases transcription rate of adjacent genes in <i>E. coli</i> chromosomes
Transport	-Hemoglobin	-transports oxygen from lungs to tissue
	-Glucose transporter	-transports glucose across cell membrane
Storage	-Casein	-most abundant milk protein, major source of nitrogen for mammalian infants
	-Ferritin	-iron binding protein used to store iron for use in important iron containing proteins ie hemoglobin
Contractile/ Motile	-Tubulin	-major component of microtubules (involved in cell division)
	-Dynein, Kinesin	-propel intracellular movement of vesicles, granules and organelles
Structural	- $\alpha$ -kerratin	-insoluble fibrous protein making up hair, horns and fingernails
	-Collagen	-strong fibrous protein found in bone, connective tissue, tendons and cartilage

	-Elastin	-important component of ligament
	-Fibroin ( $\beta$ -keratin)	-major component of cocoons (silk) and spider webs
Protective	-Immunoglobulin (antibodies)	-immune response involved in fighting off infection
	-Thrombin, Fibrinogen	-blood clotting proteins
	-Ricin	-plant toxin to thwart consumption
Exotic	-Antifreeze proteins	-present in Arctic/Antarctic fish to prevent their blood from freezing
	-Resilin	-elastic protein found in the hinges of insect wings
	-Glue proteins	-present in some marine organism to allow attachment to hard surfaces

It is worth repeating at this point that the great diversity of function in proteins, as reflected in the above table, is obtained using just twenty amino acids.

## Protease Enzymes

An important class of proteins, are those classified as protease enzymes. Protease enzymes constitute a large family of enzymes, which catalyze the hydrolysis of amide linkages in proteins and polypeptides. These enzymes are active in the digestion of food, the release of peptide hormones and neuromodulators from inactive precursors, activation of enzymes, and termination of biological responses by the degradation of the message transmitting peptide. Four classes of protease enzyme<sup>15</sup> have been identified, each with a characteristic mechanism of catalysis. These are classified as serine, aspartic, cysteine or metallo proteases based on the most significant catalytic functional group present in the enzyme active site. Illustrated below, in Table 2, are some examples of each of the classes of protease along with their associated biological function. In many medical conditions it is the inhibition of these enzymes that forms the basis for overall treatment.

Table 2 Examples of Proteases, Subdivided into Mechanistic Categories

Protease	Significant Active Site Groups	Example	Function
Serine	Ser (hydroxyl) His (imidazole) Asp (carboxyl)	chymotrypsin, trypsin, pancreatic elastase	digestion
		thrombin, plasma kallikrein, factors VIIa, IX-XIIa, activated protein C	blood coagulation
		tissue kallikrein, post protein cleaving enzyme	hormone metabolism
		elastase, cathepsin G, most cell chymases, tryptases	phagocytosis
		ATP dependent proteases	protein turnover
Metallo	Zinc	angiotensin converting enzyme (ACE), amino-peptidases, renal dipeptidases	blood pressure regulation
		carboxypeptidase	digestion
		collagenase	tissue elasticity
		macrophage elastase	blood pressure regulation, peptide metabolism
Aspartic	Asp (carboxyl)	renin	blood pressure regulation
		HIV protease	HIV replication
		pepsin, thermolysin	digestion
Cysteine	Cys (thiol)	cathepsins B, H, L, calcium activated neutral proteases	protein turnover, bone resorption

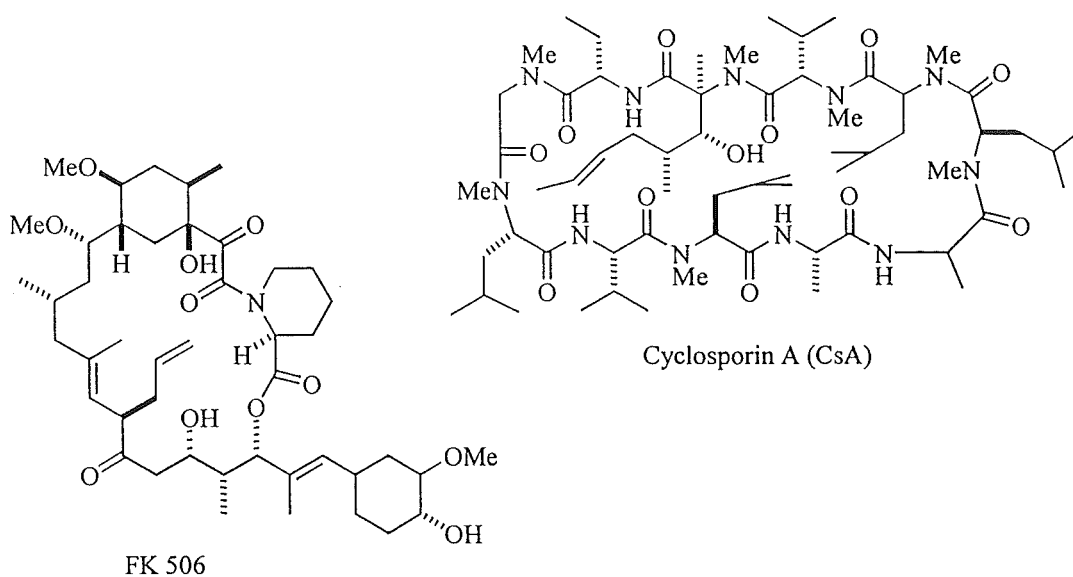
## Peptidomimetics

Peptides and their analogues have long been used in medicinal chemistry as therapeutic agents against a range of pathological conditions, generally characterized by a disruption of the interactions between an enzyme substrate or messenger and their targets, the enzymes and receptors. The use of biologically active peptides as therapeutic agents is often severely limited by their pharmacological properties. In particular, degradation in the gut and serum, poor absorption after oral ingestion and rapid excretion through the liver and kidneys restrict their usefulness as drugs. In overcoming these problems there has been an increasing tendency towards the use of so called chemical 'Trojan horses' or peptidomimetics.<sup>16</sup>

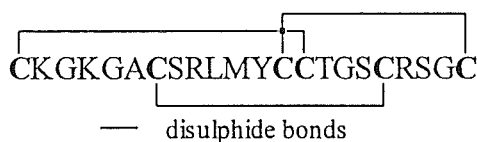
“A peptidomimetic is defined as a substance having a secondary structure as well as other structural features analogous to that of the original peptide that allow it to displace the original peptide from its receptor or enzyme. As a result, the effects of the original peptide are either inhibited (antagonist, inhibitor) or duplicated (agonist).”<sup>16</sup>

A successful peptidomimetic, while possessing all these characteristics, must also be sufficiently non-peptidic to overcome the problems of degradation and bio-availability associated with peptides, while exhibiting minimal side effects. This has led to the development of new and more effective types of treatment for a wide range of diseases.

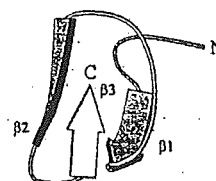
There are two sources of peptidomimetics: natural sources, and rational drug design and synthesis. Natural products have long served as a significant source of important peptidomimetics. Screening of extracts for a particular biological activity from plant, animal, microbial or fungal sources provide an invaluable guide to the identification and isolation of bio-active natural products. In this way important leads in drug discovery and development can be revealed for the potential treatment of specific diseases. This is often achieved through mass screening and relies on the assumption that a desired active compound already exists in nature. Examples of successful natural product peptidomimetics include Cyclosporin A (CsA), FK-506 and  $\omega$ -conotoxin MviiA.



CsA<sup>17</sup> and FK-506<sup>17</sup> are generally viewed as immunosuppressants, with the macrocyclic peptide CsA having emerged as the principle immunosuppressive agent for solid organ transplants. The wealth of studies surrounding the interactions of CsA, and the macrolide FK-506, with their corresponding immunophilin receptors have greatly advanced research in immunosuppressants.



ω-Conotoxin MviiA



The marine natural product ω-conotoxin MviiA<sup>18</sup> is currently in Phase II/III clinical trials as a powerful agent against intractable pain. It has high selectivity for N-type  $\text{Ca}^{2+}$  channels and with the added characteristic of being non-addictive, is touted as the future alternative to morphine. Other novel bio-active peptides, particularly of marine origin, continue to be isolated and promise to assist in the design of new therapeutic agents.

Alternatively, a peptidomimetic may be obtained through the process of rational design and synthesis. This involves obtaining a detailed knowledge of the conformational, topochemical and electronic properties of the native peptide and that of its corresponding receptor or enzyme active site.

The design of peptidomimetics as potential bio-active substances must take particular account of two structural factors.<sup>16</sup>

1) Favourable fit - this involves identifying the bio-active conformation(s) corresponding to the complementary spatial situation at the receptor or active site. If necessary, the conformation of the peptidomimetic can be stabilized by the introduction of elements conferring rigidity.

2) Favourable interactions - this involves the placement of certain functional elements (e.g. functional groups, polar and hydrophobic regions) in defined positions so that the required interactions (e.g. hydrogen bonds, electrostatic or hydrophobic interactions) can occur.

### Structural Possibilities in Peptidomimetic Design

From a structural point of view, peptidomimetics can be prepared by approaches ranging from the slight modification of the initial structure to the generation of a pure non-peptide. These approaches are categorized below.

#### Modification of the Side Chains of Amino Acid Residues

The incorporation of unnatural amino acids and amino acid surrogates into peptides by chemical methods has often been used to study structure-function relationships. The replacement of a residue by its optical isomer provides useful information regarding possible turn sites while modification of a side chain gives clues to its role in the bio-active conformation.

A recent example demonstrates that this is still important today. Modification of the tyrosine side chain, by the introduction of methyl groups at the 2', 6' and  $\beta$ -positions,<sup>19</sup> hinders free rotation about the  $C_\alpha$ - $C_\beta$  bond and can thus favour the formation of bio-active conformations. This has been used to study the effects of restricted rotation of aromatic side chains in the interior of proteins<sup>20</sup> (e.g. bovine pancreatic trypsin inhibitor),<sup>21</sup> as well as peptide-protein complexes (e.g. oxytocin and neurophysin)<sup>19</sup> and bio-active peptides (e.g. methionine-enkephalin).<sup>19</sup>



### Modification of the Peptide Backbone

Modification of a peptide backbone generally involves the exchange of units within a peptide chain with electronically and/or sterically equivalent units, as well as the incorporation of additional units. This generally leads to an increase in the biological half-life in comparison to the native peptide.

A common approach is the replacement of an amide bond with a suitable mimic. This has proved important in the development of enzyme inhibitors as it can result in a compound resistant to hydrolysis. Cases where amide bonds have been replaced with a ketoethylene ( $\text{COCH}_2$ ) or a hydroxyethylene ( $\text{CH}(\text{OH})\text{CH}_2$ ) have led to compounds that are among the most active inhibitors of Renin and HIV-1 protease. Some common examples of peptide backbone modifications are outlined below.<sup>16</sup>

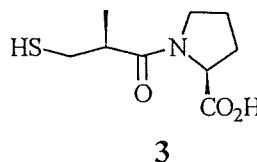
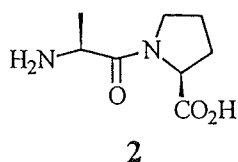
Replacement of:				Fragment addition:
-NH-	-CH(R)-	-CO-	-CO-NH-	-NH-X-CH(R)-
-O-	-N-	-CS-	-NH-CO-	-O-
-S-	-CR-	-CH <sub>2</sub> -	-CH(OH)-CH <sub>2</sub> -	-NH-
-CH <sub>2</sub> -	-BH-	-SO <sub>2</sub> -	-CH=CH-	-CH <sub>2</sub> -

The development of the therapeutic agent Captopril<sup>22</sup> (see earlier) is a simple and yet prime example of enzyme inhibition by modification of a peptide backbone. Captopril prevents the release of the hypertensive peptide hormone Angiotensin II by inhibiting the enzyme responsible for its production - Angiotensin Converting Enzyme (ACE). Lowering the Angiotensin II levels reduces chronic high blood pressure and thus the incidents of heart failure and strokes. Captopril was developed from the lead structure (2), a simple dipeptide shown to be a weak inhibitor of ACE. Replacement of the terminal amine of (3) with a mercapto group gave the potent and orally active drug



Captopril.<sup>16,23</sup> The mercapto group was introduced to coordinate to a zinc ion which is known to be located in the active site of ACE.

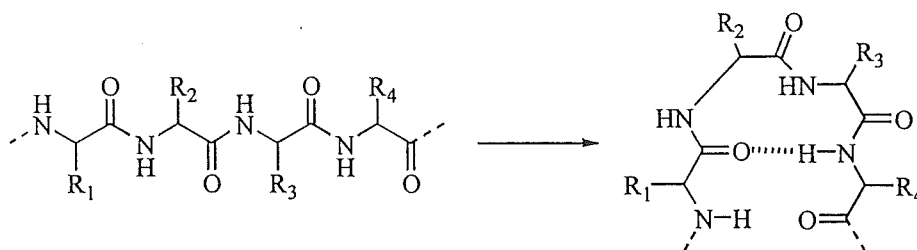
1 Asp-Arg-Val-Try-Ile-His-Pro-Phe



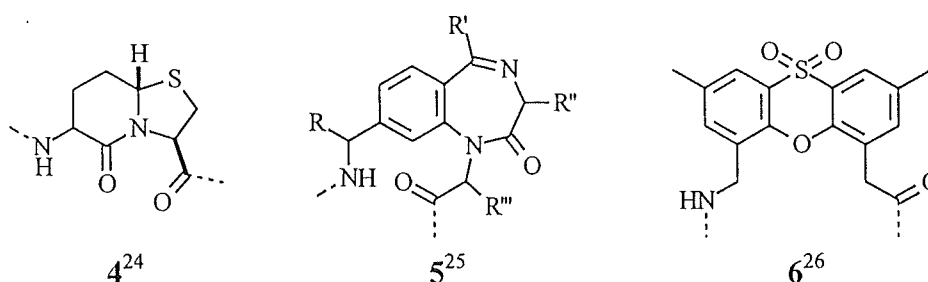
Inducement and Stabilisation of Secondary Structure ( $\beta$ -turns,  $\gamma$ -turns,  $\alpha$ -helix,  $\beta$ -sheet)

Secondary peptide structure plays an important role in biological activity.  $\alpha$ -Helices,  $\beta$ -sheet structures, turns and loops are all essential components of peptides and proteins.<sup>13,14</sup> Therefore, the retention of such structures by mimeticism is an important tool for the fixing of the bio-active conformation in a peptide containing these elements.

A good example of this is the  $\beta$ -turn, an element widely recognized as being important as a molecular recognition site. A common  $\beta$ -turn consists of 4 amino acids and is stabilized by a hydrogen bond between the carbonyl of the first and the NH group of the fourth. The main feature of a  $\beta$ -turn is the reversal of the peptide chain by approximately 180°.



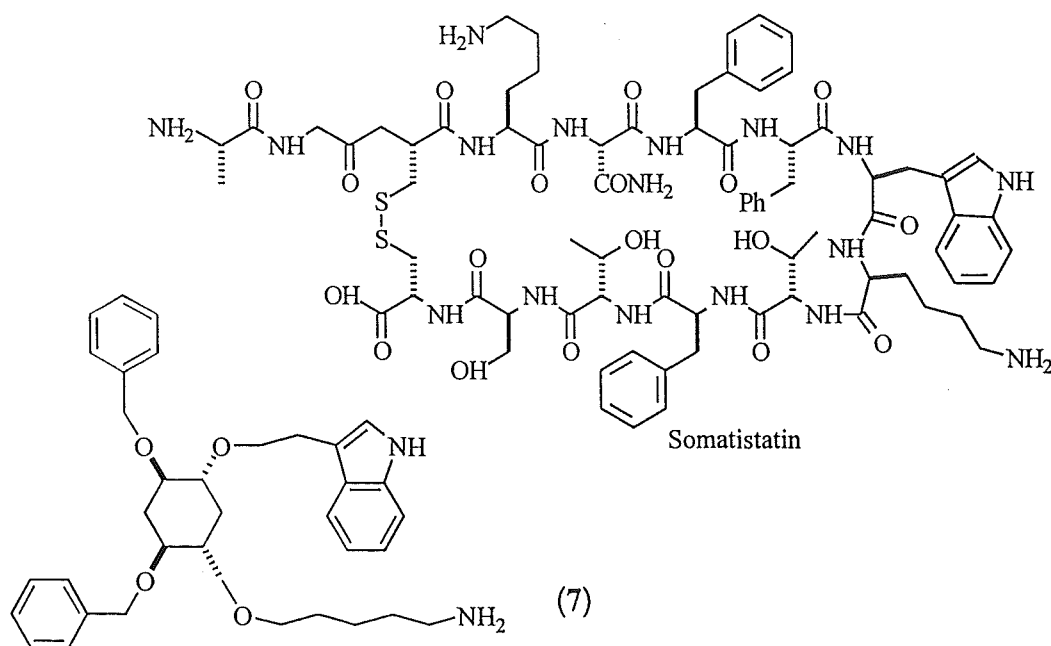
Compounds 4,5 and 6 are examples of  $\beta$ -turn mimics and demonstrate that the structural motifs involved can range from still recognizable peptide chains to completely non-peptidic components.

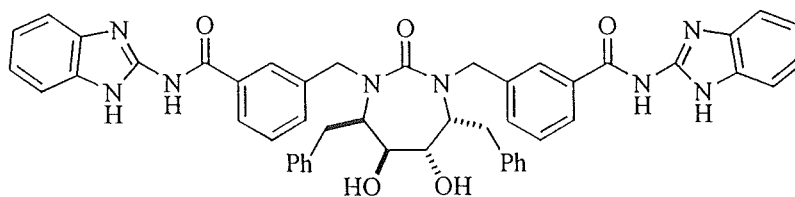


### Scaffold Peptidomimetics

This class of peptidomimetics utilizes a completely unnatural framework to support the elements of the original peptide responsible for its effectiveness. Compounds of this type appear particularly interesting with regards to their potential oral bio-availability. It is therefore possible to use frameworks or structural elements of known compounds whose oral absorption properties are established.

A successful example of this technique involved the macrocyclic hormone Somatistatin.<sup>27</sup> Extensive structure-function and conformational studies led to the discovery of a non-peptidyl partial somatistatin agonist 7, containing a  $\beta$ -D-glucose scaffold.<sup>28</sup> Another example 8, utilizes a cyclic urea scaffold and has been shown to be a potent *in vitro* inhibitor of HIV-I protease.<sup>29</sup> These examples demonstrate the ability of scaffolds to hold key functional elements in the desired bio-active conformation.

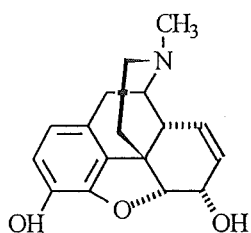




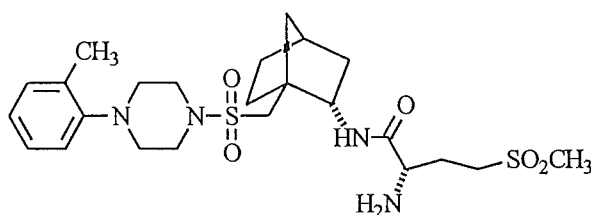
8 SD-146

### Non-Peptide Mimetics

Non-peptide mimics have generally been discovered through the course of random screening of large numbers of substances, including microbial extracts and fungal metabolites. These mimetics belong to various classes of organic compounds and bare no apparent structural resemblance to the native peptide. Once a bio-active structure has been obtained, attempts can be made to optimise its structure through systematic structure modification. The analgesic morphine **9** is an example of a completely non-peptide mimetic, while compound **10** has arisen through development of antagonists of the pituitary hormone oxytosin. It has shown to be a potent antagonist with an  $IC_{50}$  value of less than 10 nM.<sup>30</sup>



9



10

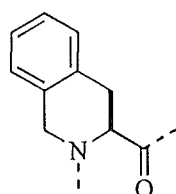
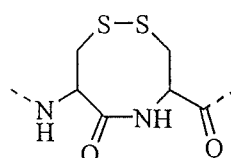
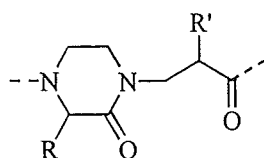
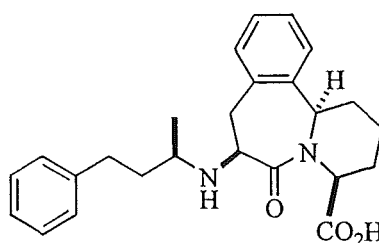
### Conformation Stabilizing Rings

The final, and perhaps the most important class of peptidomimetics, in terms of this thesis, is that of conformational stabilizing rings. This has proven to be a successful method for the development of peptidomimetics and involves the introduction of bridges

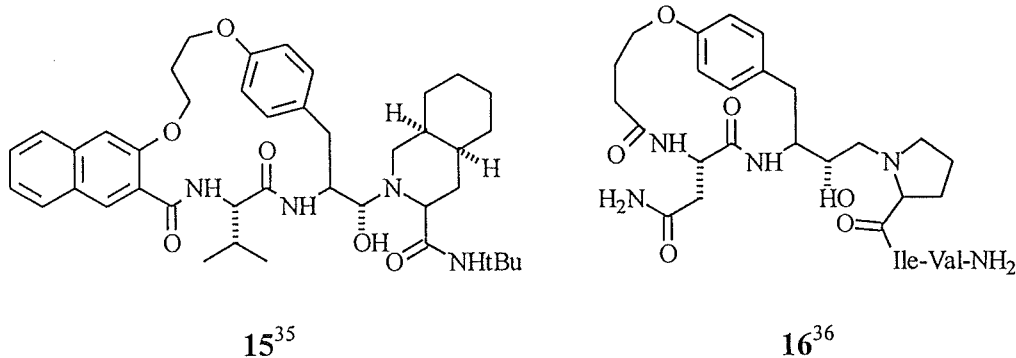
of varying lengths between different parts of the molecule, thereby making the molecule more rigid. With careful design, this has the effect of imitating the receptor bound conformation of the biologically active peptide and thus allows investigation into the relationship between peptide structure and function.

In the past most enzyme inhibitors have been based on either substrate analogues or transition state mimics. However, these inhibitors have been hampered by poor stability, bio-selectivity and bio-availability. The introduction of conformational restriction into a drug can improve potency by locking it in a bio-active state and reducing the loss of entropy that occurs upon its binding to a receptor or enzyme active site. This increased potency may in turn permit the use of fewer amino acid residues in a peptide recognition sequence, thereby lowering the molecular weight and improving bio-availability.

The specificity of the inhibitor for an enzyme may also be enhanced by excluding conformations that are capable of inhibiting other enzymes. Commonly, two adjacent amino acid residues are involved in bridging but there are many other sites where this is possible. These include within a single amino acid (**11**), between two amino acid side-chains (**12**), between backbone units (**13**) or between a backbone unit and a side chain as in the case of (**14**), a potent inhibitor of ACE.

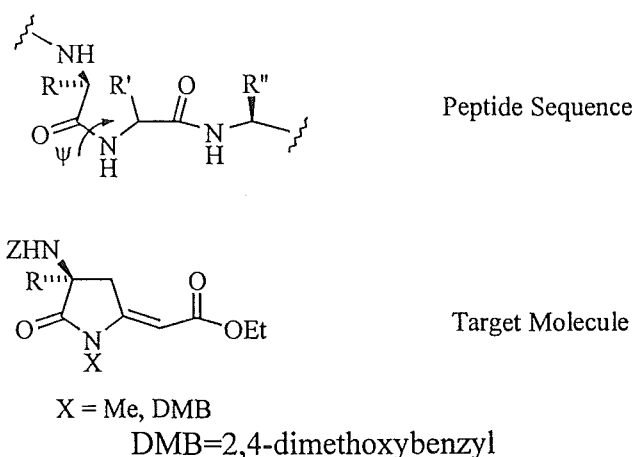
**11**<sup>31</sup>**12**<sup>32</sup>**13**<sup>33</sup>**14**<sup>34</sup>

Compounds **15** and **16** are examples of macrocyclic peptidomimetics where the P<sub>1</sub> and P<sub>3</sub> residues are linked to bring them close in space and induce biological activity. Both **15** and **16** and their derivatives are potent inhibitors (1-30 nM) of HIV protease and display enhanced metabolic stability relative to acyclic inhibitors.



## Research Described in this Thesis

This thesis is concerned with the synthesis of a new class of conformationally restricted amino acid analogue, as a potential tool in enzymatic studies. The proposed system is based on a lactam. The target molecules are illustrated in Figure 1, alongside the analogous section of peptide backbone they are intended to mimic.



**Figure 1** Target *cis*-amide bond mimics

The target peptidomimetics bridge the peptide backbone as shown. This restricts the otherwise free rotating system about the amide bond (indicated by  $\psi$ ). The result is to

lock the peptide bond in a *cis*-like conformation\* which, in many cases, is known to be biologically important. Although this does not necessarily lead to an enzyme inhibitor, by studying the change in enzyme activity, information can be obtained concerning the preferred conformation of natural substrates. These results would provide valuable insights into the optimal conformation required for biological function.

\* This assignment adopts the standard method practiced in peptide chemistry, whereby the configuration of the peptide backbone is said to be *cis* and does not recognize the higher priority of the carbonyl oxygen relative to the CN bond.<sup>37</sup>

# Peptidomimetic design

An important principle in the design and synthesis of peptidomimetics is conformational restriction (refer to Introduction). This involves the flexibility of a system being restricted in some way to promote biologically active conformations. By replacing one or more components of a target peptide with various structural modifications, thereby restricting the degree of rotational freedom along the peptide backbone, it is possible to probe the conformational requirements of the peptide for biological activity.

Modifications of this type have also been shown to improve the pharmacological properties of such analogues by reducing their peptidic character. This can lead to an increase in bio-stability and hence compounds more suitable for therapeutic use. The aim of this process is to produce a non-peptidic ligand constrained to adopt the appropriate bio-active conformation.

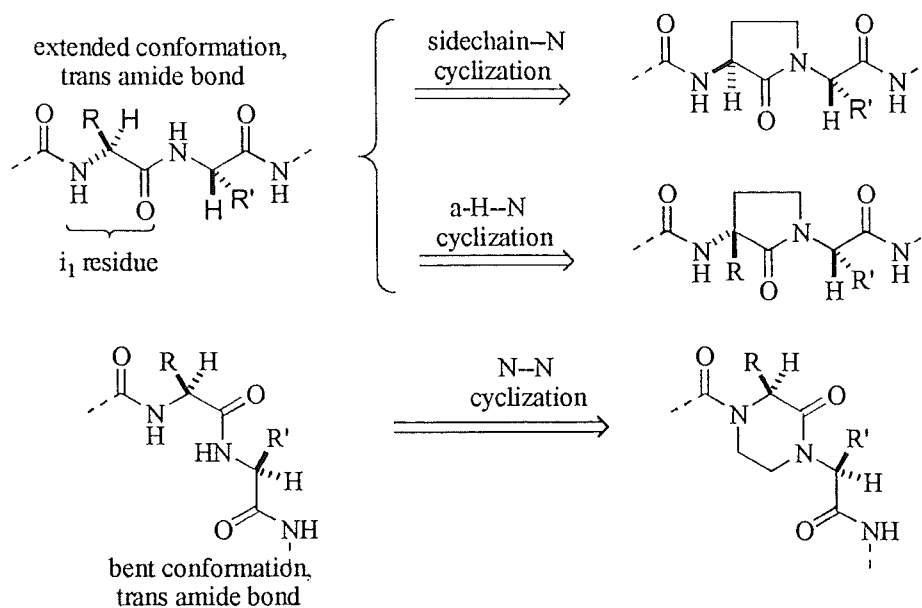
## Freidinger lactams

Conformational restriction of a peptide chain can be achieved in a number of ways. Perhaps the most successful and widely used strategy is that of cyclization. This involves the introduction of bridges of varying lengths between different parts of the molecule, thereby making the molecule more rigid. Among this most important class of compounds, Freidinger lactams, and their close relatives, have proved among the most useful in drug design.

A Freidinger lactam is derived from a peptide in which the  $\alpha$ -position of an arbitrarily chosen amino acid residue has been connected to a down-stream amide nitrogen through the addition of a carbon bridge. The original Freidinger lactam,<sup>38</sup> and the motif most commonly used, involves the formation of a ring from the position normally occupied by the  $i_1$  side-chain forming a 3-amino (or 3-amido) lactam (see Figure 2).

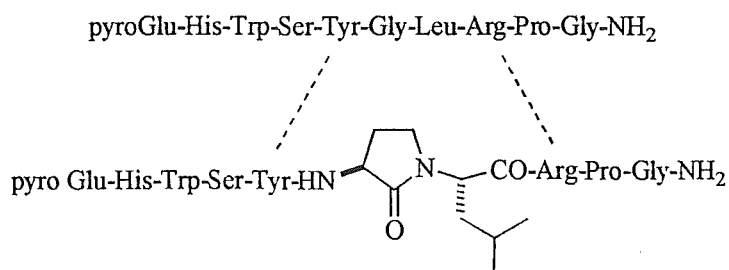
Alternatively, it is possible to replace the  $\alpha$ -hydrogen of this residue with the bridging ring while still retaining the normal side-chain. Peptidomimetics in which the nitrogens of adjacent residues are linked have also been reported (see Figure 2).<sup>33</sup>





**Figure 2** Freidinger lactams derived from a peptide

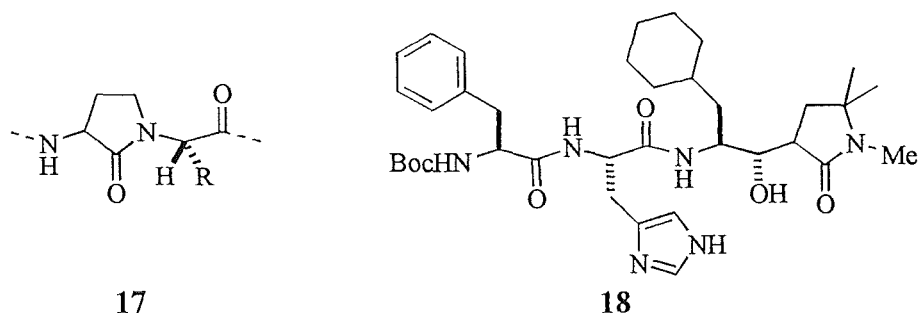
Freidinger and his colleagues at Merck first reported the use of  $C_{\alpha}$ -N cyclizations for the constraint of peptides in 1980.<sup>38</sup> By far the most influential paper published in this field concerned their design and synthesis of a potent analogue of the hypothalamic hormone luteinizing-hormone-releasing hormone (LHRH, also known as gonadotropin-releasing hormone GRH). This analogue contained a lactam constituent, that successfully constrained the peptide to imitate the Tyr-Gly-Leu-Arg type II  $\beta$ -turn in the bio-active conformation of the molecule (Figure 3). This lactam-containing analogue was found to be 8.9 times more potent as LHRH in an *in vitro* pituitary cell culture study and 2.4 times as potent in an *in vivo* rat study.



