The Synthesis and Structural Characterisation of Novel 4- and 5- Membered Nitrogen Heterocycles derived from Azoacetates.

by

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A thesis presented for the degree of Doctor of Philosophy

at

Dublin City University

Ollscoil Chathair Bhaile Átha Cliath

School of Chemical Sciences

2009

To my Parents

Crescat Scientia Vita Excolatur

(Let Knowledge grow, Let Life Be Enriched)

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Ph.D is entirely my own work, that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Neil O' Halloran

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Acknowledgements

Firstly I would like to thank my supervisor, Dr Paraic James, initially for giving me the opportunity to conduct my research under his supervision, but also for being supportive and encouraging at both an academic and personal level.

I would also like to express my gratitude to the following groups of people:

The Irish Research Council for Science, Engineering and Technology who generously provided the funding that allowed me to conduct my research, in the form of a scholarship under the Embark Initiative.

The entire staff of the School of Chemical Sciences, in particular the technical staff, Mick, Maurice, Damien, Ambrose, Veronica, Ann, John and Vinny. They were always there with equal doses of help, advice and patience when required.

The undergraduate students who contributed to this project, Samuel Bertrand and Patrick Delaney.

Some former members of the Paraic James Research Group, Dr. Mairead Sheridan, Dr. Thomas Duff, Dr. Shane O' Malley and Dr. Pat O' Malley.

All of my fellow Postgraduate students and the researchers past and present in X2-49 for their friendship, support and help.

Finally, I would like to give a special thanks to my family without whose support I would probably have spent these years homeless, broke, starved and naked. In particular my parents Ricky and Anne, any words I use here could never encapsulate the level of encouragement, support and guidance they have given me. So I will simply say "Thank You". Also the other members of my family both immediate and extended, in particular Derek, Mary and Padraig and Ricky and Alex, my nieces Katelyn, Bonny and Libby and Nephew Ethan. They always put a smile on my face when I needed it most.

Abstract

Azo-drugs are among the earliest fully synthetic chemotherapeutic agents known (Prontosil 1935). However the synthesis and application of phenylazo compounds in the area of medicinal chemistry has largely been restricted to derivatives of primary aromatic amines. This has been due, in the most part, to a perceived view that azo compounds not containing two aromatic groups are unstable. The syntheses of a number of novel heterocycles including important medicinal structures directly linked to an arylazo moiety are reported. The literature survey introduces a range of four and five membered heterocycles with established pharmaceutical activity. In particular the attention is drawn to molecules containing the azo functional group. This thesis contains a chapter detailing the introduction of a variety of alkyl and aryl groups into novel β-lactam molecules bearing the arylazo and arylazoxy functional groups. The succeeding chapter investigates the role of the azocarbinol group as an intermediate in the rearrangement of azoacetates to N-acyl hydrazides. The deacetylation reactions of azoacetates derived from L-threonine are also described. This work resulted in the synthesis of novel oxazolidinone and hydantoin species, also with phenylazo attachments. The final chapter describes the incorporation of a cyanide unit into heterocyclic compounds through intermolecular cyclisations of a range of substituted azoacetates when potassium cyanide is employed as base. It is shown that the azo group may be incorporated into the cyclic system to produce pyrazoles, or as an exocyclic pendant group attached to a 2-iminopyrrolidin-5-one; X-ray crystal structures of these compounds are reported. The 2-iminopyrrolidin-5-one was easily modified to produce the corresponding pyrrolidine-2,5-dione (succinimide) derivative. All of the heterocycles reported were generated from azoacetates derived from simple, cheap and readily available starting materials (ethylacetoacetate and L-threonine), thus showing azoacetates to be versatile and valuable building blocks in the field of heterocyclic chemistry.

List of abbreviations

^0C	Degrees Celcius
2,4-DNP	2,4-Dinitrophenyl hydrazine
4-NP	4-Nitrophenyl hydrazine
6-APA	6-Aminopenicillanic acid
7-ACA	7-Aminocephalosporanic acid
α	Alpha
β	Beta
δ	Chemical shift
Δ	Delta (Used to denote addition of heat to reaction)
Ac	Acetate
Ac_2O	Acetic Anhydride
ACAT	AcylCoA:cholesterol acyltransferase
AcOH	Acetic Acid
$AlCl_3$	Aluminium chloride.
aq	Aqueous
Bn	benzyl
Boc	*tert*-Butoxycarbonyl
bp	Boiling point
br	Broad (spectral)
Btc	N-(1-benzotriazolecarbonyl)
BTIB	Bis(trifluoroacetoxy)-iodobenzene
Bz	Benzoyl
$CaCl_2$	Calcium Chloride
CAD	Coronary artery disease
CADD	Computer aided drug discovery

Cbz (or Z)	Carbobenzyloxy
CbzCl	Benzylchloroformate
CCl_4	Carbon tetrachloride
$CDCl_3$	Chloroform-D (deuterochloroform)
CDKs	Cyclin-dependent kinases
CH_3CN	Acetonitrile
$CHCl_3$	Chloroform
cm^{-1}	Wavenumber(s) (spectral)
cm^3	Centimetre cubed (millilitre)
CO_2	Carbon Dioxide (gas)
COSY	Correlation spectroscopy
CrO_3	Chromium Trioxide
d	Doublet (spectral)
d	Density
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	Dicyclohexylurea
DEAD	Diethylazodicarboxylate
DEPT	Distortionless enhancement through polarisation transfer
DMARDs	Disease-modifying antirheumatic drugs
DMD	Dimethyldioxirane
DMF	Dimethylformamide
DMP	Dess Martin Periodinane
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
E.S.R.	Electron spin resonance

ED_{50}	The median effective dose
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	Enantiomeric excess
Et	Ethyl
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
FDA	Food and Drug Administration
Fmet	N-formylmethionine
FMOC	N-alpha-(9-fluorenylmethyloxycarbonyl)
g	Gram(s)
GABA	γ-aminobutyric acid
GISA	Glycopeptide-Intermediate *Staphylococcus aureus*
GSH	L-γ-glutamyl-L-cysteinyl-glycine;
H_2O	Water
HCl	Hydrochloric Acid
HIO_2	Hydroiodous acid
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
HOBt	1-Hydroxybenzotriazole Hydrate
HOMO	Highest Occupied Molecular Orbital
HPLC	High performance Liquid Chromatography
Hz	Hertz
I.R.	Infra Red
I_2O_5	Diiode pentaoxide
IBA	Iodosobenzoic acid

IBDA	Iodobenzene Diacetate
IBX	2-Iodoxybenzoic acid
IC_{50}	Half maximal (50%) inhibitory concentration
i-Pr	*iso*-propyl
J	Coupling constant (spectral)
K_2CO_3	Potassium Carbonate
KO^tBu	Potassium *tert*-Butoxide
L-BSO	L-buthionine-(R,S)-sulfoximine
LDA	Lithium diisopropylamide
LTA	Lead tetraacetate
m	Multiplet (spectral)
M	Molar
m.p.	Melting point
MAP	Mitogen-activated protein
Me	Methyl
MeLi	Methyl Lithium
MeOH	Methanol
MeS	Dimethyl sulphide
MES	Maximal electric shock seizure (seizure induction technique)
ml.	Millilitre
mM	Millimolar (millimoles per liter)
mmol	Millimole(s)
Mmol.	Millimole
mol	Mole(s)
mol wt	Molecular weight
mRNA	Messenger RNA

MRSA	Methicillin Resistant Staphylococcus Aureus
MsCl	Mesyl chloride
N.M.R	Nuclear Magnetic Resonance
Na_2CO_3	Sodium carbonate
$NaBF_4$	Sodium tetraflouroborate
NaCl	Sodium Chloride
$NaHCO_3$	Sodium Bicarbonate
NaOH	Sodium Hydroxide
NaO^tBu	Sodium *tert*-Butoxide
NH_4Cl	Amonium Chloride
Ns	*Meta*-nitophenylsulphonyl
Nu	Nucleophile
PABA	*Para*-Amino Benzoic Acid
PBP	Penicillin Binding Protein
PCC	Pyridinium chlorochromate
Ph	Phenyl
pH	Concentration of hydrogen ions in terms of the potenz scale
Ph_3P	Triphenylphosphine
Ph_3PO	Triphenylphosphine Oxide
$PhI(OAc)_2$	Iodobenzene Diacetate
$PhI(OCOCF_3)_2$	Bis(trifluoroacetoxy)-iodobenzene
$PhIO_2$	Iodoxybenzene
Phth	Phthaloyl
PI	Protective index = TD_{50} / ED_{50}
PCP	*Para*-chlorophenyl
PMB	*Para*-methoxybenzyl

PNP	*Para*-nitrophenyl
ppm	Part(s) per million
Pr	Propyl
PTZ	Pentylenetetrazole seizure (seizure induction technique)
q	Quartet (spectral)
RNA	Ribonucleic acid
RuO_4	Ruthenium tetroxide
s	Singlet (spectral)
SAR(s)	Structure Activity Relationship (studies)
Ser	Serine
soln	Solution
t	Triplet (spectral)
TB	Tuberculosis
TBAF	Tetrabutylammonium fluoride
t-Bu	*tert*-Butyl
t-BuLi	*tert*-Butyl Lithium
TD_{50}	The median toxic dose
TEMPO	Tetramethylpiperdinyloxy free radical
TGI	Tumor growth inhibition
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TMSCN	Trimethylsilyl cyanide
TNFα	Tumor necrosis factor alpha
TPAP	Tetra-n-Propylammonium perruthenate

U.V.	Ultra Violet
VRE	Vancomycin Resistant Enterococci
Zyvox ™	Linezolid

Table of Contents

Chapter 1

(Medicinal roles of selected heterocycles)
Literature survey

Bene legere saecla vincere

1.1 Introduction

1.1.1 The early history of antibiotics

The history of antibiotics can be traced back to observations made by tribal peoples who noticed that certain plants could be used to stave off infection. For centuries natives of South America have chewed the bark of the cinchona tree as a treatment for malaria. According to the Ebers papyrus (1500 BC), beer yeast was used on wounds by the Egyptians. The ancient Greeks and ancient Chinese also noticed that certain moulds and plants were effective treatments for infections.

Some would argue that salt was the first antibacterial agent, reference to salt being used as a food preservative can be found in the earliest documents surviving in most cultures. Most historians agree that the practice probably predates most written language. The connection between the toxic effects of NaCl on food-spoiling bacteria would not have been fully understood but the health benefits of employing the procedure of salt-based meat curing would have been obvious.

In the late 19th century some of the most significant breakthroughs were made. In 1871, Joseph Lister[1] noticed that some moulds could inhibit the growth of certain microbes. Unfortunately the significance of this work was not realised immediately and it would be decades before the full potential of this discovery was explored fully.

In 1877, Louis Pasteur showed that anthrax could be countered in animals with the injection of saprophytic bacteria found in soil.[2] Louis Pasteur's pupil Paul Vuillemin coined the term 'antibiosis' in 1889 to mean a process by which life could be used to destroy life. [3] Emmerich and Freudenreich among others made similar observations in relation to other pathogens including important contributions towards the treatment of cholera and diphtheria.[4] In 1897 Ernest Duchesne a French medical student first uncovered the potential antibiotic properties of the penicillium mould.[5] Duchesne studied the interaction between *Escherichia coli* and

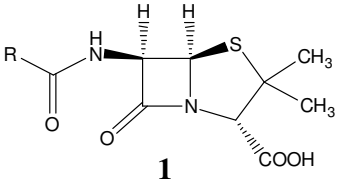
Penicillium glaucum; He had observed how stable boys kept their saddles in a dark and damp room to encourage mould to grow on them because the mould helped to heal the saddle sores on the horses. Later he found that after preparing solutions of the mould and injecting them into diseased guinea pigs, they recovered. However his discoveries went largely unnoticed by the scientific community.

But the most significant step forward came with the discovery by Alexander Fleming that a product in human tears could lyse bacterial cells. Fleming's finding, which he called lysozyme, was the first example of an antibacterial agent found in humans. Lysozyme would prove to be a dead end in the search for an efficient antibiotic, because it destroyed non-pathogenic bacterial cells. In 1928 however, Fleming discovered another antibacterial agent.[6] Upon looking through a set of old plates that he had left out, Fleming found that colonies of *Staphylococcus* which had been streaked out had lysed. The observation that lysis had occurred adjacent to some mould that had grown on the plate and the realisation that a product of the mould had caused the cell lysis, would become probably the most important observation in the history of medical research.

Through follow-up work, Fleming showed experimentally that the mould produced a small substance that diffused. He named this substance penicillin after the *Penicillium* mould that had produced it. By extracting the substance from the plates, Fleming was then able to directly show its effects. Important to its discovery was that penicillin had destroyed a common bacterium, *Staphylococcus aureus*, associated with sometimes deadly skin infections. From that time up to the present day the vast majority of commercially available antibacterial agents have been structural variations of the β-lactam core of that original penicillin skeleton.

1.1.2 The emergence and development of β-lactam antibiotics

When the structural elucidation of the active structures in penicillin (**1,** Figure 1.1) was eventually achieved in the mid 1940's the drive to find useful derivatives of the molecule began in earnest.[7] The benefits of these new drugs had been brought clearly into focus with the onset of the Second World War. In 1942 the U.S. government made an appeal to companies to join the race to see which one would develop a way to mass-produce the world's first broad-spectrum antibiotic. In response Pfizer developed a production technique that allowed large quantities of penicillin to be produced and shipped to the troops on the frontlines saving countless lives before the end of the war.



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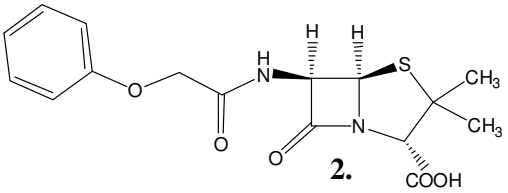

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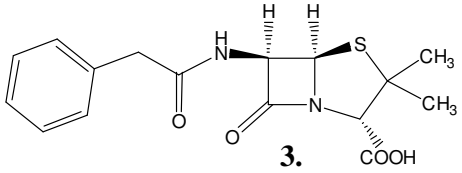
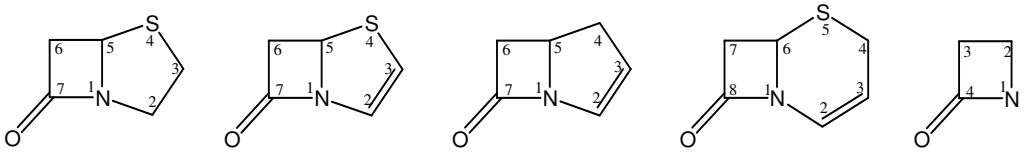


3.

Figure 1.1 General structure penicillins (**1**) (**2** = penicillin V, **3** = penicillin G)

1.1.3 Ring numbering and nomenclature

There has been some inconsistency in the use of numbering systems for β-lactam ring systems, particularly in the case of monocyclic β-lactams, for the sake of clarity the system shown below (Figure 1.2) has been adopted in this thesis and will be used throughout.



Penam Penem Carbapenem Cefem Azetidinone

Figure 1.2 β-Lactam ring numbering

1.1.4 Semi-synthetic variations of penicillin

Initial attempts at producing new useful β-lactam compounds centred around making structural changes to the side chain at position C6 on the penicillin skeleton.

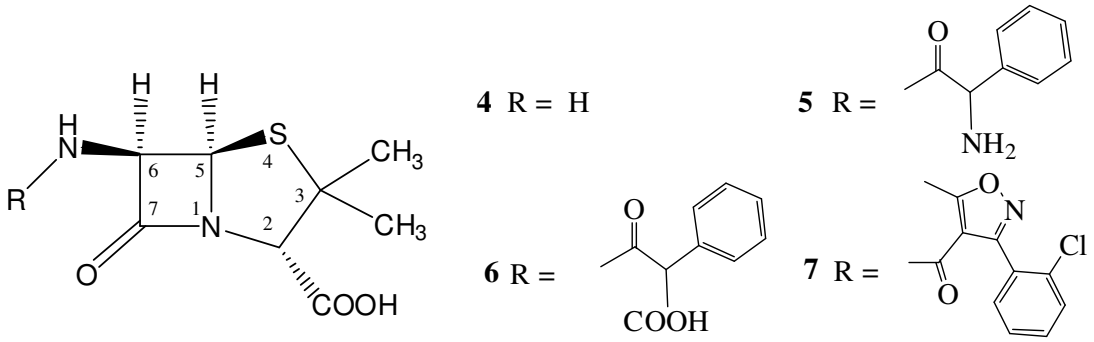


Figure 1.3 Semi-synthetic penicillin derivatives

This was initially achieved by removing the naturally occurring acyl groups to give compound **4**, 6-aminopenicillanic acid. Addition of new acyl groups afforded the new and useful semi-synthetic derivatives ampicillin[8] **5**, carbenicillin[9] **6** and oxacillin **7** (Figure 1.3).

1.1.5 Structural diversity in the β-lactam skeleton

After the preliminary studies on the activity of penicillin, analogous studies uncovered various active compounds similar in structure to the penicillins, containing the β-lactam skeleton as part of the structure. The first of these to emerge was the cephalosporins in 1948 (Figure 1.4); Giuseppe Brotzu isolated these compounds from cultures of *Cephalosporium acremonium* from a sewer in Sardinia.[10] These compounds were found to be somewhat effective against typhoid fever.

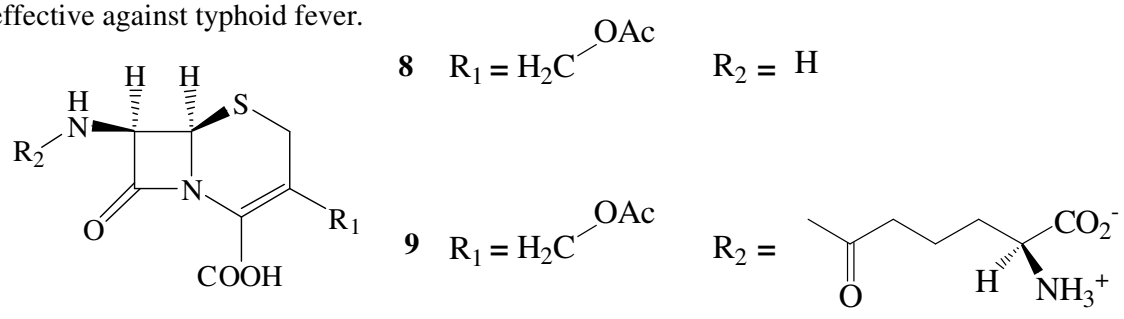


Figure 1.4 Cephalosporins

Research was carried out at the Sir William Dunn School of Pathology at the University of Oxford and the compound cephalosporin C **9** was isolated.[11] This compound was found to be less potent in terms of antibiotic activity than penicillin however it showed good stability towards β-lactamases. It was also found, in a similar fashion to penicillin, that isolation of the parent structure, 7-aminocephlosporanic acid (7-ACA, **8**, Figure 1.4) gave a synthetic route to many new and potent antibiotics. The first of these new compounds to become commercially available was the antibiotic cephalothin (cefalotin) **10** (Eli Lilly-1964).