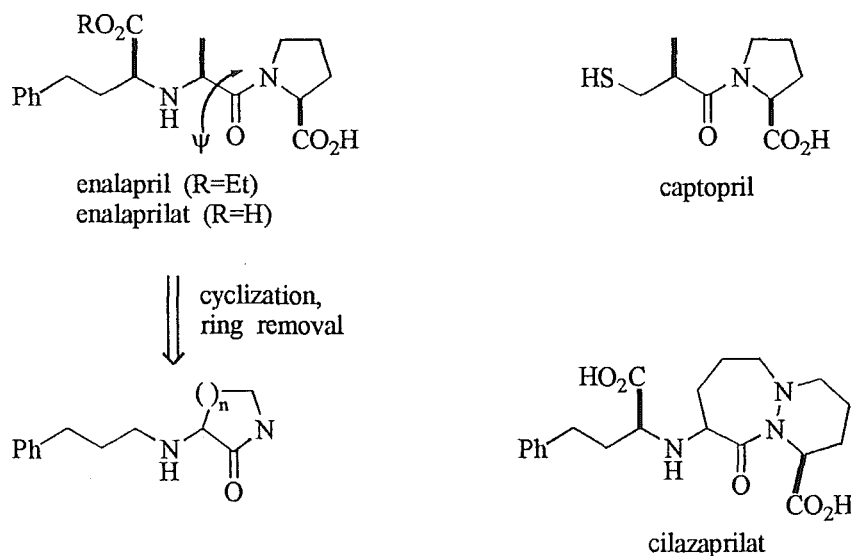
**Figure 3** Incorporation of a lactam constituent to mimic  $\beta$ -turn of LHRH

Further investigations of the uses of these lactams for several other biological targets revealed them to be a successful general tool in achieving conformational constraint of bio-active peptides.

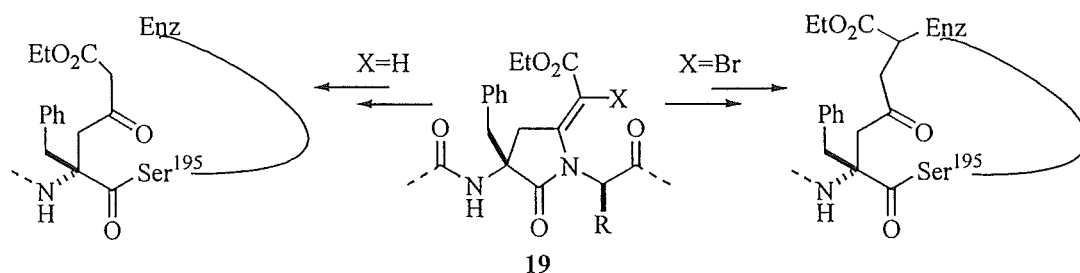
The most in-depth use of Freidinger lactams has been for the control of blood pressure, by interfering with the Renin-Angiotensin system (see introduction). The incorporation of **17** into inhibitors of Renin, led to more potent inhibitors which were resistant to degradation by chymotrypsin. For example **18** was shown to have an  $IC_{50}$  of 1.3 nM, whereas the corresponding analogue, where the lactam ring was removed, had an  $IC_{50}$  of 45 nM, a 35 fold reduction in potency.<sup>39</sup>



Attempts to follow up the enormous commercial success of the Angiotensin Converting Enzyme (ACE) inhibitor Captopril **3** (see Introduction), and enalapril, led to new classes of inhibitors containing the lactam substituent being developed for the treatment of hypertension and congestive heart failure. Studies of five-, six-, seven-, and eight-member lactams as conformationally restricted analogues of enalaprilat, an ACE inhibitor, led to the determination of  $\psi$  (the torsion angle) in the bio-active conformation of enalaprilat.<sup>40</sup> This information was then used to develop enalaprilat analogues of increased potency, such as cilazaprilat, constrained to the optimum  $\psi$  angle.<sup>41</sup>



The use of Freidinger lactams to incorporate latent reactivity has also been used in the development of mechanism-based inhibitors of the serine protease chymotrypsin. Compounds of type **19**,<sup>42</sup> which include a substituted lactam ring, can lead to the covalent binding of the inhibitor to the enzyme active site (Figure 4).



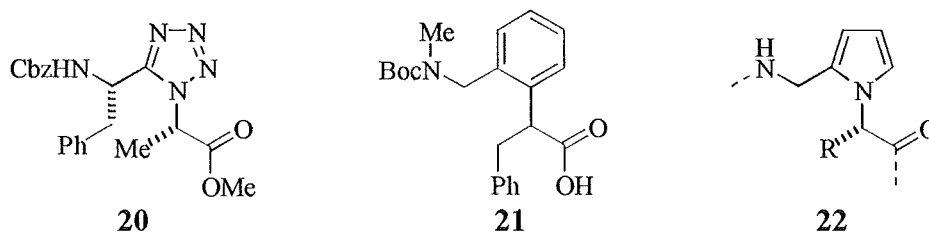
**Figure 4** Freidinger lactams as Mechanism-based Inhibitors of Serine Proteases

## cis-Amide bond mimics

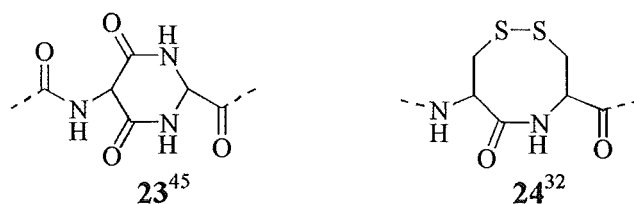
It is worth pointing out at this stage that all the examples of Freidinger lactams discussed to date, place the amide bond within the lactam ring and constrain it to a *trans* configuration, with respect to the peptide backbone. This, it would seem, has been the focus of applications of Freidinger lactam and has proved successful in the development of peptidomimetics for processes where this is a desired bio-active conformation. However, the partial double bond character of an amide bond leads to *cis* and *trans* isomers. As was discussed earlier (see Introduction), the configuration of an amide bond in a peptide is of crucial importance to the overall conformation of the molecule, and therefore to its ability to bind to a receptor. In many cases, binding to a receptor will only occur with a *cis*-configuration at certain amide bonds.

In nature, *cis*-amide bonds can occur in the form of *N*-alkylated amides, secondary structures such as turns, constrained cyclic peptides and bio-active conformations. In a normal amide bond, the *trans* configuration is thermodynamically favoured over the *cis* by approximately 10 kcal/mol, due to less steric hinderance between adjacent groups. In *N*-alkylated amides, for example, the energy difference between the *cis* and *trans* isomers is only about 2 kcal/mol, due to both isomers having similar 1,4-interactions, and the *cis* and *trans* forms can both be observed by NMR spectroscopy. *N*-Alkylated

amides are found in a variety of biologically important peptides and make the *cis*-amide isomer more energetically accessible for recognition and binding to a given receptor. Proline is the only naturally occurring amino acid that leads to *N*-alkylated amides. Many other cases exist where either local, or global interactions, favour a *cis*-amide configuration in a biologically active peptide. The biological activity of Angiotensin **1**, for example, has been shown to coincide with the isomerization of the His-Pro amide bond to the *cis* configuration.<sup>37</sup> Compounds **20**,<sup>43</sup> **21** and **22**,<sup>44</sup> are examples of dipeptide analogues that mimic *cis*-amide bonds.

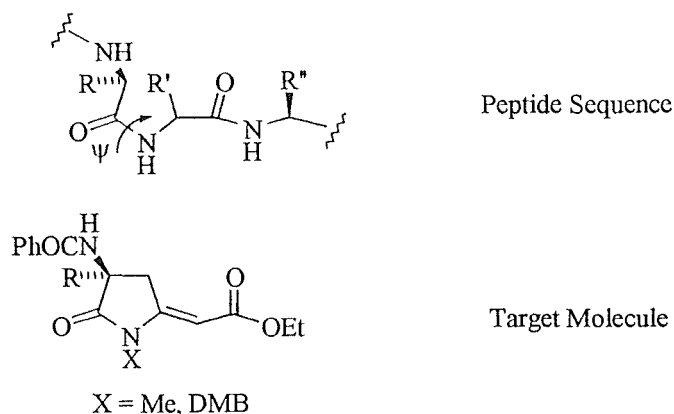


The development of *cis*-amide bond analogues that mimic this form of biologically active conformation is an important general technique in peptidomimetic design. It is with this in mind that we propose a new class of conformationally restricted *cis*-amide bond analogue incorporating a Freidinger lactam arrangement. Previously reported Freidinger lactams containing a *cis*-like amide constraint have proved to be scarce. Two examples of these are shown below.



Peptide azides such as **24**, a reduced form of Cys-Cys, have been of particular interest, as hexapeptide analogues containing such units show substance P antagonistic activity up to ten times that of the original peptide.

It is with the scarcity of *cis*-amide mimics of this type in mind, that we propose a new class of conformationally restricted *cis*-amide bond analogue incorporating a Freidinger lactam arrangement. The proposed system is based on a lactam and is illustrated in Figure 5, along with the analogous section of peptide backbone it is intended to mimic.



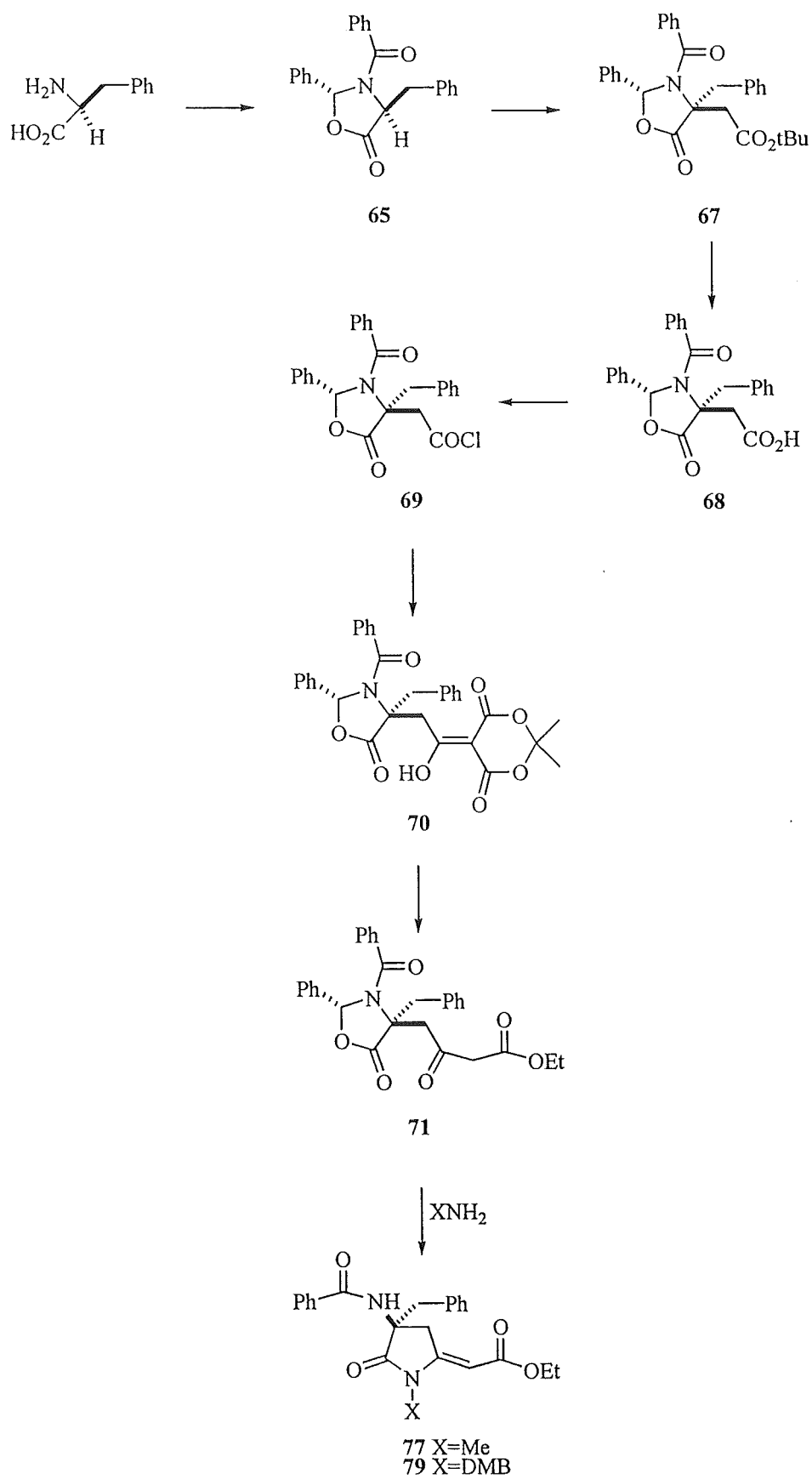
**Figure 5** Target *cis*-Amide Bond Mimics (DMB=2,4-dimethoxybenzyl)

The target peptidomimetics bridge the peptide backbone as shown, restricting the otherwise free rotation about the peptide bond (indicated by  $\psi$ ). The result is a five-membered lactam ring, containing the peptide bond, being incorporated into a peptide sequence with the peptide bond being locked in the *cis*-like configuration. Compounds of this type have been shown to be good *cis*-amide bond mimics due to the  $sp^2$  nature of the ene-lactam double bond locking the lactam ring in a planar configuration.

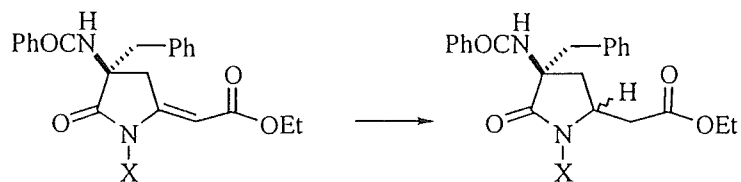
An outline of the proposed synthesis of the target molecule is shown in Scheme 1. Key steps in this sequence include the conversion of acid chloride **69**, to the  $\beta$ -keto ester **71** via an acyl Meldrum's acid intermediate **70**. In this case ethanol was used as a quenching agent to give the ethyl ester, but, as will be shown, it is also possible to use amines as a way for producing  $\beta$ -keto amides. This provides a method for extending the peptide chain in the *C*-direction in one easy step. The conversion of **71** to the cyclic acylated enamino esters **77**, and **79**, occurs by way of an imine/enamine intermediate, to form the five-membered substituted cyclic lactam ring. These two steps form the basis of this synthesis and will be described in greater detail in the following chapters.

Preparation of compounds **77** and **79** also allows the possibility of subsequent reduction of the ene-lactam double bond to form a dipeptide analogue more closely resembling the parent peptide it is intended to mimic. Hydrolysis of both the *N* and *C* protecting groups also gives rise to the potential for chain extension in both the *N* and *C* directions.

Scheme 1



Although this will not be discussed in great detail here, brief mention will be made to future synthetic possibilities, with this being an important goal of this work.



# Synthesis of $\beta$ -keto esters and amides



Since the first example of the Claisen condensation was discovered more than a century ago,  $\beta$ -keto esters have been very important intermediates in organic synthesis. This importance can be attributed to such compounds containing a unit composed of two different electrophilic carbonyls, and two carbons,  $\alpha$  to the carbonyls, which can be made to react selectively under suitable conditions.<sup>46</sup> This makes them valuable tools in the synthesis of a wide variety of molecular systems. A simple example of this use is in the preparation of Thienamycin,<sup>47</sup> a potent  $\beta$ -amino antibiotic, where the starting compound for the synthesis is a  $\beta$ -keto ester of the type described here. This chapter describes the synthesis of the succinic acid-derived  $\beta$ -keto ester **23**, via a general Meldrum's acid method, in preparation for its use in the synthesis of unsubstituted enamino esters of type **55** and **56**.

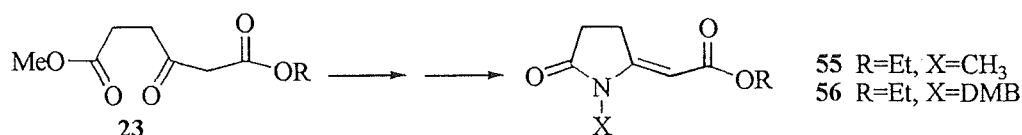


Figure 6

Also in this chapter, extension of these principles will be applied to the synthesis of the aspartic acid derived  $\beta$ -keto ester oxazolidinone **28**. This serves as a precursor to the 3-substituted enamino esters **59** and **61**, which have the potential for peptide chain extension in both the *N* and *C* directions (Figure 7).

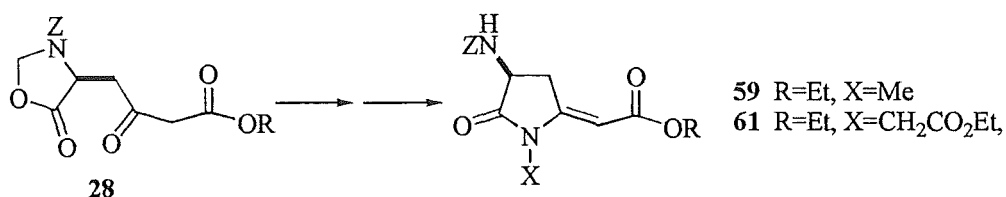


Figure 7

Compounds of this type are examples of five-membered Freidinger lactams where the *N*-substituted amide bond is locked in a *cis*-configuration. Incorporation of enamino esters into an appropriate substrate peptide has been shown to be a successful strategy for enhancing recognition by a target enzyme.

A related procedure has been reported by Pak *et al.*,<sup>48</sup> in which an acyl Meldrum's acid intermediate was refluxed with various amines, in toluene, to give the corresponding  $\beta$ -keto amide. The synthesis of compounds of this type, allows the incorporation of an amide bond, with chain extension in the C-direction, in one easy step. This has proved valuable in the synthesis of lactams of type **63** and **64**, where the ester group is replaced with a desired amine or amino acid (Figure 8).

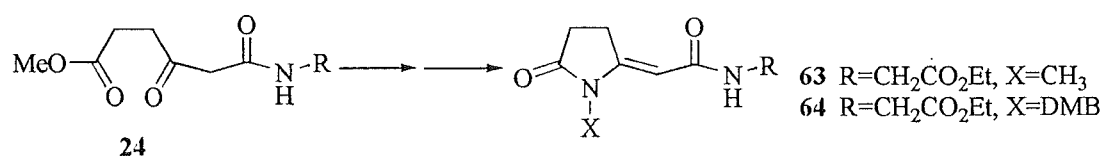
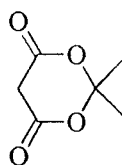


Figure 8

## The Meldrum's acid reaction

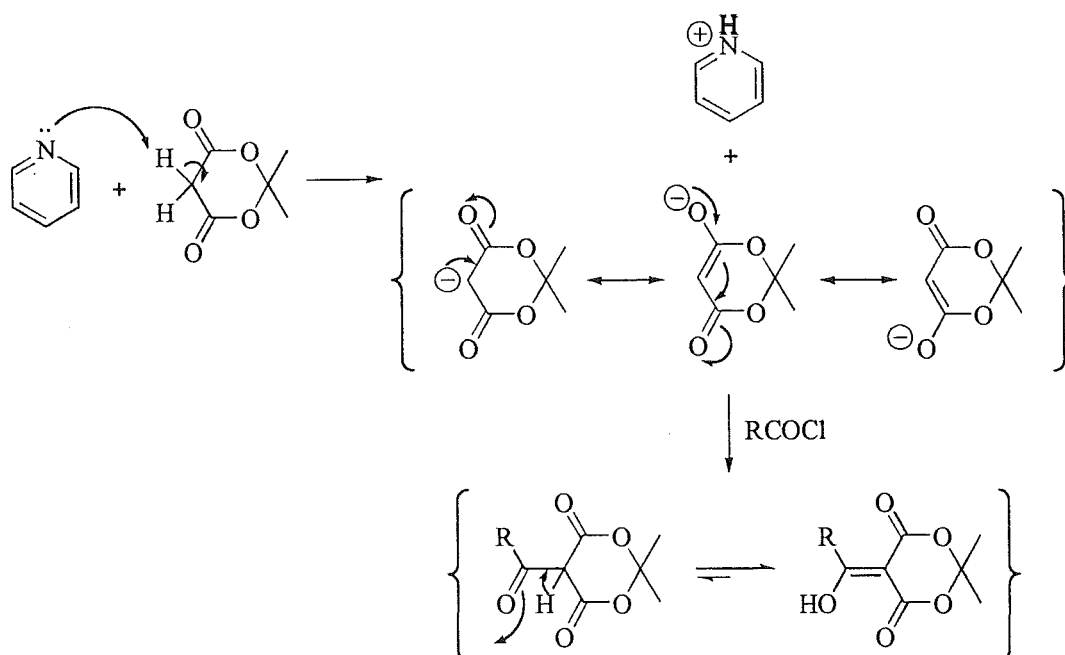
In 1908 A. N. Meldrum observed that malonic acid and acetone reacted in a cold acetic anhydride-sulphuric acid medium, to eliminate water and form a crystalline product.<sup>49</sup> Meldrum's acid, as it was called, was later characterised and found to be the cyclic bifunctional ester 2,2-dimethyl-1,3-dioxane-1,4-dione.<sup>50</sup>



Meldrum's acid

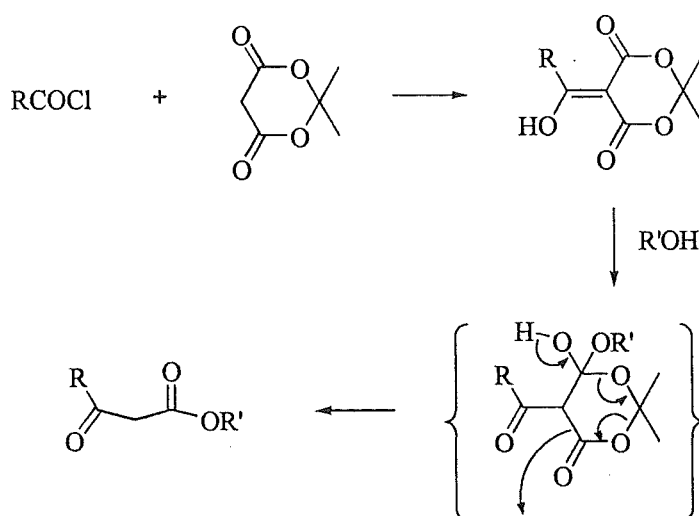
In 1978 Yonemitsu *et al* demonstrated that upon acylation with various acyl chlorides, meldrum's acid could be used to give the corresponding acyl Meldrum's acids, which readily underwent alcoholysis to give  $\beta$ -keto esters in good yield.<sup>51</sup> They further showed that Meldrum's acid readily reacted with acid chlorides even in the absence of a strong base, a requirement of many other methods of the time. This reactivity was due to its enhanced acidity ( $\text{pK}_a$  4.97) and allowed acylation to occur under relatively mild conditions. Treatment of Meldrum's acid with a mild base such as pyridine, led to the deprotonation at the methylene carbon to give a stable anion. The anion is stabilized by delocalization through both carbonyls. This anion of Meldrum's acid was shown to

carry out nucleophilic attack on an acid chloride to give an acyl Meldrum's intermediate (Figure 9).



**Figure 9** Formation of an acyl Meldrum's acid intermediate

Alcoholysis of this intermediate led to the elimination of acetone and carbon dioxide to give the  $\beta$ -keto ester in good yield. This was subsequently presented as a general and efficient method for the synthesis of  $\beta$ -keto esters of type  $\text{RCOCH}_2\text{CO}_2\text{R}'$  (Scheme 2).



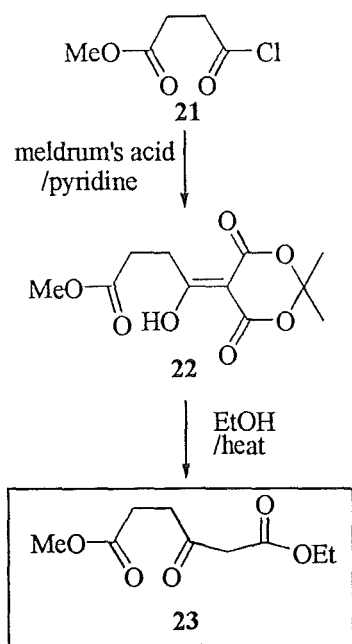
**Scheme 2** Formation of  $\beta$ -keto esters from acid chlorides via an acyl Meldrum's acid intermediate

## Synthesis of $\beta$ -keto esters

Consequently,  $\beta$ -keto ester **23**, the starting compound for the synthesis of target enamino esters **55** and **56**, was prepared from the acid chloride **21**, according to the procedure outlined by Yonemitsu *et al* (Scheme 3).<sup>51</sup>

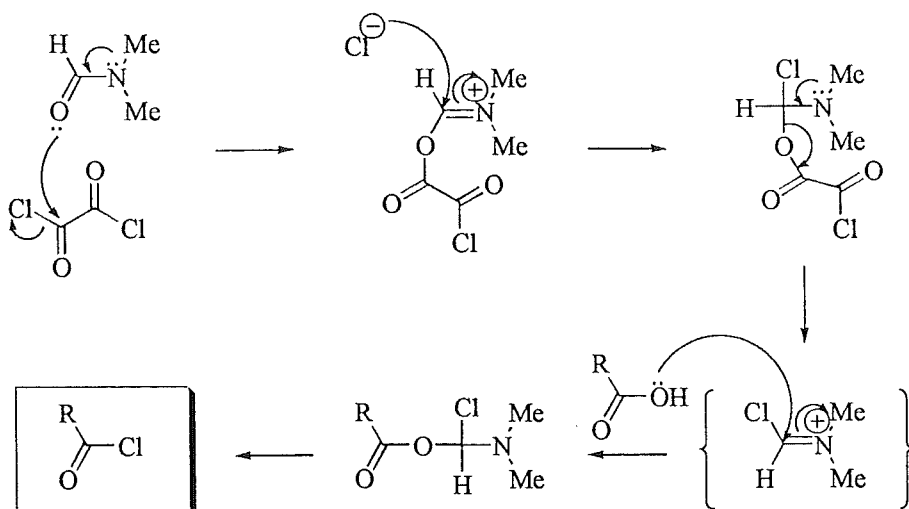
A solution of carbomethoxypropionyl chloride **21** (1 equivalent), in dichloromethane, was treated with Meldrum's acid (1.1 equivalent) and pyridine (2 equivalents), and stirred at 0° C for 50 min, and then at 20° C for 45 min to give the acyl Meldrum's acid intermediate **22**.

The acyl Meldrum's acid **22** was refluxed in ethanol for 2.5 hr, whereupon the solvent was removed and the residue given a base wash (NaHSO<sub>4</sub>) to give the  $\beta$ -keto ester **23** in high yield (84%), and purity.



**Scheme 3** Synthesis of Succinic Acid-derived  $\beta$ -Keto Ester **23**

The acid chloride used in this reaction was prepared from the corresponding acid, on reaction with oxalyl chloride (5 equivalents) and DMF, in dichloromethane. This was employed in the subsequent preparation of all acid chlorides described here and has the added advantage of producing gaseous by-products, which can be easily removed. The mechanism for the formation of **23** is shown in Figure 10. Note that DMF is used catalytically to generate a species more susceptible to chlorination.



**Figure 10** Preparation of an Acid Chloride from an Acid using Oxalyl Chloride and DMF

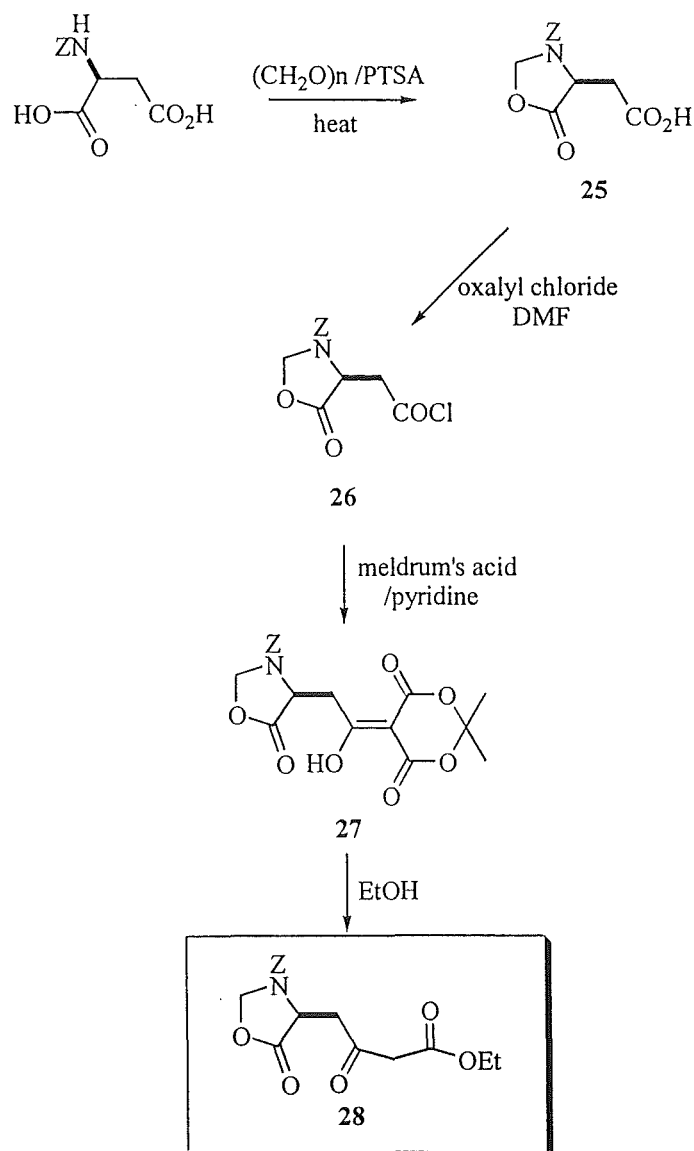
An extension of these principles allowed the synthesis of the aspartic acid-derived  $\beta$ -keto ester **28** (Scheme 4), an oxazolidinone compound used in the preparation of the substituted enamino esters **59** and **61** (Figure 7). The starting compound for this pathway was the acid chloride **26**, which was itself prepared from the reaction of the corresponding acid **25**, with oxalyl chloride (5 equivalents) and DMF, in dichloromethane.

The acid **25** was obtained via the procedure set forth by Scholtz and Bartlett<sup>52</sup>, whereby a solution of *N*-benzyloxycarbonyl (Cbz)-(*S*)-aspartic acid, paraformaldehyde (2 equivalents) and PTSA (0.06 equivalents), in benzene, was refluxed for 1 h with azeotropic removal of water.

The resulting oxazolidinone **25** effectively protects the  $\alpha$ -carboxylic acid group of *S*-Cbz-aspartic acid, and allows subsequent reactions to occur at the unprotected side chain  $\beta$ -carboxylic acid. Conversion of the acid to the acid chloride **26** allows its use in the preparation of the  $\beta$ -keto ester **28** (Scheme 4), via the same method described above for the succinic acid-based  $\beta$ -keto ester **23** (refer to Scheme3).

That is, acid chloride **26** (1 equivalent), pyridine (2 equivalents), and Meldrum's acid, dissolved in dichloromethane, were stirred at 0° C for 50 min, then at 20° C for 45 min. The solvent was removed under reduced pressure and the residue was refluxed in

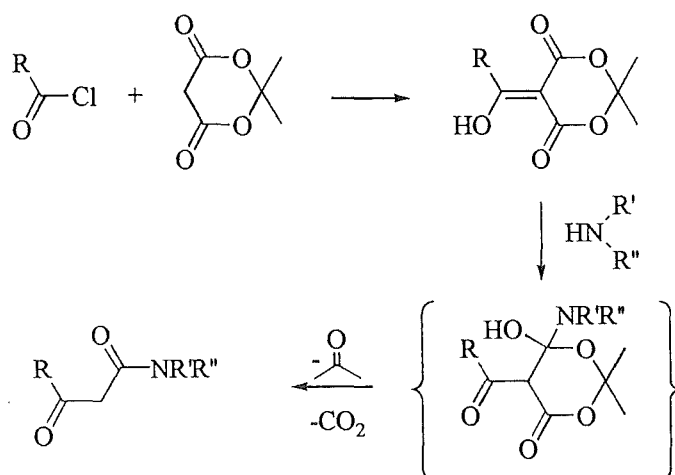
ethanol for 2.5 h. Purification by radial chromatography gave the  $\beta$ -keto ester **28** as a yellow oil (87%).



**Scheme 4** Synthesis of Aspartic Acid-derived  $\beta$ -Keto Ester **28**

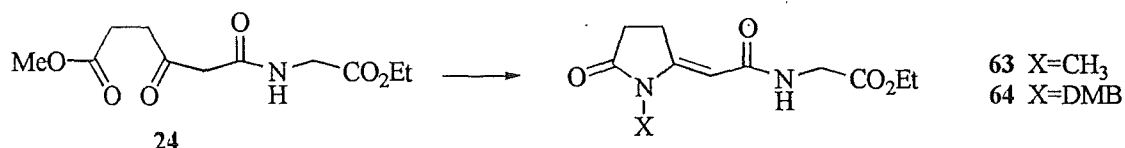
## Synthesis of $\beta$ -keto amides

In 1992, Pak *et al* reported that the meldrum's acid methodology, used by Yonemitsu *et al* for the synthesis of  $\beta$ -keto esters, could be applied to the preparation of  $\beta$ -keto amides.<sup>48</sup> These compounds have also proved to be very versatile intermediates in organic synthesis. There are a number of synthetic methods for the preparation of these compounds, however many of these suffer from limitations of one kind or another. Pak *et al* showed that by using an amine in place of an alcohol, the direct aminolysis of acyl meldrum's acid afforded a convenient and versatile synthesis of  $\beta$ -keto amides (Figure 11).



**Figure 11** Pak *et al*'s Methodology for the Synthesis of  $\beta$ -Keto Amides

Among the many amines used by Pak and his colleagues, was glycine ethyl ester ( $H_2NCH_2CO_2Et$ ). Amination of acyl Meldrum's acid using this amine gave a  $\beta$ -keto amide containing a carboxyl-protected amino acid residue. Application of this method has been used here in the synthesis of the  $\beta$ -keto amide **24** (Figure 12).



**Figure 12**

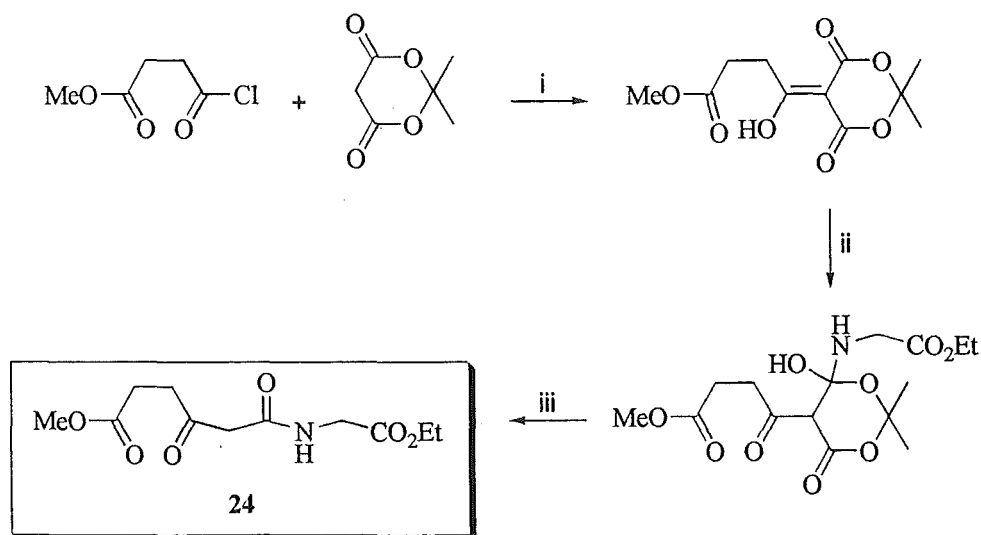
This has been used in the preparation of lactams of type **63** and **64**, where extension of the molecule in the *C*-direction, in the form of an amide bond, has already been

achieved. This also allows the possibility of incorporating various amino acids or peptide sequences into the molecule to allow a closer resemblance to adjacent residues in the parent peptide, a factor of great importance in peptidomimetic design.

Compounds of this type can also be prepared from the cyclic enamino ester equivalents **55** and **56** (Figure 6), via hydrolysis of the ethyl ester and subsequent coupling of an amino acid. However, where possible, direct aminolysis of Meldrum's acid negates the need for this step and leads to a reduction in the total number of steps in the synthesis of the desired product.

Therefore, the preparation of  $\beta$ -keto amide **24** from the corresponding acid chloride **21**, was carried out according to the procedure described by Pak *et al* (Scheme 5). A solution of the acid chloride **21** (1 equivalent), in dichloromethane was treated with meldrum's acid (1.1 equivalents), and pyridine (2 equivalents) and stirred at 0° C for 50 min, and at 20° C for 45 min, to give the acyl meldrum's acid intermediate **22**.

A solution of acyl Meldrum's acid **22**, glycine ethyl ester hydrochloride (1.1 equivalents) and triethylamine (1.1 equivalents), in benzene, was then refluxed for 4 h. Purification by radial chromatography gave the  $\beta$ -keto amide **24** as a yellow oil (47%).

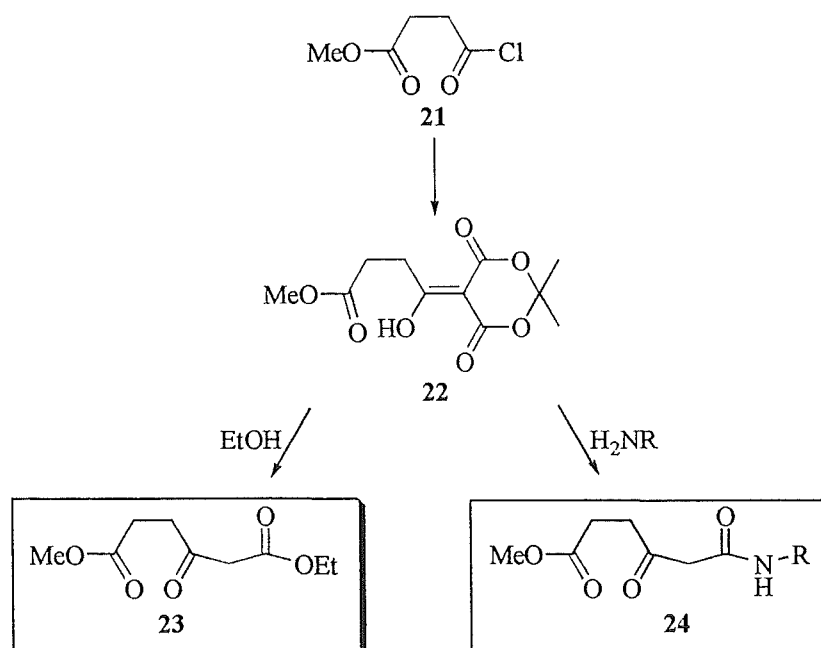


**Scheme 4** i pyridine, dichloromethane, ii HCl.H<sub>2</sub>NCH<sub>2</sub>CO<sub>2</sub>Et, Et<sub>3</sub>N, iii 150° C/1 mm

By combining both Yonemitsu's and Pak's methodologies, this now gave us a convenient method for the preparation of succinic acid-derived  $\beta$ -keto esters and  $\beta$ -keto

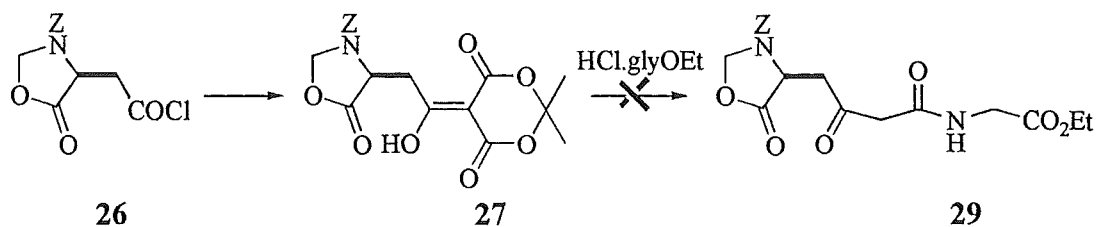


amides, via a common intermediate **22**. By varying the nucleophile used to quench the acyl Meldrum's acid, it was possible to extend the molecule via an ester linkage or an amide linkage (Figure 13). In this case, extension in the form of an amide was preferable, as it allowed direct incorporation of a C-protected amino acid into the molecule. However, extension of the molecule via an ester linkage was still an attractive option due to it being a very high yielding reaction.



**Figure 13** Synthesis of Succinic Acid-derived  $\beta$ -Keto Esters and  $\beta$ -Keto Amides from a Common Intermediate **22**

Examination of these processes led us to believe that a similar method could be adopted for the preparation of the corresponding aspartic acid-derived  $\beta$ -keto amide oxazolidinone **29** (Scheme 6).



**Scheme 6**

Again this would invoke the use of an acyl Meldrum's acid intermediate, and involve direct amination as before. However, when this process was attempted, it was unsuccessful and gave only a trace of the desired product. This was reasoned to occur due to the oxazolidinone ring acting as an activated ester and hence being the preferred site of attack for aminolysis. This led to a fragmentation of the molecule, with no recognizable products being observed by NMR. This process was repeated several times without success.

Despite the unsuccessful nature of this reaction, the synthesis of the aspartic acid derived  $\beta$ -keto ester **28** still gave a convenient route to the formation of enamino esters (Figure 14). Removal of the ester group and addition of an amino acid via a peptide coupling reaction could still be carried out at a later stage.

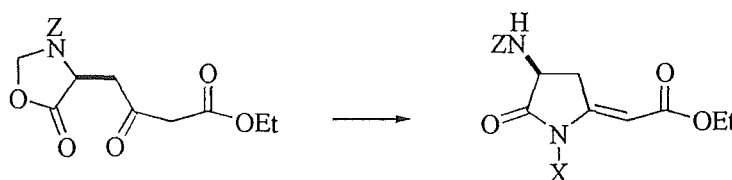
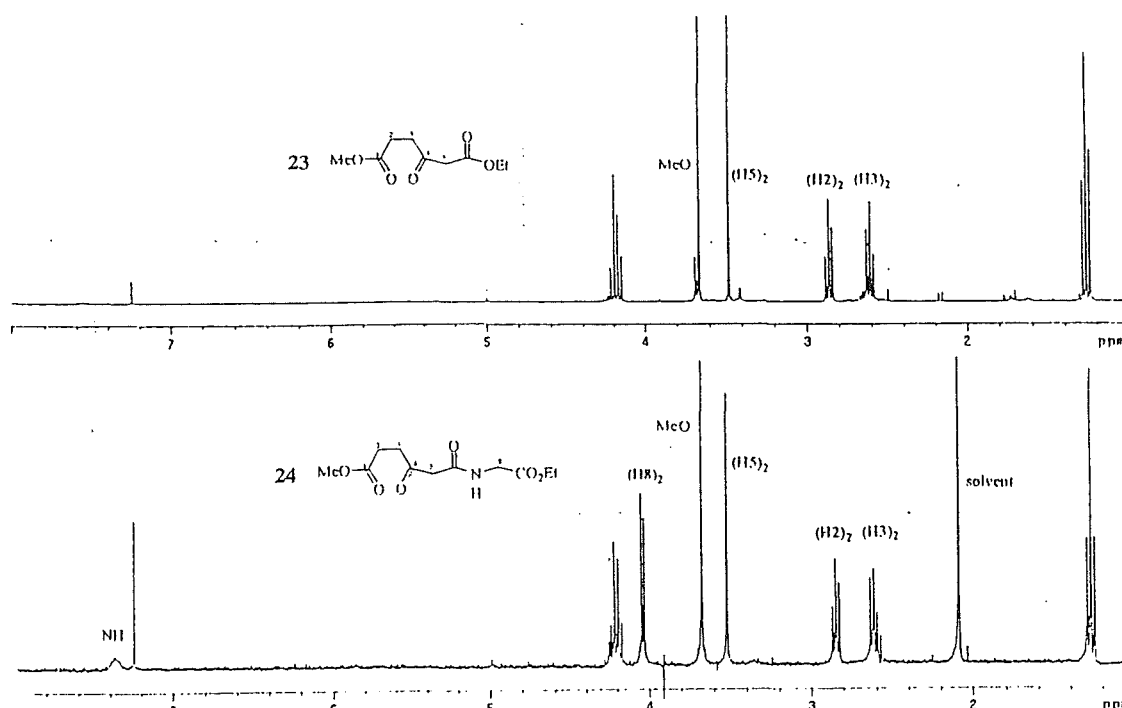


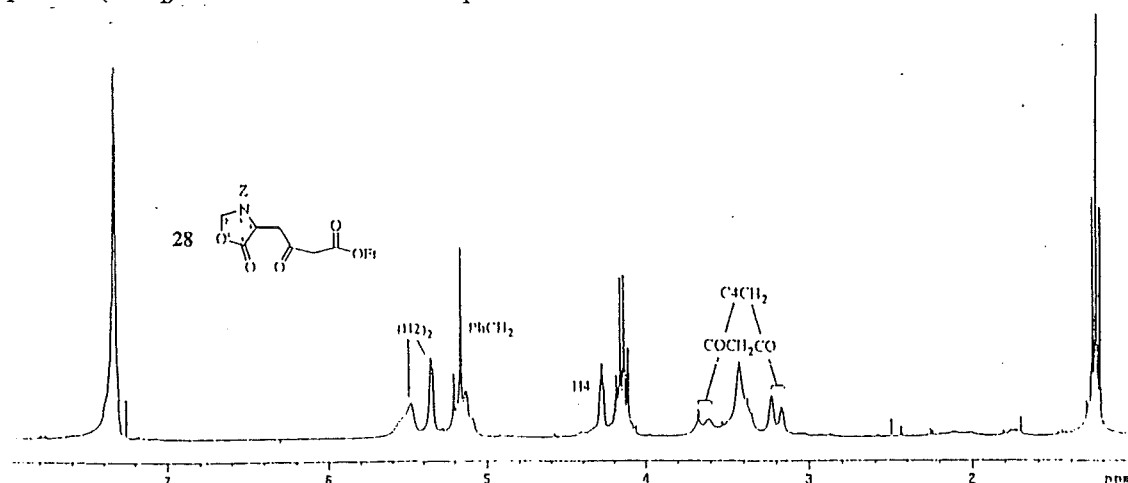
Figure 14

## Structural Assignment and NMR data

The  $^1\text{H}$  NMR spectra of the succinic acid-derived  $\beta$ -keto ester **23**, and  $\beta$ -keto amide **24**, gave rise to standard proton coupling signals and hence will not be discussed in detail here. A simple numbering system was used for both compounds to allow easy comparison of each proton signal (this differs from the systematic name in the case of **24**). The methylenes at positions 2 and 3 each gave rise to a triplet due to mutual spin-spin coupling. A characteristic singlet was observed for the two protons at position 5, they being adjacent to two carbonyls, and therefore not available for short-range proton-proton coupling. The two protons at position 8 in compound **24**, appeared as a doublet due to coupling with the adjacent amide proton.



In the preparation of the aspartic acid-derived  $\beta$ -keto oxazolidinone **28**, the introduction of a stereogenic centre into the molecule gave rise to the possibility of diastereotopic protons. That is, protons attached to the same carbon, which exist in different electronic environments due to the chiral nature of the molecule. In the case of **28**, C4 is a stereogenic centre which leads to the two protons associated with  $(\text{H}2)_2$  being in different environments, as are the  $\text{PhCH}_2$  protons of the Cbz group. Hence they are referred to as diastereotopic. This gives rise to a splitting of the signals associated with each proton. As each proton can exist in either of two spin states, a doublet of doublets results, with the inner peak's intensity of each increasing, the smaller the frequency difference is between the two protons. This is commonly referred to as an AB quartet (ABq) and is evident in the spectra below.



The protons associated with  $C4CH_2$  also display signal splitting, as they too are diastereotopic. It is clear that both are associated with the same carbon, however they have been reported here as 2 doublets due to the broad nature of the signals. In this compound, the resonance associated with  $COCH_2CO$  appeared as a broad singlet, but, as will be discussed later, in compounds where substitution of the oxazolidinone is introduced, signal splitting occurs here also. The  $CH_2$  of the ethyl ester, although part of an ester linkage, is too far away from the chiral centre to display any signs of the environment of the protons being affected. Hence, splitting of this signal does not occur.

## Summary

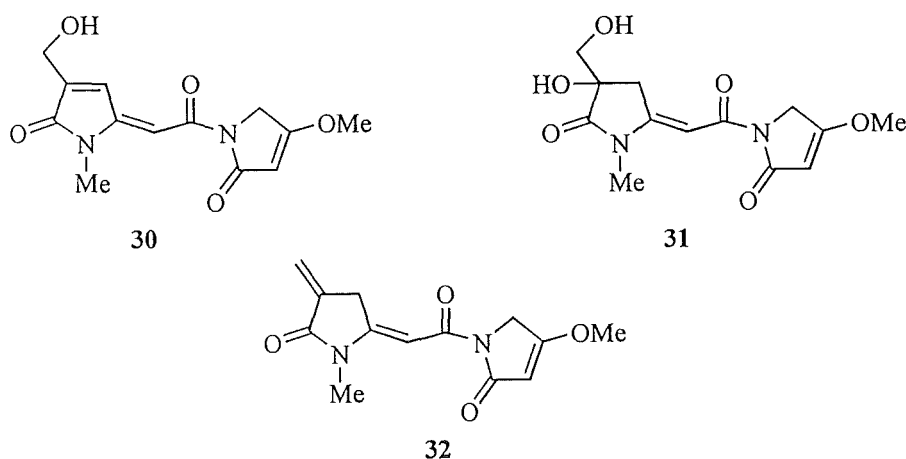
Using the meldrum's acid method developed by Yonemitsu *et al*, it was possible to synthesize  $\beta$ -keto ester **23** (Scheme 3), in preparation for its reaction with an amine to form simple unsubstituted enamino esters of type **55** and **56**. This method was also applied to the preparation of the aspartic acid derived  $\beta$ -keto ester oxazolidinone **28** (Scheme 4). Preparation of **23** and **28** were carried out under relatively mild conditions, and gave a convenient and consistently high yielding method for synthesis of compounds of this type.

Extension of these principles using the methodology developed by Pak *et al*, led to the preparation of  $\beta$ -keto amide **24**. This allowed the incorporation of an amide bond, with chain extension in the *C*-direction, in one easy step. Although preparation of the corresponding aspartic acid derived  $\beta$ -keto amide proved unsuccessful, due to the reactivity of the oxazolidinone ring, this method still afforded a convenient method for the preparation of simple  $\beta$ -keto amides.

Compounds **23** and **24** gave rise to characteristic  $^1H$  NMR resonances, with the introduction of a stereogenic centre into compound **28** leading to signal splitting, due to the nature of a number of the protons being diastereotopic

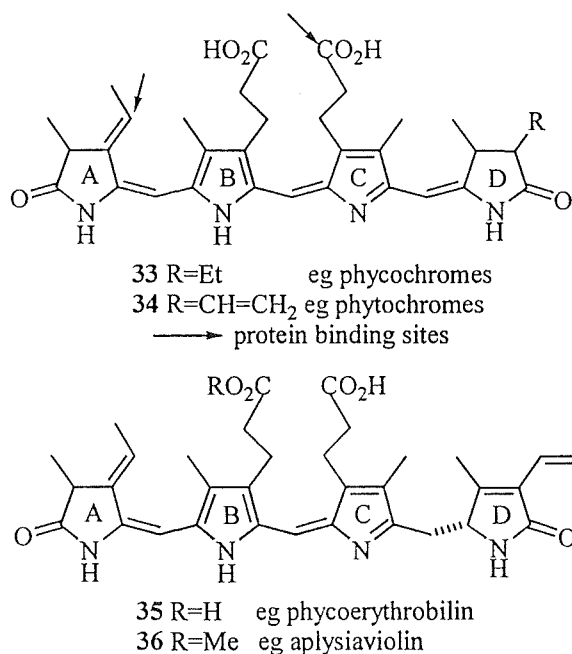
# Synthesis of cyclic enamino esters/amides

Enamino esters and enamino amides are sub-classes of an important group of compounds known as ene-lactams. Ene-lactams are found in nature, in both plant and animal life, and serve a number of important purposes. An example of this is in the case of *Lyngbya majuscula*,<sup>53</sup> a toxic shallow water variety of marine blue algae. Certain strains of this algae are known to be responsible for the contact dermatitis known as 'swimmers itch'. Samples of *Lyngbya majuscula* containing such irritants have also been found to contain a class of compounds known as Pukeleimides. Exemplified by pukeleimide A **30**, pukeleimide C **31**, and pukeleimide E **32**,<sup>54</sup> pukeleimides are an example of a naturally occurring class of compounds containing an ene-lactam unit.



There are numerous other examples in nature where compounds of this type, containing one or more ene-lactam units, can be found. Perhaps the most widespread and important examples in which this functionality is utilised, is in the skeleton of macrocyclic tetrapyrroles of the chlorin, isobacteriochlorin and corrin oxidation level.<sup>55</sup> Members of this latter class serve important biological functions as vitamins, co-factors, and light absorbing pigments. Compounds such as hemoglobin, myoglobin, cytochromes and chlorophylls are derived from this class and contain tetrapyrrole components that form the essential basis for their biological function. A good example of this are biliproteins,<sup>56</sup> a family of chromophores consisting of a linear tetrapyrrole covalently bound to a protein. Found in algae, cyanobacteria, cryptophytes, mosses and other higher green plants, biliproteins are extremely important in photosynthesis and are involved in the photoregulatory functions, such as photomorphogenesis, of many species. As such they have been of considerable interest for over a hundred years. This

may also be, in part, a result of the many brilliant and intense colours associated with compounds of this type. Rings A (**33,34**) and D (**35,36**) of the biliproteins are ene-lactams.

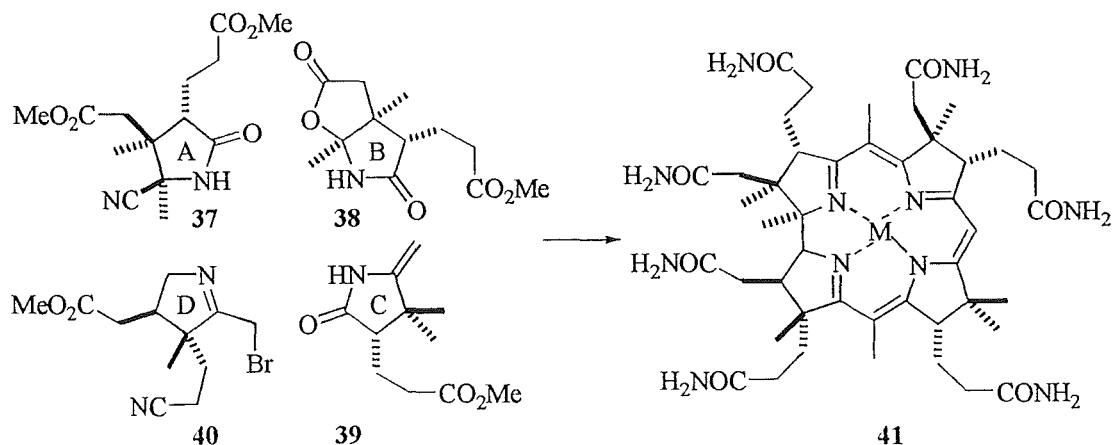


Pigments such as **33** and **34**, are important for photosynthesis and are found in nearly all forms of plant life as well as photosynthetic organisms.<sup>57</sup> These so-called accessory light-harvesting pigments assist chlorophylls a and b, the major light gathering pigments, in trapping light energy for use in the cell. These accessory pigments, along with others, are also responsible for the magnificent colours associated with autumn. They persist longer after leaf death than the more labile chlorophylls and account for the many yellow, orange and red leaf colours seen at this time of the year.

In some cases, members of the animal kingdom have taken advantage of such plant pigments to aid in their survival. *Aplysia*, a species of sea hare with a voracious appetite for red algae, when disturbed, will excrete a thick, purple ink from a specialized ink gland. Aplysiaviolin **36**, as it was called, turned out to be a modified form of phycoerythrobilin **35**, the red pigment associated with the red algae off which it fed.<sup>58</sup>

As well as being important components in nature, ene-lactams have also proved to be a valuable functionality in natural products chemistry. They have been utilized in either free or protected form, in the synthesis of many compounds such as prostaglandin

analogues,<sup>59</sup>  $\gamma$ -lactam antibiotics,<sup>60</sup> peptide mimics,<sup>61</sup> angiotensin II antagonists,<sup>62</sup> and many other biologically active compounds. An example of this was in the first total synthesis of cobyrinic acid **41**, a naturally occurring corrin and a known precursor of vitamin B<sub>12</sub>. Early work by Woodward<sup>63</sup> and Eschenmoser, led to the publishing, by Eschenmoser *et al*, of an improved synthesis of **41** which made use of ene-lactam derivatives **37-39**, and pyrroline **40**, for the assembling of the corrin skeleton.<sup>64</sup>

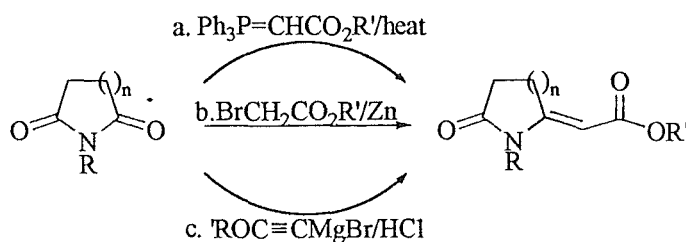


This has led to the use of ene-lactams, in the synthesis of a wide variety of biologically active compounds. Cyclic enamino esters have proven to be valuable intermediates in these processes, a few of which are shown below.<sup>53, 65, 66, 60, 67</sup>

Enamino Ester Intermediate	Target Molecule	Structure of Target molecule
	chromophore of billproteins	<b>35</b>
	analogues of pukeleimide A	<b>30</b>
	prostaglandin analogues	
	carbapenem analogues	
	corrins (eg vitamin B <sub>12</sub> ) and corrin analogues	<b>41</b>

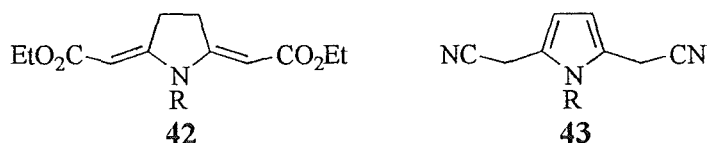


Therefore, cyclic enamino esters represent an attractive synthetic target. Traditionally, cyclic enamino esters have been prepared from corresponding imides, via a Wittig (pathway a, Scheme 7),<sup>68, 69, 70</sup> Reformatsky (pathway b, Scheme 7),<sup>70, 71</sup> or Grignard reaction (pathway c, Scheme 7).<sup>70, 71</sup>

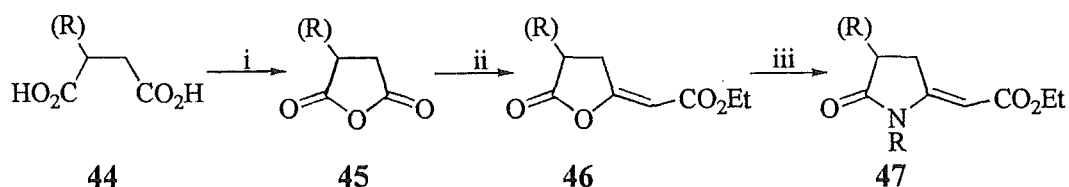


Scheme 7

However, reactions of this type suffer from low yields, harsh reaction conditions, and the tendency to undergo undesirable side reactions, such as bis-adduct formation e.g. **42**, and subsequent isomerization to pyrroles e.g. **43**.