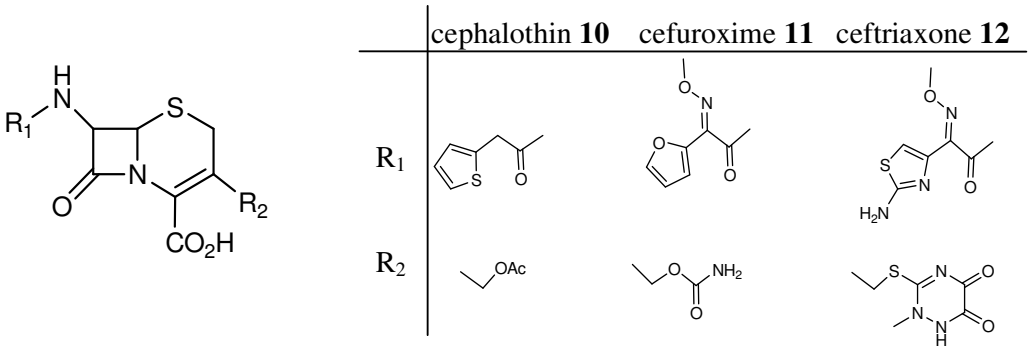


Figure 1.5 Structural diversity in cephalosporins (1st, 2nd and 3rd generation)

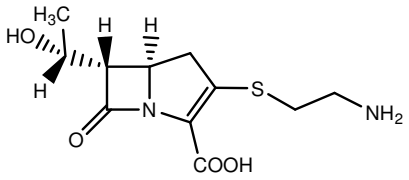
Subsequently large arrays of cephalosporin based antibiotics were introduced to the pharmaceutical market. In 1995 cephalosporins were the largest selling type of antibiotic worldwide, generating almost 8.5 billion US$ in revenue for the vendors, with penicillins being the second largest seller (4.4 billion US$).[12] The development of cephalosporins has given rise to several generations of subsequent derivatives, examples of which include, cephalothin **10** (first generation),[13] cefuroxime **11** (second generation), and ceftriaxone **12** (third generation) (Figure 1.5).

1.1.6 Other important bicyclic β-lactams

1.1.6.1 Carbapenems

Another important addition to the bicyclic β-lactams came with the discovery of carbapenems in the late 1970s at Beecham and Merck.[14] Thienamycin (**13,** Figure 1.6), which was isolated from the mould, *Streptomycin cattlea*, was found not only to be an extremely

potent antibiotic but also an effective β-lactamase inhibitor. The main feature that distinguishes thienamycin from similar bicyclic β-lactams is the presence of the sulphur atom exocyclic to the five membered ring system. Thienamycin could not be released onto the market despite initial promising biological results. It was found that although the compound was a potent antibiotic it had a tendency to undergo an intra-molecular reaction involving the amide group and the β-lactam ring at certain concentration levels. The problem was overcome by chemically altering the amide functionality to an imine, a group that did not interfere with the β-lactam ring and did not have a detrimental effect on the potency of the compound. The new drug Imipenem (**14**, Figure 1.6) was successfully marketed as an antibiotic and also has good activity against β-lactamases. However imipenem is rapidly degraded by the renal enzyme dehydropeptidase if administered alone, for this reason the drug is co-administered with cilastatin, a dehydropeptidase inhibitor, cilastatin blocks the effects of the renal enzyme.



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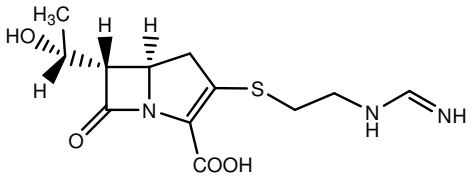
Thienamycin



14

Imipenem

Figure 1.6 Carbapenems

Since the release of imipenem a number of useful carbapenems have been developed and they have been found to be among the broadest spectrum anti-microbial agents available.

1.1.6.2 Penems

Penems evolved out of the desire by synthetic chemists to incorporate some of the structural features that were known to convey biological activity in penicillins and cephalosporins into a molecule more readily synthesised in the lab. A team at the Woodward

Research Institute in Basel achieved the synthesis of such a molecule containing both the bicyclic structure and a double bond in 1975.[15] The initial compounds synthesised were of the general type shown (Figure 1.7).

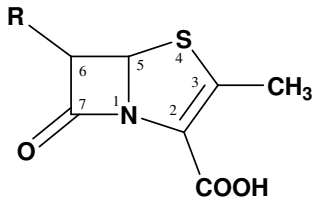


Figure 1.7 General structure of first synthetic penems

It was the discovery of clavulanic acid (**19**, Figure 1.13) as a potent β-lactamase inhibitor and of thienamycin as a powerful broad-spectrum antibiotic that rose hopes that interesting biological activity in compounds with a penem skeleton. However the challenge facing researchers was finding the delicate balance between the inherent reactivity of the β-lactam system and reasonable levels of stability within the systems being manufactured. Interestingly the first attempts at addressing this problem of balance centred around the synthesis of compounds with alkyl groups attached to position 6 on the ring (Figure 1.8).

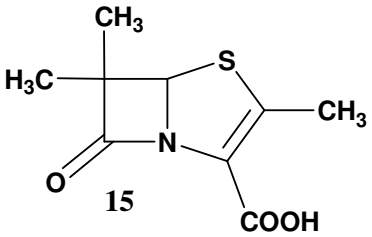


Figure 1.8 Early penem showing activity towards β-lactamases

The penem **15** was synthesised with the intention of providing a route to active antibiotics, however it was found that the molecule had little or no antibiotic activity but was a promising candidate as a β-lactamase inhibitor, showing good activity towards the β-lactamases from *P. aeruginosa* 18SA and *Enterobacter* P99.[16] In results similar to clavulanic acid and thienamycin it was found that the antibiotic activity imparted by the amide group on the ring was not a vital element in the observed activity against β-lactamases.

1.1.7 Mode of action of β-lactams

β-Lactam antibiotics are bacterial cell wall biosynthesis inhibitors, active against mainly Gram-positive bacteria. To understand how β-lactams disrupt the synthesis of the cell wall, a basic knowledge of the make up of the bacterial cell wall is necessary (Figure 1.9). The Gram-positive bacterial cell wall is made up of petidoglycan layers usually 50-100 molecular layers thick.

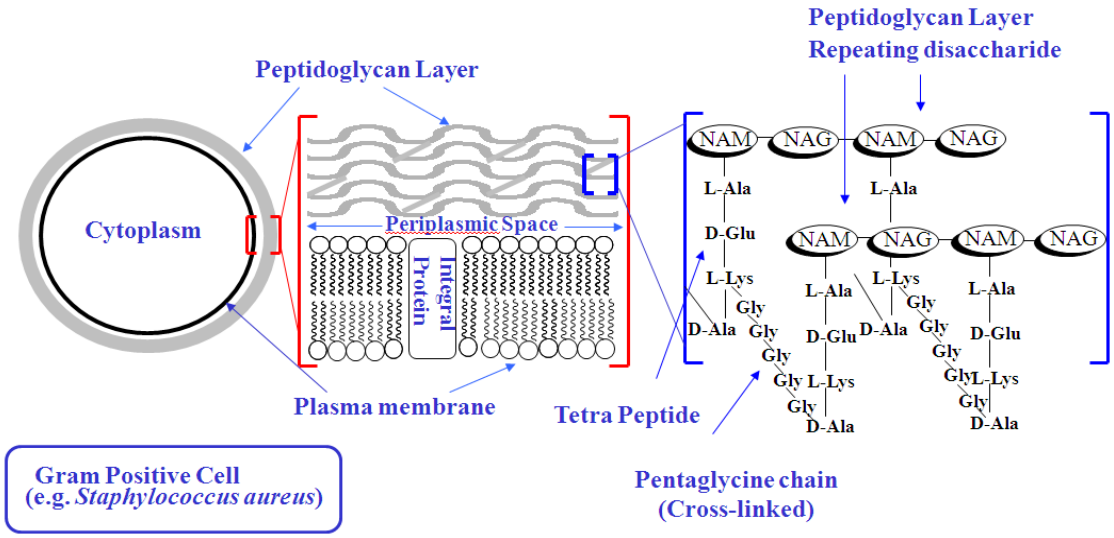
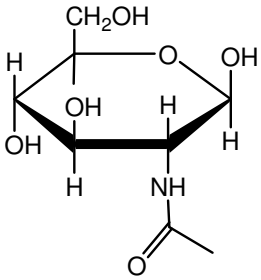
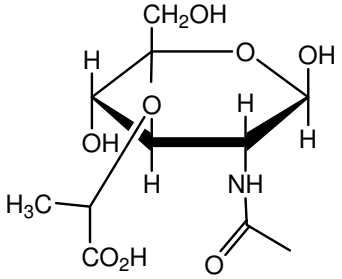


Figure 1.9 Gram positive bacterial cell wall

The peptidoglycan layer gives strength and rigidity to the cell and determines its shape. The peptidoglycan layer is made up of a repeating unit of disaccharide (NAG **16** and NAM **17** Figure 1.10) linked to a tetrapeptide. These peptides are, in turn, cross-linked by a peptide chain, (e.g. *Staphylococcus aureus* is cross-linked by a pentaglycyl peptide chain Figure 1.9).



N-Acetylglucosamine (**NAG**) **16**



N-Acetylmuramic acid (**NAM**) **17**

Figure 1.10 Sugars making up the disaccharide-repeating unit of peptidoglycan

It is at the point of formation of these essential cross linkages that inhibition of cell wall synthesis by β-lactams takes place. β-Lactams compete for the binding site on the enzyme that catalyses the cross linking process. There are several of these binding sites within the bacterial cell known as penicillin binding proteins (PBPs). [17] The PBP discussed here is commonly known as PBP-1, and contains a serine residue that facilitates the cross-linking procedure. In the case of its reaction with β-lactam molecules it produces a bacteriocidal effect resulting in cell lysis. The β-lactam ring is a highly strained entity and as such is a highly reactive moiety. It has been proposed that the cyclic nature of the β-lactam ring contributes to its reactivity relative to similar acyclic amides; this is due to a reduced ability to allow delocalisation of the nitrogen lone pair. A feature of the β-lactam amide is shortened carbonyl bond length together with an elongated C—N bond. The difference between the types of amide can be seen in the I.R. carbonyl stretch of β-lactams (ν(C==O) 1775-1795 cm^{-1}) and acyclic amide (ν(C==O) 1640 – 1690 cm^{-1}).[18]

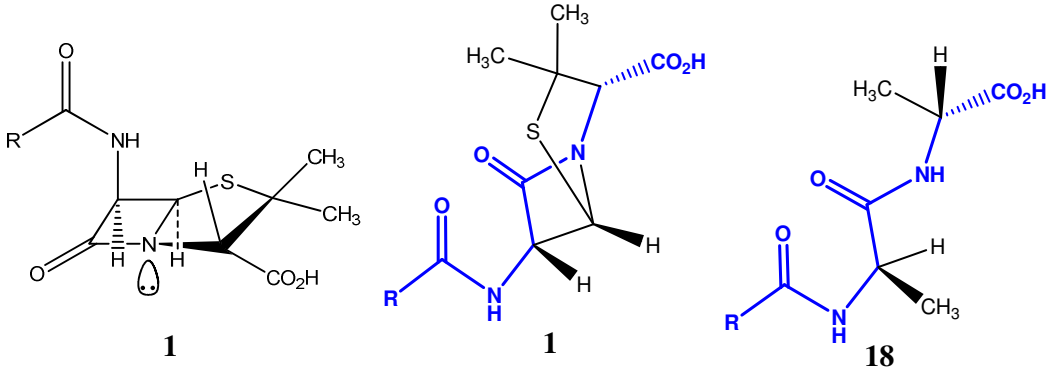


Figure 1.11 Comparison between penicillin and D-Ala-D-Ala peptide terminus

This difference is directly attributable to the ring strain common in these smaller rings and is the nature of the susceptibility of β-lactams to nucleophilic attack. Another feature of penicillins considered to be a factor in their activity is the structural similarity to the D-Ala-D-Ala terminus **18** of the peptide chain in peptidoglycan (Figure 1.11). This capacity to

mimic the enzyme-binding site coupled with the inherent reactivity of the β-lactam ring is seen as the source of the activity of penicillins (Figure 1.12). It should be noted however that some potent β-lactam antibiotics do not share all of these characteristics associated with penicillins. Monocyclic β-lactams for instance do not usually share the same level of structural similarity to the D-Ala-D-Ala terminus **18** but do bind effectively to PBP-3. As a result the Structure Activity Relationship (SAR) has been revised many times in accordance with new information about the system.

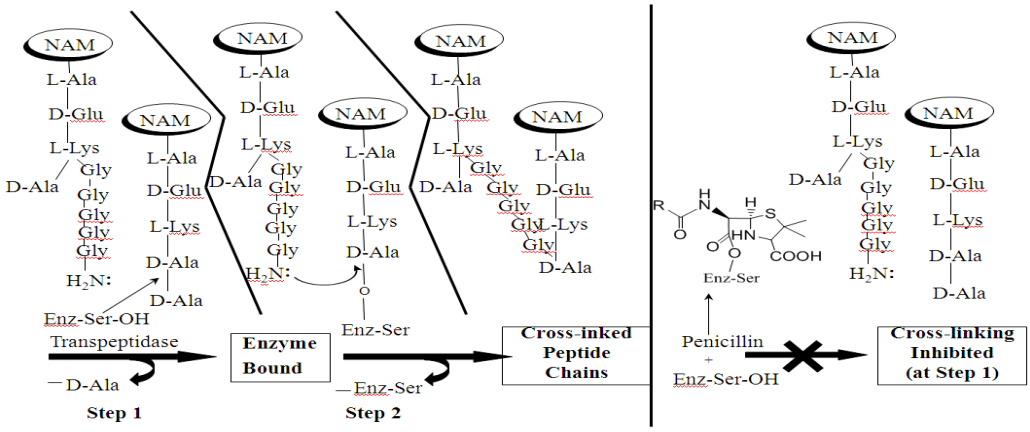
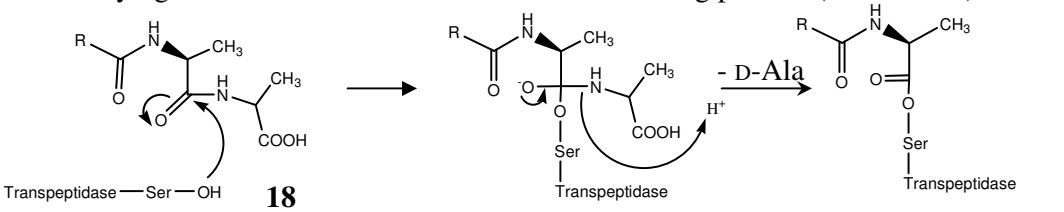
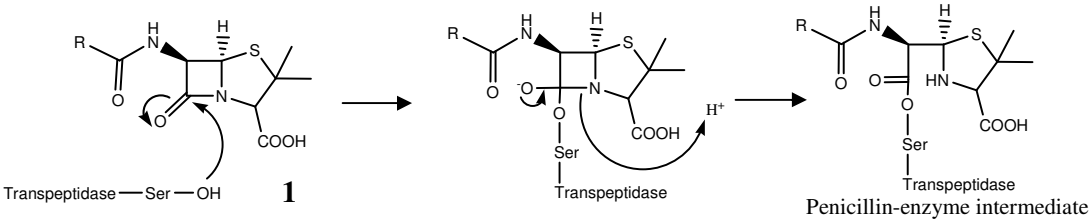


Figure 1.12 Inhibition of cross-linking process in Gram-positive bacterial cell wall

As mentioned previously β-lactams function as bacteriocidal inhibitors. They bind irreversibly to the serine residue of the transpeptidase enzyme thereby blocking the enzyme from carrying out its essential function in the cross-linking process (Scheme 1.1).



Scheme 1.1a Action of transpeptidase enzyme on Terminal D-Ala-D-Ala Residue



Scheme 1.1b Action of transpeptidase enzyme on Penicillin

1.1.8 Development of resistance to β-lactam antibiotics (β-lactamases).

The development of resistance to antibiotics by bacterial cells was in many ways an inevitable development. Observations made by Darwin long before the development of modern antibiotics could be seen to auger the development of resistance.[19] His observation that in every environment competition dictates the selection of the strain most likely to survive in that environment has turned out to be exactly what has been observed over and over in the domain of bacteria. The large numbers of bacterial cells, combined with the short generation times facilitate the development of mutants. In a typical bacterial population of 10^{11} bacterial cells (i.e. in an infected patient) there can easily be 1000 mutants. If a mutant confers a selective advantage upon the bacterium (i.e. the ability to survive in the presence of an antibiotic) then that resistant bacterium will be selected and continue to grow while its neighbours perish. This can happen in a matter of days in patients being treated with antibiotics. These resistant species multiply, until the entire population is composed of resistant bacteria. This type of action of the bacteria is known as the selection of resistant mutants. For example approximately 10 years after the introduction of penicillin, penicillin-resistant *Staphylococcus aureus* was observed. In response modified penicillins were produced that were resistant to this specific strain of β-lactamases. Before long, strains of bacteria containing β-lactamases with different mechanisms of action were selected and soon became the predominantly observed strain. This cycle has been repeated since the introduction of antibiotics and remains the predominant problem facing researchers trying to stay one step ahead of the development of bacteria.

Several mechanisms of resistance to antibiotics exist, the most effective are:

(i) The prevention of build up of antibiotic in the cell (decrease in uptake, or increase in efflux),

(ii) Alteration of the target site to reduce the affinity for the antibiotic by mutation or modification,

(iii) Inactivation of the antibiotic by hydrolysis or modification

Although the first two mechanisms are a considerable problem the most troublesome in terms of β-lactam antibiotics is the inactivation by hydrolysis due to the presence in the cell of β-lactamases.

It has been shown that the activity of the β-lactam antibiotics is due to their inhibitory effect on the transpeptidase enzyme, combining with the PBP to inactivate the enzyme and thus causing cell death due to weakened cell walls. The presence of β-lactamases in the cell provides another target for penicillin with a similar binding site, the success of the β-lactamase is dependant on the propensity of the β-lactam to bind preferentially to the β-lactamase over the transpeptidase. Both contain a serine residue that is central to the activity of the enzyme, this structural similarity combined with the similar mechanism of action suggests that the β-lactamase may have developed as a defence mechanism directly from the PBP.

The preference for reaction with the β-lactamase can be accounted for in terms of the rate of deacylation of the intermediate formed between the β-lactam and the β-lactamase, this occurs relatively rapidly to return the intact β-lactamase enzyme.

1.1.9 β-Lactamase Inhibitors

Probably the most important member of this group is clavulanic acid (**19**, Figure 1.13) which was isolated from *Streptomyces clavuligerus* in 1976.[20] Clavulanic acid was the first suicide inhibitor of β-lactamases to be described in the literature. The clavam structure was the first naturally occurring bicyclic β-lactam described that did not possess a penicillin or cephalosporin nucleus. This is significant in that it led the way for later research into the

potential of β-lactams without the structural features hitherto considered essential for imparting activity.

The similarity in chemical structure allows the molecule to act as a competitive inhibitor of β-lactamases secreted by certain bacteria to confer resistance to β-lactam antibiotics. The clav ring is similar to the penicillins except at three moieties: at ring position 4 oxygen replaces sulphur, at position 3 a hydroxyethylidene group replaces two methyl groups, and at position 6 there is no substituent.