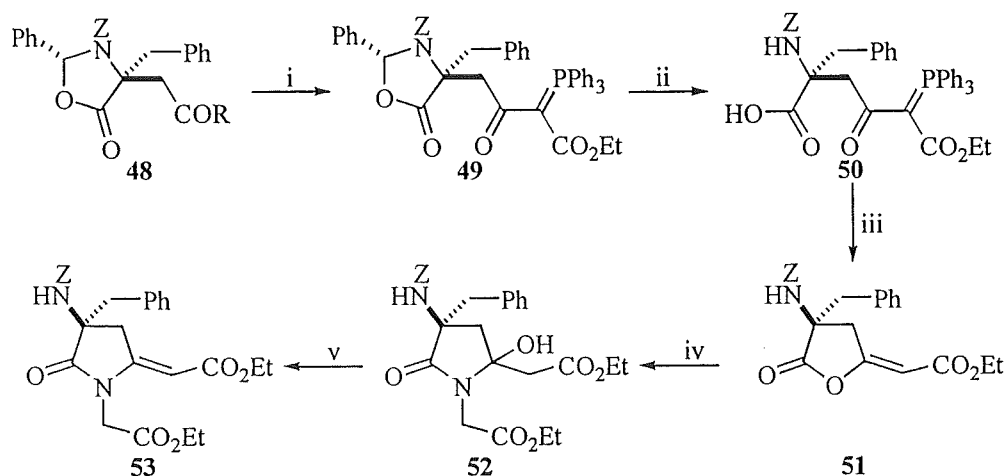
More recently, it has been reported that cyclic enamino esters can be prepared from the reaction of an enol lactone, e.g. **46**, with an amine, via an insertion reaction (Scheme 8).<sup>61</sup> Initially, enol lactones of type **46** were prepared from the corresponding anhydride **45**, via a Wittig reaction. However, synthesis of anhydrides of this type was not trivial and required large-scale preparation from the corresponding diacid **44**, something that was not readily available. Subsequent distillation of the anhydride product **45**, also presented a problem thereby making preparations of such compounds quite difficult.



Scheme 8

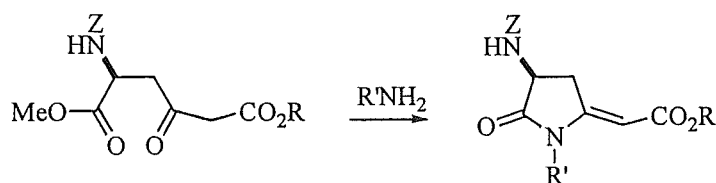
In 1993, Abell *et al* overcame many of these problems by reporting a modified use of the classic Wittig reaction.<sup>61</sup> This modification, the Schlosser modification, or  $\alpha$ -

substitution plus carbonyl olefination via  $\beta$ -oxido phosphorus ylides (SCOOPY) reaction, involved the lactonization of a keto acid phosphorane, eg **49**, to give the corresponding enol lactone **51** in high yield (Scheme 9). The introduction of an amine or amino acid into the enol lactone, via an insertion reaction, gave the cyclic acylated enamino ester **53**. The keto acid phosphorane **49**, was prepared from the alkylated oxazolidinone **48**, upon treatment with  $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$ . The methodology for this process is illustrated in Scheme 9, and provided a general and convenient synthesis of cyclic enamino esters.



**Scheme 9** i,  $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$  1.5 h,  $20^\circ\text{C}$  4.5 h; ii,  $\text{LiOH}$ , THF, MeOH reflux 4 h; iii,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0-20^\circ\text{C}$ ; iv,  $\text{gly-OEt.HCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $20^\circ\text{C}$  18 h; v, PTSA, 1,2-DCE reflux 3.5 h

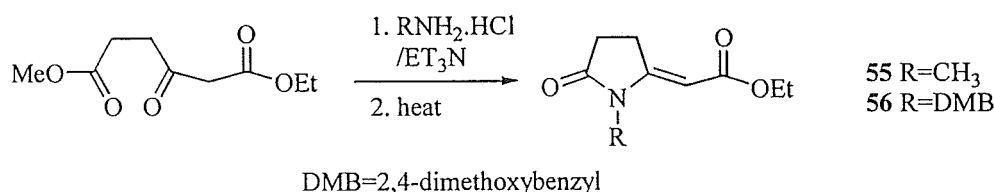
Along with this work, it has also been shown that cyclic enamino esters can be prepared from  $\beta$ -keto esters.<sup>60</sup> This involves the treatment of a  $\beta$ -keto ester with  $\text{RNH}_2\cdot\text{HCl}/\text{Et}_3\text{N}$ , followed by heating under reduced pressure. A cyclization process occurs by way of an imine/enamine intermediate to give cyclic enamino esters of the type shown below (Scheme 10).



**Scheme 10**

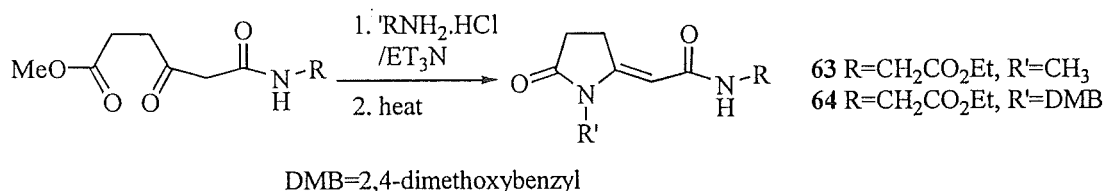
It immediately became clear that the application of this process to a 3,3-disubstituted  $\beta$ -keto ester oxazolidinone, derived from the corresponding alkylated oxazolidinone **48**, would allow a more direct synthesis of cyclic enamino esters of type **53**. The *N*-methylated and *N*-protonated forms would give rise to Freidinger lactams that could be incorporated into a peptide sequence to mimic a *cis*-amide bond.

This chapter describes the use of this method in the preparation of succinic acid-derived, non-substituted compounds of this type, where the amide nitrogen is alkylated (Scheme 11). 2,4-Dimethoxybenzylamine (DMB) was used as a protected form of ammonia which, if desired, can later be removed to give the protonated form of the amide nitrogen.



**Scheme 11**

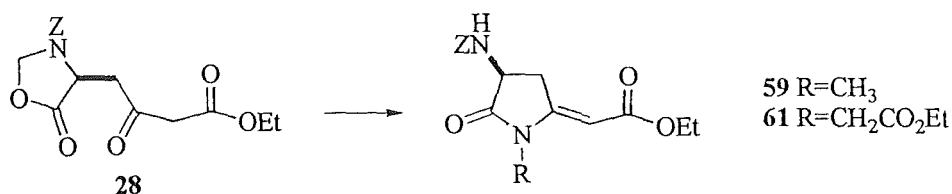
Application of this technique was also applied to the preparation of succinic acid-derived enamino amides, using  $\beta$ -keto amides as a precursor (Scheme 12).



**Scheme 12**

This allows extension of the molecule in the form of an amide bond while again negating the need for the ester cleavage and peptide coupling reactions referred to earlier.

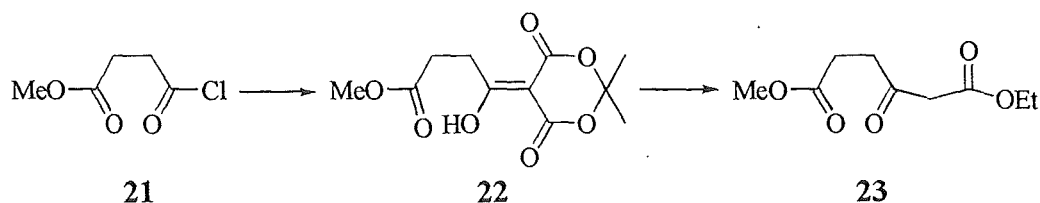
Also in this chapter, the  $\beta$ -keto ester route is extended to the synthesis of aspartic acid derived 3-substituted enamino esters **59** and **61** (Scheme 13). These compounds were prepared from the oxazolidinone  $\beta$ -keto ester **28** and have the potential for chain extension in both the *N* and *C* directions.

**Scheme 13**

The final synthesis of 3,3-disubstituted cyclic enamino esters will be discussed in chapter 5.

## Synthesis of cyclic enamino esters

The preparation of  $\beta$ -keto esters has been described previously in chapter 3 (Schemes 3 and 4). The  $\beta$ -keto ester **23**, the starting compound for the synthesis of enamino esters **55** and **56** (Scheme 11), was prepared from the acid chloride **21** according to the procedure reported by Yonemitsu *et al* (see chapter 3). This method involved the alcoholysis of an acylated Meldrum's acid intermediate **22** to give the  $\beta$ -keto ester **23** in good yield (Scheme 14).

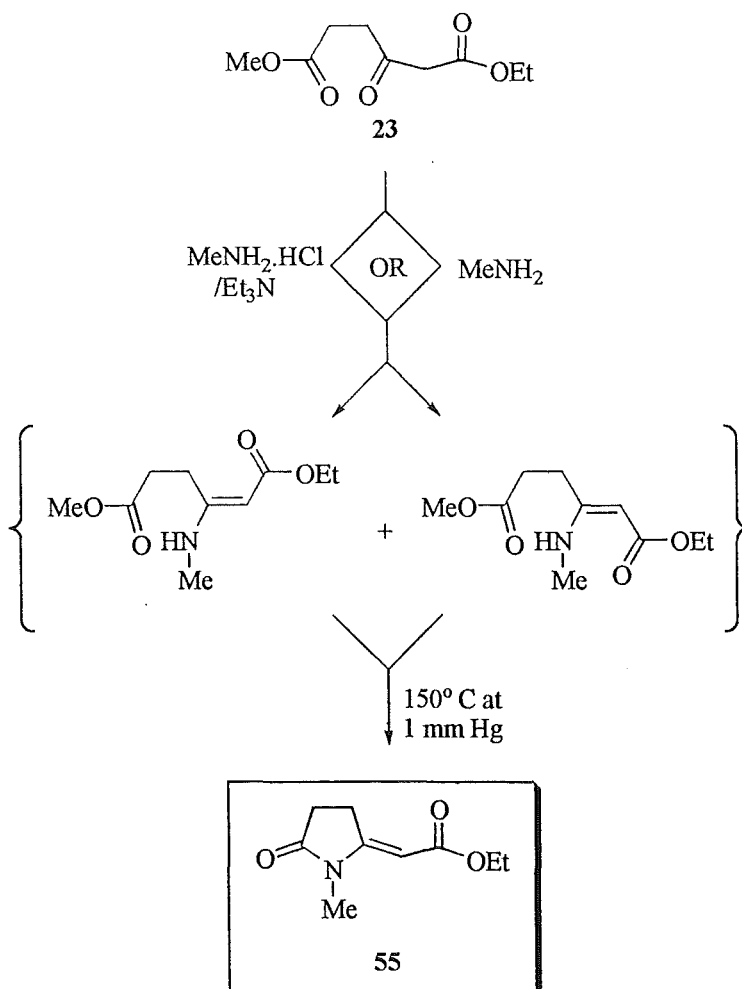
**Scheme 14**

In 1988, Hashiguchi *et al*, in synthesizing analogues of Thienamycin and other carbapenems, showed that cyclic enamino esters could be prepared by refluxing, with azeotropic removal of water, a toluene solution of a  $\beta$ -keto ester, with an amine.<sup>60</sup> Heating of the residue, under reduced pressure, induced the formation of the corresponding cyclic enamino ester. Hence enamino esters **55** and **56** were prepared by a similar method to that reported by Hashiguchi *et al*.

The enamino ester **55** was prepared by refluxing a solution of the  $\beta$ -keto ester **23** in 1,2-dichloroethane, containing methylamine hydrochloride (9 equivalents), and triethylamine. After 90 min, the solution was filtered and the solvent was removed under reduced pressure. The residue was then heated at 150° C, under reduced pressure,

(1 mm of Hg) for 1 h. Purification by radial chromatography gave **55**, as a white solid, in 26% yield.

In an attempt to increase the yield of this reaction, an alternative method for the synthesis of **55** was attempted, involving the use of the free amine rather than the hydrochloride salt (Scheme 9). A solution of methylamine in 1,2-dichloromethane was used and refluxed with the  $\beta$ -keto ester **23** in 1,2-dichloroethane in a similar manner as before. Purification of the crude product by radial chromatography gave the enamino ester **55**, however, in a similar yield to that obtained using the previous method. Hence, the use of the free amine in the reaction mixture was seen as no great advantage. In fact it was seen as somewhat of a disadvantage due to the free amine being extremely volatile and difficult to handle. Therefore, where possible, the hydrochloride salt was used in such reactions.



Scheme 15

Preparation of **55** is therefore carried out in a two step process via an enamine intermediate. An  $^1\text{H}$  NMR spectrum of this intermediate indicated that both the *E* and *Z* isomers were present. However, this did not appear to affect the overall reaction as, upon heating at  $150^\circ\text{C}$  at 1 mm Hg, the target enamino ester **55** was formed relatively cleanly. Isolation of intermediates of this type has proved difficult and no attempt to isolate the enamine intermediates was made here. Purification of the final product by radial chromatography yielded **55** exclusively as the *E* isomer, confirming that isolation of the enamine intermediate was not necessary.

## Mechanism of enamino ester formation

The mechanism for the formation of enamino ester **55** involves two stages (Figure 15). First, the formation of an enamine intermediate **54** via attack of the free amine, generated *in situ*, on the  $\beta$ -carbonyl of the  $\beta$ -keto ester **23**. Secondly, heating of the residue to bring about attack of the enamine N on the methyl ester carbonyl. This second stage is a one step process and leads to ring closure and the formation of the 5-membered enamino ester ring.

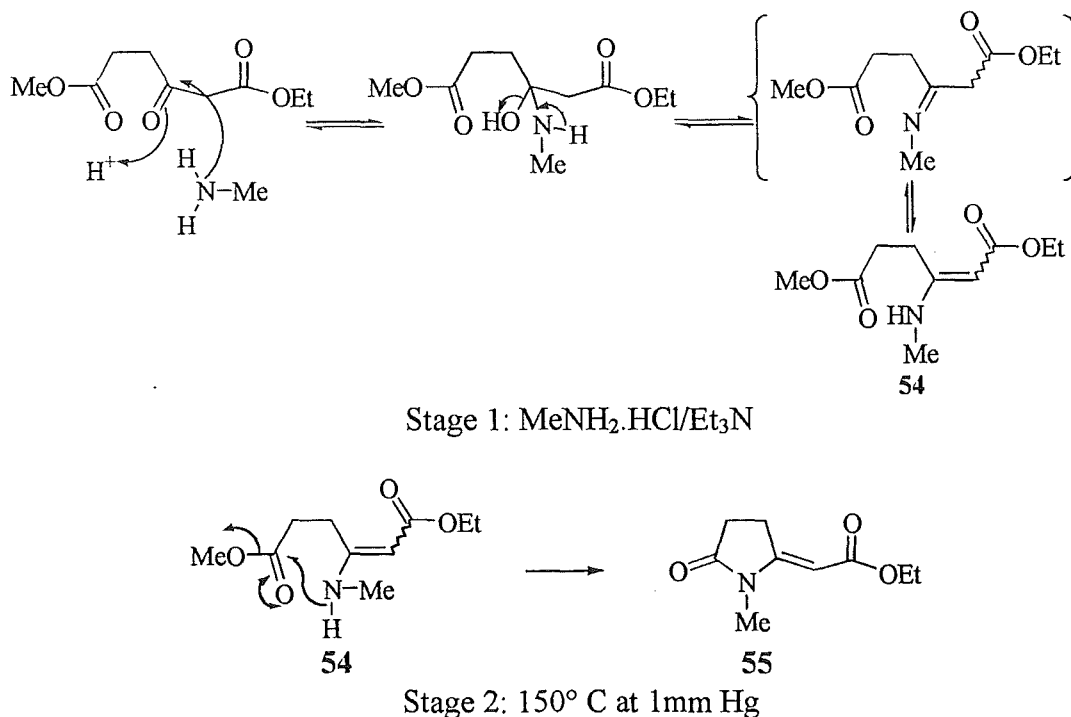
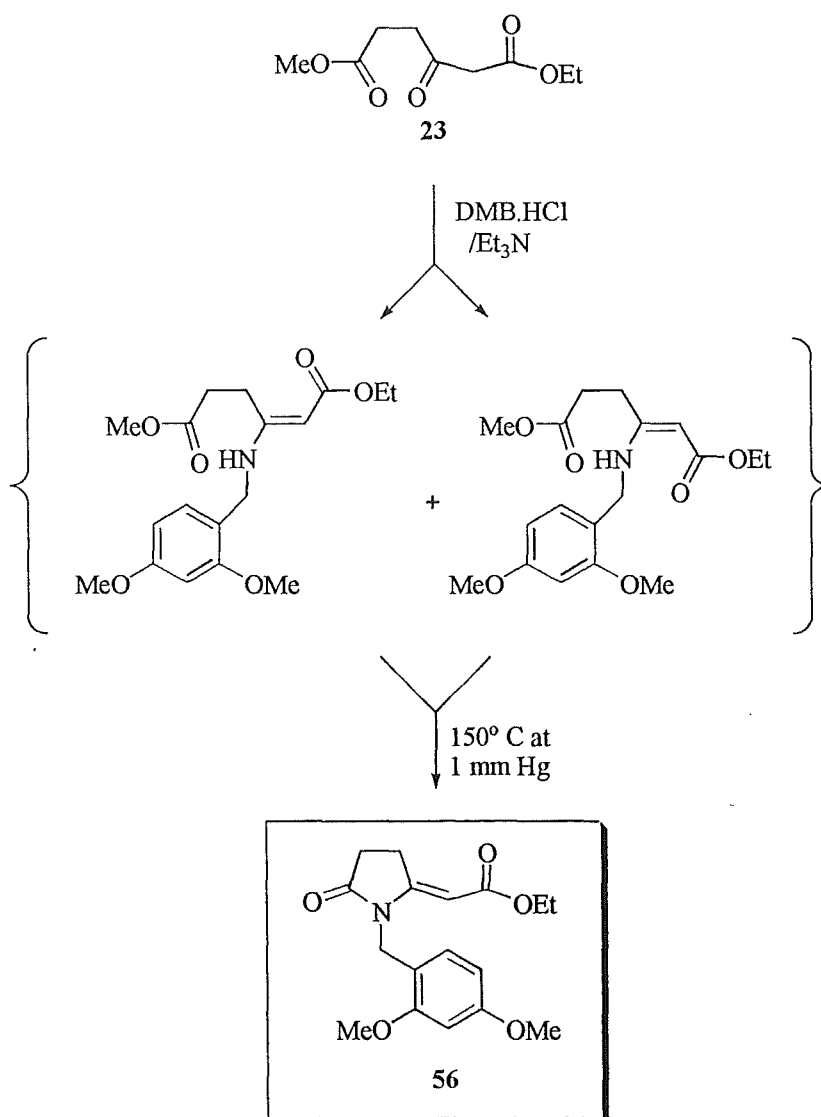


Figure 15 Mechanism of Formation of Enamino Ester **55**

Generation of the enamine intermediate **54** also gives rise to the possibility of imine/enamine tautomerism. However, as no direct evidence of this effect was observed by  $^1\text{H}$  NMR in the preparation of **55** and **56**, it will not be discussed further at this stage. Imine/enamine formation will be discussed in more detail later in the preparation of 3-substituted enamino esters **59** and **61**, where the  $^1\text{H}$  NMR spectra of the intermediates are complex and unable to be assigned. This suggests that not only were the *E* and *Z* enamines present, but the corresponding *E* and *Z* imines as well.

The enamino ester **56** was prepared in a similar manner to that described for **55**, and involved the treatment of **23** with 2,4-dimethoxybenzylamine hydrochloride (1.2 equivalents) and triethylamine (1.2 equivalents) (Scheme 16).

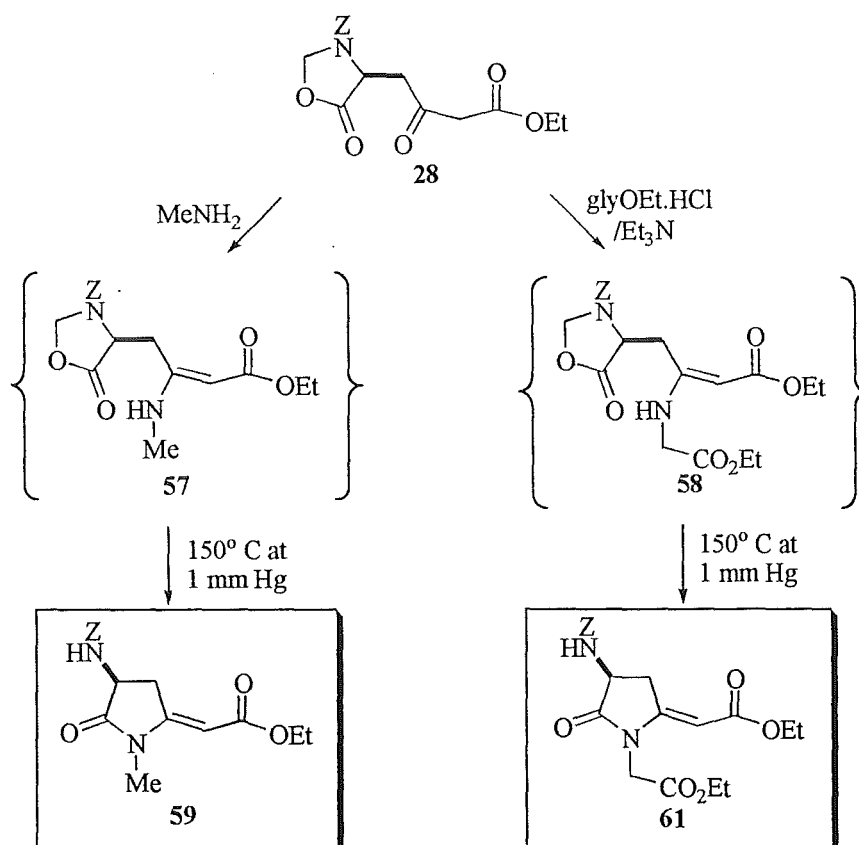


Scheme 16

Purification by radial chromatography gave **56** as a white solid in 42% yield. The mechanism for this reaction is analogous to that described in figure 15 for **55**, and gives the *N*-protected enamino ester **56** exclusively as the *E* isomer.

## Synthesis of 3-substituted enamino esters

The same methodology outlined for the preparation of simple enamino esters **55** (Scheme 15) and **56** (Scheme 16), was used to prepare 3-substituted enamino esters **59** and **61**. The starting compound for these syntheses was the oxazolidinone  $\beta$ -keto ester **28** (Scheme 17).

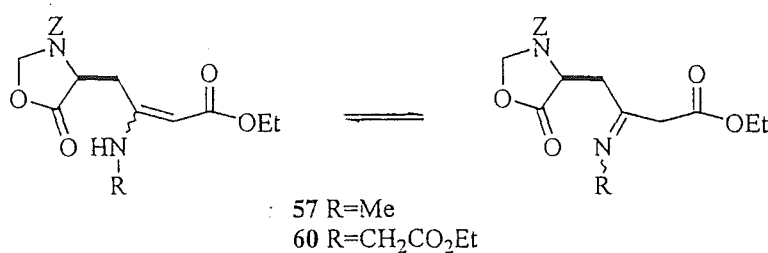


**Scheme 17** Synthesis of Aspartic Acid-derived Enamino Esters **59** and **61**

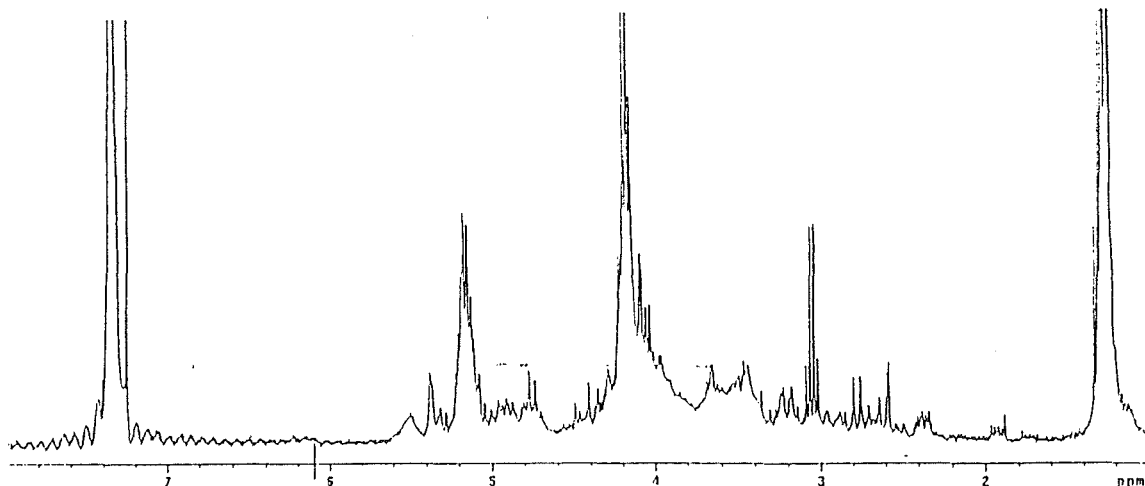
Enamino esters **59** and **61**, were prepared by refluxing, with azeotropic removal of water, a solution of the  $\beta$ -keto ester **28** in 1,2-dichloroethane, with either methylamine (20 equivalents), or glycine ethyl ester hydrochloride (3 equivalents) and triethylamine (3 equivalents). After 90 min, the mixture was filtered and the solvent was removed



under reduced pressure. By analogy to the succinic acid-based systems **55** and **56**, the compound present at this stage should be the enamines **57** and **60** (Scheme 17). However, the  $^1\text{H}$  NMR of both intermediate residues were complex and unable to be assigned. This suggested a mixture of *E*- and *Z*-enamines, and possibly also the corresponding *E*- and *Z*-imines, had formed (Figure 16).

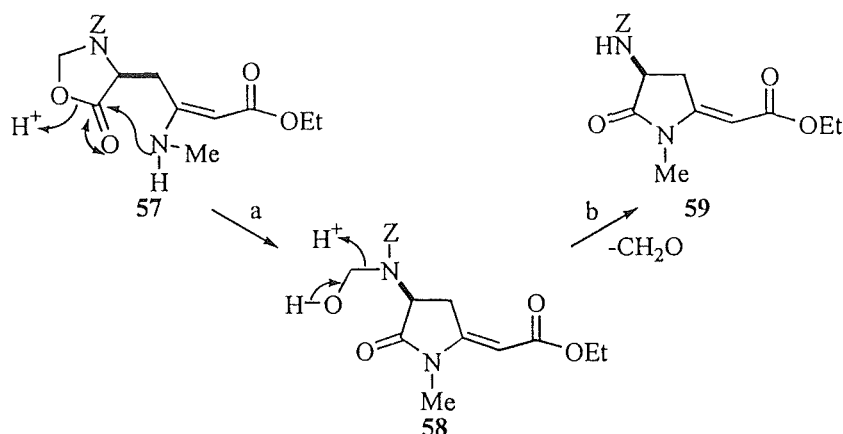


**Figure 16** Imine/Enamine Tautomerism exhibited by **57** and **60**



This did not appear to affect the overall reaction as upon heating the residues at  $150^\circ\text{C}$ , under reduced pressure (1 mm of Hg), for 1 h, the target enamino esters were formed relatively cleanly. Purification by radial chromatography gave **59** and **61**, as yellow oils, in 70% and 63% yields respectively.

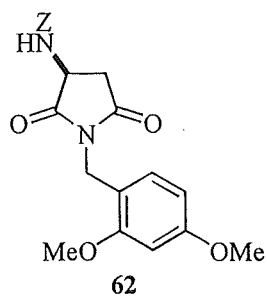
Mechanistically, the formation of enamino esters **59** and **61** involves two steps. Figure 17 illustrates these steps in the formation of enamino ester **59**. The first step involves the formation of the 5-membered enamino ester ring, via attack of the enamine N on the oxazolidinone carbonyl (step a, Figure 17), while the second step leads to the loss of the formaldehyde protecting group (step b, Figure 17).



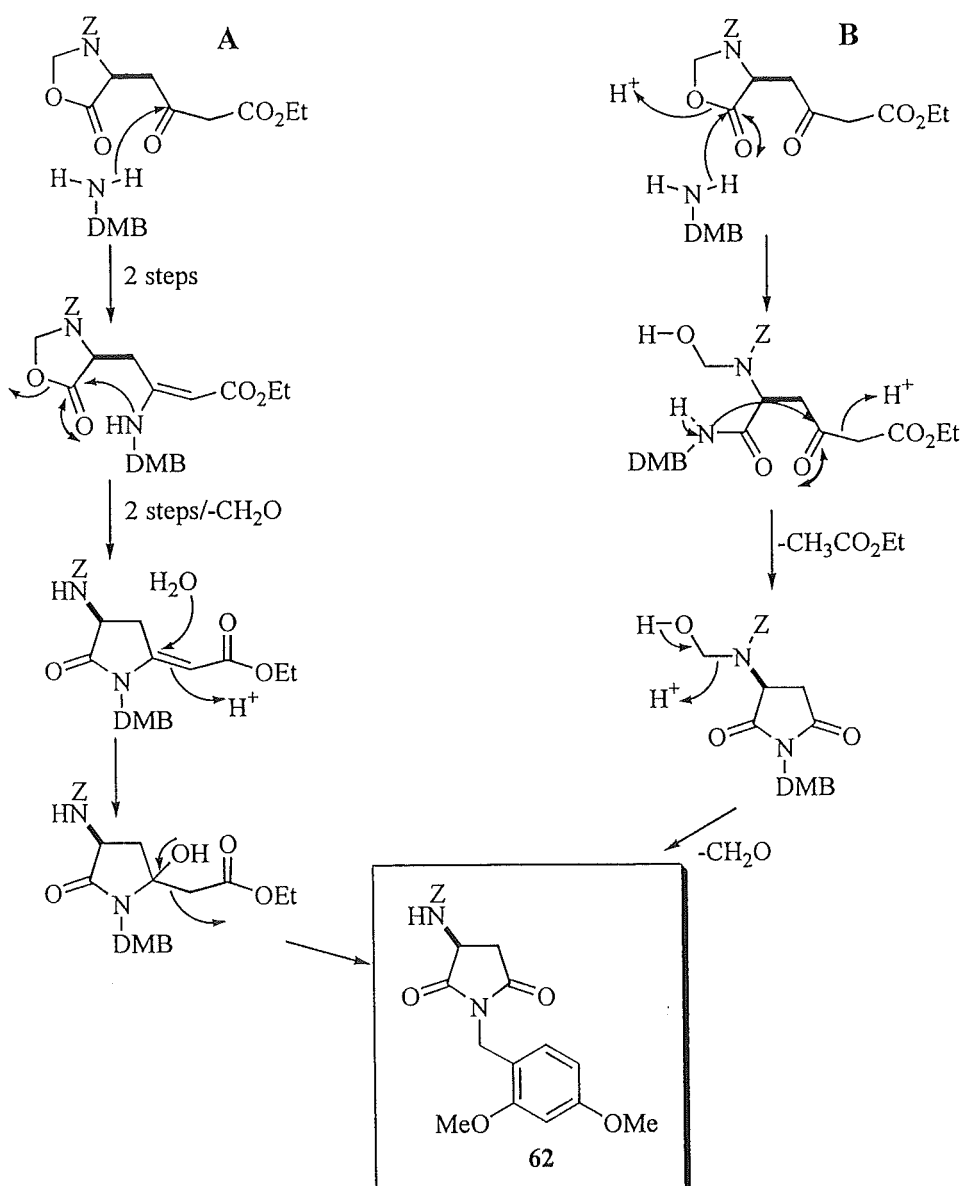
**Figure 17** Mechanism of Formation of Aspartic Acid-derived Enamino Ester **59**

## Elimination product

Attempts to synthesis the corresponding 3-substituted enamino ester using 1,2-dimethoxybenzylamine were unsuccessful. Use of both the free amine, and the protected hydrochloride salt, did not allow formation of the desired enamino ester. As in the case of  $\beta$ -keto amide formation, this was postulated to be due to the preferred attack of the amine on the oxazolidinone ring. This was subsequently confirmed by the isolation of a 3-substituted succinimide derivative **62**, where the alkyl side chain of the enamino ester had been eliminated.



Purification by radial chromatography gave **62** as a yellow oil in 77% yield. Two proposed mechanisms for the formation of this elimination product are outlined in Figure 18.

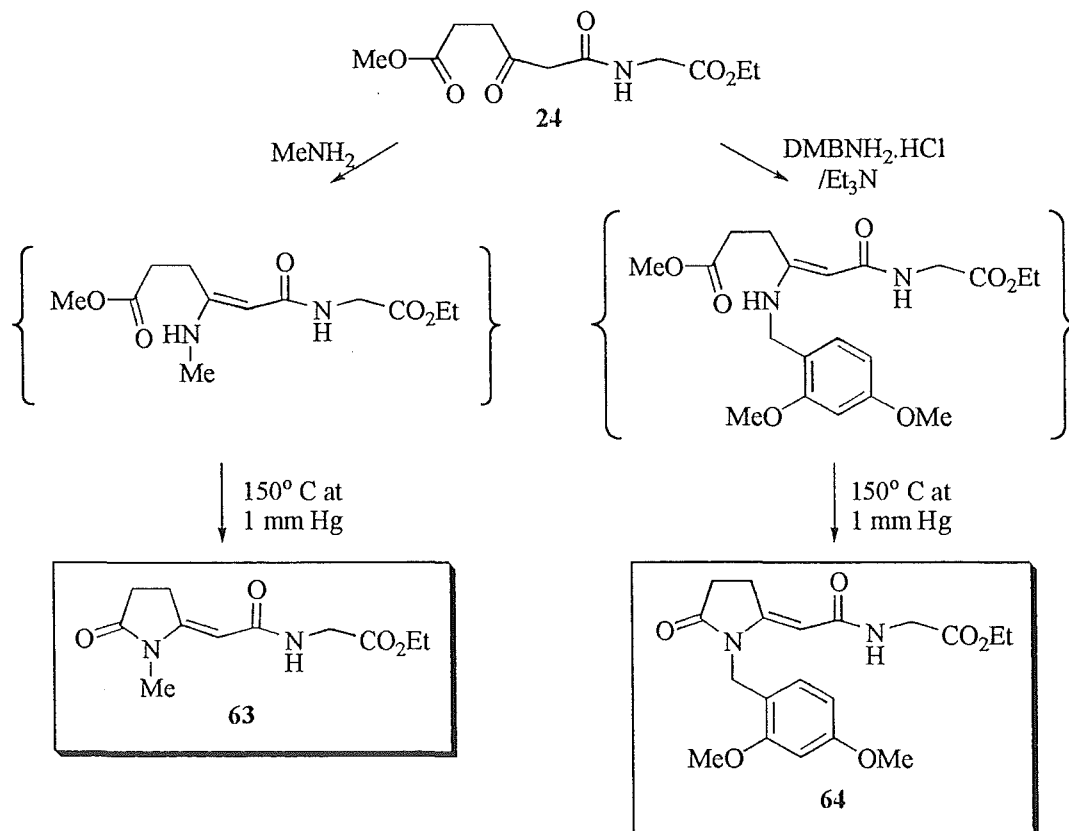


**Figure 18** Two Proposed Mechanisms for the formation of Elimination Product 62

## Synthesis of enamino amides

This technique was also applied to the preparation of enamino amides from the corresponding  $\beta$ -keto amide, in the event it would lead to a convenient method for chain extension in the *C*-direction. Synthesis of these compounds was achieved via the same conditions as that for enamino esters 63 and 64, only using the  $\beta$ -keto amide 24 as the starting compound (Scheme 18).

The  $\beta$ -keto amide **24**, the starting compound for the synthesis of enamino amides **63** and **64**, was prepared from the acid chloride **21**, via a meldrum's acid reaction with glycine ethyl ester hydrochloride (Scheme 5, Chapter 3).

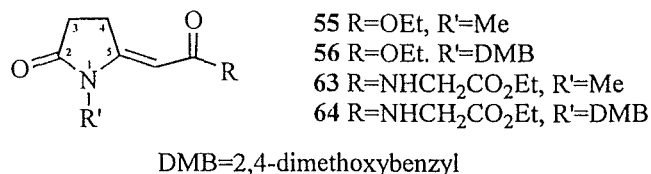


**Scheme 18** Synthesis of Enamino Amides from  $\beta$ -Keto Amides

Enamino esters **63** and **64**, were subsequently prepared by refluxing, with azeotropic removal of water, a solution of  $\beta$ -keto amide **24** in 1,2-dichloromethane, with methylamine (20 equivalents), or 1,2-dimethoxybenzylamine hydrochloride (3 equivalents) and triethylamine (3 equivalents). After 90 min, the solution was filtered and the solvent was removed under reduced pressure. The residue was then heated at  $150^\circ\text{C}$ , under reduced pressure (1 mm of Hg), for 1 h. Purification by radial chromatography gave the enamino amides **63** and **64**, as white solids in 25% and 39% yields respectively.

## Structural assignment and NMR data

The structure of enamino esters **55**, **56**, **59**, **61** and enamino amides **63** and **64**, were primarily assigned on the basis of  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, IR spectroscopy, and high resolution mass spectrometry. Figure 19 illustrates the structural assignment of enamino esters **55**, **56**, **59**, **61** and enamino amides **63** and **64**.



**Figure 19**

$^1\text{H}$  NMR spectroscopy was used to assign the *E/Z* configuration. The most diagnostic resonances were those due to (H4)<sub>2</sub> and =CH. The ethyl ester in the *E* configuration is known to deshield (H4)<sub>2</sub> in succinimide-based enamino esters.<sup>72</sup> Therefore, enamino esters **55**, **56**, **59**, **61**, and enamino amides **63** and **64**, were assigned the *E* configuration on the basis of the downfield shift of (H4)<sub>2</sub> being similar to known literature compounds of this type.<sup>70, 73</sup> Both (H3)<sub>2</sub> and (H4)<sub>2</sub> resonances appear as coupled triplets, with (H4)<sub>2</sub> displaying additional signal splitting due to long range coupling with the =CH proton. Table 3 summarizes the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data of enamino esters **55**, **56** and enamino amides **63** and **64**.

Compound	$\delta$ (H3) <sub>2</sub> , m	$\delta$ (H4) <sub>2</sub> , m	$\delta$ =CH, t	$\delta$ =CH	$\delta$ C5
<b>55</b>	2.55	3.23	5.18	91.6	160.6
<b>56</b>	2.63	3.25	5.32	92.6	160.4
<b>63</b>	2.53	3.28	5.12	93.0	166.6
<b>64</b>	2.60	3.28	5.17	94	166.8

**Table 3** Significant  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR of succinic acid-derived enamino esters **55**, **56** and enamino amides **63** and **64**.

In the case of aspartic acid-derived 3-substituted enamino esters **59** and **61** (Scheme 17), the introduction of a stereogenic centre at C3 gives rise to an increase in complexity of the resonances associated with (H4)<sub>2</sub>, and CH<sub>2</sub>CO<sub>2</sub>Et where R'=CH<sub>2</sub>CO<sub>2</sub>Et. The  $^1\text{H}$

NMR and  $^{13}\text{C}$  NMR spectra indicated a single isomer in both cases. These were both assigned the *E* configuration as the (H4)<sub>2</sub> resonances were in a characteristic downfield position, reflecting the deshielding effect of the CO<sub>2</sub>Et group. Characteristic  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data for the 3-substituted enamino esters **59** and **61** is summarized in Table 4.

Compound	R'	$\delta$ H3	$\delta$ (H4) <sub>a</sub>	$\delta$ (H4) <sub>b</sub>	$\delta$ =CH	$\delta$ =CH	$\delta$ C5
<b>59</b>	Me	4.38	3.05	3.82	5.19	93.0	166.8
<b>61</b>	CH <sub>2</sub> CO <sub>2</sub> Et	4.43	3.11	3.91	5.12	93.5	166.5

**Table 4** Significant  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data of the aspartic acid-derived enamino esters **59** and **61**.

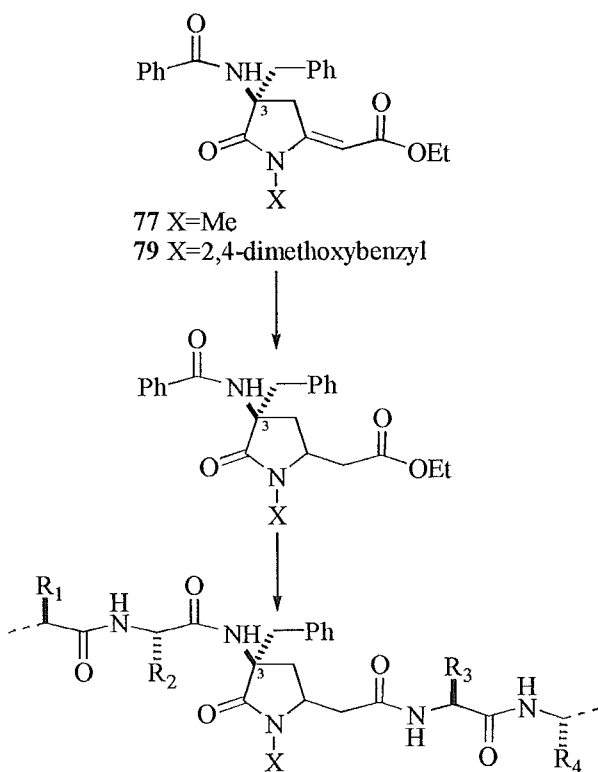
## Summary

Using the method described by Hashiguchi *et al*, for the synthesis of cyclic enamino esters, it was possible to prepare succinic acid-derived enamino esters **55** and **56**, as well as the corresponding enamino amides **63** and **64**. The starting compounds for these preparations were the  $\beta$ -keto ester **23**, and the  $\beta$ -keto amide **24** respectively. The 3-substituted enamino esters **59** and **61**, were prepared via the same method from the aspartic acid derived  $\beta$ -keto ester **28**. Enamino esters **59** and **61** are examples of 3-substituted Freidinger lactams where the amide bond is locked in a *cis*-like conformation (refer Chapter 2).

Synthesis of enamino esters of this type proceeds via an imine/enamine intermediate, before cyclizing to give the five-membered lactam ring. The assignment of these compounds exclusively as the *E* isomer was based on  $^1\text{H}$  NMR data and comparison of that data with known literature compounds.

# **Synthesis of phenylalanine-derived 3,3-disubstituted enamino esters**

This chapter describes the synthesis of phenylalanine derived 3,3-disubstituted enamino esters **77** and **79**. These compounds have the potential for incorporation into a peptide sequence to mimic a *cis*-amide bond. Enamino esters **77** and **79** represent a new class of conformationally restricted amino acid analogues for use as a potential tool in enzymatic studies (see Chapter 2).



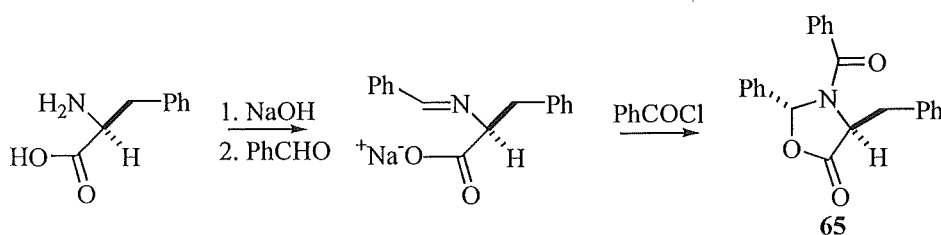
The 3,3-disubstituted enamino esters **77** and **79**, unlike the enamino esters discussed in chapter 4, contain an amino acid R group at position 3, in this case the benzyl group of phenylalanine. This particular R group is required for recognition by such target enzymes as chymotrypsin.<sup>74, 75</sup> Methodology largely developed by Seebach *et al* for the asymmetric synthesis of  $\alpha,\alpha$ -disubstituted amino acids,<sup>76</sup> was used to allow the introduction of the benzyl group, with stereo-control, into enamino esters **77** and **79**. Although (*S*)-phenylalanine was used here to allow the introduction of the benzyl group, extension of this work should allow the introduction of any amino acid R group at position 3.



## Synthesis of benzoyl oxazolidinones

### Synthesis of 4-substituted oxazolidinone **65**

The syntheses of the oxazolidinone precursors to enamino esters **77** and **79** are summarized in Schemes **19** and **20**. The key oxazolidinone **65** was prepared via the procedure reported by Seebach *et al* (Scheme 19),<sup>77</sup> and involved the treatment of the Schiff base salt of (*S*)-phenylalanine, with benzoyl chloride (1 equivalent). The resulting mixture was stirred at -20° C, under nitrogen, for 12 h, and then at 4° C for 3 days. An <sup>1</sup>H NMR spectrum of the crude oxazolidinone product revealed there to be a single epimer which was assigned as the *trans* isomer (see later for *cis/trans* assignment). Purification by radial chromatography, and subsequent recrystallisation, gave the benzoyl oxazolidinone **65**, as a white solid, in 33% yield.



Scheme 19

### Synthesis of 4,4-disubstituted oxazolidinones

The sequence for the synthesis of 4,4-disubstituted benzoyl oxazolidinones is summarized in Scheme 20. The benzoyl oxazolidinone **65** was alkylated, with a high degree of specificity, using the method pioneered by Seebach.<sup>76</sup> A solution of oxazolidinone **65**, in THF, was cooled to -78° C, and treated with 1.2 equivalents of lithium hexamethyldisilazide (LiHMDS) in THF. The resulting solution was stirred at -78° C for 7 min. The alkylating agent, BrCH<sub>2</sub>CO<sub>2</sub>tBu, was added and the solution was stirred at -78° C for 2 h, and then allowed to warm to 20° C over 16 h. An <sup>1</sup>H NMR spectrum of the product again revealed a single epimer, the (2*R*, 4*S*)-epimer, and purification by radial chromatography, and subsequent recrystallisation, gave the *tert*-butyl oxazolidinone **67**, as a white solid, in 87% yield.

The alkylating agent used here,  $\text{BrCH}_2\text{CO}_2\text{tBu}$ , differs from that previously used in reactions of this type. The *tert*-butyl ester of bromoacetic acid was used in preference to the benzhydryl ester<sup>61</sup> since it allows easy hydrolysis to give the free acid **68**. It has the added advantage of being readily available, requiring no prior preparation, and hence is the choice of alkylating agent in this case.

The formation of the 4,4-disubstituted oxazolidinone **67**, from oxazolidinone **65**, proceeds via the unstable, planar enolate **66** (Scheme 20). The C4 stereogenic centre of the enolate controls the stereochemical outcome of the alkylation reaction. The alkylating group, in this case  $\text{BrCH}_2\text{CO}_2\text{tBu}$ , approaches from the least sterically hindered face of the enolate, that is, opposite the C2-phenyl group. The configuration at C4 was initially dictated by the configuration of (*S*)-phenylalanine, and the overall stereochemical outcome of the alkylation process results in an inversion of configuration. Note that even though the benzyl group is inverted, an (*S*) assignment is still given in this case, due to priority of substituents about C4. Therefore, the amino acid stereogenic-centre controls the relative stereochemistry of the alkylation, and hence the absolute configuration of the final product. The assignment of configuration is discussed later (see Assignment of *R/S* Configuration to the Oxazolidinones). The use of a short reaction time, typically 7 min, between the addition of the base and the electrophile, minimizes the formation of self-addition by-products associated with reactions of this type.<sup>78</sup>

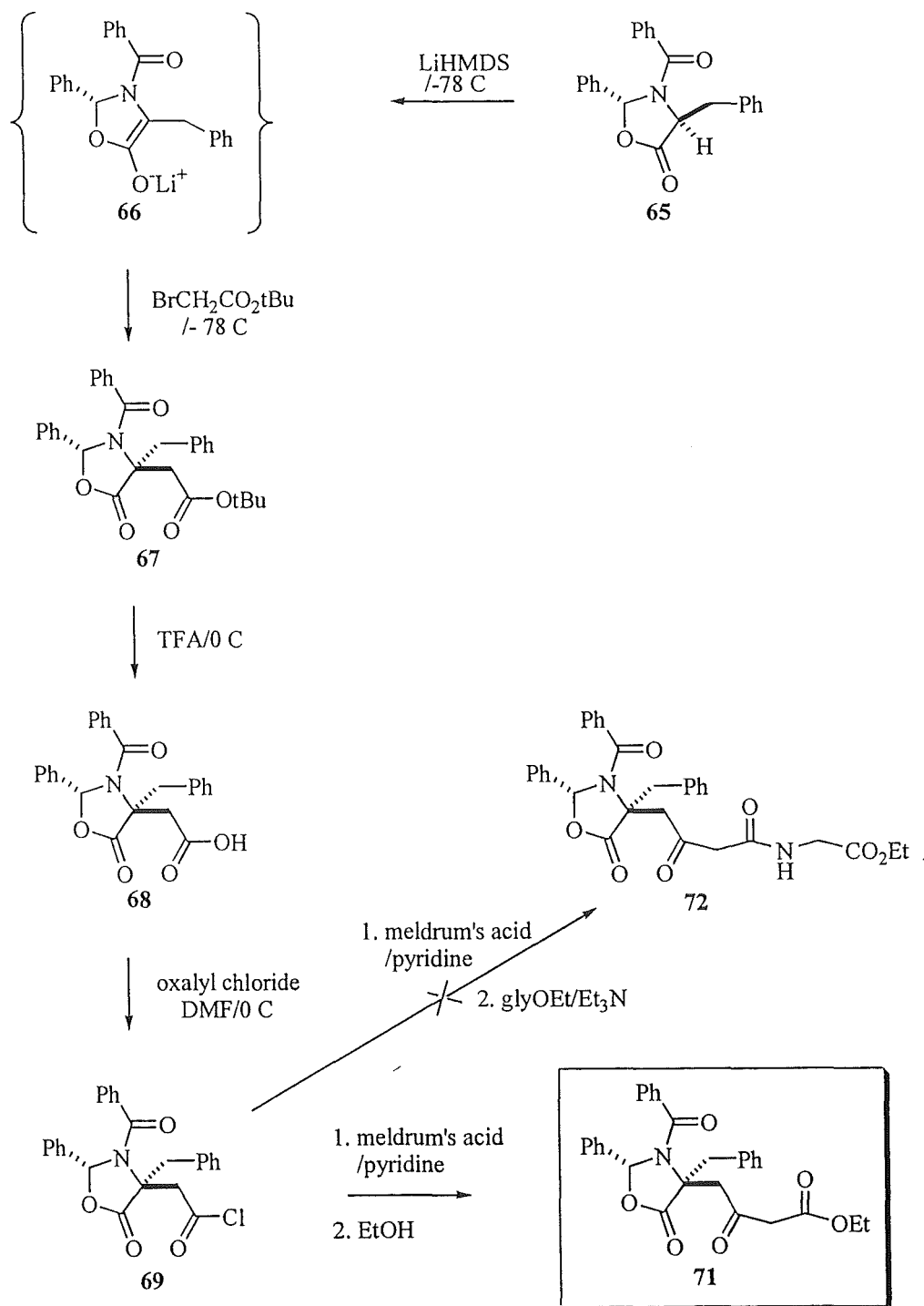
The Seebach alkylation reaction has been carried out with oxazolidinones derived from most amino acids, using a number of different alkylating agents and bases, and has proved to be a convenient method for the preparation of 4,4-disubstituted oxazolidinones of this type.

The *tert*-butyl group of **67** was removed via treatment with a large excess of TFA, at 0° C. Purification by extraction into aqueous  $\text{NaHCO}_3$  resulted in emulsification, but eventually led to the isolation of the purified acid **68**, as a white solid, in 41% yield. Attempts to increase this yield were carried out using the method described by S. Torii *et al.*<sup>90</sup> This involved the treatment of oxazolidinone **67** with HCl (0.5 equivalent), using phenol as a solvent. However, this gave the acid in only 19% yield and hence was not incorporated as a viable route for its preparation.

A solution of the acid **68** in dichloromethane was cooled to 0° C and treated with oxalyl chloride (5 equivalents) and a catalytic amount of DMF to give the corresponding acid chloride **69**, in 100% yield.

The benzoyl  $\beta$ -keto ester oxazolidinone **71** (Scheme 20), was prepared from the treatment of the acid chloride **69** with meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) and pyridine, in dichloromethane. The mixture was stirred at 0° C for 50 min and then at 20° C for 45 min. The resulting acyl meldrum's intermediate was refluxed in ethanol for 4 h and the crude product was purified by radial chromatography to give the benzoyl  $\beta$ -keto ester oxazolidinone **71**, as an oil, in 73% yield.

Attempts to synthesize the corresponding  $\beta$ -keto amide **72** (Scheme 20) via reaction of the acyl meldrum's intermediate of **69**, with glycine ethyl ester hydrochloride and triethylamine, proved unsuccessful. It was originally thought that further substitution, at C2, would sterically hinder the attack of the amine on the oxazolidinone ring, thereby allowing reaction at the alkyl side chain of the meldrum's acid intermediate to give the desired  $\beta$ -keto amide. However, this did not prove to be the case. Nevertheless, peptide chain extension is still possible via hydrolysis of the ethyl ester of **71** and the subsequent coupling of an amino acid, to give the desired  $\beta$ -keto amide **72**.

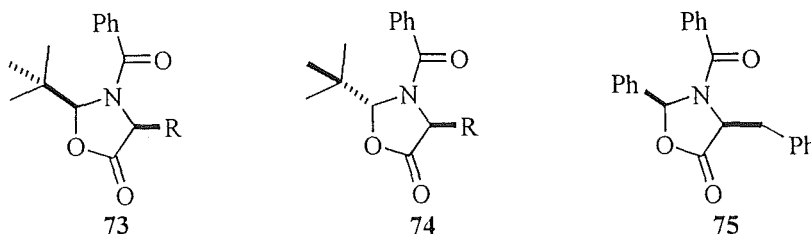


Scheme 20

## Assignment of R/S configuration to the oxazolidinones

The benzoyl oxazolidinone **65** (Schemes 19 and 20), was assigned the *trans* configuration on the basis of the melting point, IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR data which was identical to that reported in the literature.<sup>82</sup>

The original literature assignment of the *trans* configuration to **65** was based on the comparison of the chemical shifts of the protons at **H2** and **H4** with corresponding chemical shifts of *cis* and *trans* *tert*-butyl oxazolidinones **73** and **74**.<sup>77, 83</sup> The **H2** and **H4** resonances are characteristically downfield in the *trans* isomer, relative to the corresponding *cis* isomer.

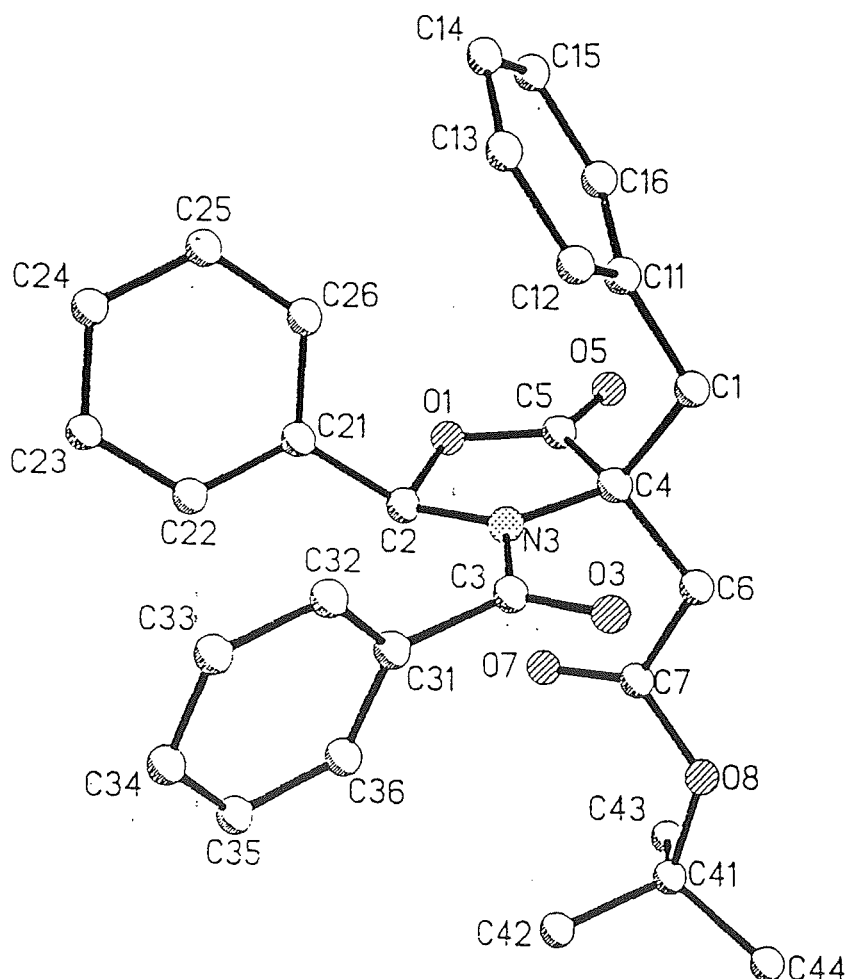


R	Compd	Cis		Compd	Trans	
		$\delta$ H2	$\delta$ H4		$\delta$ H2	$\delta$ H4
Me	a	6.13	4.07	a	6.25	4.37
$\text{CH}_2\text{CH}_2\text{SMe}$	b	6.10	4.20	b	6.21	4.52
	<b>75</b>	-	4.92	<b>65</b>	5.81	5.21

The assignment of the *trans* configuration to **65** was also confirmed by the observance of a nuclear Overhauser enhancement (NOE) between **H2** and  $\text{CH}_2\text{Ph}$ .<sup>78</sup> Therefore, having used (*S*)-phenylalanine as the starting material to fix the configuration at C4, this allowed the assignment of the absolute configuration of oxazolidinone **65** as the (2*R*, 4*S*) isomer.