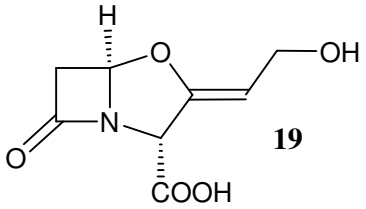


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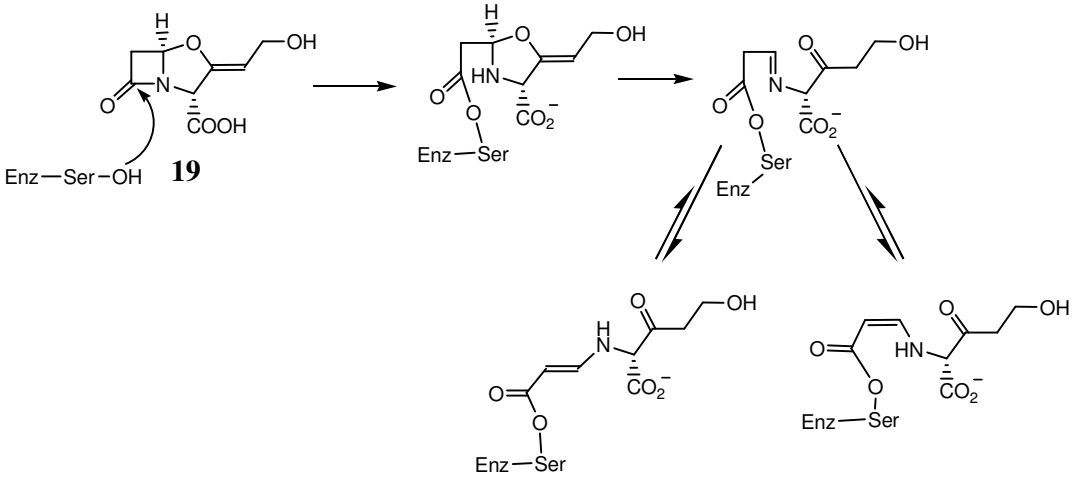
Figure 1.13 Structure of clavulanic acid

The exact mechanism of action of clavulanic acid is thought to be quite complex, including several different processes in which the β-lactamase enzyme can be either reversibly or irreversibly inhibited. However, in general terms the main reaction of clavulanic acid with the β-lactamase molecule is similar to that observed with penicillin (Scheme 1.2).[21]



Scheme 1.2 Mechanism of inhibition of β-lactamase by clavulanic acid

However, unlike penicillin, the initial acyl-enzyme intermediate can rearrange into a chemically inert enamine, whose further reaction the enzyme is unable to catalyse.[22]

Clavulanic acid has been shown to be a potent inhibitor of β-lactamases produced by staphylococci and plasmid-mediated β-lactamases of *E. coli*, as well as examples from *Klebsiella*, *Proteus*, and *Hemophilus*. Although it has proven to be one of the most successful β-lactamase inhibitor yet discovered the molecule does not possess sufficient activity against transpeptidase molecules for it to be administered unaccompanied. The most common formulations of this drug are in combination with another antibiotic, (e.g. Augmentin, amoxicillin and clavulanic acid). The inhibitory effects of the clavulanic acid allow enhanced performance of the co-administered drug.

1.1.10 Monocyclic β-lactams (Monobactams and Azetidinone)

In the overall scheme of β-lactam research, the emergence of chemical and biosynthetic pathways to the monocyclic β-lactam compounds is a relatively new field. The first reported isolation of nocardicins from the fermentation broth of a strain of *Actinomycetes* (strain WS 1571, was identified as *Nocardia uniformis* subsp. tsuyamanensis ATCC 21806)[23] was followed in quick succession by alternative syntheses of both the core structure (3-ANA) 3-aminonocardicinic acid **20** and nocardicin A **21** (Figure 1.14). [24,25]

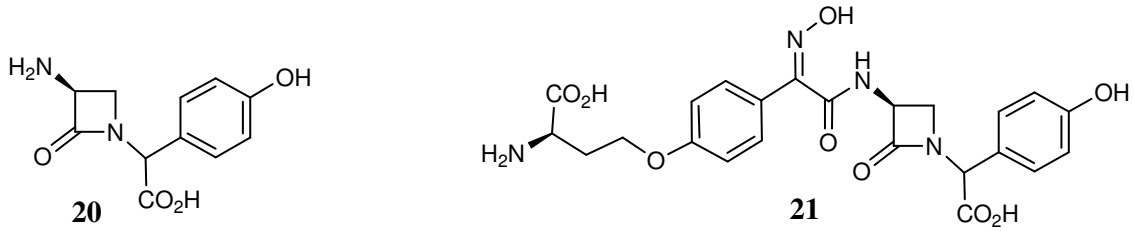


Figure 1.14 Structure of nocardicins, (3-ANA) **20** and nocardicin A **21**

Unfortunately the nocardicins proved to have limited effectiveness against pathogenic bacteria. Nocardicin A was found to show selective antibacterial activity, with moderate activity against a range of Gram-negative bacteria including *Proteus* and *Pseudomonas*. However it had no inhibitory effect on *Staphylococcus*, *Mycobacterium*, fungi and yeast.

Shortly after the nocardicins came to light, the isolation of another group of monocyclic β-lactam derivatives was reported in a series of papers by researchers at Squibb.[26] The so-called monobactams isolated from strains of *Chromobacterium violaceum*, *Acetobacter sp.*, and *Agrobacterium radiobacter* were characterized by a central β-lactam ring with N-acyl side chains at position 3 and a sulphate moiety attached to the ring nitrogen (Figure 1.15). Most naturally occurring monobactams also bear a methoxy group at ring position 3, but examples where it is not present have also been isolated.[27] The initial exploitation of the penicillins and the consequent synthesis of derivatives through the modification of the side chain were wholly dependant on the development of efficient fermentation procedures for the mass production of the parent compound. In the case of the monobactams the relative simplicity of the monocyclic system allowed access to the core structure through chemical synthesis in good yield. The synthesis of (3-AMA) 3-aminomonobactamic acid (**22,** Figure 1.15) the central unit, to which subsequent modifications were made was achieved by Squibb chemists in 1982.[28]

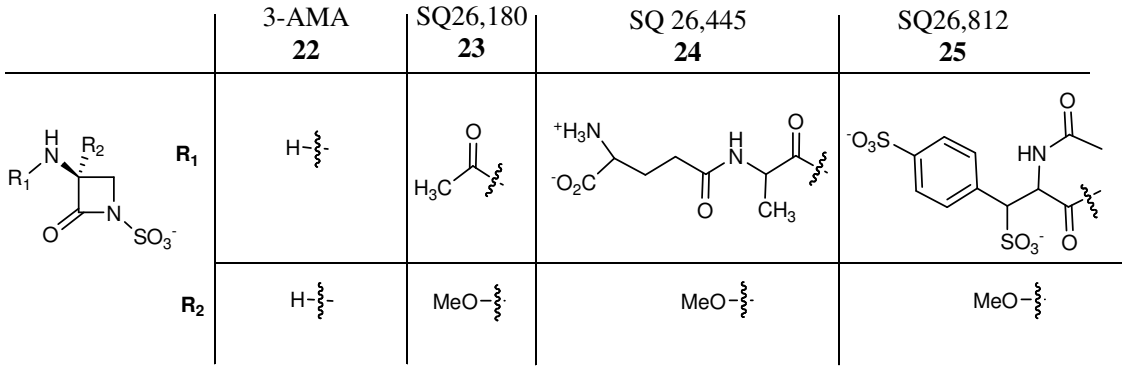


Figure 1.15 Structures of 3-AMA, SQ 26,180, SQ 26,445, and SQ 26,812.

Unfortunately much like the naturally occurring nocardicins, the isolated monobactams SQ 26,180 **23**, SQ 26,445 **24**, and SQ 26,812 **25** (Figure 1.15) showed little potential as antibiotic. However after much experimentation with side chain modifications aztreonam (Azactam®, **26**, Figure 1.16) was first synthesised. This potent antibiotic was a

combination of the core 3-aminomonobactamic acid with the side-chain of the cephalosporin ceftazidime. The compound is active against Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Haemophilus influenzae*, but was found to perform poorly against Gram-positive organisms. A further attractive characteristic was found to be high degree of resistance to enzymatic hydrolysis by most of the common β-lactamases.

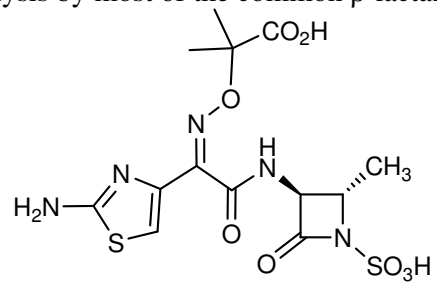


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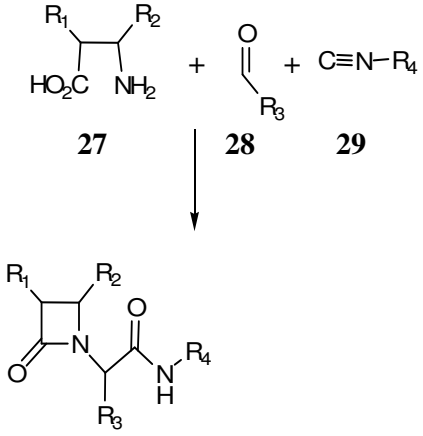
Figure 1.16 Aztreonam: β-lactamase resistant Gram negative antibiotic

Until recently, interest in newly generated β-lactams tended to focus solely on their ability to induce bacterial cell death due to their historically successful application in this role. However β-lactams have now come to the attention of researchers in a diverse range of medicinal fields. Compounds of a combinatorial monocyclic β-lactam library synthesised using the Ugi 4CC four-component condensation reaction were found to be apparently uncompetitive inhibitors of HIV-1 protease, providing lead compounds for a new class of HIV protease inhibitors.[29,30]



27 **28** **29**

	30	**31**	**32**
R_1	H	H	H
R_2	COOBn	COOBn	(CO)NH(CH)(Bn)(COOMe)
R_3	Ph	Ph	Ph
R_4	CH_2-($C_6N_3H_4$)	CH_2-COOMe	CH_2-($C_6N_3H_4$)

Scheme 1.3 Ugi 4CC multi-component synthesis of β-lactam based HIV protease inhibitors

The combinatorial library produced a total of 126 compounds based on the starting β-amino acid **27**, aldehyde **28** and isocyanide **29** (Scheme 1.3). High throughput screening of those compounds showed three, **30, 31** and **32** (Scheme 1.3) to be active HIV protease inhibitors showing greater than 60% inhibition.

In yet another role, monocyclic β-lactam analogues have been proven effective in human clinical trials as agents capable of inhibiting the absorption of cholesterol. The goal of the work was the generation of an agent for the prevention of atherosclerotic coronary artery disease (CAD) through the generation of AcylCoA: cholesterol acyltransferase (ACAT) inhibitors, blocking the formation of intestinal cholesteryl esters. *In vitro* testing showed the azetidinones to have a modest inhibitory effect on ACAT, however when *in vivo* testing in hamsters was completed the studies indicated that the action of the substrate was at the intestinal wall to inhibit cholesterol absorption through a mechanism not involving ACAT inhibition. [31]

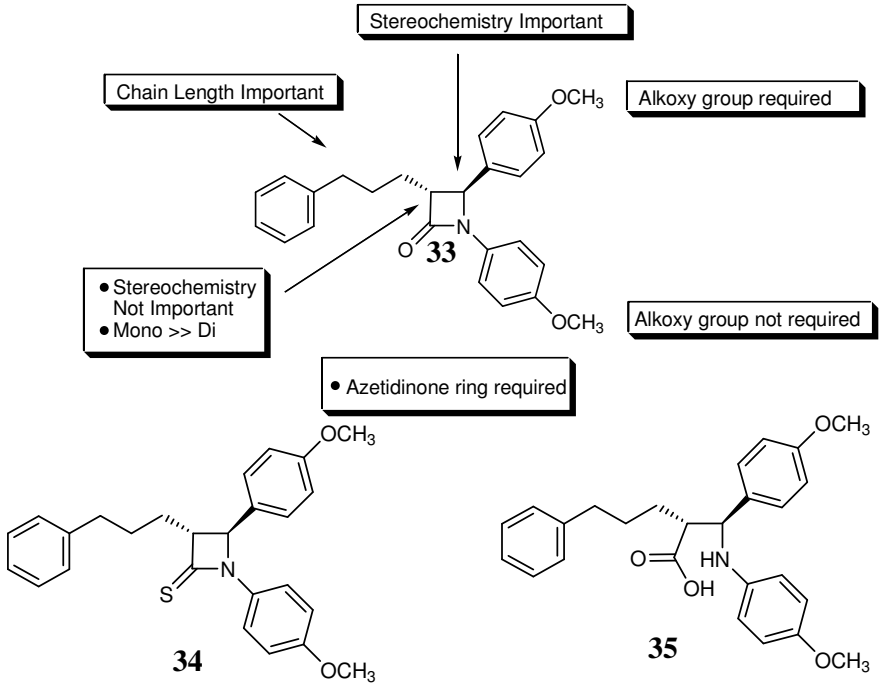
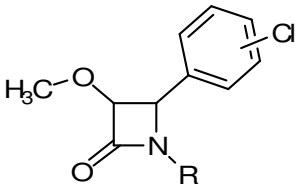


Figure 1.17 Basic pharmacophore for β-lactam based ACAT inhibitor and structurally similar inactive analogues.

Preliminary SAR studies showed that the azetidinone ring was an essential element in the most active compound **33** (Figure 1.17). The analogous thioazetidinone **34** and uncyclised β-amino acid **35** were found to be ineffective. Additional features necessary for the conferral of activity were identified and a basic pharmacophore was developed (Figure 1.17).

1.1.11 Anti-cancer activity of β-lactams

Due to the toxic nature of many of the currently available anti-cancer agents, the search for reagents equally effective, but without the detrimental effects on the patient has been ceaseless. Recently the search has focused on β-lactam containing molecules. This has come about partly due to the recent abundance of novel β-lactams appearing in the literature in response to the search for more resilient compounds capable of withstanding the resistance developing in many strains of bacteria.[32] The proliferation of these compounds has led to their being reassessed as possible agents capable of bringing about apoptosis in cancerous cells. The β-lactam structure has been found to date to have very low toxicity in most cases and this characteristic makes them an excellent candidate for further development if it can be shown that the potential exists to create ant-cancer compounds with similar efficacy and low toxicity levels. Recently strides have been made towards achieving this goal, with an increasing number of publications showing promising results in this area. In 2002 for the first time the ability of β-lactams to induce cell death in cancerous cells was reported.[33]



36, *o*-Chloro, R = S-CH_3
37, *o*-Chloro, R = H
38, *o*-Chloro, R = S-CH_2-CH_3
39, *o*-Chloro, R = S-$(CH_2)_3$-CH_3
40, *m*-Chloro, R = S-CH_3
41, *p*-Chloro, R = S-CH_3
42, *o*-Chloro, R = S-CH_2-C_6H_5

Figure 1.18 β-lactam molecules showing anti-cancer activity

Some basic SAR and mechanistic studies were carried out on the compounds, and although the direct target of the lactam substrate was not identified, valuable insight into the processes resulting in cancer cell death was gathered. It was found that compounds with an N-methylthio group were able to induce DNA damage and inhibit DNA replication in human leukaemic (Jurkat T) cells within a 2-h treatment. This was followed by p38 mitogen-activated protein (MAP) kinase activation, S phase arrest, and apoptotic cell death. The p38 (MAP) kinase was found to play a central role in β-lactam induced apoptosis. It was found that the rank of potencies of the lactams to induce DNA damage matches precisely the order for activation of p38 MAP kinase and the N-methylthio group was a required element for activity. In other words increasing the number of carbons on the N-thio constituent of the lactams was accompanied by a stepwise decrease in the ability of these compounds to induce DNA damage and also directly correlated to the ability to cause p38 phosphorylation, **36** (S-CH_3) > **38** (S-CH_2-CH_3) > **39** (S-CH_2-CH_2-CH_3) (Figure 1.18).

Apoptosis can be thought of as a means of inducing the target cell to actively commit suicide. Apoptosis is characterized by shrinkage of the cell, reorganization of the nucleus, active membrane blebbing, and fragmentation of the cell into membrane-enclosed vesicles. The most potent β-lactam selected in this study, was able to induce apoptosis in human leukaemic (Jurkat T), breast (MCF7, MDA-MB-231), prostate (PC-3), and head and neck (PCI-13) cancer cell lines. The best results observed were those associated with apoptosis in human leukaemic (Jurkat T) cells where the β-lactam bearing the *o*-chlorophenyl and N-thiomethyl moieties **39** achieved an IC_{50} value of 32μM (Figure 1.18).

After initial investigations by Banik *et al* on the potential of certain acyclic polyaromatic amine derivatives as novel anticancer agents it was decided to investigate the synthesis and activity of a cyclic analogue based on the β-lactam skeleton.[34] The potent biological effects of the commercially available β-lactams compared to similar acyclic

systems has long been known to be linked to the reactivity imparted by ring strain associated with β-lactam based structures. It was hoped that the combination of some of the structural elements shown to be effective in the acyclic system (**43**, Figure 1.19) would be further enhanced in the strained β-lactam ring.

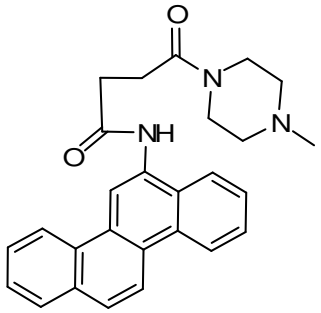


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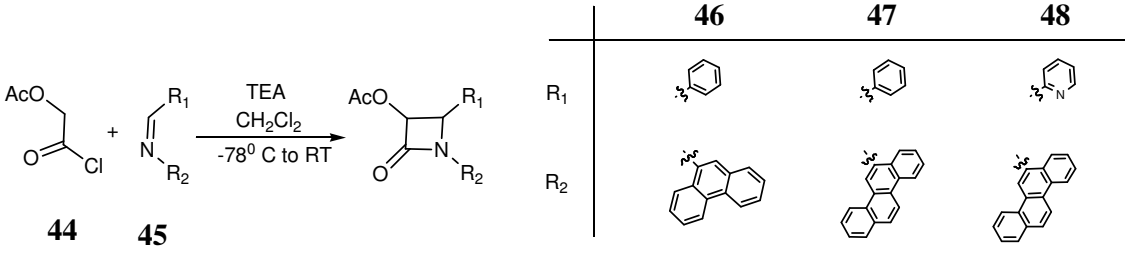
Figure 1.19 acyclic polyaromatic model systems for β- lactam synthesis

The Staudinger reaction was used to condense previously synthesized polyaromatic imines with the appropriate acid chlorides to give β-lactam as product (Scheme 1.4).[35] (Note: Syntheses of 21 azetidinone compounds with a range of polyaromatic appendages are reported; only those showing anticancer activity are described here). Although the synthesized compounds did not possess all of the elements present in the acyclic analogues, the desired β-lactam polyaromatic amines system was achieved.



Scheme 1.4 Anticancer β-lactams *via* the Staudinger reaction

In vitro anticancer testing on the synthesized compounds showed that they had good activity for a range of cancer cell lines. As anticipated, it was found that in general the β-lactam based compounds showed significantly improved activity over the similarly substituted acyclic

systems. However, no efforts were made to identify the target or the mechanism of the subsequent anti-tumour activity of these novel agents. In the tests carried out cisplatin was used as a comparison for the compounds being tested.

1.2 Alternatives to the penicillins

At a certain point in the history of β-lactam antibiotics it became clear that the rate of proliferation of resistant strains of bacteria would eventually render the search for new variations of β-lactams ineffective. This realisation gave rise to a renewed interest in developing other antibiotic molecules. The research followed two general trends. The first was the continued search for new natural products preferably with mechanisms of action differing from that of the β-lactams, thus minimising (or somewhat slowing) the development of bacterial strains resistant to the new molecules. The strategy was generally the same as that adopted for penicillin and the β-lactams, the arduous search for a lead compound with good activity followed by Structure-Activity Relationship (SAR) studies. After the elements necessary to confer activity are identified, an abundance of structurally similar substances are synthesised and assessed for any discernable change in the effects or side effects compared to the lead compound (Summary in Table 1.2). In addition to the natural product approach to drug design, there has been a history of purely synthetic drug design and testing that has led ultimately to the synthesis of innovative compounds such as linezolid.

Early synthetic Anti-biotics (Prontosil)

There is a long history of using the azo linkage in the pharmaceuticals industry. Gerhard Domagk is generally credited with the discovery of the antimicrobial potential of sulphonamides for which he received the Nobel Prize in medicine (1939). Prontosil **49**, the

world's first commercially available antibiotic was originally synthesised by Josef Klarer and Fritz Mietzsch, colleagues of Domagk at the Bayer Company in Germany. [36]

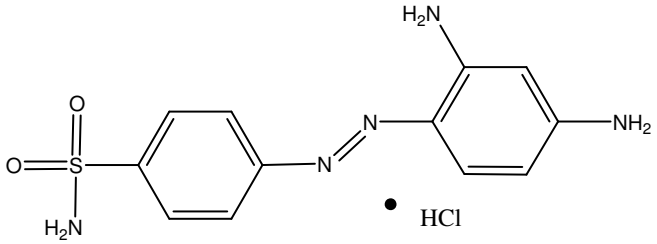


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Figure 1.20 Prontosil

In 1932 while testing a large number of dyes and azo-compounds produced by Bayer, Domagk uncovered the great potential of Prontosil as an antibiotic. It was not until 1935, after Bayer had received a patent on Prontosil, that Domagk published his results. It was quickly discovered that Prontosil was a pro-drug, and that the active part of the molecule was in fact the simple compound sulfanilamide **50** that is released upon reduction in the digestive system when taken orally.

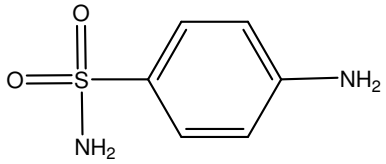

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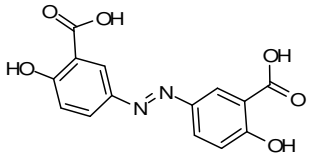
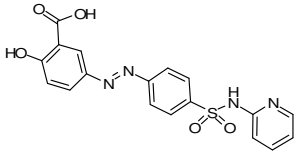
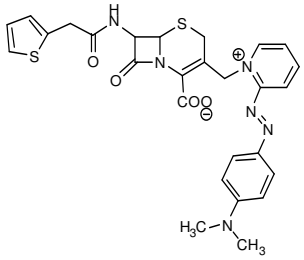
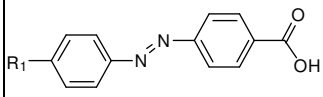
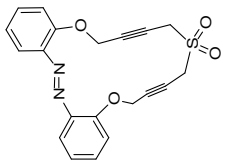
Figure 1.21 Sulfanilamide

The major indicators that Prontosil was not the direct cause of the antibiotic activity were its relative insolubility, the observed activity *in vivo* and total lack of activity *in vitro* and the fact that it was sulphanilamide that was excreted from the body and not Prontosil. Since the discovery that sulphanilamide was biologically active compound was made quite quickly, the commercial success of Prontosil was limited. Following to the development of Prontosil many useful biological compounds have been isolated and studied that utilise azo bond cleavage brought about by intestinal azo-reductase enzymes (**51, 52** and **54**, Table 1.1).

Table 1.1 Phenylazo drugs

Name	Structure	Clinical uses	Mode of action	No.
Olsalazine[37,38]		Anti-inflammatory	Azo-reductase activation	**51**
sulfasalazine[39,40]		Anti-inflammatory Anti-cancer	Azo-reductase activation	**52**
Pyridine-2-azo-p-dimethylaniline cephalosporin (PADAC) [41,42]		β-lactamase detection	β-lactamase activated	**53**
NCS 79068, R_1 = Et[43] NCS 59492, R_1 = Me		Anti-cancer	Azo-reductase activation	**54a and b**
Cyclic-phenylazo sulfone [44,45,46]		Anti-cancer	Garratt–Braverman rearrangement	**55**

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95

1.2.1 The search for synthetic alternatives

The period between 1941 and 1962 is often thought of as the golden era of antibiotic discovery. During this period it is estimated that more than 7000 compound showing antibacterial activity were discovered; the majority through the process of whole cell screening of fermentation broths of cultured organisms. However, by the late 1980s motivation to pursue this avenue of approach was low due to the large array of antibiotic drugs already on the market, the increased difficulty and cost of drug discovery by traditional methods (screening, purification and structural characterisation). During the late 1980s and

early 1990s many pharmaceutical companies abandoned the search for new antibiotics due to the abundance of generic drugs available on the market. The threat from Gram-positive bacteria was considered low during this period due to the success of vancomycin, with no signs of the resistant strains from 1956 up until 1988.

Table 1.2 Post-penicillin natural product derived antibiotics [47]

Antibiotic Class →	Aminoglycosides	Tetracyclines	Cyclic peptides	Macrolide antibiotics
Year of Discovery	1943	1945	1956	Isolated 1949 Launched commercially 1952
First Example	Streptomycin	Chlortetracycline	Vancomycin	Erythromycin
Discoverer	Selman A Waksman	Dr Benjamin Duggar Modified by Lloyd H. Conover 1952	Pittenger and Brigham	Submitted by A. Aguilar Developed by J. M. McGuire At Eli Lilly
Natural Source	actinobacterium Streptomyces griseus	Found in the soil bacteria Streptomyces aureofaciens. Sanborn fields USA	Found in the soil bacteria Nocardia orientalis in India	Streptomyces erythreus
Other examples	Gentamycin, Tobramycin, Amikacin	Doxycycline Demeclocycline Tetracycline	Streptogramins, Polymyxins	azithromycin clarithromycin dirithromycin
Mechanism of action	Bacteriostatic	Bacteriostatic	Bacteriocidal	Bacteriostatic
Mode of Action	Protein synthesis inhibitor (binds to the 23S rRNA of the bacterial ribosome)	Protein synthesis inhibitor (Binds to the 30S subunit of the bacterial ribosome)	Cell wall biosynthesis inhibitor	Protein synthesis inhibitor Inhibits elongation at transpeptidation step.
Active Against	Gram-negative bacteria, Pseudomonas, Acinetobacter, and Enterobacter	Chlamydia (Trachoma, Psittacosis, salpingitis, Non-specific urethritis and Lymphogranuloma venereum)	Staphylococcus pseudomembranous colitis	Legionella pneumophila Mycoplasma pneumoniae Anaerobic cocci & bacilli
Resistance first Observed	1959	1953	1988	1988

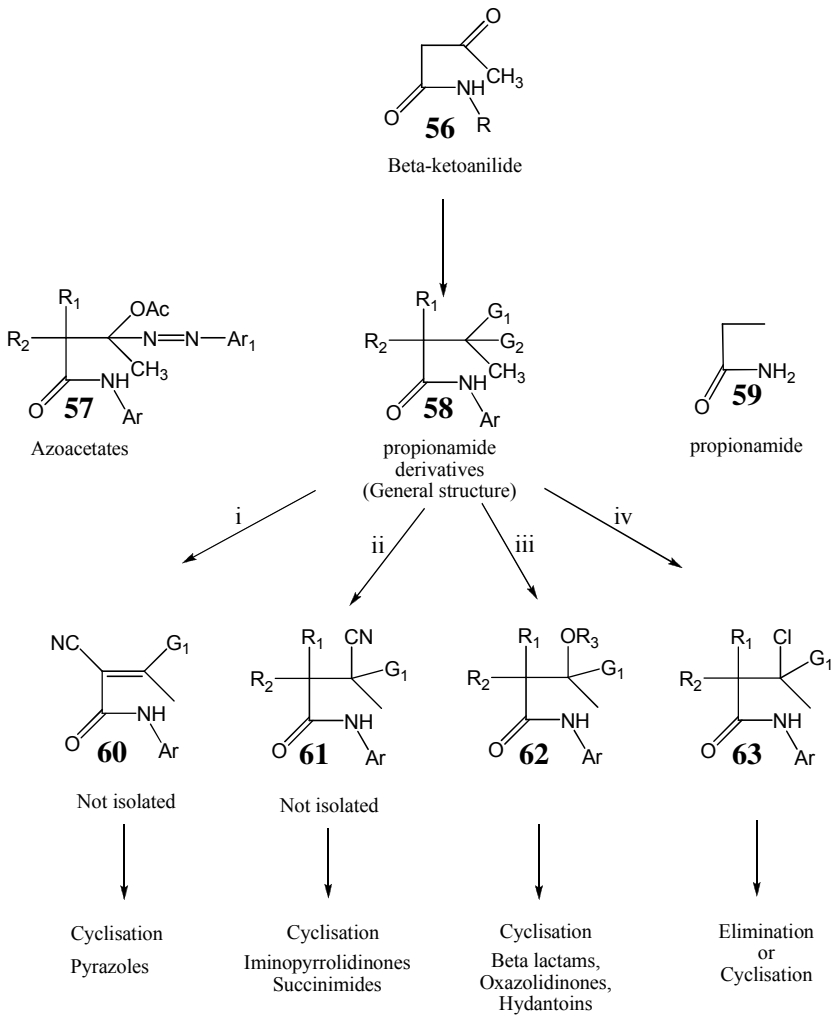
During this period greater emphasis was placed on the search for compounds active against Gram-negative bacteria. The perception by clinicians that the pharmaceutical industry had reached a point where treatments were available for the majority of serious bacterial infections was a major contributing factor in the subsequent development of resistant strains of bacteria. This period of complacency and reduced vigilance saw the emergence of VRE (Vancomycin Resistant *Enterococci),* a strain of bacteria resistant to the super drug once seen as the last line of defence in the battle against serious bacterial infections. This development in combination with the development of MRSA (Methicillin-Resistant *Staphylococcus Aureus*) does not augur well for ongoing conflict between bacteria and the treatment of

bacterial infections. The relentless use of vancomycin as the drug of choice in the treatment of MRSA produces a scenario where vancomycin resistance in the *Enterococci* realistically threatens transfer of vancomycin resistance to the *Staphylococci*, and a vancomycin- and multidrug-resistant *Staphylococcus* strain threatens to be essentially untreatable.

Another factor that has to be taken into consideration when comparing present rates of drug discovery and release with those in the 50's and 60's is the increased levels of safety and testing required in the modern era. For instance streptomycin was discovered in 1943 and was introduced to clinics in 1944, by comparison the potential antibiotic activity of oxazolidinones was discovered in 1979 however it was not until 2001 that linezolid appeared on the market as research had been totally abandoned for several years due to safety concerns regarding early versions of the drug.

1.3 Background chemistry

Throughout this thesis the cyclisation reactions of azo compounds derived from simple β-ketoesters and anilides are described, and the reactions of these compounds are novel in terms of azoacetate chemistry. However if the presence of the azo group (G_1, **51-56**, Scheme 1.5) is ignored briefly, it becomes clear that in most cases the chemistry is familiar and has been previously reported for functionalised propionamide derivatives. The majority of the azoacetates investigated in this thesis have a configuration similar to the basic propionamide structure, and these similarities have been exploited by our group in order to adapt this fundamental chemistry to our needs. The introduction of neighbouring functional groups used to facilitate these cyclisations is at the core of the work presented in this thesis. How these groups have been introduced at the appropriate positions is discussed within the relevant synthetic chapters of the thesis.



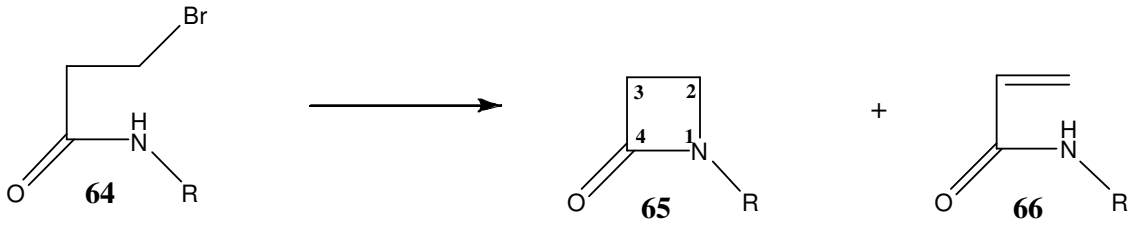
i, R_1= R_2 =H, KCN, n-PrOH, ii, R_1= R_2 =Me, KCN, n-PrOH, iii, R_1 = H R_2 =NH-CBz (Threonine derivatives), R1= R2 = CH_3 (Acetoacetanilide derivatives), KCN, n-PrOH, iv,R_1=R_2 =Me, *t*-BuOCl.

Scheme 1.5 General overview of propionamide reactions used.

However, for a fuller understanding of how these groups have been used in our work a discussion of their chemistry and how they have been previously been used will follow. In many cases variations of the chemistry of interest to us has been previously used with simpler substrates to produce useful therapeutic compounds in a diverse range of fields. It was therefore of great interest to us to capitalise on the known elements of propionamide chemistry in order to expand the range of attainable compounds based on the reactions of azoacetates rather than simple propionamides.

1.3.1 Cyclisation of halopropionamides

The syntheses presented in chapter 2 of this thesis are primarily concerned with the generation β-lactams through the N_1-C_2 bond formation. Procedures for this cyclisation using a range of leaving groups have been previously reported. Of these one of the most versatile and frequently used is the cyclisation of halopropionamides. Reports of the synthesis of β-lactams **65a** from haloamide **64a** by the treatment of strong bases have been appearing in the literature since the late 50's.[48] When the procedure was investigated using various base solvent systems in the 1960's by Manhas and Jeng they found that the desired β-lactam could be isolated with a yield of 96 % when NaH/DMSO was used. [49]

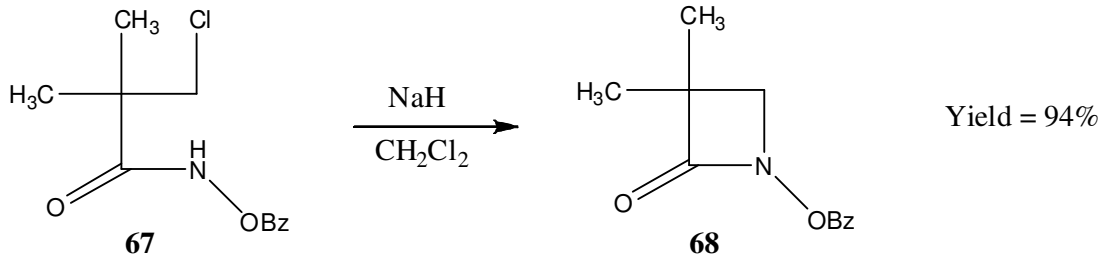


64a to **65a**, R = Ph, **65a** yield = 96 % using (NaH/DMSO)
64b to **65b**, R = CH_2-PMP, **65b** yield = 60 %, **66b** yield = 6 %, using (NaH/DMF/CH_2Cl_2)
PCP = *Para*-methoxyphenyl

Scheme 1.6 Cyclisation of halopropionamides to β-lactams

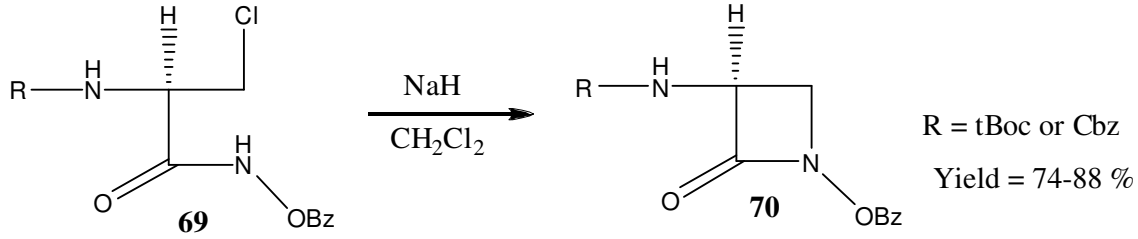
The success of these procedures is principally dependant on the relative acidity of the amido proton and the tolerance of other functional groups present to the strong basic condition. Although the formation of elimination products were not reported in this case, in general, if the amido proton is not sufficiently acidic, competing elimination reactions tend to be the predominately observed processes when other ionisable groups are present. Wasserman et al in similar studies on these systems found that when NaH in DMF/CH_2Cl_2 was used β-elimination was the predominately observed process in the majority of cases (**66**, Scheme 1.6). However after variations of the dilution, rate of addition of the substrate to the base and choice of halide as leaving group were examined, they found that the desired β-lactam (**65b**, Scheme 1.6) could be isolated as the major product.[50] A significant breakthrough in the

search for a reliable procedure for the synthesis of these systems was made when it was discovered by Miller et al that heteroatom activation of the N-H bond considerably improved selectivity for ionisation at this position. It was also found that if this was the only ionisable position the reaction gave the cyclisation product (**68**, Scheme 1.7) in very high yield.[51]



Scheme 1.7 High yielding cyclisation using heteroatom activator at N-H

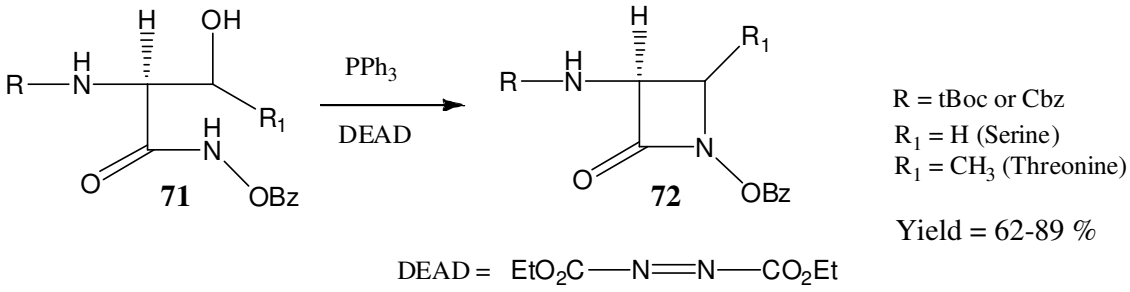
When the scope of the investigation was broadened to include systems where competition between ionisable protons was possible **69**, it was similarly found that the desired β-lactam (**70**, Scheme 1.8) products could be isolated selectively. [52]



Scheme 1.8 Stereoselective cyclisation without competitive elimination

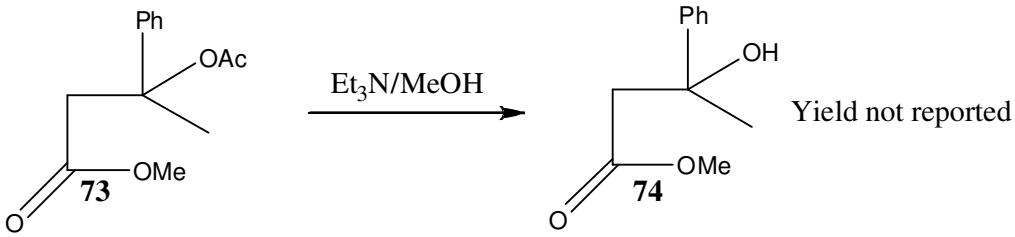
1.3.2 Cyclisation via Mitsunobu reaction

The cyclisation of β-lactams using the Mitsunobu reaction is another commonly used method of N_1-C_2 bond formation and was first reported by Miller et al in the same paper as their work with halopropionamides. When β-hydroxyhydroxamates (**71**, Scheme 1.9) were used as substrates the desired β-lactam (**72**, Scheme 1.9) could be isolated without competing elimination products and with retention of chirality.