The relative *trans*-configuration, of the 4,4-disubstituted benzoyl oxazolidinone **67** was assigned on the basis of  $^1\text{H}$  NMR data and confirmed by single crystal x-ray structure analysis (Figure 20). This result also confirmed that the alkylation had occurred from the least sterically hindered face of the enolate **66** (Scheme 20). As the

absolute stereochemistry of the precursor oxazolidinone **65** was known, this again allowed assignment of the absolute configuration of the 4,4-disubstituted oxazolidinone **67** as the (2*R*, 4*S*) isomer.



**Figure 20** X-ray Molecular Structure of **67** with Crystallographic Numbering Scheme

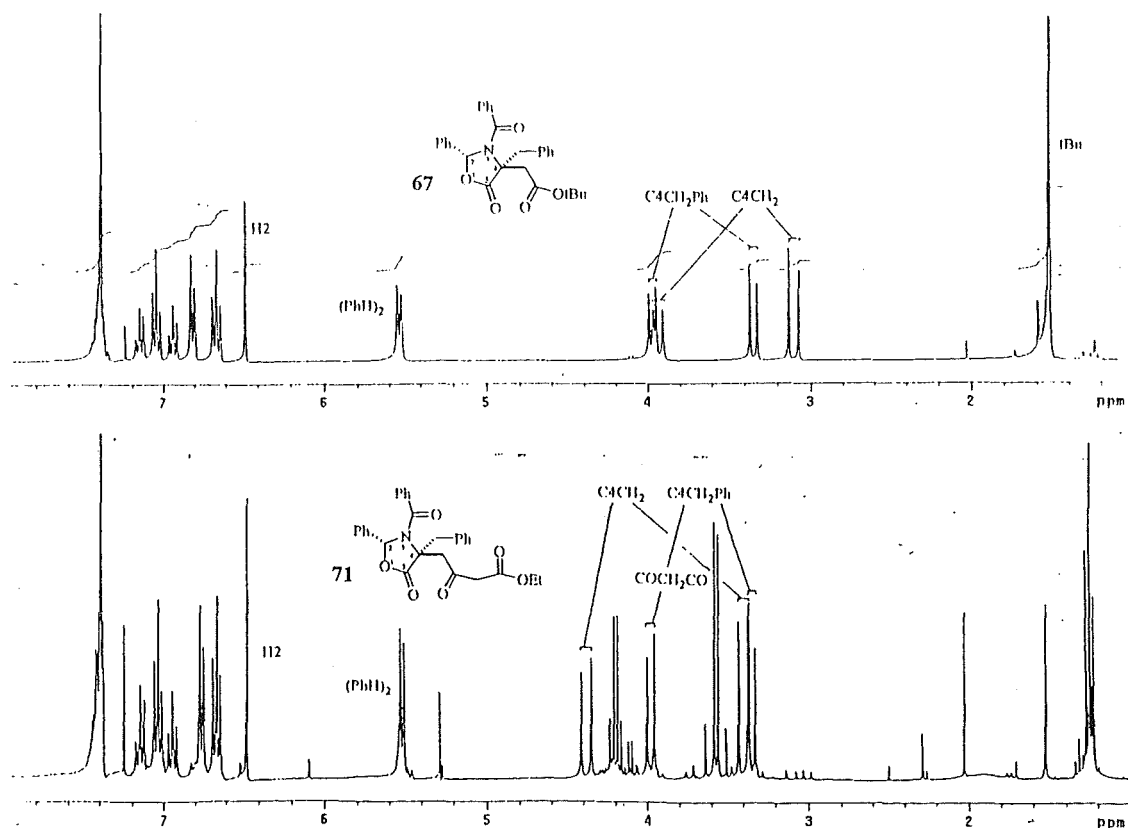
## X-ray molecular structure of compound **67**

A perspective drawing of compound **67**, with atom labeling, is shown in Figure 20. The C2 *tert*-butyl analogues of oxazolidinones of type **73** and **74** (previous page), have been shown to adopt a slight envelope conformation for the oxazolidinone ring, with C2 out of the plane of the other four atoms of the ring.<sup>84, 85</sup> These reports also reveal pyramidalization of the amide N-atom and that the *N*-acyl carbonyl O-atom and the

acetal substituent are in an *s-cis*-conformation.<sup>84</sup> The pyramidalization is thought to contribute to the steric bias of the oxazolidinone ring towards an incoming electrophile in Seebach alkylations of the type shown in Scheme 20.<sup>84</sup> The structure of compound **67** is a product of C4 alkylation and has an essentially planar oxazolidinone ring, little or no pyramidalization of the amide N-atom, and does not have the *N*-acyl carbonyl O-atom in an *s-cis*-position relative to the acetal C2-substituent (see Figure 20). It should also be noted that the C21-26 and C11-16 aromatic rings are orthogonal to each other giving rise to the possibility of deshielding of protons associated with the C2-phenyl ring (see later).

## Structural assignment and NMR data of oxazolidinones

The introduction of chirality, at C2 and C4, into oxazolidinones comprising the series **65-71**, gives rise to the possibility of multiple signal splitting due to the diastereotopic nature of some protons. <sup>1</sup>H NMR spectra for compounds **67** and **71** respectively, illustrate that AB quartets are seen for the diastereotopic protons associated with C4CH<sub>2</sub>Ph and the C4CH<sub>2</sub> of the alkyl group, as well as for the COCH<sub>2</sub>CO of the β-keto ester oxazolidinone **71**.



It is also interesting to note the chemical shift of the doublet at  $\delta$  5.55. This resonance corresponds to two equivalent aromatic protons, associated with the C2Ph, being deshielded by the aromatic ring of the C4CH<sub>2</sub>Ph. This deshielding effect causes an upfield shift of the resonance associated with these two protons. This was observed in the solid state conformation as determined by single crystal x-ray analysis which clearly showed the two phenyl rings orthogonal to each other in the solid state (Figure 20). Significant <sup>1</sup>H and <sup>13</sup>C NMR spectral data for oxazolidinones **65**, **67**, **68** and **71** is summarized in Table 5.

Table 5

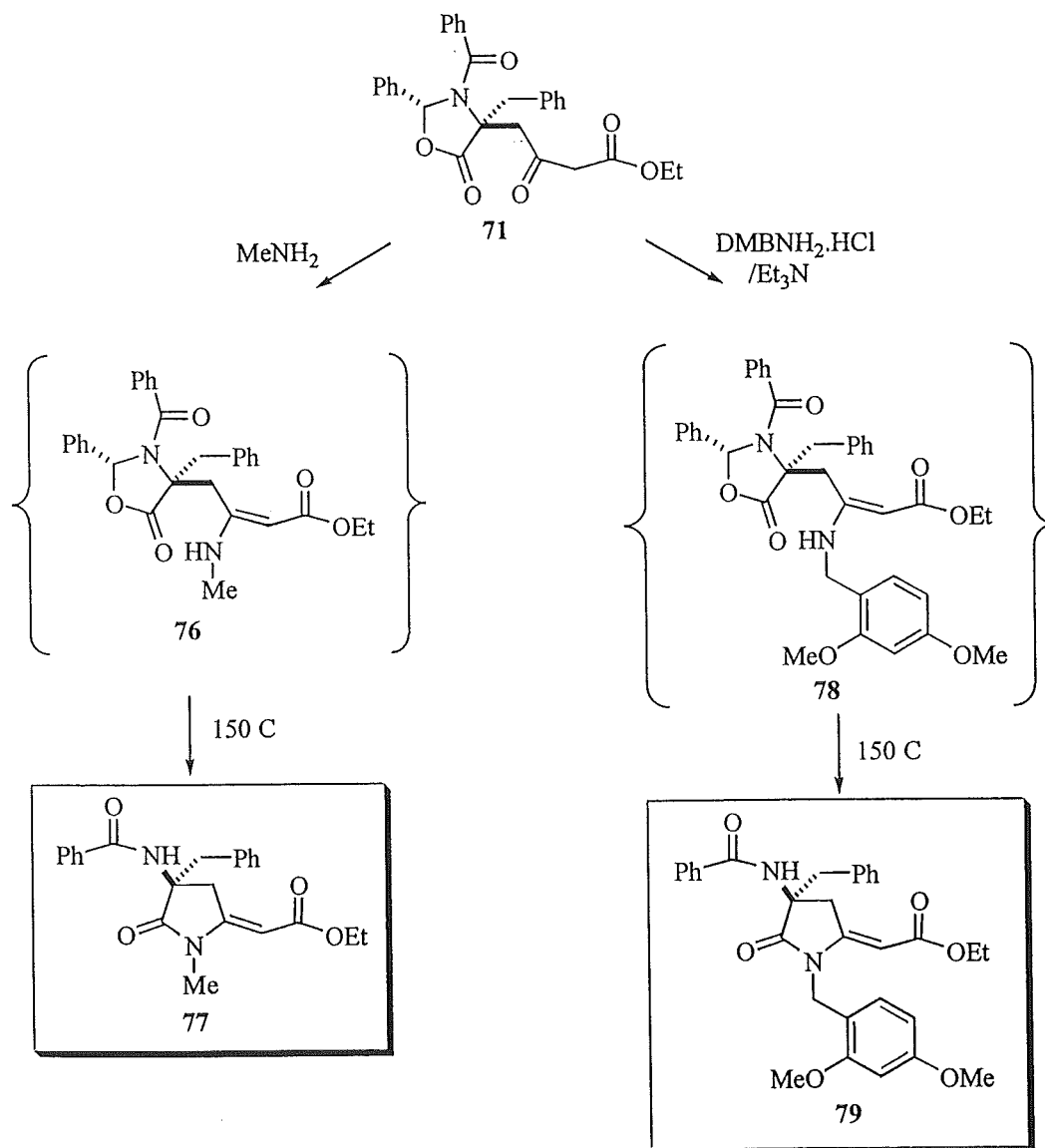
<sup>1</sup> H NMR data							
Compd	$\delta$ H2	C4CH <sub>2</sub> Ph Abq			C4CH <sub>2</sub> R ABq		
		$\delta$ H <sub>a</sub>	$\delta$ H <sub>b</sub>	J <sub>AB</sub> Hz	$\delta$ H <sub>a</sub>	$\delta$ H <sub>b</sub>	J <sub>AB</sub> Hz
<b>65</b>	5.81	3.41	3.79		N/A		
<b>67</b>	6.49	3.35	3.98	13.6	3.11	3.95	17.8
<b>68</b>	6.46	3.41	4.04	13.4	3.25	4.13	18.1
<b>71</b>	6.49	3.34	3.96	13.5	3.39	4.39	19.0
<sup>13</sup> C NMR data							
Compd	C2	C4	C4CH <sub>2</sub> Ph	C4CH <sub>2</sub> R	PhCO	C5	
<b>65</b>	91.3	57.8	34.9	N/A	169.3	171.2	
<b>67</b>	91.5	66.3	42.3	40.3	169.7	173.2	
<b>68</b>	91.7	66.1	42.3	38.5	170.2	173.1	
<b>71</b>	91.6	65.4	42.2	48.0	169.9	173.0	

Comparison of the <sup>1</sup>H NMR data of literature oxazolidinones with those in Table 5 showed that H2 characteristically resonated at  $\delta$  5.29-6.51. In the alkylated oxazolidinones **67**, **68** and **71**, C4CH<sub>2</sub>Ph and C4CH<sub>2</sub>R appeared as AB quartets with  $J \approx 13$  Hz and  $J \approx 18$  Hz, respectively. <sup>13</sup>C NMR resonances for C2, C4, C5, C4CH<sub>2</sub>Ph, C4CH<sub>2</sub>R, and PhCO, appeared at characteristic chemical shifts for all examples. C2 typically resonates at  $\delta$  91 ppm for all examples, despite the downfield shift of H2 for the disubstituted oxazolidinone compounds **67**, **68** and **71**.



## Synthesis of the target enamino esters

The target enamino esters **77** and **79** were synthesized from benzoyl  $\beta$ -keto ester oxazolidinone **71** via the process developed by Hashiguchi *et al* (Scheme 21).<sup>60</sup>



**Scheme 21**

Enamino esters **77** and **79** were prepared by refluxing a solution of the benzoyl  $\beta$ -keto ester oxazolidinone **71**, in 1,2-dichloroethane, with methylamine (20 equivalents), or 2,4-dimethoxybenzylamine hydrochloride (5 equivalents) and triethylamine (5 equivalents). After 90 min, the solution was filtered and the solvent removed under

reduced pressure. The residue was then heated at 150° C, under reduced pressure (1 mm of Hg), for 1 h. Purification by radial chromatography gave **77**, as a white solid, in 43% yield. Attempted purification of enamino ester **79**, by radial chromatography, using 2:3 ethyl acetate/petroleum ether as the elutant, gave a mixture of the desired product, as well as other unidentified material. Crystallization of this mixture using ethyl acetate/petroleum ether gave **79**, as a tan solid, in 8% yield.

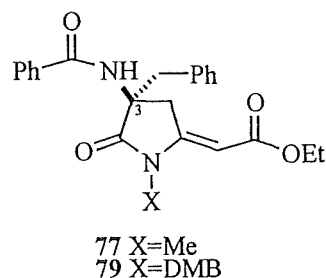
The enamino esters **77** and **79** probably form via a mechanism analogous to that shown in Figure 15 (Chapter 4) for the preparation of 3-substituted enamino esters **59** and **61**.

It should be noted that in the preparation of **77** and **79**, the corresponding imide products were not observed. This was proposed to be due to the increased substitution, at C4 and C2, of the acyclic oxazolidinone precursor **71**. This represents an example of a general effect commonly known as the 'Thorpe-Ingold' or '*gem*-dialkyl' effect. It has long been known in the literature that, for a given ring size, alkyl substituents on an acyclic system promote cyclization and favour the ring form in an equilibrium involving the opening and closing of a ring. To explain these observations, Thorpe and Ingold suggested that a decrease in the internal angle of a small ring, led to a spreading apart of the external angle.<sup>79</sup> This, in turn, relieved steric interactions between substituents attached to the same carbon, thus favouring the ring form over the open chain form.

Although this was shown to be true for five-membered rings such as **77** and **79**, in 1960 Allinger and Zalkow proposed a more general theory to account for this increased rate in ring formation.<sup>81</sup> This was termed the "*gem*-dialkyl effect" and was described in terms of the enthalpies and entropies of open-chain vs ring compounds. It was found that there were fewer extra gauche interactions in rings than there were in the corresponding open chains. This meant that, compared with an unsubstituted chain, the substituted chain (e.g. **71** Scheme 21) had a more favourable enthalpy of ring closure. In addition, there was an entropy effect, due to branching, based on the fact that branching reduced the rotational entropy of open-chain compounds, something that could not occur in the ring form due to its rigidity. Therefore, this increased entropy factor, where entropy is not lost, also favours ring closure for the more branched compounds. Since branching both reduces the enthalpy, and increases the entropy of ring closure, it therefore decreases the free energy of ring closure and leads to an equilibrium more

favourable to the ring structure.<sup>80</sup> In the case of the formation of **77** and **79** (Scheme 21), this effect adds to the stability of the form of the 3,3-disubstituted oxazolidinones, making them less susceptible to ring opening via attack by an amine. This results in imide formation (via mechanism B, Figure 18, Chapter 4) being suppressed, thereby favouring the formation of the enamino esters **77** and **79** (Scheme 21). This effect is not seen for the monosubstituted oxazolidinone **28**, and the corresponding imide **62** was isolated (Figure 18).

## Structural assignment and NMR data of target enamino esters



The configuration of the double bond of enamino esters **77** and **79** was assigned on the basis of  $^1\text{H}$  NMR spectroscopy. Both **77** and **79** were assigned as the (*E*) isomer on the basis of similar chemical shifts for the ( $\text{H4}$ )<sub>2</sub> resonance, to those observed in earlier unsubstituted and 3-substituted enamino esters (see Chapter 4). This reflects the deshielding influence of the  $\text{CO}_2\text{Et}$  group on ( $\text{H4}$ )<sub>2</sub> and the result was used to assign the *E/Z* configuration of simpler unsubstituted enamino esters discussed in chapter 4,<sup>70</sup> as well as compounds of this type described in the literature. It should also be noted that in all the *N*-acylated enamino esters and *N*-acylated enamino amines described here, the *E* isomer is invariably favoured.

Key  $^{13}\text{C}$  NMR resonances of the enamino ester **77**, were assigned following an NMR proton-carbon heteronuclear correlation experiment. The  $^1\text{H}$ - $^{13}\text{C}$  NMR correlation experiment showed that the  $^1\text{H}$  NMR resonance at  $\delta$  4.91 was coupled to the  $^{13}\text{C}$  NMR resonance at  $\delta$  91.8, characteristic of  $=\text{CH}$ . The  $^1\text{H}$  NMR resonance at  $\delta$  6.74 did not show coupling to any  $^{13}\text{C}$  NMR resonance and was hence assigned as  $\text{NH}$ . Comparison

of these resonances with those of the enamino ester 79, showed that NH was downfield from =CH for both compounds.

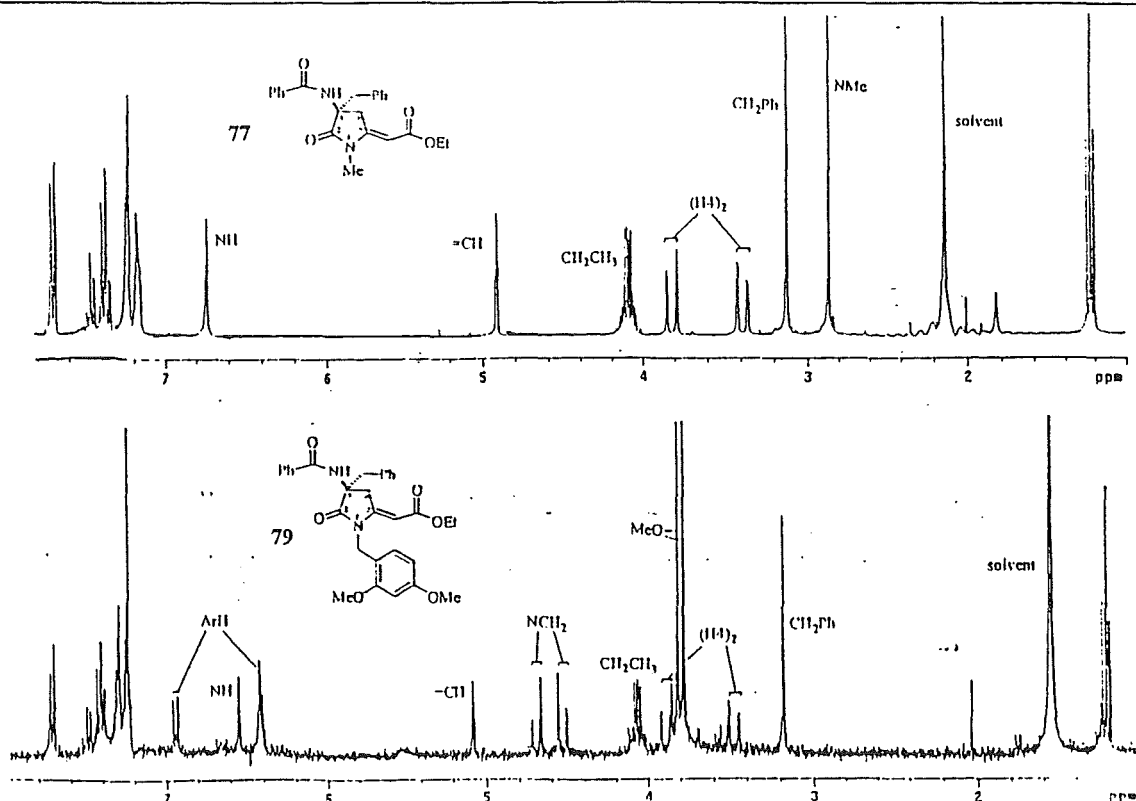
The  $^1\text{H}$  NMR spectrum of enamino esters 77 and 79 were found to again display signal splitting due to the stereogenic centre at C3. The resonances for  $(\text{H4})_2$  and  $\text{OCH}_2\text{CH}_3$  appear as an AB quartet and a multiplet respectively, while the resonance for  $\text{CH}_2\text{Ph}$  appears as a narrow multiplet, upfield of that for  $(\text{H4})_2$ . The  $\text{NCH}_2$  resonance for enamino ester 79 also appears as an AB quartet characteristic of the diastereotopic nature of these protons. Characteristic  $^1\text{H}$  and  $^{13}\text{C}$  NMR data is summarized in Table 6 with the  $^1\text{H}$  NMR spectra of both compounds shown below.

Table 6

$^1\text{H}$ NMR					
Compd	=CH	C4 $\text{CH}_2\text{Ph}$	$\text{OCH}_2\text{CH}_3$	$\text{NCH}_2$	NH
77	4.91 t	3.12 m	4.09 m	N/A	6.74
79	5.07	3.12 m	4.09 m	4.61 Abq $J=16.1\text{Hz}$	6.56

$^{13}\text{C}$ NMR					
Compd	=CH	C4 $\text{CH}_2\text{Ph}$	C3	C4	C5
77	91.8	42.8	59.5	36.5	156.4
79	93.1	42.9	59.4	36.6	154.1



Mass spectra of enamino esters **77** and **79**, like that of oxazolidinones **65-71**, displayed a characteristically large signal at  $M-91$ , corresponding to the  $M-CH_2Ph$  fragment.

## Conclusion and future work

A general Meldrum's acid method was used to synthesize the succinimide-based  $\beta$ -keto ester **23**, as well as the aspartic acid-derived  $\beta$ -keto ester **28**, as precursors to the synthesis of cyclic enamino esters. This method was also used to prepare the  $\beta$ -keto amide **24**, which allowed chain extension in the  $C$ -direction in one easy step. The attempted synthesis of the corresponding aspartic acid-derived  $\beta$ -keto amide **29** was unsuccessful and this was proposed to be due to the susceptibility of the oxazolidinone ring to attack by an amine.

Compounds **23** and **24** were then used in the preparation of cyclic enamino esters **55**, **56**, **59**, **61**, **63** and **64**, via their reaction with an amine, with **59** and **61** being examples of Freidinger lactams where the amide bond is locked in a *cis*-like conformation.

The phenylalanine-derived 3,3-disubstituted enamino esters **77** and **79** were also synthesized to allow the incorporation of an amino acid R group ( $CH_2Ph$ ) at the 3-position of the succinimide ring. These *cis*-amide bond mimics contained an *N*-alkylated amide bond (Me, DMB), and have the same configuration as the natural peptide substrates. The key configuration at C3 of **77** and **79**, was controlled by the configuration of the starting amino acid used in the syntheses. The configuration of these compounds was determined by  $^1H$  NMR and confirmed by the obtaining of a single crystal x-ray structure of **67**, a key compound in the synthesis of enamino esters **77** and **79**. Peptidomimetics of this type can be incorporated into peptide sequences to mimic a *cis*-amide bond configuration. By studying the effect that this has on the activity of a corresponding enzyme or receptor, information can be gained as to the bio-active conformation of a given peptide.

The method of synthesizing the enamino esters has shown to be versatile and in the future could be adapted to incorporate the mimics into different peptides so that maximum interaction with the desired target is achieved. Also, different recognition

groups could be incorporated at the 3-position to allow other enzymes and receptors to be targeted.

Use could be made of different forms of protected ammonia, other than 2,4-dimethoxybenzylamine, in an effort to increase the overall yield, purity and ease of synthesis of compounds of this type. Further, methods for the hydrolysis of the ethyl ester and the reduction of the ene-lactam double bond of **77** and **79** need to be investigated, to allow chain extension in the *C*-direction and systems that more closely resemble the geometry of the natural substrate. Preliminary results indicate that this could be achieved via hydrogenation, although further investigation is still required.

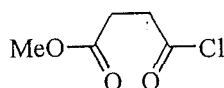
# Experimental

### General Experimental

All solvents used throughout the work described in this thesis were freshly distilled prior to use, and petroleum ether applies to the fraction with boiling point 60-70° C. Flash chromatography was performed using 250-400 mesh grade 923 silica gel on 18-20 cm columns, under a positive air pressure. Thin layer chromatography was performed on plastic backed silica plates. Radial chromatography was performed on a Harrison and Harrison chromatotron using 2 mm silica plates. Melting points were measured using an Electrothermal melting point apparatus and are uncorrected.

Proton NMR spectra were recorded on a Varian Unity 300 spectrometer in CDCl<sub>3</sub>, using Me<sub>4</sub>Si as an internal reference, recorded as (multiplicity, coupling constants, number of protons, assignment). <sup>13</sup>C NMR spectra were recorded on a Varian XL-300 spectrometer referencing through CDCl<sub>3</sub>. Infrared absorption spectra were recorded, using KBr plates, on a Shimadzu FTIR-8201 PC spectrometer. Mass spectroscopy results were obtained from a Kratos MS80RFA spectrometer.

#### Preparation of 3-Carbomethoxypropionyl chloride **21**



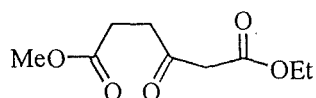
A solution of succinic anhydride (28.430 g, 0.285 mol) in methanol (14 ml) was refluxed over a steam bath for 35 min. The mixture was removed from the steam bath and swirled frequently for 15 min until homogeneity resulted, and the solution was then heated on a steam bath for an additional 30 min. The methanol was evaporated to leave methyl hydrogen succinate **20**, as a white crystalline solid (37.10 g, 98.6%). mp 56-58° C (lit mp 57-58° C).<sup>89</sup>

A solution of methyl hydrogen succinate **20** (20.191 g, 0.153 mol), and thionyl chloride (23 ml, 0.315 mol, 2.1 equiv), was warmed at 30-40° C for 3 h. The mixture was distilled under reduced pressure to give 3-carbomethoxypropionyl chloride **21** (14.329 g, 62%). bp 58-65° C at 1mm Hg (lit bp 92-93° C at 18 mm Hg).<sup>89</sup>



$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.68 (t,  $J=6.6\text{Hz}$ , 2H,  $\text{CH}_2\text{CO}_2\text{Me}$ ), 3.22 (t,  $J=6.6\text{Hz}$ , 2H,  $\text{CH}_2\text{COCl}$ ), 3.72(s, 3H, Me).

Preparation of 1-Ethyl-6-methyl-3-oxohexandioate **23**



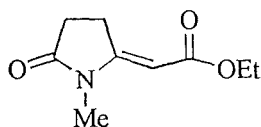
2,2-Dimethyl-1,3-dioxan-4,6-dione (Meldrum's acid) (1.970 g, 0.014 mol, 1.1 equiv) was dissolved in dichloromethane (5 ml) and the solution was cooled to  $0^\circ\text{C}$ . Dry pyridine (2.69 ml, 0.033 mol, 1.1 equiv) was added over 10 min, followed by 3-carbomethoxypropionyl chloride **21** (1.645 ml, 0.013 mol, 1 equiv) over 90 min. After stirring at  $0^\circ\text{C}$  for 50 min, and at  $20^\circ\text{C}$  for 45 min, the mixture was poured into 2N aqueous HCl (2 ml) containing crushed ice. The organic layer was removed and the aqueous layer was extracted with dichloromethane (2x0.5 ml). The combined dichloromethane extracts were washed successively with 2N HCl (2x0.5 ml), saturated aqueous NaCl (0.8 ml), dried ( $\text{MgSO}_4$ ), and evaporated to give the acyl meldrum's acid intermediate **22**, as a dark red solid (2.385 g), which was used in the next step without further purification.

The acyl meldrum's acid intermediate **22** (2.385 g, 9.24 mmol), was refluxed in dry ethanol (40 ml) for 2.5 h to yield a red/brown oil. The oil was dissolved in dichloromethane and successively washed with cold saturated aqueous  $\text{NaHCO}_3$  (10 ml), water (10 ml), dried ( $\text{MgSO}_4$ ), and evaporated to yield the  $\beta$ -keto ester **23** as an oil (2.205 g, 84%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.24 (t,  $J=7.1\text{Hz}$ , 3H,  $\text{CH}_2\text{CH}_3$ ), 2.58 (t,  $J=6.6\text{Hz}$ , 2H,  $\text{CH}_2\text{CO}_2\text{Me}$ ), 2.84 (t,  $J=6.6\text{Hz}$ , 2H,  $\text{CH}_2\text{CO}$ ), 3.46 (s, 2H,  $\text{COCH}_2\text{CO}$ ), 3.64 (s, 3H, OMe), 4.16 (q,  $J=7.1\text{Hz}$ , 2H,  $\text{CH}_2\text{CH}_3$ ).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  13.57, 27.16, 36.88, 48.69, 51.24, 60.79, 166.53, 172.31, 200.73.  
IR (KBr) 1739, 1720  $\text{cm}^{-1}$ .

HRMS Found 202.0841 (Calcd for  $\text{C}_9\text{H}_{14}\text{O}_5$  202.0841).