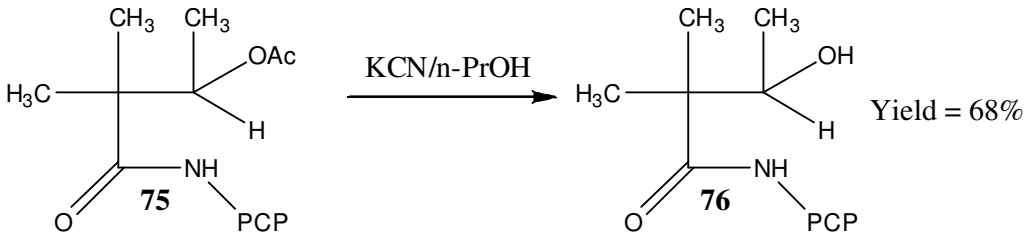


Scheme 1.9 Stereoselective cyclisation using Mitsunobu conditions.

This procedure became popular because it did not require the strong basic conditions necessary for the cyclisation of halopropionamides and therefore allowed a more diverse range of functional groups to be present before cyclisation. It was found that the procedure worked equally well with both seryl and threonyl derivatives.



Scheme 1.10 Deacetylation of 3-acetoxy-3-phenylpropanoate



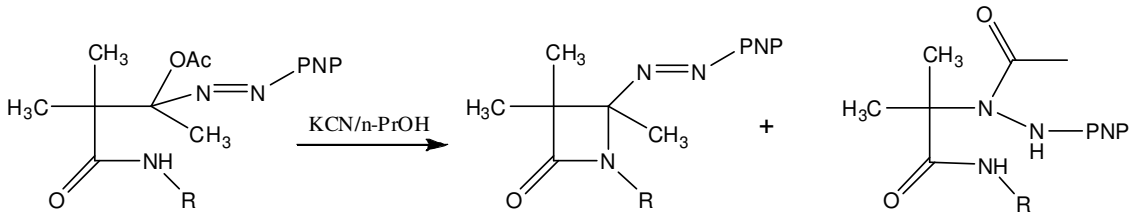
PCP = *Para*-chlorophenyl

Scheme 1.11 Deacetylation of N-(4-chlorophenyl)-2,2-dimethyl-3-acetoxybutanamide

Techniques for the generation of these hydroxy substrates through the deacetylation of the appropriate acetate analogues using triethylamine in methanol were adapted from the work of Garay, and Cabaleiro (Scheme 1.10).[53] Within our group a KCN/propanol system was used to produce similar substituted 3-hydroxyproipinamide derivatives (Scheme 1.11).[54]

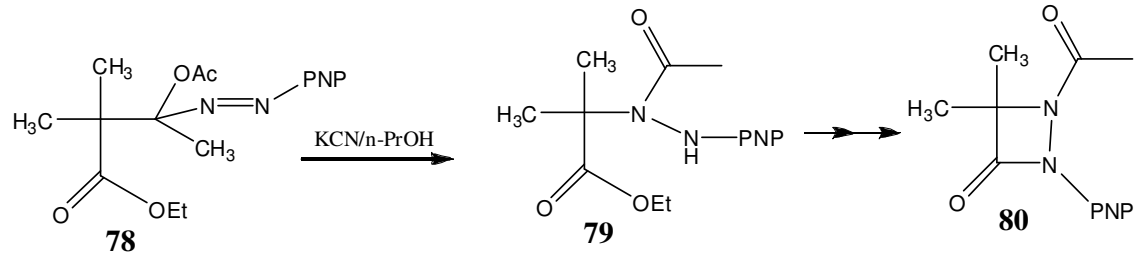
1.3.3 Cyclisation of azoacetates

When the deacetylation procedures described in the previous section were applied to the azoacetates **133** of interest to our group it was found that the substrate underwent lactamisation to **134** (Note: See chapter 2 for details of synthesis of **133** and **134**), accompanied by rearrangement to an N-acyl hydrazide derivative (**77,** Scheme 1.12). The rearrangement product **79** isolated from the reaction of the azoacetate ester **78** was subsequently cyclised (in several steps) to form an aza-β-lactam (**80,** Scheme 1.13).



133 f or j **134 f or j** **77f or j**

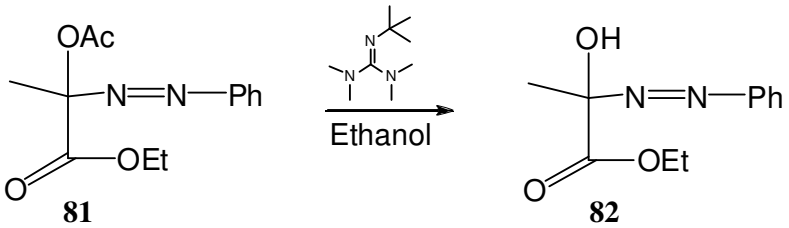
f, R = Ph or **j**, PCP, (PCP = *Para*-chlorophenyl, PNP = *Para*-nitrophenyl)
Scheme 1.12 Lactamisation and rearrangement of azoacetates anilide derivatives



78 **79** **80**

Scheme 1.13 Rearrangement of azoacetates ester derivatives

The rearrangement resulting in the N-acyl hydrazide is thought to have come about after initial deacetylation followed by a 1,2 carbon to nitrogen migration. Also, the rearrangement was found to occur irrespective of whether an ester or anilide was present. These results were published in a brief communication by the group in 1992.[55]



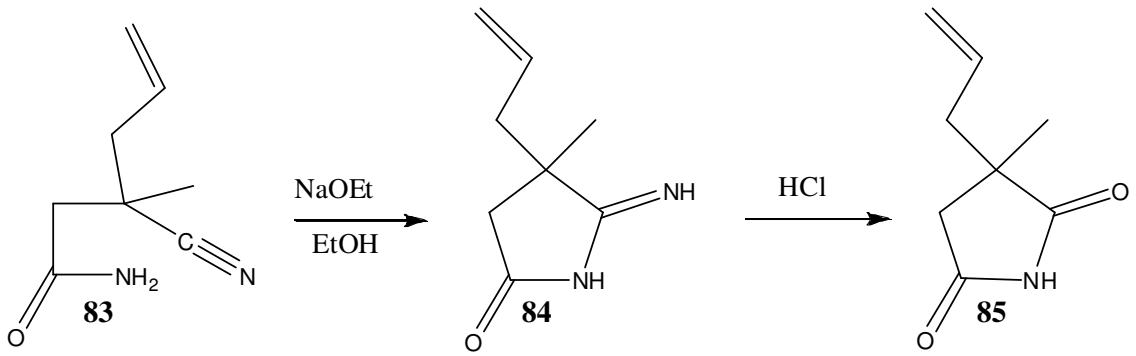
81 **82**

Scheme 1.14 Barton deacetylation of azoacetate

Although the reaction was assumed to proceed via initial deacetylation, the azocarbinol intermediate was not isolated. However in studies carried out by Barton et al on similar substrates it was found that by using a sterically hindered base (2-*t*-butyl-1,1,3,3-tetramethylguanidine) it was possible to isolate the azocarbinol product (**82,** Scheme 1.14) of the deacetylation.[56]

1.3.4 Cyclisation of cyanoamide to 2-iminopyrrolidin-5-ones and succinimides

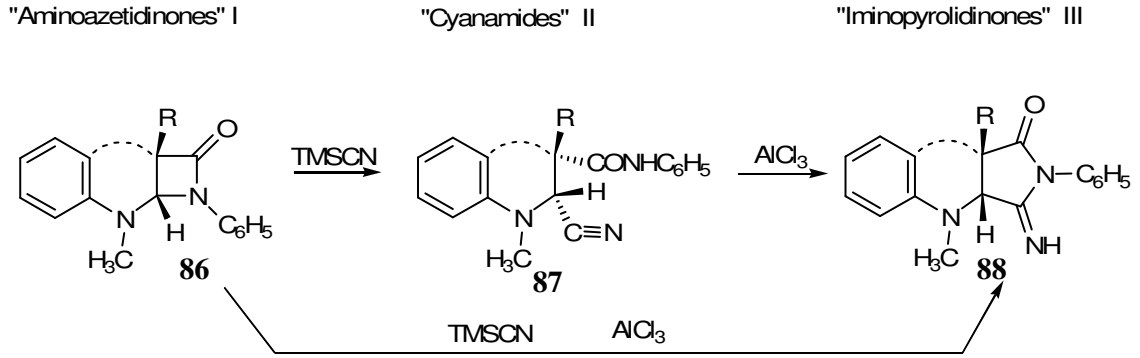
The use of relatively simply substituted 3-cyanopropionamide derivatives in the construction of succinimide rings has been previously reported (Refer to reactions of **61** in scheme 1.5). While investigating biosynthetic research on vitamin $B_{12,}$ Battersby and Westwood identified 2-(2-methoxycarbonylethyl)-3-methoxycarbonylmethyl-3-methylsuccinimide, (usually called the ring-B imide) as a key building block and set about its synthesis.[57] Central to this synthesis was the construction of the succinimide ring **85**. Several routes to these compounds were investigated including via initial formation of 2-iminopyrrolidin-5-one **84** from the appropriate cyanamide **83** followed by acid hydrolysis to the succinimide **85**.



Scheme 1.15 Barton deacetylation of azoacetate

Another reaction mechanism was investigated by Nisole *et al*. The ring expansion of some β-lactams (**86**, Scheme 1.16) has been reported using trimethylsilyl-cyanide (TMSCN) in the presence of $AlCl_3$ in a one-pot synthesis of 2-iminopyrrolidin-5-ones (**88**, Scheme 1.16).

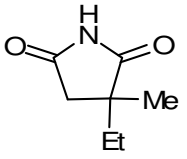
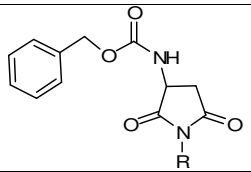
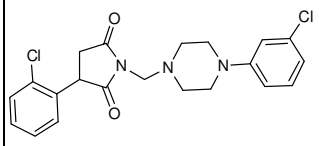
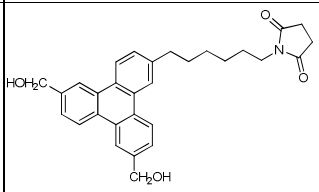
However the stepwise reaction involving initial formation of a cyanamide (**87**, Scheme 1.16) using (TMSCN) followed by cyclisation using $AlCl_3$ was also investigated. [58]



Scheme 1.16 One and two step reaction pathways from β-lactam to 2-iminopyrrolidin-5-one

Succinimide (pyrrolidine-2,5-dione) derivatives have been investigated in a wide and diverse range of roles within the field of medicinal chemistry as well as being commonly encountered as synthetic intermediates.

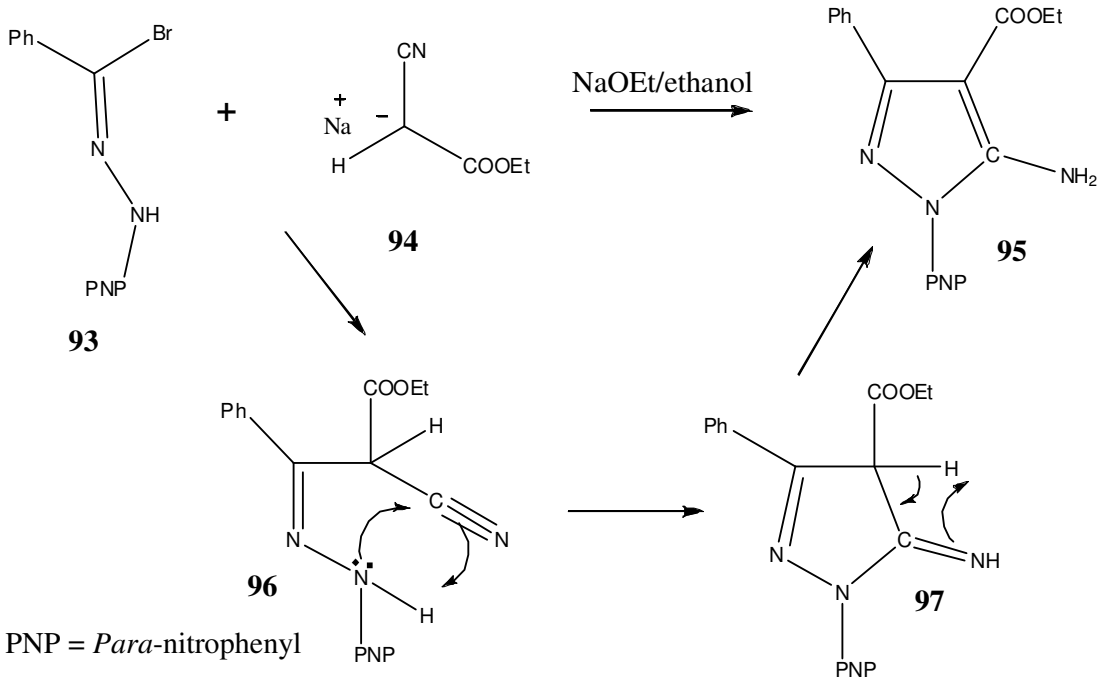
Table 1.3 Succinimide based drugs

Name	**Structure**	**Clinical uses**	**Mode of action**	**No.**
Ethosuximide[59,60,61] (Zarontin® Emeside®)		anticonvulsant agent	T-type calcium channel blocker	**89**
Succinimide-3-benzylcarbamate[62]		anticonvulsant agent	unknown	**90**
3-(2-chlorophenyl)-1-((4-(3-chlorophenyl) piperazin-1-yl)methyl) succinimide[63]		anticonvulsant agent	unknown	**91**
TDS2[64]		HIV protease inhibitor	Tat HIV-1 inhibitor[65]	**92**

Within the medicinal chemistry sphere, succinimides are probably best known as anticonvulsant agents (**89**, **90** and **91** Table 1.3).[66] However they have received some attention recently as a possible source of HIV protease inhibitors (**92,** Table 1.3).

1.3.5 Pyrazole synthesis

The synthesis of 5-amino pyrazoles **95** has been previously achieved through the condensation of ethyl cyanoacetate **94** with a hydrazidic halide **93** in a sodium ethoxide/ethanol system followed by spontaneous cyclisation (Refer to reactions of **60** in scheme 1.5).[67]

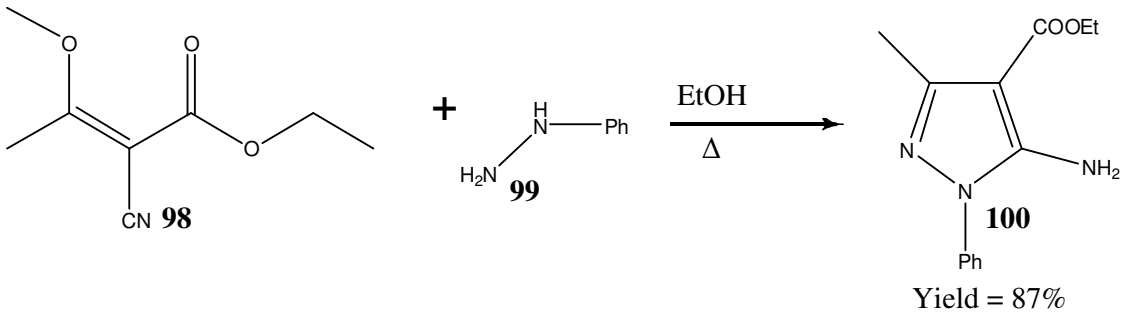


PNP = *Para*-nitrophenyl

Scheme 1.17 Pyrazole synthesis from ethyl cyanoacetate/hydrazidic halide condensation

More recently the synthesis of similar compounds (**100,** Scheme 1.18) have been reported using the more commonly reported type of condensation involving condensation of a hydrazine with an appropriate ketone,[68] in this case involving (Z)-2-Cyano-3-methoxy-but-2-enoic acid ethyl ester **98** and phenylhydrazine **99**.[69] Pyrazoles and their derivatives are an important class of heteroaromatic ring systems that find extensive use in the pharmaceutical and agrochemicals industries. Substituted pyrazoles are important synthetic targets because

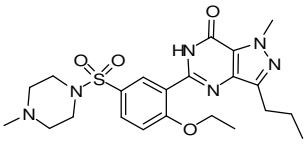
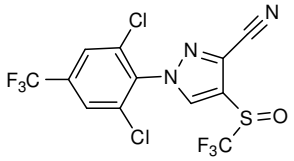
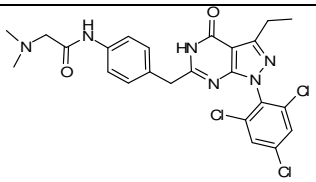
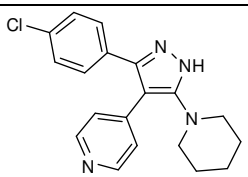
the pyrazole ring makes up the core structure of important biological compounds including drugs such sildenafil (Viagra™, **101,** Table 1.4)



Scheme 1.18 Pyrazole from phenylhydrazine

Another important medicinal role to have emerged for pyrazole based systems (**104,** Table 1.4) is that of inhibitors of the p38 mitogen-activated protein (MAP) kinase. Since the discovery of p38 kinase, it has been intensively pursued as a target for the development of disease-modifying antirheumatic drugs (DMARDs).

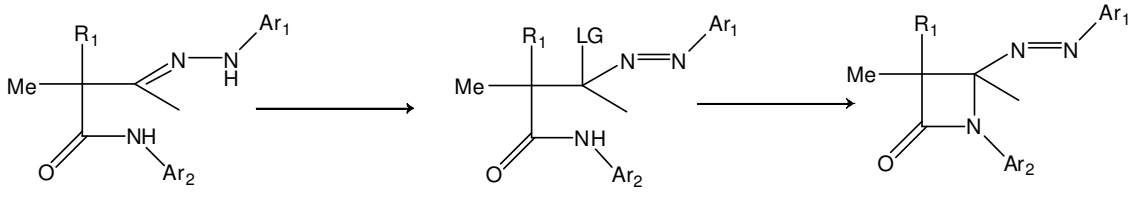
Table 1.4 Examples of biologically active pyrazoles

Name	**Structure**	**Clinical uses**	**Mode of action**	**No.**
sildenafil [70] Viagra™		Anti-impotence	PDE5 enzyme inhibitor	**101**
Fipronil®[71,72]		insecticide	GABA-gated chloride channel inhibition	**102**
2-(dimethylamino)-N-(4-((3-ethyl-4-oxo-1-(2,4,6-trichlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)methyl)phenyl)acetamide[73,74]		Anti-cancer	CDK4/cyclin D1 inhibitor	**103**
4-(3-(4-chlorophenyl)-5-(piperidin-1-yl)-1H-pyrazol-4-yl)pyridine[75,76]		disease-modifying antirheumatic drugs (DMARD)	p38 MAP kinase inhibitor	**104**

1.3.6 Thesis statement

As introduced in the previous section, our group had identified that certain azoacetates could be cyclised to form β-lactams with a phenylazo group attached through reactions similar to those commonly used in the cyclisation of N-aryl propionamide derivatives. Though the reactions of our group used the acetate moiety as the leaving group the realisation that the cyclisation was unaffected by the presence of the phenylazo group prompted the search for propionamide cyclisation reactions that might be similarly adapted. After a review of the relevant literature, several potentially suitable reactions were identified. Of particular interest to our group were those reactions resulting in the generation of small heterocycles from families of compounds known to exhibit medicinal effects.

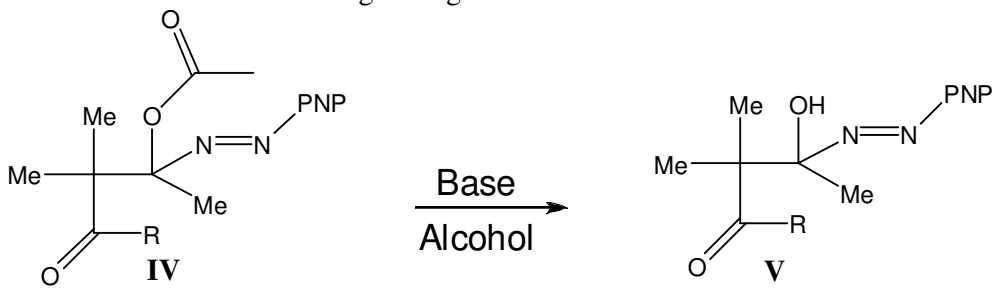
Our initial interest was on follow-up work on the original proof of concept studies. It was anticipated that if a series of phenylazo compounds containing the basic N-aryl propionamide structure could be synthesised that these compounds might provide a starting point for a series of studies investigating the cyclisation to the β-lactam. A review of the relevant literature suggested that known reactions from the chemistry of hydrazones provide a means of introducing potential alternative leaving groups (LG, **II**, scheme 1.8) while preserving the phenylazo group intact. The introduction of other attachments of interest (R_1, Ar_1, Ar_2, scheme 1.8) is made possible by making simple alterations to the reaction sequences used by the original researchers. The β-lactam syntheses reactions are dealt with in chapter 2 of the thesis.



I **II** **III**

Scheme 1.19 General scheme outlining β-lactam synthetic strategy

The other important discovery identified by the original researchers was a rearrangement product that accompanied the β-lactam cyclisation process. It had been proposed that the reaction took place via initial deacetylation followed by a 1,2 carbon to nitrogen migration. However several years after the publication of our groups' results (1992) a group led by Sir Derek Barton (1996) published a paper involving the deacetylation reactions of similar azoacetates under similar reaction conditions (i.e. base and alcoholic solvent, scheme 1.14). [56] However in that case it was found that the isolation of azocarbinol products was possible. This encouraged an interest in re-examining the deacetylation of our azoacetates with a view to isolating analogous azocarbinols from our azoacetate substrates.



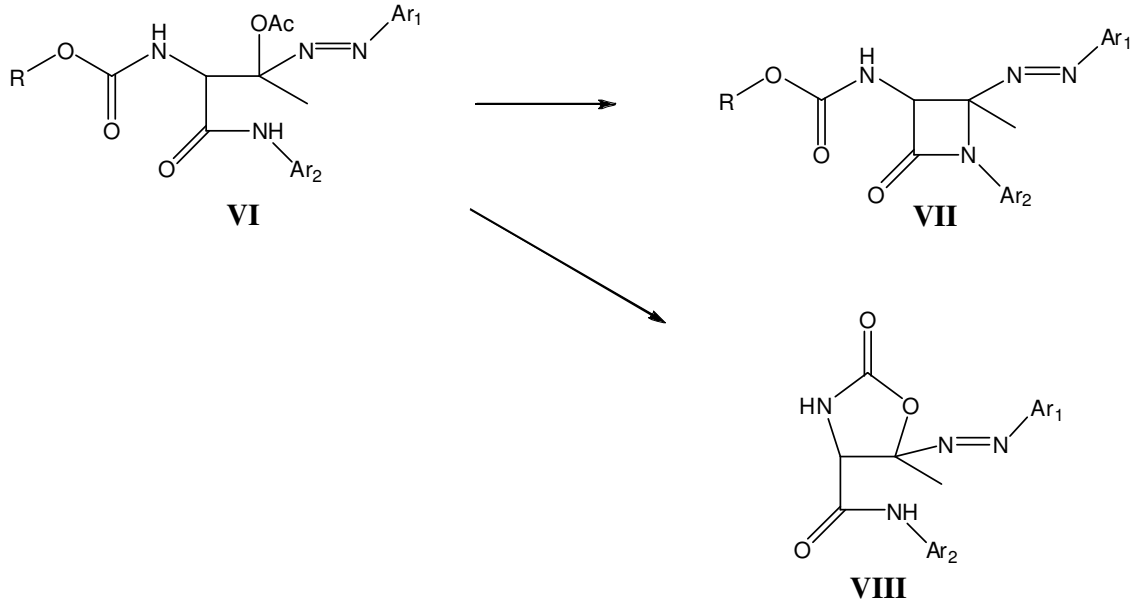
R = Ph, PCP or OEt (PCP = *Para*-chlorophenyl, PNP = *Para*-nitrophenyl)

Scheme 1.20 Proposed route to azocarbinols

The aim of this section of the research (chapter 3) was to explore whether or not it would be possible to isolate azocarbinols from our azoacetates substrates using reaction conditions previously found to be successfully used for this transformation.

The second part of chapter 3 is concerned with the further exploration of the deacetylation reactions of azoacetates in an environment where cyclisation to oxazolidinones was the most likely outcome. From an examination of what is known about the cyclisation of simple protected threonyl anilides it seemed that the most likely outcome of the base treatment of these compounds might be the formation of β-lactams or oxazolidinones, both possibilities were of interest to our group. The synthesis of β-lactams (**VII**, scheme 1.21) would expand the range of phenylazo substituted examples of these compounds synthesised

by our group. The synthesis of oxazolidinones (**VIII**, scheme 1.21) would be another example of the deacetylation of the acetate group in azoacetates. Cyclisation reactions of protected threonyl compounds in general are well known, but again the cyclisation reactions of analogous azoacetate compounds **VII** have not been previously studied.



Scheme 1.21 Interesting potential cyclisation reactions of threonyl azoacetates

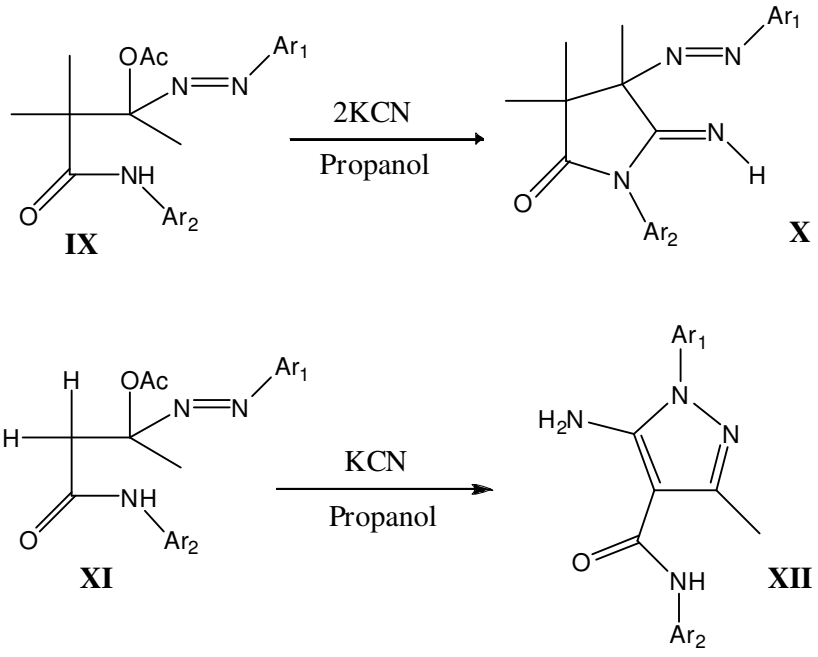
The synthesis of the azoacetates (**VI**, scheme 1.21) necessary for cyclisation studies was not trivial. Neither the peptide chemistry literature nor previously published hydrazone chemistry give specific information as to the full synthesis of these compounds. To the best of our knowledge investigations into the oxidation of hydrazones to azoacetates of this type (**VI**, scheme 1.21) has never before been undertaken. These azoacetates synthesised from N-protected threonyl anilides, once again, have the central propionamide shape (Scheme 1.5) that has been the focus of our other work.

The many repetitions of the cyclisation reaction used in the synthesis of β-lactams led to the discovery that a further product could be isolated from the reaction. Chapter 4 of this thesis focuses heavily on the isolation, identification and characterisation of these newly discovered phenylazo substituted 2-iminopyrolidin-5-one derivatives (**X**, scheme 1.22). The

formation of these compounds was unexpected as it had not been reported by members of our group carrying out similar studies. It would seem that the ratio of KCN to substrate in the reaction vessel drastically affects the role of the cyanide ion in the reaction. Higher concentrations of KCN in the reaction mixture were found to favour the formation of nucleophilic substitution products with a unit of cyanide being incorporated into the product (Scheme 1.22).

2-iminopyrolidin-5-ones are most commonly encountered in the chemistry literature as precursors to succinimides (Scheme 1.15). This synthetic exploitation of the 2-iminopyrolidin-5-ones in that role was also of interest to us due to the many reported medicinal applications of succinimides.

The 2-iminopyrolidin-5-ones **X** initially proved difficult to characterise from their spectra. The NMR spectra proved particularly difficult to interpret due to unusual line broadening and signal dampening effects observed in the both the ^{1}H NMR and ^{13}C NMR spectra. These unusual spectra and the measures taken to elucidate the structure of the products from them are one of the main focal points of chapter 4.



Scheme 1.22 Main reactions from chapter 4

The discovery that cyanide can act as a nucleophile in substitution reactions with azoacetates prompted the assessment of other azoacetates under similar conditions, ultimately producing pyrazole products (**XII**, Scheme 1.21). The incorporation of the cyanide unit in both of these systems (2-iminopyrolidin-5-ones **X** and pyrazole **XII**, Scheme 1.21) are novel results in terms azoacetate chemistry and therefore have been studied in some detail.

1.4 Conclusion

The development of antibiotics effective against pathogenic bacteria has gone through several phases of evolution, prompted initially by pure necessity and more recently as a result of the development of highly virulent and resistant strains of bacteria. The process of drug discovery in this area has seen a shift in focus from the traditional sources of lead compounds. The aim of this chapter has been to highlight the diverse range of medicinal roles attributed to heterocyclic compounds similar to the compounds synthesised and characterised within this thesis. It has also been shown that one of the responses to modern requirements has been the reinvestigation of known classes of compounds in previously unexplored roles.

From a review of the literature it seemed clear that there was great potential to adapt reactions that are relatively well known in the chemistry of propionamide for use with the azoacetates of interest to our group. The chemistry of these compounds dealing specifically with the azoacetate group has been thoroughly explored within our group. However the structural similarity between the specific azoacetates of interest to us and functionalised propionamides allowed us to begin the investigation of the reactions of these compounds from a different perspective than the previous studies.

Of interest amongst the reactions reviewed were those involving cyclisations of propionamides as these might provide further insight into the formation of the products previously published by our group.[44] The deacetylation reactions of azoacetates were also of

interest since it was suspected that this reaction plays a role in the formation of the novel N-acylhydrazide rearrangement product published by our group. The process was chosen for further study because since the original work had been carried out, several insightful and relevant works dealing with similar systems had been published by other groups. [56]

Chapter 2

Synthesis and characterisation of the novel phenyl-azo bearing β-lactams

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contraria contrariis curantur

2.1 Introduction

The focus of the studies carried out in this chapter is the further development of work done towards the synthesis of β-lactams from azoacetates by previous members of our group as introduced in chapter 1. The original researchers had proved the concept that substituted azoacetates derived from acetoacetanilides could be cyclised to give β-lactams using the acetate moiety as leaving group. However the work resulted in the production of only two structurally similar examples of these compounds (**134**, Chapter 1) and until now no further studies had been undertaken. The syntheses of molecules incorporating the azo linkage have proved to be attractive targets for prodrug synthesis due to the affinity of this group for reduction upon the action of intestinal azo-reductase enzymes (Chapter 1).

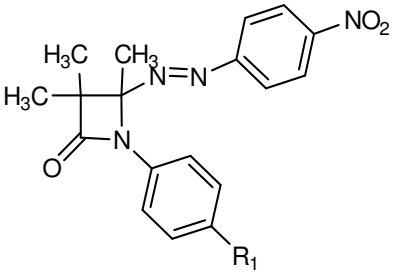
Due to the novel nature of these compounds they seemed worthy of further study. The area of most interest to us initially was the further investigation of the early sequence of reactions ultimately leading to the cyclisation of the β-lactam (Scheme 2.1). Each of these steps involves the introduction of new groups into the system, and each is a relatively well known reaction. This offered the opportunity to investigate the introduction of groups alternative to those previously used, and also the potential for the expansion of a new series of compounds into a library for future biological testing. With similar motivation, the modification of the phenylazo group on the β-lactam to the corresponding phenylazoxy moiety was undertaken. This is a structure that, to date, has not been previously reported in the medicinal chemistry literature.

Investigations into the use of alternative leaving groups for the cyclisation were also carried out in the hopes of improving the existing procedure. Several potential alternative substrates for cyclisation were synthesised, in particular it was hoped that the well documented procedures for cyclisation of halopropionamides could be adapted for use with our system.

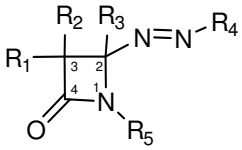
2.2 Results and Discussion

2.2.1 Synthetic strategy towards β-lactam analogues

The synthesis of monocyclic β-lactams bearing the phenyl-azo functional group had been previously achieved, however, it remained unclear whether not the range of compounds synthesised could be expanded. This chapter includes work to investigate some of these issues. Examination of the basic structure previously investigated allows for functional group diversification within the system (Figure 2.1).

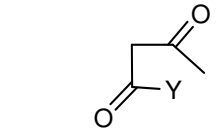


134f, R_1 = H
134j, R_1 = Cl
Previously made β-lactams



105

Core β-lactam structure showing possible points for new group insertion ($R_1 - R_5$)



106 Y = OEt
107 Y = HN R

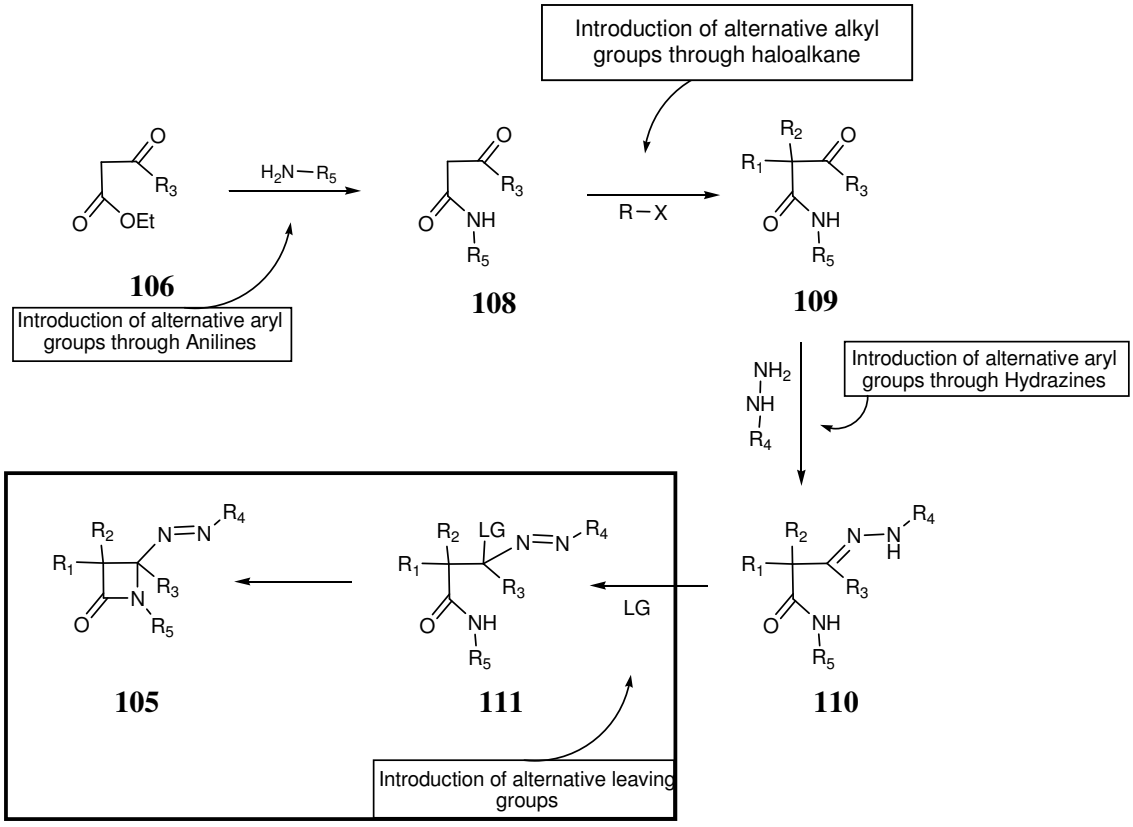
Starting materials

Figure 2.1 Overview of target structures compared to starting materials.

It was decided to attempt to systematically alter the groups present at each position. It seemed prudent to initially attempt to modify the substituents of the system individually whilst keeping the remaining substituents as close to the original structure as possible.

The aim of the original research (Schemes 1.12 and 1.13) was to develop procedures to synthesise potential biological molecules using facile procedures and simple, cheap and readily available starting materials.[55] For this reason it was decided that the methyl group at ring position 2 (**105** $R_3 = CH_3$ Figure 2.1) would remain unchanged since it was an inherent element of both the ethyl acetoacetate and acetoacetanilide starting materials. Whereas raw materials analogous to the acetoacetanilide starting materials with alternative groups at this

position are available commercially, they are significantly more expensive and less readily available (in SigmaAldrich™ rare chemicals library).



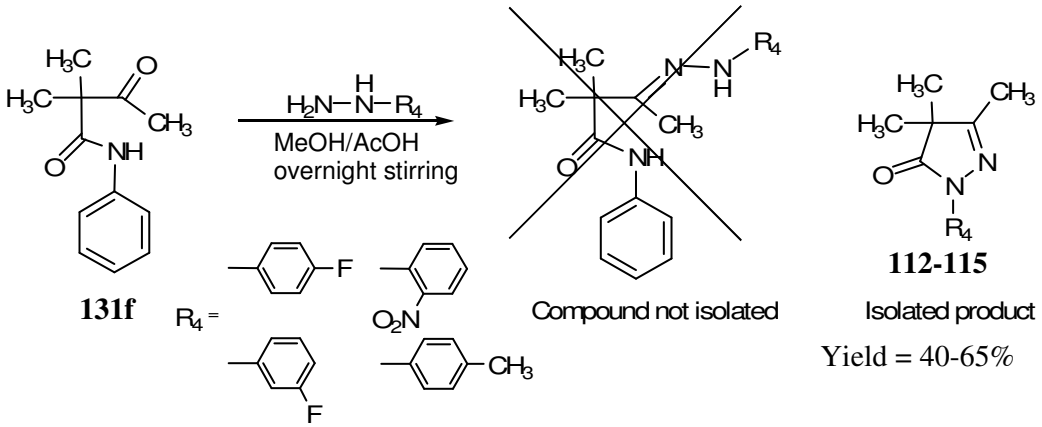
Scheme 2.1 Synthetic route to β-lactams.

Scheme 2.1 shows a general synthetic pathway to the β-lactams of interest. Highlighted are the targeted insertion points for the introduction of functionalities alternative to those previously investigated. The introduction of different leaving groups and the cyclisation will be dealt with separately as these processes ultimately do not lead to any change in the groups present in the final product. Since the synthetic steps used to produce the original β-lactams had been tried and tested, it was decided that the strategy adopted would be the one that adhered most closely to the original procedures. For this reason the processes where functional group diversity was introduced last were the first to be tested.

Introduction of alternative functional groups at R_4 (Figure 2.1)

The first challenge undertaken was an attempt to alter the groups present on the phenyl azo ring system (R_4). To this end a simple di-methylated analogue of acetoacetanilide (**131f,** scheme 2.2) was synthesised as a starting point (synthesis of **131f** and analogous compounds described in section **2.2.4**). At this point attempts were made to synthesise a number of phenyl-hydrazone derivatives of this basic structure by substituting a variety of phenyl hydrazines into the synthetic procedure in place of the 4-nitrophenyl hydrazine hitherto used throughout.