Preparation of (*E*)-5-Ethoxycarbonylmethylidene-1-methylpyrrolidin-2-one 55**Method A**

To a solution of the  $\beta$ -keto ester **23** (100 mg, 0.5 mmol, 1 equiv), in 1,2-dichloroethane (10 ml), was added methylamine hydrochloride (300 mg, 4.5 mmol, 9 equiv) and triethylamine (0.6 ml, 4.5 mmol, 9 equiv), and the mixture was refluxed, under nitrogen, for 90 min with azeotropic removal of water. The solution was filtered and the solvent was removed by rotary evaporation. The residue was then heated, at 150° C, at 1mm Hg for 1 h. Purification of the residue by radial chromatography using 1:1 ether/petroleum ether gave the product **55** as a white solid (23 mg, 26%). mp 113-114° C (lit mp 118° C).<sup>73</sup>

<sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.28 (t,  $J=7.1$ Hz, 3H, CH<sub>2</sub>CH<sub>3</sub>), 2.55 (m, 2H, (H<sub>3</sub>)<sub>2</sub>), 2.99 (s, 3H, NMe), 3.23 (m, 2H, (H<sub>4</sub>)<sub>2</sub>), 4.15 (q,  $J=7.1$ Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.18 (t,  $J=1.9$ Hz, 1H, =CH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  14.39 (CH<sub>2</sub>CH<sub>3</sub>), 24.53 (C<sub>3</sub>), 26.89 (NMe), 28.02 (C<sub>4</sub>), 59.52 (CH<sub>2</sub>CH<sub>3</sub>), 91.65 (=CH), 160.59, 167.23, 176.79 (3xCO).

IR (KBr) 1730, 1625 cm<sup>-1</sup>.

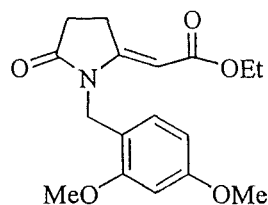
HRMS Found 183.0895 (Calcd for C<sub>9</sub>H<sub>13</sub>NO<sub>3</sub> 183.0895).

**Method B**

To a solution of the  $\beta$ -keto ester **23** (100 mg, 0.5 mmol, 1 equiv), in 1,2-dichloroethane (10 ml), was added methylamine (1.6 ml of 6M solution in 1,2-dichloroethane, 0.01 mol, 20 equiv) and the mixture was refluxed, under nitrogen, for 90 min with azeotropic removal of water. The solvent was removed by rotary evaporation and the residue was heated at 150-160° C at 1mm for 1 h. Purification by radial chromatography using 1:1 ether/petroleum ether gave **55** as a white solid (16 mg, 18%). mp 112-114° C

NMR data as above.

Preparation of (*E*)-1-(2,4-Dimethoxybenzyl-5-ethoxycarbonylmethylidenepyrrolidin-2-one **56**



To a solution of the  $\beta$ -keto ester **23** (50 mg, 0.248 mmol, 1 equiv), in 1,2-dichloroethane (5 ml), was added 2,4-dimethoxybenzylamine hydrochloride (51 mg, 0.3 mmol, 1 equiv) and triethylamine (0.34 ml, 0.3 mmol, 1 equiv) and the mixture was refluxed, under nitrogen, for 45 min. The solvent was removed under reduced pressure and the residue heated at 150-160° C at 1mm Hg for 1 h. The resulting oil was purified by radial chromatography using 1:1 ethyl acetate/petroleum ether, and the main fraction was recrystallized to give the pyrrolidinone **56** (21 mg, 28%). mp 112-113° C.

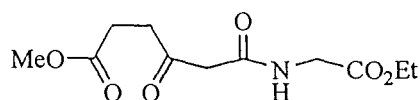
$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.24 (t,  $J=7.1\text{Hz}$ , Me), 2.63 (t,  $J=7.6\text{Hz}$ , 2H, (**H4**)<sub>2</sub>), 3.25 (t,  $J=7.6\text{Hz}$ , 2H, (**H3**)<sub>2</sub>), 3.79 and 3.86 (s, 2x3H, 2xOMe), 4.10 (q,  $J=7.0\text{Hz}$ , 2H,  $\text{CH}_2\text{CH}_3$ ), 4.68 (s, 2H,  $\text{NCH}_2\text{Ar}$ ), 5.32 (s, 1H,  $=\text{CHCO}$ ), 6.43 (d,  $J=8.5\text{Hz}$ , 1H, ArH), 6.45 (s, 1H, ArH), 6.95 (d,  $J=8.5\text{Hz}$ , 1H, ArH).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.39 ( $\text{CH}_2\text{CH}_3$ ), 24.59 (**C3**), 28.14 (**C4**), 38.32 and 55.34 (2xOMe), 59.40 ( $\text{CH}_2\text{CH}_3$ ), 92.63 ( $=\text{CH}$ ), 98.44, 104.44, 115.27, 128.69, 157.85, 159.31, 160.40, 167.48, 177.19.

IR (KBr) 1735, 1618  $\text{cm}^{-1}$

HRMS Found 319.1420 (Calcd for  $\text{C}_{17}\text{H}_{21}\text{NO}_5$  319.1420).

Anal. Calcd for  $\text{C}_{17}\text{H}_{21}\text{NO}_5$ : C, 63.94; H, 6.63; N, 4.39. Found: C, 63.96; H, 6.70; N, 4.52.

Preparation of Methyl-5-(N-Ethoxycarbonylmethylcarbamoyl)-4-oxo-pentanoate 24

Meldrum's acid (500 mg, 3.5mmol) was dissolved in dichloromethane (2 ml) and the solution was cooled to 0° C. Dry pyridine (0.68 ml, 3.5 mmol) was added over 10 min, followed by 3-carbomethoxypropionyl chloride **21** (0.42 ml, 3.3 mmol) in dichloromethane (1 ml), over 60 min. The mixture was stirred at 0° C for 50 min and at 20° C for 45 min. The resulting red/brown solution was poured into 2N aqueous HCl (2 ml) containing crushed ice. The organic layer was removed and the aqueous layer was extracted with dichloromethane (2 ml). The combined dichloromethane extracts were successively washed with 2N aqueous HCl (2x0.5 ml), saturated aqueous NaCl (1 ml), dried (MgSO<sub>4</sub>) and evaporated to give the acyl meldrum's acid intermediate **22** (0.716g).

A solution of the acyl meldrum's acid intermediate **22** (0.716 g, 2.8 mmol), glycine ethyl ester hydrochloride (0.42 g, 3 mmol) and triethylamine (.0.42ml, 3 mmol) was refluxed, under nitrogen, in dry benzene (25 ml) for 4 h. The solvent was evaporated under reduced pressure and the residue was purified by radial chromatography using 5:3 petroleum ether/ethyl acetate, to give **24** as an oil (383 mg, 47%).

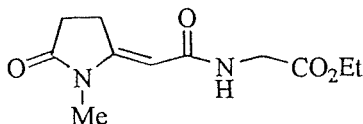
<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.29 (t, *J*=7.0Hz, 3H, CH<sub>2</sub>CH<sub>3</sub>), 2.63 (t, *J*=6.4Hz, 2H, CH<sub>2</sub>CO<sub>2</sub>Me), 2.87 (t, *J*=6.4Hz, 2H, CH<sub>2</sub>CO), 3.54 (s, 2H, COCH<sub>2</sub>CO), 3.69 (s, 3H, OMe), 4.05 (d, *J*=5.6Hz, 2H, NCH<sub>2</sub>), 4.22 (q, *J*=7.0Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 7.42 (br s, 1H, NH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 13.53, 27.00, 37.39, 40.89, 48.22, 51.39, 61.00, 165.32, 175.45, 203.74.

IR (KBr) 3354, 1739 (br), 1674 cm<sup>-1</sup>.

HRMS Found 259.1056 (Calcd for C<sub>11</sub>H<sub>17</sub>NO<sub>6</sub> 259.1056).

Preparation of (*E*)-1 Methyl-5-(*N*-ethoxycarbonylmethylcarbamoyl)-methylidene-pyrrolidin-2-one **63**



To a solution of **24** (65 mg, 0.25 mmol, 1 equiv), dissolved in 1,2-dichloroethane (10 ml), was added methylamine (0.83 ml of 6M solution in 1,2-dichloroethane, 15 mmol, 20 equiv), and the mixture was refluxed, under nitrogen, for 90 min with azeotropic removal of water. The solvent was removed by rotary evaporation and the residue heated at 150° C at 1mm Hg for 1 h. Purification by radial chromatography using 7:3 ethyl acetate/dichloromethane gave **63** as a white solid (22 mg, 25%). mp 129-130° C.

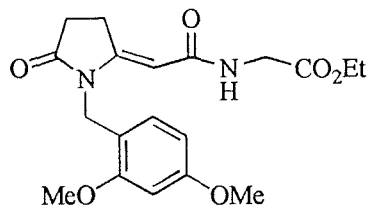
<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.28 (t, *J*=7.1Hz, 3H, CH<sub>2</sub>CH<sub>3</sub>), 2.53 (m, 2H, (H<sub>3</sub>)<sub>2</sub>), 2.98 (s, 3H, NMe), 3.28 (m, 2H, (H<sub>4</sub>)<sub>2</sub>), 4.07 (d, *J*=4.9Hz, 2H, NHCH<sub>2</sub>), 4.22 (q, *J*=7.1Hz, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.12 (t, *J*=1.7Hz, 1H, =CH), 5.81 (s, 1H, NH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.14 (CH<sub>2</sub>CH<sub>3</sub>), 24.36 (C<sub>3</sub>), 26.85 (NMe), 28.17 (C<sub>4</sub>), 41.22 (NHCH<sub>2</sub>), 61.53 (CH<sub>2</sub>CH<sub>3</sub>), 93.03 (=CH), 158.33 (CO), 166.63 (C<sub>5</sub>), 170.42 (CO), 176.85 (C<sub>2</sub>).

IR (KBr) 1738, 1665, 1605 cm<sup>-1</sup>.

HRMS Found 240.1110 (Calcd for C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub> 240.1110).

Preparation of (*E*)-1-2,4-(Dimethoxybenzyl)-5-[(*N*-ethoxycarbonylmethylcarbamoyl)-methylidenepyrrolidin-2-one **64**



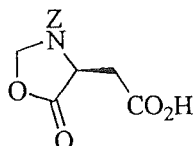
To a solution of **24** (100 mg, 0.4 mmol, 1 equiv), dissolved in 1,2-dichloroethane (10 ml), was added 2,4-dimethoxybenzylamine hydrochloride (236 mg, 1.2 mmol, 3 equiv) and triethylamine (161  $\mu$ l, 1.2 mmol, 3 equiv), and the mixture was refluxed, under nitrogen, for 90 min with azeotropic removal of water. The solution was filtered and the solvent removed by rotary evaporation. The residue was then heated at 150° C at 1mm Hg for 1 h. Purification by radial chromatography using 1:100 methanol/ether gave **64**, 95% pure by NMR, as an oil (56 mg, 39%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.26 (t,  $J=7.1\text{Hz}$ , 3H,  $\text{CH}_2\text{CH}_3$ ), 2.60 (m, 2H, (**H3**)<sub>2</sub>), 3.30 (m, 2H, (**H4**)<sub>2</sub>), 3.77 and 3.86 (s, 2x3H, 2xOMe), 3.99 (d,  $J=5.4\text{Hz}$ , 2H,  $\text{NHCH}_2$ ), 4.18 (q,  $J=7.1\text{Hz}$ , 2H,  $\text{CH}_2\text{CH}_3$ ), 4.65 (s, 2H,  $\text{NCH}_2$ ), 5.17 (t,  $J=1.9\text{Hz}$ , 1H, =CH), 5.67 (br s, 1H, NH), 6.39 (dd, 2.4 and 8.3Hz, 1H, ArH), 6.44 (d,  $J=2.4\text{Hz}$ , 1H, ArH), 6.87 (d,  $J=8.3\text{Hz}$ , 1H, ArH),

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.08 ( $\text{CH}_2\text{CH}_3$ ), 24.32 (**C3**), 28.21 (**C4**), 38.35 ( $\text{NCH}_2$ ), 41.13 ( $\text{NHCH}_2$ ), 55.32 and 55.37 (2xOMe), 61.39 ( $\text{CH}_2\text{CH}_3$ ), 94.00 (=CH), 98.48, 104.40, 115.12, 128.14, 156.88, 157.67, 160.30, 166.78, 170.38, 177.17.

IR (KBr) 3325, 1732, 1668, 1616  $\text{cm}^{-1}$ .

HRMS Found 376.1634 (Cald for  $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_6$  376.1635).

Preparation of 4(*S*)-3-Benzylloxycarbonyl-5-oxo-oxazolidin-4-yl acetic acid **25**

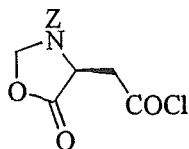
A mixture of N-Cbz-(*S*)-aspartic acid (3.15 g, 11.8 mmol), paraformaldehyde (0.708 g, 23.5 mol) and PTSA (0.135 g, 0.7 mmol) was refluxed in benzene (90 ml) for 1 h with azeotropic removal of water. The resulting solution was washed successively with ethyl acetate (12 ml), 0.3 M aqueous K<sub>2</sub>CO<sub>3</sub> (10 ml), water (3x5 ml), dried (MgSO<sub>4</sub>) and the solvent was evaporated to give 4(*S*)-3-benzylloxycarbonyl-5-oxo-4-oxazolidine-4-yl acetic acid **25** as a white solid (2.007 g, 61%). mp 86-88° C (lit mp 87-88.5° C).<sup>52</sup>

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.05-3.15 (br d, *J*=18.1Hz, 1H, CH<sub>a</sub>CO), 3.25-3.35 (br s, 1H, CH<sub>b</sub>CO), 4.36 (br s, 1H, H<sub>4</sub>), 5.19 (m, 2H, PhCH<sub>2</sub>), 5.30 (br d, *J*=3.4Hz, 1H, (H<sub>2</sub>)<sub>a</sub>), 5.55 (br s, 1H, (H<sub>2</sub>)<sub>b</sub>), 7.37 (m, 5H, ArH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 34.1, 51.3, 68.1, 78.2, 128.3, 128.6, 135.0, 152.7, 171.5, 174.9, 178.2.

IR (KBr) 3500-2600, 3010, 1805, 1720 cm<sup>-1</sup>.

[α]<sub>D</sub><sup>20</sup>=+125.7° (c=3.53 methanol).

Preparation of 4(*S*)-3-Benzylloxycarbonyl-5-oxo-oxazolidin-4-yl ethanoyl chloride **26**

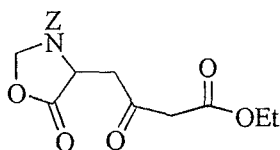
The acid **25** (1.75 g, 6.3 mmol) was dissolved in dichloromethane (40 ml) and the solution was cooled to 0° C. Freshly distilled oxalyl chloride (2.72 ml, 31.3 mmol, 5 equiv) and a catalytic quantity of dimethylformamide were added and the mixture was

stirred at 0° C for 2 h and at 20° C for 16 h. The solvent was removed under reduced pressure. Dichloromethane (2 ml) was added and then removed under reduced pressure. This was repeated twice more. Final traces of oxalyl chloride were removed at 1mm to give the acid chloride **26** as a brown oil (1.87 g, quant). This was used in subsequent steps without purification.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.50-3.60 (br d, *J* = 18.6 Hz, 1 H, (H2)<sub>a</sub>), 3.80-3.95 (br s, 1 H, (H2)<sub>b</sub>), 4.33 (br s, 1 H, H4), 5.19 (m, 2 H, PhCH<sub>2</sub>), 5.32 (br d, *J* = 3.5 Hz, 1 H, CH<sub>a</sub>COCl), 5.50 (br s, 1 H, CH<sub>b</sub>COCl), 7.37 (m, 5 H, PhH).

[α]<sub>D</sub><sup>20</sup> = +94° (dichloromethane).

Preparation of 4(*S*)-Ethyl 4-(3-benzyloxycarbonyl-5-oxo-2-phenyl-1,3-oxazolidin-4-yl) 3-oxobutanoate **28**



Meldrum's acid (51 mg, 0.35 mmol, 1 equiv) was dissolved in dichloromethane (2 ml) and the solution was cooled to 0° C under nitrogen. Dry pyridine (0.136 ml, 1.7 mmol) was added drop-wise over 5 min.

The acid chloride **26** (100 mg, 0.32 mmol), dissolved in dichloromethane (2ml), was added drop-wise to the mixture over 10 min. The mixture was stirred at 0° C for 50 min and then at 20° C for 45 min. The resulting red/purple solution was poured into 2N aqueous HCl (2 ml) containing crushed ice. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 ml). The combined dichloromethane extracts were washed with 2N HCl (2 ml), followed by saturated aqueous NaCl (1 ml), and then dried over MgSO<sub>4</sub>. The solvent was removed by rotary evaporation to give the meldrum's acid intermediate **27** (0.133 g, 98%).

The meldrum's acid intermediate **27** (0.133 g) was refluxed in dry ethanol under a nitrogen atmosphere for 2.5 h. The solvent was removed under reduced pressure to give



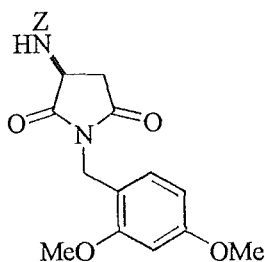
a red/purple oil. Purification by radial chromatography using 4:1 dichloromethane/ ethyl acetate gave **28** as a yellow oil (0.102 g, 87%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.28 (t,  $J=7.0\text{Hz}$ , 3H,  $\text{CH}_2\text{CH}_3$ ), 3.00-3.18 (br d,  $J=18.3\text{Hz}$ , 1H,  $\text{C4CH}_a$ ), 3.28-3.40 (br s, 1H,  $\text{C4CH}_b$ ), 3.42 (s, 2H,  $\text{COCH}_2\text{CO}$ ), 4.24 (q,  $J=7.0\text{Hz}$ , 2H,  $\text{CH}_2\text{CH}_3$ ), 4.36 (br s, 1H, **H4**), 5.19 (m, 2H,  $\text{PhCH}_2$ ), 5.30 (br d,  $J=3.5\text{Hz}$ , 1H, (**H2**)<sub>a</sub>), 5.51 (br s, 1H, (**H2**)<sub>b</sub>), 7.37 (m, 5H, **PhH**).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  13.88, 40.86, 46.04, 51.36, 61.82, 68.08, 78.36, 128.22, 128.57, 128.62, 135.06, 152.73, 166.88, 170.85, 199.95.

IR (KBr) 1801, 1717 (br)  $\text{cm}^{-1}$ .  $[\alpha]_D^{20}=+100^\circ$  (dichloromethane).

Preparation of 3(*S*)-3-Benzyloxycarbonylamino-1-(2,4-Dimethoxybenzyl)-succinimide **62**



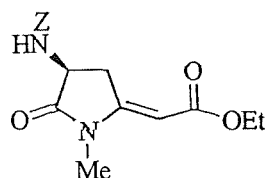
A mixture of the  $\beta$  keto ester **28** (50 mg, 0.14 mmol, 1 equiv) and 2,4-dimethoxybenzylamine hydrochloride (32 mg, 0.2 mmol, 1.1 equiv) and triethylamine (22  $\mu\text{L}$ , 0.2 mmol, 1.1 equiv) in toluene (3 ml) was refluxed under a nitrogen atmosphere for 35 min. The solvent was removed by rotary evaporation and the residue was heated at 150-160° C under reduced pressure (20mm Hg) for 1 hr. The product was purified by radial chromatography using 1:1 ethyl acetate/petroleum ether to give **62** as a yellow oil (44 mg, 77%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.80 (dd,  $J=5.4$  and  $17.1\text{Hz}$ , 1H, (**H4**)<sub>a</sub>), 3.11 (dd,  $J=9.7$  and  $18.5\text{Hz}$ , 1H, (**H4**)<sub>b</sub>), 3.78 and 3.79 (s, 2x3H, 2xOMe), 4.34 (m, 1H, **H3**), 4.68 (ABq,  $J_{AB}=14.2\text{Hz}$ , 2H,  $\text{NCH}_2$ ), 5.11 (ABq,  $J_{AB}=6.5\text{Hz}$ , 2H,  $\text{PhCH}_2$ ), 5.44 (br s, 1H, **NH**), 6.41 (m, 2H, **ArH**), 7.18 (d,  $J=9.3\text{Hz}$ , 1H, **ArH**), 7.35 (m, 5H, **ArH**).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  35.87 (C4), 37.75 ( $\text{NCH}_2$ ), 50.27 (C3), 55.30 and 55.38 (2xOMe), 67.36 ( $\text{PhCH}_2$ ), 98.46, 103.94, 115.49, 128.15, 128.28, 128.33, 128.56, 130.12 (Aromatic C's), 155.84, 158.16, 160.58, 173.76, 175.28.

LRMS (M) Found 398.1 (Calcd for  $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_6$  398.1478).

Preparation of 3(*S*)-(*E*)-3-Benzoyloxycarbonylamino-5-ethoxycarbonylmethyl-idene-1-methylpyrrolidin-2-one **59**



A solution of  $\beta$  keto ester **28** (21 mg, 0.06 mmol, 1 equiv), and methylamine (170  $\mu\text{l}$  of a 7.1M solution of methylamine in 1,2-dichloroethane, 1.2 mmol, 20 equiv), were refluxed in 1,2-dichloroethane (10 ml) for 90min. The imine/enamine mixture was then heated at  $150^\circ\text{C}$  at 1mm Hg for 1h. Purification by radial chromatography using 4:1 dichloromethane/ethyl acetate gave **59**, as an oil (14 mg, 70%).

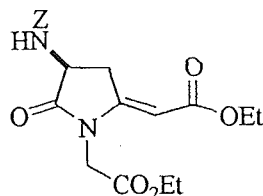
$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.28 (t,  $J=7.3\text{Hz}$ , 3H,  $\text{CH}_2\text{CH}_3$ ), 2.97-3.10 (m, 4H,  $\text{NMe}$  and ( $\text{H4}$ )<sub>a</sub>), 3.82 (dd,  $J=18.7$  and  $9.3\text{Hz}$ , 1H, ( $\text{H4}$ )<sub>b</sub>), 4.18(q,  $J=7.3\text{Hz}$ , 2H,  $\text{CH}_2\text{CH}_3$ ), 4.38 (m, 1H, **H3**), 5.12 (AB<sub>q</sub>,  $J_{\text{AB}}=6.4\text{Hz}$ , 2H,  $\text{PhCH}_2$ ), 5.19 (s, 1H,  $=\text{CH}$ ), 5.49 (d,  $J=5.4\text{Hz}$ , 1H, **NH**), 7.34 (br s, 5H, **PhH**).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.37 ( $\text{CH}_2\text{CH}_3$ ), 27.37 ( $\text{NMe}$ ), 32.69 (C4), 50.20 (C3), 59.74 ( $\text{CH}_2\text{CH}_3$ ), 67.26 ( $\text{PhCH}_2$ ), 93.03 ( $=\text{CH}$ ), 128.14, 128.27, 128.53, 137.97 (Aromatic C's), 155.21, 156.44, 166.80, 173.97 (3xCO and  $\text{C=}$ ).

HRMS  $m/z$  ( $\text{M}^+$ ) 91.1, 137.0, 179.0, 224.1, 287.1, 332.1

Found 332.1374 (Calcd for  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5$  332.1373).

Preparation of 3(*S*)-(*E*)-3-Benzoyloxycarbonylamino-1-ethoxycarbonylmethyl-5-ethoxycarbonylmethylidenepyrrolidin-2-one **61**



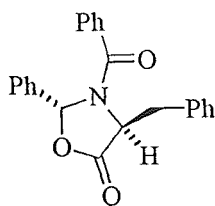
Glycine ethyl ester hydrochloride (73 mg, 0.53 mmol, 3 equiv) and triethylamine (73  $\mu$ l, 0.53 mmol, 3 equiv) were added to  $\beta$  keto ester **28** (61 mg, 0.18 mol, 1 equiv), dissolved in benzene (10 ml), and the mixture refluxed, with azeotropic removal of water, for 90min. The mixture was filtered and the solvent removed by rotary evaporation. The residue was then heated at 150° C, at 1mm, for 1 h. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 4:1 dichloromethane/ethyl acetate gave the enamino ester **61** as an oil. (44mg, 63%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.29 (t,  $J=7.1\text{Hz}$ , 6H,  $2\times\text{CH}_2\text{CH}_3$ ), 3.11 (dd,  $J=4.9$  and  $18.5\text{Hz}$ , 1H, (**H4**)<sub>a</sub>), 3.91 (dd,  $J=9.4$  and  $19\text{Hz}$ , (**H4**)<sub>b</sub>), 4.11-4.24 (m, 6H,  $2\times\text{CH}_2\text{CH}_3$  and  $\text{NCH}_2$ ), 4.43 (m, 1H, **H3**), 5.12 (m, 3 H,  $\text{PhCH}_2$  and  $=\text{CH}$ ), 5.39 (br s, 1 H, **NH**), 7.35 (m, 5 H, **ArH**).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.07, 14.36, 32.85, 42.11, 50.06, 59.92, 62.12, 67.39, 93.50, 128.19, 128.33, 128.58, 128.64, 154.84, 155.78, 166.27, 166.49, 173.74.

FTIR (KBr) 3350, 1801, 1714, 1632, 1530  $\text{cm}^{-1}$ .

HRMS (M) Found 404.1577 (Calcd for  $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_7$  404.1583).

Preparation of (2*R*, 4*S*)-3-Benzoyl-4-benzyl-2-phenyl-1,3-oxazolidin-5-one **65**

(*S*)-Phenylalanine (5 g, 30 mmol) was treated with 1M aqueous NaOH (30 ml, 30 mmol) to give the corresponding Schiff base salt. A solution of the Schiff base salt, in 1,2-dichloroethane (60 ml), was treated with benzaldehyde (5 ml, 45 mmol, 1.5 equiv) and the solution was refluxed, with azeotropic removal of water, for 24 h. The mixture was cooled to -20° C under nitrogen and benzyl chloroformate (2.1 ml, 12.2 mmol) was added. The mixture was stirred at -20° C for 12 h and then at 4°C for 3 days. The solvent was removed under reduced pressure and the residue partitioned between ethyl acetate (60 ml) and 5% aqueous NaHCO<sub>3</sub> (50 ml). The organic layer was extracted, washed successively with 5% aqueous KHSO<sub>4</sub> (50ml) and water (50 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to yield an oil. Purification by radial chromatography using 10:3 petroleum ether/ethyl acetate gave the *anti*-oxazolidinone **65**, as a white solid (3.479g, 33%). mp 186-187° C (lit mp 184.3° C).<sup>82</sup>

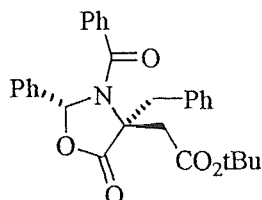
<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.41 (br s, 1H, CH<sub>4</sub>Ph), 3.79 (br s, 1H, CH<sub>2</sub>Ph), 5.21 (s, 1H, **H**<sub>4</sub>), 5.81 (s, 1H, **H**<sub>2</sub>), 7.06-7.41 (m, 15H, PhH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 34.89, 57.76, 91.25, 126.64, 127.72, 128.52, 128.85, 129.88, 130.82, 135.22, 136.13, 169.22, 171.23.

[α]<sub>D</sub><sup>20</sup> = +302° (c=1.0, chloroform).

HRMS (M) Found 357.1365 (Calcd for C<sub>23</sub>H<sub>19</sub>NO<sub>3</sub> 357.1365).

Preparation of (2*R*, 4*S*)-*tert*-Butyl 3-Benzoyl-4-benzyl-5-oxo-2-phenyl-1,3-oxazol-idin-4-yl ethanoate 67



The oxazolidinone **65** (200 mg, 0.56 mmol, 1 equiv) was dissolved in THF (20 ml) and the solution was cooled to  $-78^{\circ}\text{C}$ . LiHMDS (0.68 ml, 0.68 mmol, 1.2 equiv) was added and the resulting yellow solution was stirred at  $-78^{\circ}\text{C}$  for 7min.

*Tert*-Butyl-bromoacetate (0.1 ml, 0.62 mmol, 1.1 equiv) was added and the solution was stirred at  $-78^{\circ}\text{C}$  for 2h and was then allowed to warm to  $20^{\circ}\text{C}$  over 16h. The solution was partitioned between saturated  $\text{NH}_4\text{Cl}$  solution (15 ml) and ether (10 ml). The aqueous layer was separated and extracted with ether (10 ml). The combined ether extracts were washed with water (2x5 ml), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to give **67** as a pale yellow solid (217 mg, 87%). mp  $180\text{--}181^{\circ}\text{C}$ .

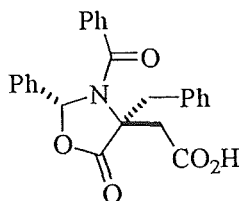
$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.54 (s, 9H, **tBu**), 3.11 and 3.95 ( $\text{AB}_{\text{q}}$ ,  $J_{\text{AB}}=17.8\text{Hz}$ , 2H,  $\text{CH}_2\text{CO}_2$ ), 3.35 and 3.98 ( $\text{AB}_{\text{q}}$ ,  $J_{\text{AB}}=13.6\text{Hz}$ , 2H,  $\text{C4CH}_2\text{Ph}$ ), 5.55(d,  $J=7.8\text{Hz}$ , 2H, **ArH**), 6.49 (s, 1H, **H2**), 6.67 (t,  $J=7.8\text{Hz}$ , 2H, **PhH**), 6.82 (d,  $J=7.4\text{Hz}$ , 2H, **PhH**), 6.95 (t,  $J=7.6\text{Hz}$ , 1H, **PhH**), 7.06 (t,  $J=7.4\text{Hz}$ , 2H, **PhH**), 7.17 (t,  $J=7.4\text{Hz}$ , 1H, **PhH**), 7.41 (m, 5H, **PhH**).  
 $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  28.04 ( $\text{C}(\text{CH}_3)_3$ ), 40.27 ( $\text{CH}_2\text{CO}_2\text{tBu}$ ), 42.26 ( $\text{CH}_2\text{Ph}$ ), 66.28 (**C4**), 82.20 ( $\text{C}(\text{CH}_3)_3$ ), 91.54 (**C2**), 125.26, 127.68, 127.74, 127.89, 128.18, 128.90, 128.09, 129.33, 130.89, 134.61, 135.30, 136.58, 169.71 (**PhCO**), 170.07 ( $\text{CO}_2\text{tBu}$ ), 173.18 (**C5**).