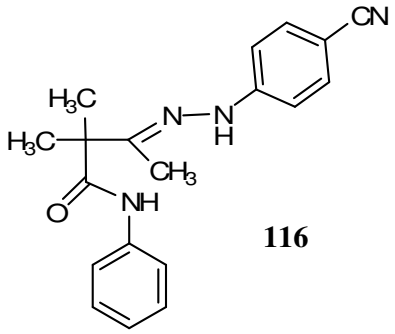
This approach yielded mixed results; it was found that in the majority of cases that the product formed was not the desired hydrazone, but in fact a cyclised by-product of the hydrazone reaction. The creation of the pyrazolone derivatives is due to cyclisation of the hydrazone immediately upon formation. This is a well known detrimental reaction to the formation of phenylhydrazones from β-ketoesters.[77] The formation of these compounds was investigated in some detail by previous researchers and were not explored further. [78]



Scheme 2.2 Attempted synthesis of hydrazones resulting in pyrazolones.

The only successfully isolated hydrazone from this set of experiments was the 4-cyano analogue (**116**, Figure 2.2). Interestingly this seems to suggest an important role for the strong electron withdrawing group in a position *para*- to the azo group for the stabilization of

the newly formed hydrazone.[79] Especially in light of the failure to isolate the *ortho*-nitro substituted species which might have been expected to yield similar results to the *para*-nitro analogue. It would seem that the acidic conditions necessary for the condensation of the hydrazine with the substituted acetoacetanilide favours spontaneous cyclisation unless the electron withdrawing group is in a *para* position. Also of note is the dominance of the cyclisation even when an acetoacetanilides are used as substrates as opposed to β-ketoesters.

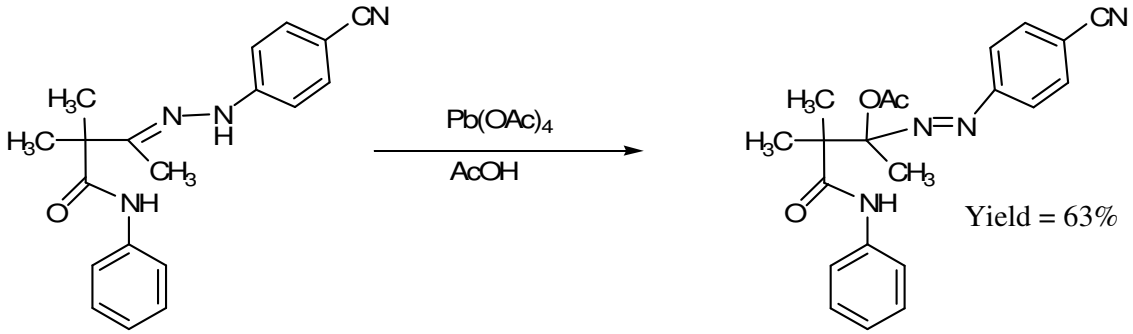


116

Yield = 60%

Figure 2.2 4-Cyanophenylhydrazone condensation product.

The subsequent formation of the corresponding azoacetate was also achieved using the procedure employed previously for the generation of the analogous 4-nitrophenyl derivatives.[78] Stirring the substrate overnight with a 1.1 molar excess of lead tetraacetate gave the desired azoacetate (**117**, Scheme 2.3).[80]



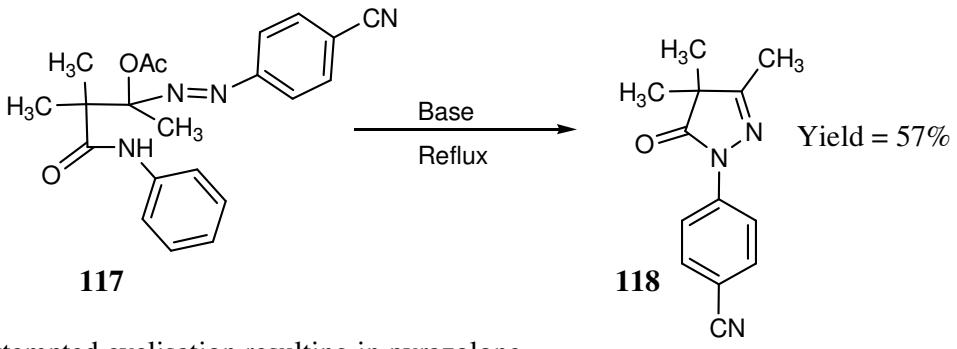
116 **117**

Yield = 63%

Scheme 2.3 Azoacetate of 4-cyanophenylhydrazone.

Attempts at the cyclisation of the azoacetate **117** to the β-lactam using the previously established base induced ring closure produced the pyrazolone (**118**, Scheme 2.4) analogous

to those previously observed (**112-115**) to form from the other substituted hydrazones. Similar transformations from azoacetates have also been previously observed by Gladstone *et al*.[81] Attempts at altering the basic media (K_2CO_3/Acetone, KCN/Propanol and *t*BuOK/Acetonitrile) failed to produce the desired β-lactam.

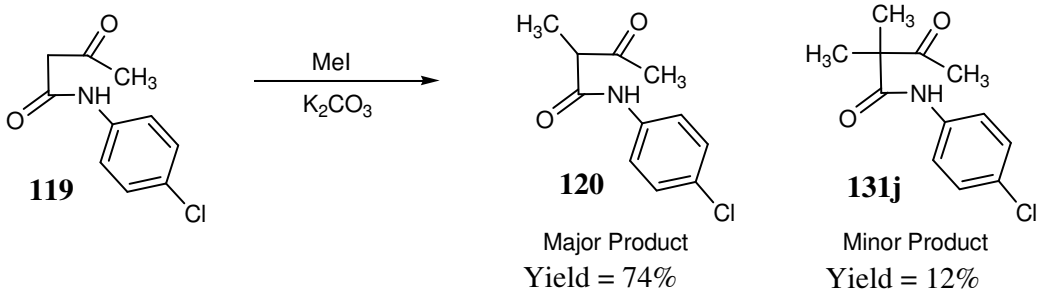


117 **118**

Scheme 2.4 Attempted cyclisation resulting in pyrazolone.

2.2.2 Introduction of alternative functional groups at R_2 and R_3 (Figure 2.1).

The introduction of an aryl group at position R_2 or R_3 was seen as the next logical step to test the robustness of the system towards modifications. The benzyl group was chosen over a phenyl substituent due to the advantage of a carbon spacer reducing the possibility of steric factors having a detrimental effect on the reaction or subsequent reactions in the series. As a starting point a mono-methyl analogue of 4-chloroacetoacetanilide was prepared. Mono-methyl chloroacetoacetanilide was prepared by slow addition of 1 molecular equivalent of methyl iodide to a refluxing solution of the substrate in acetone with 1 molecular equivalent of K_2CO_3 suspended. It was found that a reasonable yield (71%) of the desired product (**121** Scheme 2.5) could be isolated from the dimethyl by-product after several recrystallizations.

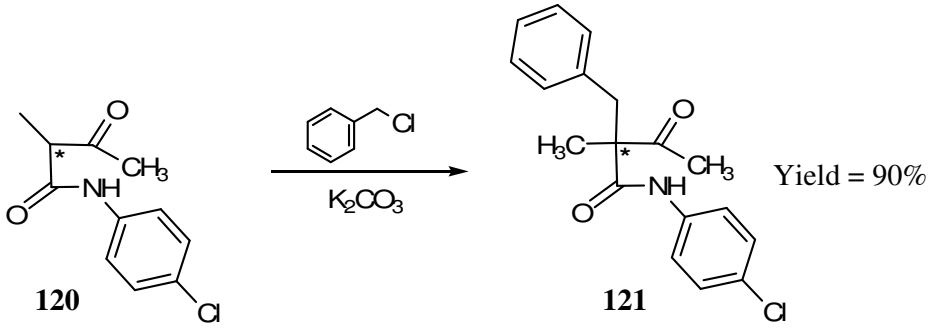


119 **120** **131j**

74.4% Major Product Yield = 74% Minor Product Yield = 12%

Scheme 2.5 Monomethylation of 4-chloroacetoacetanilide

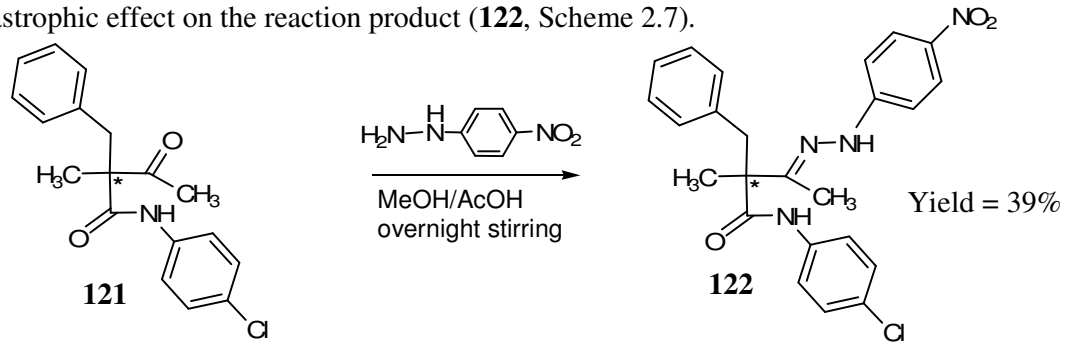
Reaction of the mono-methylated species under similar alkylating condition using benzyl chloride produced the desired benzylated product (**120**, Scheme 2.6). The introduction of this group saw the creation of a chiral centre that would be present in the final cyclisation product.



120 **121**

Scheme 2.6 Benzylation of α-methyl-4-chloroacetoacetanilide

In line with the synthetic strategy outlined previously (Scheme 2.1), 4-nitrophenylhydrazone analogues were used in all subsequent syntheses. The reaction conditions used previously to produce the 4-nitrophenyl hydrazone of the di-methylated compound were employed.[82] The desired hydrazone was isolated in reasonable yield (39%) after overnight stirring. Although the yield was slightly lower than that of the di-methyl hydrazones (Scheme 2.10), the steric bulk of the benzyl substituent was not seen to have a catastrophic effect on the reaction product (**122**, Scheme 2.7).

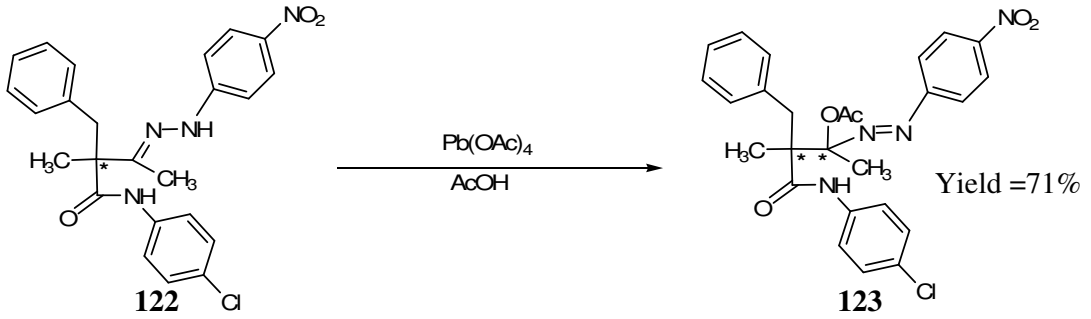


121 **122**

Scheme 2.7 Formation of 4-nitrophenylhydrazone

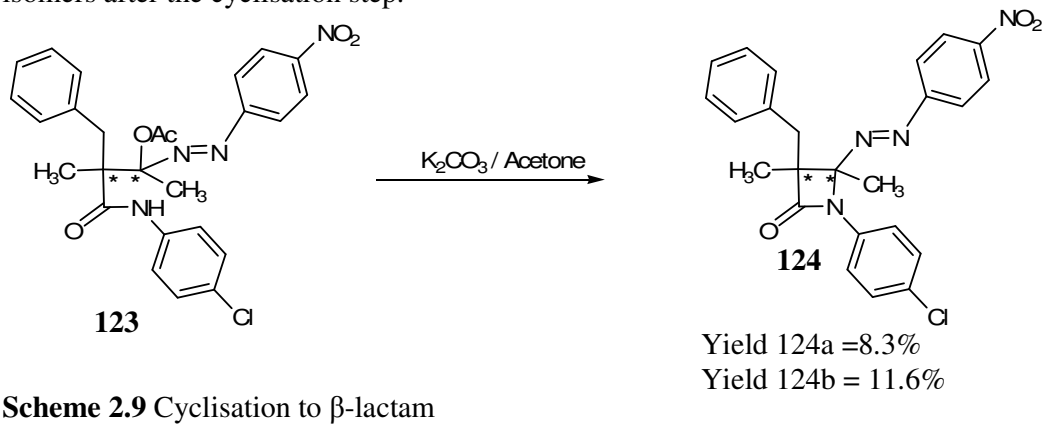
The production of the azoacetate **123** from the reaction of the hydrazone **122** with lead tetraacetate (Scheme 2.8) was synthetically consistent with the previously observed

results. A yield of 56% once again suggested that the procedure was not drastically affected by the presence of the steric bulk of the benzyl group.



Scheme 2.8 Formation of azoacetate

Since in this case the substrate leading to the formation of the azoacetate already had a chiral centre present, the product was isolated as a mixture of diastereomers. Examination of the TLC of the reaction showed the presence of two product spots with very similar R_f values. At this point a decision was made not to attempt the isolation of the individual isomeric pairs before the cyclisation step. It was deemed that the chromatographic process necessary to isolate such closely related compounds would, in all likelihood, have a drastic effect on the quantity of compound available for cyclisation. Since the products of the cyclisation (**124 a** and **b** Scheme 2.9) were unlikely to be affected by the presence of the diasteromeric mixture of starting materials it seemed prudent to attempt the separation of the isomers after the cyclisation step.



Scheme 2.9 Cyclisation to β-lactam

After the cyclisation step, the β-lactam product consisting of a diastereomeric mixture of products which could be chromatographically separated into the constituent enantiomeric pairs. The R,R and S,S enantiomeric pair and the R,S and S,R enantiomeric pair (Figure 2.4). The most prominent spectral feature that distinguished these isomeric pairs was a shift in the proton spectrum associated with the non-aromatic benzyl protons (Figure 2.3). The characteristic splitting patterns observed in these enantiotopic protons, can be observed in the precursor molecules containing this functionality. However the large chemical shift observed between the two diastereomeric forms is due to the proximity of this group to the adjacent chiral centre.

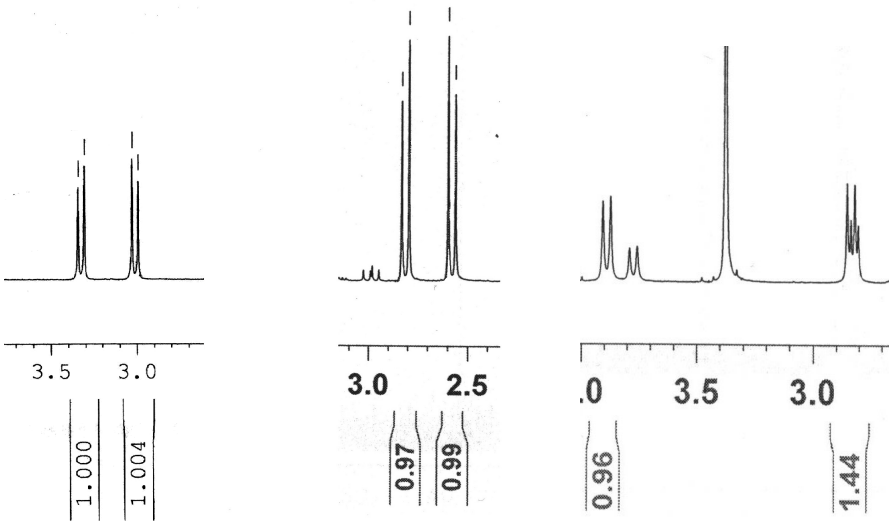


Figure 2.3 ^{1}H NMR signals of non aromatic benzyl protons of β-lactam diastereomeric pairs (**a** and **b),** and the corresponding proton signals from the mixture of azoacetate (**c**).

The conformational locking of these centres within a constrained ring system would also seem to be an important factor in the magnitude of the observed shift. As mentioned earlier the azoacetate precursor of these cyclised products were not chromatographically separated prior to the reaction. The ^{1}H NMR spectrum of this compound did show support of the presence of the two component elements of the mixture (Figure 2.3 c). This was evidenced by a relatively small chemical shift difference between the two isomeric components (Figure 2.3, **c**).

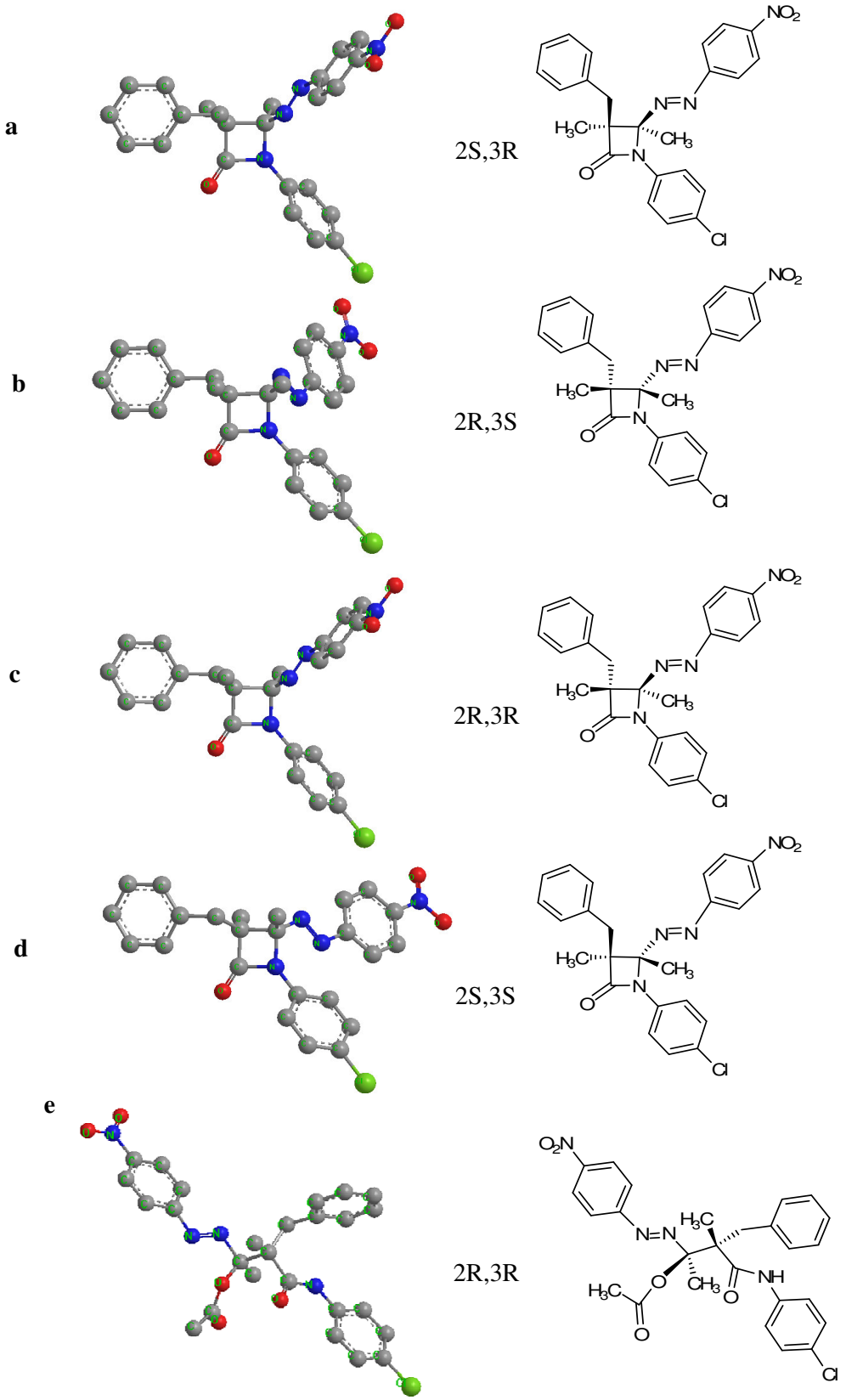


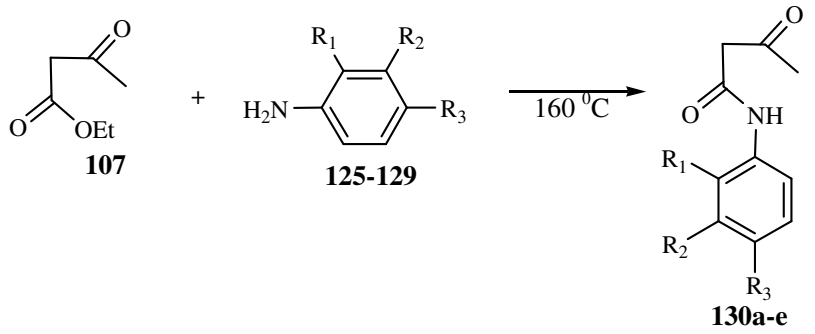
Figure 2.4 3D ball and stick models and 2D diagrams of the isomeric conformations of β-lactam **124** (**a-d**) and 1 isomer of the azoacetate precursor **123** (**e**).