IR (KBr) 1791.7, 1720.4,  $1654.8\text{ cm}^{-1}$ .

HRMS (M) Found 471.2053 (Calcd for  $\text{C}_{29}\text{H}_{29}\text{NO}_5$  471.2047)

Anal. Calcd for  $\text{C}_{29}\text{H}_{29}\text{NO}_5$ : C 73.87; H 6.20; N 2.97. Found: C 74.07; H 6.24; N 2.85.

Preparation of (2*R*, 4*S*)-*tert*-Butyl 3-Benzoyl-4-benzyl-5-oxo-2-phenyl-1,3-oxazolidin-4-yl acetic acid **68**



**Method A.**

*Tert*-Butyl oxazolidinone **67** (500 mg, 1.1 mmol, 1 equiv) was dissolved in dichloromethane (5 ml) and cooled to 0° C. TFA (13.7 ml, 0.18 mol, 168 equiv) was added and the solution was stirred at 0° C for 5 min. The solution was then diluted with dichloromethane (1 ml), washed with water (3x11.5 ml) and extracted with 5% aqueous NaHCO<sub>3</sub> solution (2x10 ml). The NaHCO<sub>3</sub> extracts were combined, cooled to 0° C, acidified to pH 1-3 with 1N HCl and extracted with ethyl acetate (3x15 ml). The combined ethyl acetate extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent evaporated to yield oxazolidinone **68** as a white solid (179 mg, 41%). mp 207-210° C. (lit mp 207.5-211° C).<sup>78</sup>

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.25 and 4.13 (AB<sub>q</sub>, *J*<sub>AB</sub>=18.1Hz, 2H, CH<sub>2</sub>CO<sub>2</sub>H), 3.41 and 4.04 (AB<sub>q</sub>, *J*<sub>AB</sub>=13.4Hz, 2H, CH<sub>2</sub>Ph), 5.54 (d, *J*=7.4Hz, 2H, PhH), 6.46 (s, 1H, H2), 6.68 (t, *J*=7.8Hz, 2H, PhH), 6.83 (d, *J*=7.3Hz, 2H, PhH), 6.96 (t, *J*=7.5Hz, 1H, PhH), 7.07 (t, *J*=7.5Hz, 2H, PhH), 7.18 (t, *J*=7.5Hz, 1H, PhH), 7.42 (m, 5H, PhH).

<sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 38.53 (CH<sub>2</sub>CO<sub>2</sub>H), 42.33 (CH<sub>2</sub>Ph), 66.09 (C4), 91.70 (C2), 125.29, 127.79, 127.89, 127.96, 128.32, 129.09, 129.26, 129.57, 130.92, 134.28, 135.00, 136.12, 170.21 (PhCO), 172.85 (CO<sub>2</sub>H), 173.13 (C5).

IR (KBr) 1797, 1726, 1612, 1595 cm<sup>-1</sup>.

[α]<sub>D</sub><sup>20</sup> = +64° (c 0.9; dichloromethane).

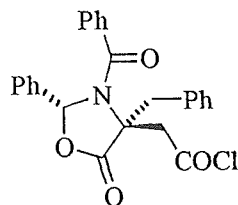
HRMS (M+1) Found 416.1498 (Calcd for C<sub>25</sub>H<sub>21</sub>NO<sub>5</sub> 415.1421).

**Method B.**

A mixture of *tert*-Butyl oxazolidinone **67** (100 mg, 0.21 mmol, 1 equiv) and phenol (400 mg, 4.2 mmol, 20 equiv) was heated to 45°C. When the phenol had melted, aqueous HCl (53  $\mu$ L of 2N solution, 0.1 mmol, 0.5 equiv) was added and the mixture was stirred at 45° C for 3 h. The mixture was then diluted with ethyl acetate (10 ml) and extracted with saturated aqueous NaHCO<sub>3</sub> (5 ml). The aqueous extract was cooled in an ice bath, acidified to pH 1 with 10% aqueous HCl and extracted with ethyl acetate (2x5 ml). The combined ethyl acetate extracts were dried over MgSO<sub>4</sub> and the solvent was removed under reduced pressure to give the product **68** as a white solid (16mg, 19%).  
mp 208-210° C

NMR data as above.

Preparation of (2*R*, 4*S*)-3-Benzoyl-4-benzyl-5-oxo-2-phenyl-1,3-oxazolidin-4-yl ethanoyl chloride **69**

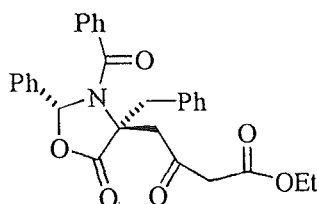


The acid **68** (240 mg, 0.58 mmol, 1 equiv) was dissolved in dichloromethane (10 ml) and the solution was cooled to 0° C. Freshly distilled oxalyl chloride (0.28 ml, 2.9 mmol, 5 equiv) and a catalytic quantity of DMF were added. The mixture was stirred at 0° C for 2h and at 20° C for 16h. The solvent was removed by rotary evaporation and more dichloromethane (2 ml) was added. This was repeated 3 times. Final traces of oxalyl chloride were removed at 1mm to yield the acid chloride **69** as a solid (262mg, quant), which was used in subsequent steps without further purification.

<sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  3.38 and 3.97 (AB<sub>q</sub>,  $J_{AB}$ =13.2Hz, 2H, CH<sub>2</sub>Ph), 3.74 and 4.64 (AB<sub>q</sub>,  $J_{AB}$ =19.2Hz, 2H, CH<sub>2</sub>COCl), 5.51 (dd,  $J$ =7.3Hz, 2H, PhH), 6.37 (s, 1H, H2), 6.68 (m,

2H, PhH), 6.80 (m, 2H, PhH), 6.96 (m, 1H, PhH), 7.08 (m, 2H, PhH), 7.17 (m, 2H, PhH), 7.35-7.46 (m, 5H, PhH).

Preparation of (2R, 4S) Ethyl 4-(3-Benzoyl-4-benzyl-5-oxo-2-phenyl-1,3-oxazol-idin-4-yl) 3-oxobutanoate 71



Meldrum's acid (96 mg, 0.67 mmol, 1.1 equiv) was dissolved in dichloromethane (2 ml) and the solution cooled to 0° C under nitrogen. Dry pyridine (154  $\mu$ L, 1.51 mmol, 2.5 equiv) was added drop-wise over 5 min followed by a solution of the acid chloride **69** (262 mg, 0.61 mmol, 1 equiv) dissolved in dichloromethane (2 ml) which was added drop-wise over 10 min. The mixture was then stirred at 0° C for 50 min and at 20° C for 45 min. The resulting solution was poured into aqueous 2N HCl (2 ml) containing crushed ice. The organic layer was removed and the aqueous layer was extracted with dichloromethane (2 ml). The combined dichloromethane extracts were washed successively with aqueous 2N HCl (1 ml), saturated aqueous NaCl (1 ml) and dried over  $\text{MgSO}_4$ . The solvent was removed by rotary evaporation to give the acyl meldrum's compound **70**.

Acyl meldrum's compound **70** (315 mg, 0.57 mmol) was refluxed under a nitrogen atmosphere in dry ethanol for 2.5h. Purification by radial chromatography using 3:7 ethyl acetate/petroleum ether gave the  $\beta$  keto ester **71** as a yellow oil (203 mg, 73%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.26 (t,  $J=7.3\text{Hz}$ , 3H,  $\text{CH}_2\text{CH}_3$ ), 3.34 and 3.96 ( $\text{AB}_q$ ,  $J_{\text{AB}}=13.5\text{Hz}$ , 2H,  $\text{C4CH}_2\text{Ph}$ ), 3.39 and 4.39 ( $\text{AB}_q$ ,  $J_{\text{AB}}=19.0\text{Hz}$ , 2H,  $\text{C4CH}_2\text{CO}$ ), 3.59 ( $\text{AB}_q$ ,  $J_{\text{AB}}=15.6\text{Hz}$ , 2H,  $\text{CH}_2\text{CO}_2\text{Et}$ ), 4.22 (q,  $J=7.3\text{Hz}$ , 2H,  $\text{CH}_2\text{CH}_3$ ), 5.54 (t,  $J=7.3\text{Hz}$ , 2H, PhH), 6.49 (s, 1H, H2), 6.68 (m, 2H, PhH), 6.78 (m, 2H, PhH), 6.96 (m, 1H, PhH), 7.05 (m, 2H, PhH), 7.17 (m, 1H, PhH), 7.38-7.44 (m, 5H, PhH).

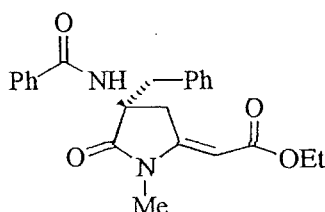


$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.03, 42.23, 48.02, 49.04, 61.87, 65.36, 91.61, 125.32, 127.74, 127.90, 127.95, 128.22, 129.00, 129.21, 129.39, 130.91, 134.45, 135.02, 136.30, 166.12, 169.91, 172.98, 201.30.

IR 1794, 1743, 1716, 1651  $\text{cm}^{-1}$ .

HRMS (FAB) ( $M+1$ ) Found 486.1917 (Calcd for  $\text{C}_{29}\text{H}_{28}\text{N}_6\text{O}_6$  486.1917).

Preparation of 3(*S*)-(*E*)-3-Benzoylamino-3-benzyl-5-ethoxycarbonylmethylidene-1-methylpyrrolidin-2-one 77



To a solution of  $\beta$ -keto ester **71** (50 mg, 0.1 mmol, 1 equiv) in 1,2-dichloroethane (10 ml) was added methylamine (340  $\mu\text{L}$  of 6M solution in 1,2-dichloroethane, 2 mmol, 20 equiv) and the mixture refluxed under nitrogen for 90 min. The solvent was removed by rotary evaporation and the residue heated at 150° C at 1mm for 1 h. Purification by radial chromatography using 9:1 dichloromethane/ethyl acetate gave the product **77** as a white solid (17mg, 43%). mp 231-232° C.

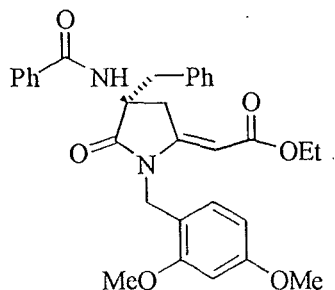
$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.23 (t,  $J=7.3\text{Hz}$ , 3H,  $\text{CH}_2\text{CH}_3$ ), 2.87 (s, 3H,  $\text{NMe}$ ), 3.12 (m, 2H,  $\text{PhCH}_2$ ), 3.39 (dd,  $J=18.8$  and  $2.0\text{Hz}$ , 1H, ( $\text{H4}$ )<sub>a</sub>), 3.81 (dd,  $J=20.5$  and  $1.0\text{Hz}$ , 1H, ( $\text{H4}$ )<sub>b</sub>), 4.09 (m, 2H,  $\text{CH}_2\text{CH}_3$ ), 4.91 (br t,  $J=1.5\text{Hz}$ , 1H,  $=\text{CH}$ ), 6.74 (s, 1H,  $\text{NH}$ ), 7.16-7.26 (m, 5H,  $\text{PhH}$ ), 7.38(m, 2H,  $\text{PhH}$ ), 7.46 (m, 1H,  $\text{PhH}$ ), 7.70 (dd,  $J=6.9$  and  $1.5\text{Hz}$ , 2H,  $\text{PhH}$ ).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.37 ( $\text{CH}_2\text{CH}_3$ ), 27.12 ( $\text{NMe}$ ), 36.53 ( $\text{C4}$ ), 42.81 ( $\text{CH}_2\text{Ph}$ ), 59.46 ( $\text{C3}$ ), 59.73 ( $\text{CH}_2\text{CH}_3$ ), 91.81 ( $=\text{CH}$ ), 127.01, 127.84, 128.57, 128.63, 129.94, 131.99, 133.09, 133.20, 156.37 ( $\text{C5}$ ), 166.63, 166.82, 175.76 ( $3\times\text{CO}$ ).

IR (KBr) 1736, 1692, 1655, 1618  $\text{cm}^{-1}$ .

HRMS Found 392.1736 (Calcd for  $\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_4$  392.1737).

Preparation of 3(*S*)-(*E*)-Benzoylamino-3-benzyl-5-ethoxycarbonylmethylidene-1-2,4-dimethoxybenzylamino-pyrrolidin-2-one 79



To a solution of  $\beta$  keto ester **71** (75 mg, 0.15 mmol, 1 equiv) dissolved in 1,2-dichloroethane (10 ml) was added 2,4-dimethoxybenzylamine hydrochloride (157 mg, 0.75 mmol, 5 equiv) and triethylamine (107  $\mu$ L, 0.75 mmol, 5 equiv) and the mixture was refluxed for 90 min. The mixture was filtered and the solvent removed by rotary evaporation. The resulting residue was heated at 150°C at 1mm for 1h. Purification by radial chromatography using 2:3 ethyl acetate/petroleum ether gave a mixture of product **79** and other unidentifiable material. Crystallization from ethyl acetate/petroleum ether gave **79**, as a tan solid (6mg, 8%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.21 (t,  $J=7.1\text{Hz}$ , 3H,  $\text{CH}_2\text{CH}_3$ ), 3.18 (s, 2H,  $\text{PhCH}_2$ ), 3.48 (dd,  $J=18.5$  and  $2.5\text{Hz}$ , 1H, ( $\text{H4}$ )<sub>a</sub>), 3.78 and 3.81 (s, 6H, 2xOMe), 3.88 (dd,  $J=18.5$  and  $2.5\text{Hz}$ , 1H, ( $\text{H4}$ )<sub>b</sub>), 4.07 (m, 2H,  $\text{CH}_2\text{CH}_3$ ), 4.61 (ABq,  $J_{\text{AB}}=16.1\text{Hz}$ , 2H,  $\text{NCH}_2$ ), 5.08 (br t,  $J=1.5\text{Hz}$ , 1H, =CH), 6.42 (m, 2H, ArH), 6.58 (s, 1H, NH), 6.93 (d,  $J=9.3\text{Hz}$ , 1H, ArH), 7.26-7.32 (m, 5H, PhH), 7.41 (m, 2H, PhH), 7.50 (m, 1H, PhH), 7.72 (dd,  $J=8.3$  and  $1.5\text{Hz}$ , 2H, PhH).

Selected  $^{13}\text{C}$  NMR data  $\delta$  14.39 ( $\text{CH}_2\text{CH}_3$ ), 36.62 (C4), 39.13 ( $\text{NCH}_2$ ), 42.88 ( $\text{CH}_2\text{Ph}$ ), 55.29 (2xOMe), 59.42 (C3), 59.58 ( $\text{CH}_2\text{CH}_3$ ), 93.14 (=CH), 98.30, 104.44, 114.87, 121.91, 154.13 (C5).

# Appendix

## Crystallographic Data for compound 67

Table 1. Crystal data and structure refinement.

*Crystal data*

Empirical Formula	C <sub>29</sub> H <sub>29</sub> NO <sub>5</sub>
Formula weight	471.53
Crystal description	Block
Crystal colour	Transparent
Crystal system	Orthorhombic
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit cell dimensions	a = 9.7037 (2) Å    α = 90° b = 21.5236 (6) Å    β = 90° c = 11.9756 (3) Å    γ = 90°
F(000)	1000
Volume	2501.21 (11) Å <sup>3</sup>
Z	4
Density (calculated)	1.252 Mg/m <sup>3</sup>
Radiation	MoK $\alpha$ λ = 0.71073 Å Fine-focus sealed tube, graphite monochromator
Linear absorption coefficient	0.085 mm <sup>-1</sup>
Temperature of measurement	293 (2)

*Data collection*

Diffractometer	Siemens P4
Temperature of data collection	293 (2) K
Diffraction measurement method	ω scans
Absorption correction	none
Reflections collected	11635
Independent reflections	3555 (R <sub>int</sub> = 0.0702)
θ range for data collection	1.89° to 23.31°
Range of h, k, l	-10 ≤ h ≤ 4, -22 ≤ k ≤ 23, -12 ≤ l ≤ 13

*Refinement*

Refinement method	Full-matrix least-squares on $F^2$
Data/restraints/parameters	3555 / 0 / 319
Goodness-to-fit on $F^2$	1.086
Final R indices [ $I > 2\theta(I)$ ]	$R_1 = 0.0389$ , $wR_2 = 0.0869$
R indices (all data)	$R_1 = 0.0411$ , $wR_2 = 0.0883$
Absolute structure parameter	1.1 (10)
Largest diff. Peak and hole	0.127 and $-0.166\text{eA}^{-3}$
Extinction method	none

*Computer processing*

Data collection	Siemens XSCANS
Data reduction	Siemens SHELXTL
Cell refinement	Siemens XSCANS
Structure solution	SHELXS-86 (Sheldrick 1990)
Structure refinement	SHELXS-93 (Sheldrick 1993)
Graphics by	Siemens SHELXTL
Computer publication	Siemens SHELXTL

Of the 11635 reflections obtained, 3555 were unique ( $R_{\text{int}} = 0.0702$ ) and were used in the full-matrix least-squares refinement [ SHELXL-93 (Sheldrick, 1993) ]<sup>86</sup>. The structure was solved by direct methods [ SHELXS-86 (Sheldrick, 1990) ]<sup>87</sup>. Hydrogens were fixed in idealized positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton<sup>88</sup>.

Table 2. Atomic coordinates [  $\times 10^4$  ] and equivalent isotropic displacement parameters [  $\text{\AA}^2 \times 10^3$  ] for 1.U (eq) is defined as one third of the trace of the orthogonalized  $U_{ij}$  tensor.

	x	y	z	U (eq)
N	-1881 (2)	-1076 (1)	3302 (1)	23 (1)
O1	-425 (1)	-1789 (1)	2510 (1)	28 (1)
O2	47 (2)	-1322 (1)	890 (1)	37 (1)
C2	-1103 (2)	-1653 (1)	3554 (2)	24 (1)
O3	-3323 (2)	-264 (1)	3800 (1)	38 (1)
C4	-1480 (2)	-810 (1)	2216 (2)	26 (1)
O4	1103 (1)	-686 (1)	3371 (1)	39 (1)
O5	917 (1)	364 (1)	3331 (1)	31 (1)
C5	-525 (2)	-1316 (1)	1776 (2)	27 (1)
C6	-2663 (2)	-754 (1)	4048 (2)	26 (1)
C7	-2832 (2)	-1025 (1)	5194 (2)	27 (1)
C8	-1796 (2)	-974 (1)	5993 (2)	32 (1)
C9	-2017 (2)	-1198 (1)	7065 (2)	40 (1)
C10	-3262 (3)	-1476 (1)	7333 (2)	43 (1)
C11	-4282 (2)	-1523 (1)	6549 (2)	43 (1)
C13	-1924 (2)	-2217 (1)	3893 (2)	25 (1)
C14	-1701 (2)	-2490 (1)	4932 (2)	30 (1)
C15	-2368 (2)	-3037 (1)	5213 (2)	33 (1)
C16	-3239 (2)	-3319 (1)	4461 (2)	33 (1)
C17	-3484 (2)	-3047 (1)	3432 (2)	34 (1)
C18	-2822 (2)	-2498 (1)	3155 (2)	29 (1)
C19	-2709 (2)	-715 (1)	1401 (2)	29 (1)
C20	-363 (2)	-1273 (1)	1243 (2)	26 (1)
C21	-4816 (2)	-1351 (1)	1874 (2)	36 (1)
C22	-5644 (2)	-1876 (1)	1726 (2)	47 (1)
C23	-5280 (3)	-2321 (1)	957 (2)	46 (1)
C24	-4118 (2)	-2249 (1)	312 (2)	40 (1)
C25	-3306 (2)	-1726 (1)	458 (2)	31 (1)
C26	-703 (2)	-193 (1)	2314 (2)	29 (1)
C27	536 (2)	-213 (1)	3068 (2)	28 (1)
C28	2162 (2)	497 (1)	4016 (2)	33 (1)
C29	3428 (2)	291 (1)	3382 (2)	49 (1)
C30	2088 (2)	1199 (1)	4121 (2)	40 (1)
C31	2036 (3)	195 (1)	5153 (2)	50 (1)

Table 3. Selected bond lengths [Å] and angles [°].

N-C6	1.362 (2)	N-C4	1.473 (2)
N-C2	1.485 (2)	O1-C5	1.349 (2)
O1-C2	1.442 (2)	O2-C5	1.197 (2)
C2-C13	1.508 (3)	O3-C6	1.223 (2)
C4-C5	1.524 (3)	C4-C26	1.533 (3)
C4-C19	1.554 (3)	O4-C27	1.212 (2)
O5-C27	1.332 (2)	O5-C28	1.488 (2)
C6-C7	1.500 (3)	C7-C12	1.388 (3)
C7-C8	1.392 (3)	C8-C9	1.388 (3)
C9-C10	1.386 (3)	C10-C11	1.368 (3)
C11-C12	1.381 (3)	C13-C18	1.380 (3)
C13-C14	1.392 (3)	C14-C15	1.386 (3)
C15-C16	1.376 (3)	C16-C17	1.385 (3)
C17-C18	1.386 (3)	C19-C20	1.510 (3)
C20-C21	1.386 (3)	C20-C25	1.392 (3)
C21-C22	1.398 (3)	C22-C23	1.375 (4)
C23-C24	1.376 (3)	C24-C25	1.384 (3)
C26-C27	1.504 (3)	C28-C29	1.510 (3)
C28-C31	1.514 (3)	C28-C30	1.517 (3)
C6-N-C4	121.9 (2)	C6-N-C2	125.2 (2)
C4-N-C2	111.71 (14)	C5-O1-C2	112.27 (14)
O1-C2-N	103.03 (14)	O1-C2-C13	108.2 (2)
N-C2-C13	117.35 (14)	N-C4-C5	100.85 (14)
N-C4-C26	113.5 (2)	C5-C4-C26	110.3 (2)
N-C4-C19	113.8 (2)	C5-C4-C19	110.1 (2)
C26-C4-C19	108.1 (2)	C27-O5-C28	122.4 (2)
O2-C5-O1	122.5 (2)	O2-C5-C4	126.5 (2)
O1-C5-C4	111.0 (2)	O3-C6-N	121.8 (2)
O3-C6-C7	120.6 (2)	N-C6-C7	117.6 (2)
C12-C7-C8	119.4 (2)	C12-C7-C6	119.2 (2)
C8-C7-C6	121.3 (2)	C9-C8-C7	119.8 (2)
C10-C9-C8	119.9 (2)	C11-C10-C9	120.3 (2)
C10-C11-C12	120.3 (2)	C11-C12-C7	120.3 (2)
C18-C13-C14	119.1 (2)	C18-C13-C2	120.8 (2)
C14-C13-C2	119.8 (2)	C15-C14-C13	120.1 (2)
C16-C15-C14	120.2 (2)	C15-C16-C17	120.1 (2)
C16-C17-C18	119.7 (2)	C13-C18-C17	120.8 (2)
C20-C19-C4	115.4 (2)	C21-C20-C25	118.2 (2)
C21-C20-C19	121.3 (2)	C25-C20-C19	120.5 (2)
C20-C21-C22	120.3 (2)	C23-C22-C21	120.1 (2)
C22-C23-C24	120.5 (2)	C23-C24-C25	119.2 (2)
C24-C25-C20	121.7 (2)	C27-C26-C4	114.5 (2)

O4-C27-O5	125.8 (2)	O4-C27-C26	124.5 (2)
O5-C27-C26	109.7 (2)	O5-C28-C29	109.0 (2)
O5-C28-C31	110.4 (2)	C29-C28-C31	113.1 (2)
O5-C28-C30	101.5 (2)	C29-C28-C30	111.8 (2)
C31-C28-C30	110.4 (2)		

Symmetry transformations used to generate equivalent atoms:

Table 4 Anisotropic displacement parameters [ $\text{\AA}^2 \times 10^3$ ]. The anisotropic displacement factor exponent takes the form:  $-2\pi^2 [ (ha^*)^2 U_{11} + \dots + 2hka^*b^*U_{12} ]$

	U11	U22	U33	U23	U13	U12
N	24 (1)	21 (1)	24 (1)	1 (1)	-2 (1)	1 (1)
O1	29 (1)	22 (1)	33 (1)	1 (1)	6 (1)	2 (1)
O2	38 (1)	37 (1)	35 (1)	-1 (1)	12 (1)	3 (1)
C2	25 (1)	22 (1)	27 (1)	-1 (1)	0 (1)	4 (1)
O3	44 (1)	30 (1)	41 (1)	2 (1)	4 (1)	13 (1)
C4	29 (1)	21 (1)	27 (1)	2 (1)	0 (1)	-1 (1)
O4	35 (1)	26 (1)	58 (1)	4 (1)	-11 (1)	-2 (1)
O5	30 (1)	24 (1)	39 (1)	-2 (1)	-7 (1)	-4 (1)
C5	26 (1)	25 (1)	32 (1)	0 (1)	-1 (1)	-5 (1)
C6	24 (1)	22 (1)	33 (1)	-4 (1)	-2 (1)	1 (1)
C7	30 (1)	20 (1)	30 (1)	-6 (1)	3 (1)	2 (1)
C8	31 (1)	31 (1)	35 (1)	-7 (1)	1 (1)	4 (1)
C9	49 (1)	40 (1)	32 (1)	-6 (1)	-5 (1)	13 (1)
C10	62 (2)	37 (1)	30 (1)	2 (1)	10 (1)	4 (1)
C11	50 (1)	40 (1)	40 (1)	-4 (1)	15 (1)	-14 (1)
C12	37 (1)	35 (1)	36 (1)	-6 (1)	1 (1)	-6 (1)
C13	26 (1)	20 (1)	28 (1)	-2 (1)	2 (1)	3 (1)
C14	34 (1)	30 (1)	28 (1)	-2 (1)	2 (1)	3 (1)
C15	39 (1)	30 (1)	31 (1)	7 (1)	4 (1)	4 (1)
C16	32 (1)	26 (1)	41 (1)	2 (1)	11 (1)	-1 (1)
C17	32 (1)	31 (1)	29 (1)	-1 (1)	0 (1)	-5 (1)
C18	33 (1)	26 (1)	28 (1)	3 (1)	-2 (1)	0 (1)
C19	34 (1)	26 (1)	28 (1)	2 (1)	-4 (1)	0 (1)
C20	30 (1)	26 (1)	23 (1)	3 (1)	-6 (1)	2 (1)
C21	31 (1)	48 (1)	27 (1)	-6 (1)	-6 (1)	0 (1)
C22	35 (1)	67 (1)	38 (1)	9 (1)	-5 (1)	-18 (1)
C23	59 (1)	39 (1)	41 (1)	5 (1)	-23 (1)	-21 (1)
C24	51 (1)	33 (1)	34 (1)	-3 (1)	-16 (1)	0 (1)
C25	34 (1)	33 (1)	27 (1)	-1 (1)	-6 (1)	2 (1)
C26	32 (1)	22 (1)	33 (1)	2 (1)	-3 (1)	-4 (1)



C27	29 (1)	24 (1)	32 (1)	2 (1)	3 (1)	-3 (1)
C28	31 (1)	28 (1)	41 (1)	-1 (1)	-9 (1)	-4 (1)
C29	32 (1)	43 (1)	71 (1)	-6 (1)	2 (1)	-3 (1)
C30	44 (1)	31 (1)	44 (1)	-4 (1)	-9 (1)	-8 (1)
C31	^1 (1)	44 (1)	44 (1)	8 (1)	-16 (1)	-12 (1)

Table 5. Hydrogen coordinates (  $\times 10^4$  ) and isotropic displacement parameters (  $\text{\AA}^2 \times 10^3$  )

	x	y	z	U (eq)
H2	-407 (2)	-1566 (1)	4126 (2)	29
H8	-957 (2)	-792 (1)	5808 (2)	39
H9	-1331 (2)	-1160 (1)	7604 (2)	48
H10	-3404 (3)	-1632 (1)	8048 (2)	52
H11	-5114 (2)	-1712 (1)	6734 (2)	43
H12	-4788 (2)	-1318 (1)	4962 (2)	43
H14	-1104 (2)	-2304 (1)	5439 (2)	36
H15	-2227 (2)	-3214 (1)	5912 (2)	40
H16	-3664 (2)	-3693 (1)	4644 (2)	39
H17	-4089 (2)	-3232 (1)	2930 (2)	40
H18	-2985 (2)	-2316 (1)	2463 (2)	35
H19B	-3265 (2)	-373 (1)	1673 (2)	35
H19A	-2345 (2)	-595 (1)	678 (2)	35
H21	-5063 (2)	-1052 (1)	2398 (2)	43
H22	-6441 (2)	-1924 (1)	2149 (2)	56
H23	-5824 (2)	-2674 (1)	872 (2)	56
H24	-3881 (2)	-2547 (1)	-215 (2)	47
H2	-2522 (2)	-1677 (1)	20 (2)	37
H26A	-408 (2)	-66 (1)	1575 (2)	35
H26B	-1334 (2)	121 (1)	2590 (2)	35
H29A	4239 (2)	411 (7)	3787 (8)	73
H29B	3436 (10)	484 (7)	2659 (6)	73
H29C	3414 (10)	-152 (1)	3297 (13)	73
H30A	2864 (9)	1345 (1)	4543 (11)	60
H30B	1251 (8)	1313 (1)	4495 (12)	60
H30C	2104 (16)	1382 (1)	3390 (2)	60
H31A	2704 (13)	373 (6)	5651 (5)	74
H31B	2198 (18)	-243 (2)	5086 (3)	74
H31C	1127 (7)	265 (7)	5444 (7)	74

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