The diastereomeric forms of the azoacetate did not show the same level of variation in chemical shift as the isolated β-lactam isomeric pairs (Figure 2.3, **a** and **b**), lending weight to the assertion that the rigid ring system has a marked effect on the electronic properties of the different components. This is a function of the magnetic shielding effect of the adjacent groups. The appearance of the azoacetate proton signals at a relatively low field would be expected, due the possibility of free rotation of the groups surrounding the benzyl protons in order to orientate themselves in solution to give maximum shielding effect. When the conformation is locked in a ring system the possible orientations are reduced, with essentially two possibilities, "more favourable for shielding" and "less favourable for shielding". Figure 2.4 shows the possible conformations of the β-lactam molecule, in the 3D representations it can be seen clearly that the benzyl group attached to the β-lactam molecule is restricted to orientations allowed by rotation around the benzyl CH_2. One isomer of the azoacetate precursor is also shown to highlight the possibility of rotation around the central carbon-carbon bond between the chiral centres.

2.2.3 Introduction of alternative functional groups at R_5

In contrast to the attempts to alter the phenyl substituents at R_4, introduction of alternative phenyl groups at R_5 was relatively straightforward. This involved using alternative acetoacetanilides as starting materials. Where possible commercially available acetoacetanilides were used as starting materials, the remainder were generated by simply heating the appropriately substituted aniline (**125-129**) (1 molar equivalents) with ethyl acetoacetate (**107**) (5 molar equivalents) to 160 ºC until the evolution of ethanol had ceased, after removing the bulk of the remaining ethyl acetoacetate by vacuum distillation the desired acetoacetanilides could be easily crystallized by scratching the reaction vessel (Table 2.1).[83] The crude acetoacetanilides were further purified by recrystallization from EtOAc:Hex.

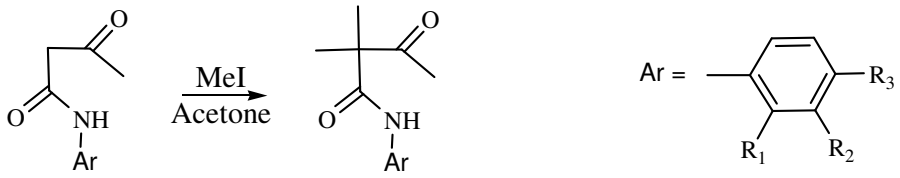
Table 2.1 Synthesis of acetoacetanilides from ethyl acetoacetate



Substrate	R_1	R_2	R_3	Product	Yield
125	H	H	Br	**130a**	90
126	H	H	OMe	**130b**	88
127	Me	Me	H	**130c**	91
128	H	H	Me	**130d**	85
129	H	H	CO_2Et	**130e**	77

Dimethylation of the acetoacetanilides (**119** and **131a-i**) was achieved by refluxing overnight in acetone with an excess of methyl iodide (6 molar equivalents) and potassium carbonate (3 molar equivalents) (Table 2.2).

Table 2.2 Dimethylation of anilides

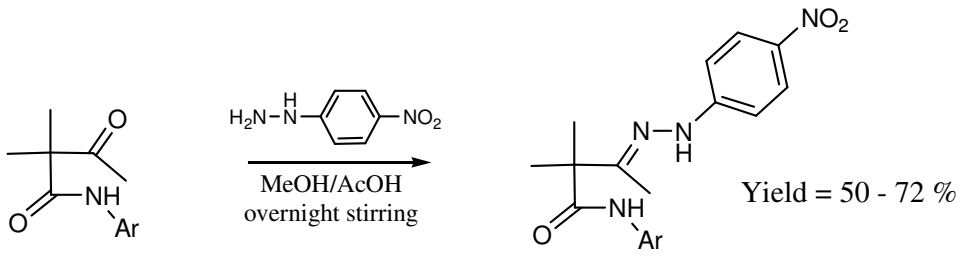


119 and 130a-i **131a-j**

Substrate	Ar			Product	Yield (%)
	R_1	R_2	R_3		
130a	H	H	Br	**131a**	79
130b	H	H	OMe	**131b**	78
130c	Me	Me	H	**131c**	80
130d	H	H	Me	**131d**	83
130e	H	H	COOEt	**131e**	70
130f [b]	H	H	H	**131f**	80
130g [b]	Me	H	Me	**131g**	67
130h [b]	Me	H	H	**131h**	75
130i [b]	OMe	H	H	**131i**	74
119 [b]	H	H	Cl	**131j**	69

[b]Starting materials were commercially available acetoacetanilides

4-Nitrophenylhydrazone derivatives were prepared for all of the α,α-disubstituted acetoacetanilides (**131a-j)** (Scheme 2.10) by stirring the substrates in a slight excess (1.1 molar equivalents) of 4-nitrophenylhydrazine in MeOH under mild acidic conditions (~5% acetic acid). This procedure routinely furnished the desired hydrazones in reasonable yields (50-72%).

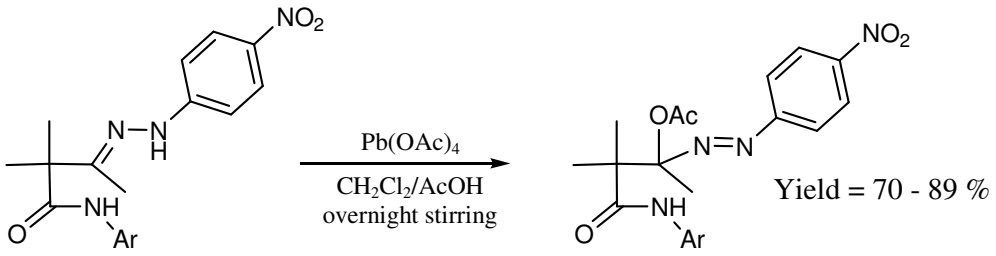


131 a-j **132 a-j**

(With reference to aromatic group, Ar, substitutions at R_1, R_2 and R_3 are as shown in Table 2.2)

Scheme 2.10 Formation of 4-nitrophenylhydrazones **132a-j**

The synthesis of the azoacetates of these hydrazones was found to be equally facile (Scheme 2.11), once again it was found that merely stirring the hydrazones overnight in a mildly acidic CH_2Cl_2 solution (10% acetic acid) containing a slight excess (1.1 molar equivalents) of $Pb(OAc)_4$ was sufficient to effect the desired transformation. After a standard work-up, flash chromatography was used to separate the azoacetates from any persistent lead salt residues.



132 a-j **133 a-j**

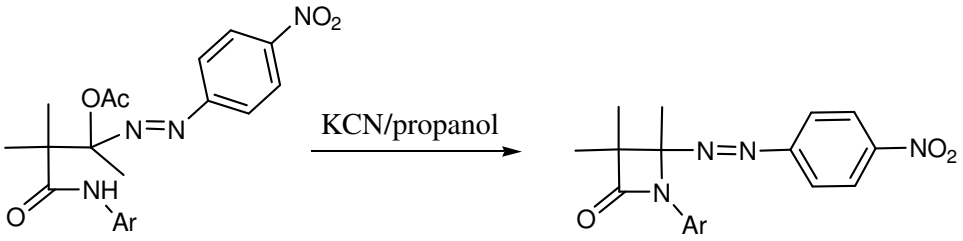
(With reference to aromatic group, Ar, substitutions at R_1, R_2 and R_3 are as shown in Table 2.2)

Scheme 2.11 Formation of azoacetates **133a-j**

After some experimentation with possible base/solvent systems, based on results by the previous researchers in this group (K_2CO_3/acetone, *t*-BuOK/acetonitrile), cyclisation of the

azoacetates was found to be best achieved using KCN/propanol. The results achieved with this system were found to be the most consistent (Table 2.3).

Table 2.3 Synthesis of β-lactams **134a-j**

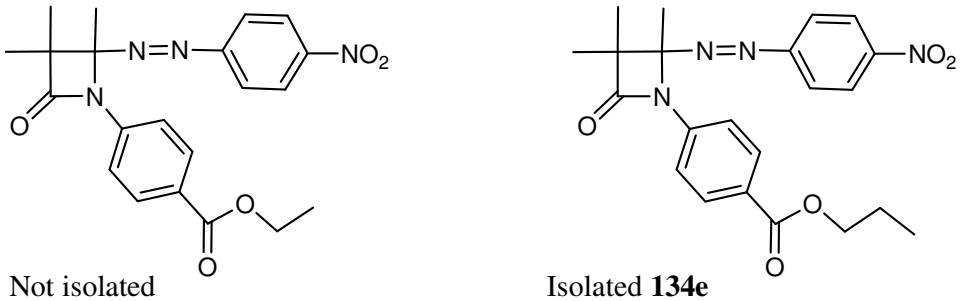


133 a-j **134 a-j**

Substrate	Ar			Product	Yield (%)
	R_1	R_2	R_3		
133a	H	H	Br	**134a**	16
133b	H	H	OMe	**134b**	20
133c	Me	Me	H	**134c**	25
133d	H	H	Me	**134d**	15
133e	H	H	COOEt	**134e**	11
133f	H	H	H	**134f**	12
133g	Me	H	Me	**134g**	25
133h	Me	H	H	**134h**	22
133i	OMe	H	H	**134i**	16
133j	H	H	Cl	**134j**	20

The cyclisation of the azoacetates to β-lactams using this system had been previously reported to produce N-acyl hydrazide derivatives as rearrangement products.[44] The formation of these compounds is discussed later in chapter 3. It was also found that in some cases 2-iminopyrrolidine-5-ones could be isolated from the reaction mixture. The formation of these compounds was found to be as a result of the intermolecular reaction of the azoacetate with the cyanide ion. The formation of 2-iminopyrrolidine-5-ones is discussed fully in chapter 4. Noteworthy amongst the β-lactam products isolated was the formation of the propanol transesterification product of β-lactam (**134e**, Figure 2.5). Since cyanide is known to promote

the transesterification process the formation of this compound using propanol as solvent was not entirely unexpected.



Not isolated Isolated **134e**

Figure 2.5 Isolation of β-lactam transesterification product **134e**.

2.2.3.1 NMR studies of phenyl-azo β-lactams

The reaction series resulting in the synthesis of the azo-substituted β-lactams also produced some interesting features in the ^{1}H NMR spectra of the related compounds. The ^{1}H NMR spectra of the di-methylated acetoacetanilide derivatives and also the subsequent 4-nitrophenylhydrazones of these compounds both show the dimethyl peaks as a single signal appearing as a singlet and integrating as 6 protons relative to the other signals in the spectrum. However in the case of the azoacetate and β-lactam where the carbon adjacent to the α-carbon bearing the methyl groups is a chiral centre, two singlet signals appear each integrating as 3 protons relative to the other signals (Figure 2.7). These observed ^{1}H NMR features are due to structural features inherent to each of the molecules (Figure 2.6).

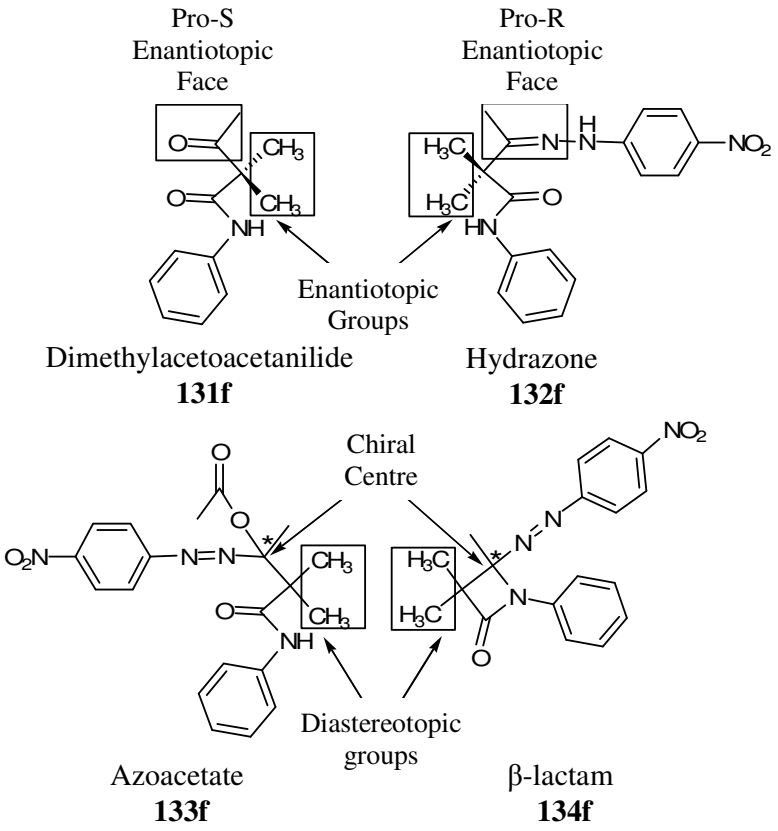


Figure 2.6 Inherent structural features of β-lactam and precursor molecules

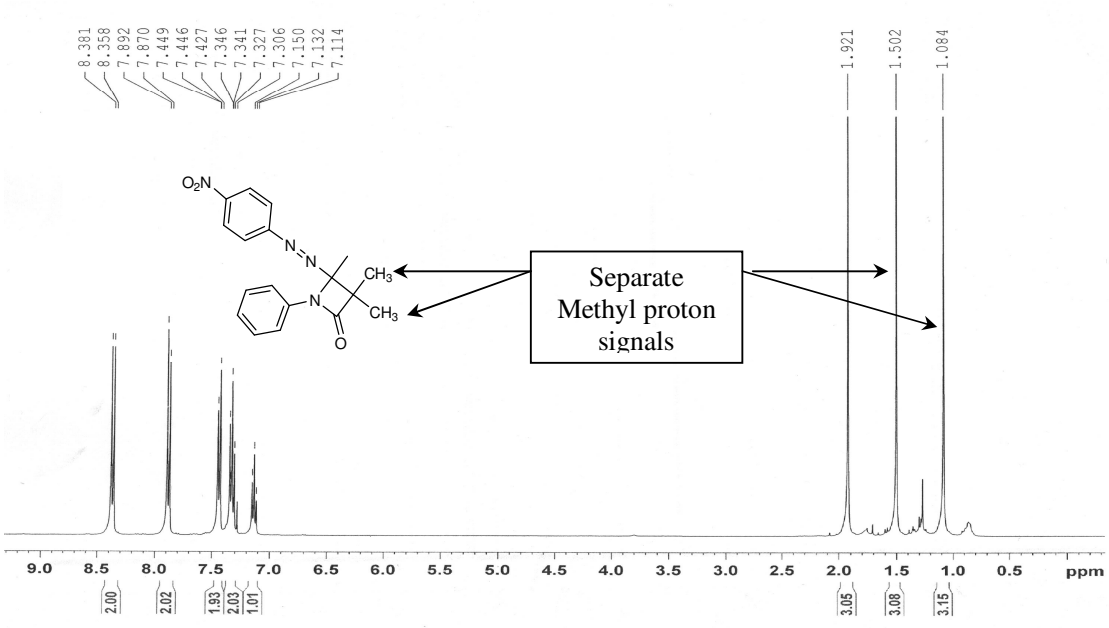


Figure 2.7 ^{1}H NMR spectrum of **134f**

In the case of the acetoacetanilide and phenylhydrazone derivatives the methyl groups are enantiotopic and are therefore chemically equivalent and indistinguishable unless in a chiral environment. This is due to an internal plane of symmetry that exists within the molecule (Figure 2.8).

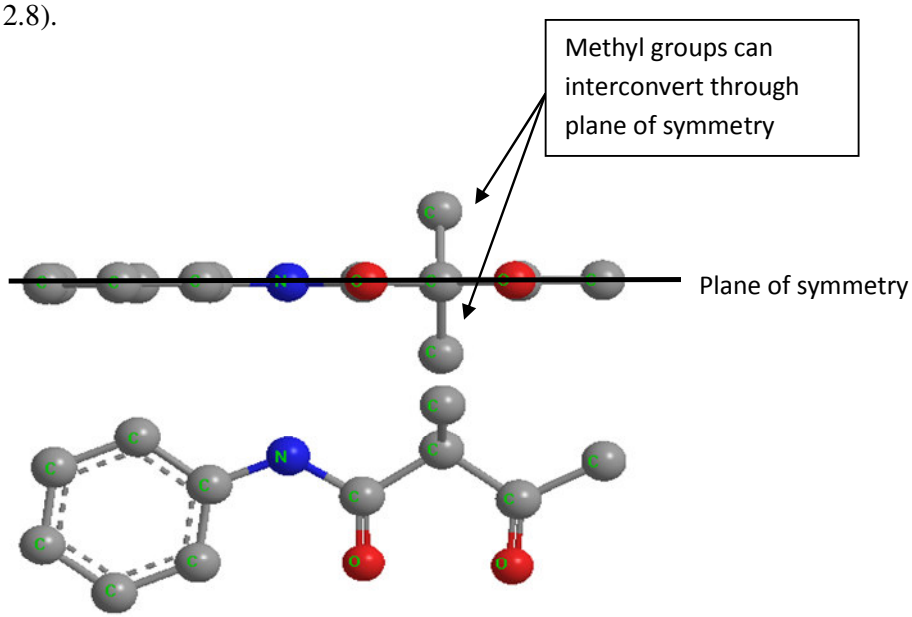


Figure 2.8 3D diagram of α,α-Dimethyl acetoacetanilide **131f** without hydrogen's showing internal plane of symmetry.

Since it is possible to interconvert the methyl groups through a symmetry operation they are equivalent in all meaningful ways. In contrast the β-lactam final product and its immediate precursor azoacetate have no such internal plane of symmetry due to the presence of a chiral centre adjacent to the methyl groups (Figure 2.9). The reaction of the phenylhydrazone with lead tetraacetate which produced the azoacetate resulted in the transformation of the enantiotopic face into a chiral centre and in so doing destroyed the plane of symmetry. The methyl groups in the product are therefore diastereotopic and not chemically equivalent. Since these groups are not equivalent they are distinguishable by ^{1}H NMR. In addition to this experimentally observed NMR feature, it would be expected that the diastereotopic methyl

groups would also exhibit different chemical properties such as reactivity, bond length and bond dissociation energies

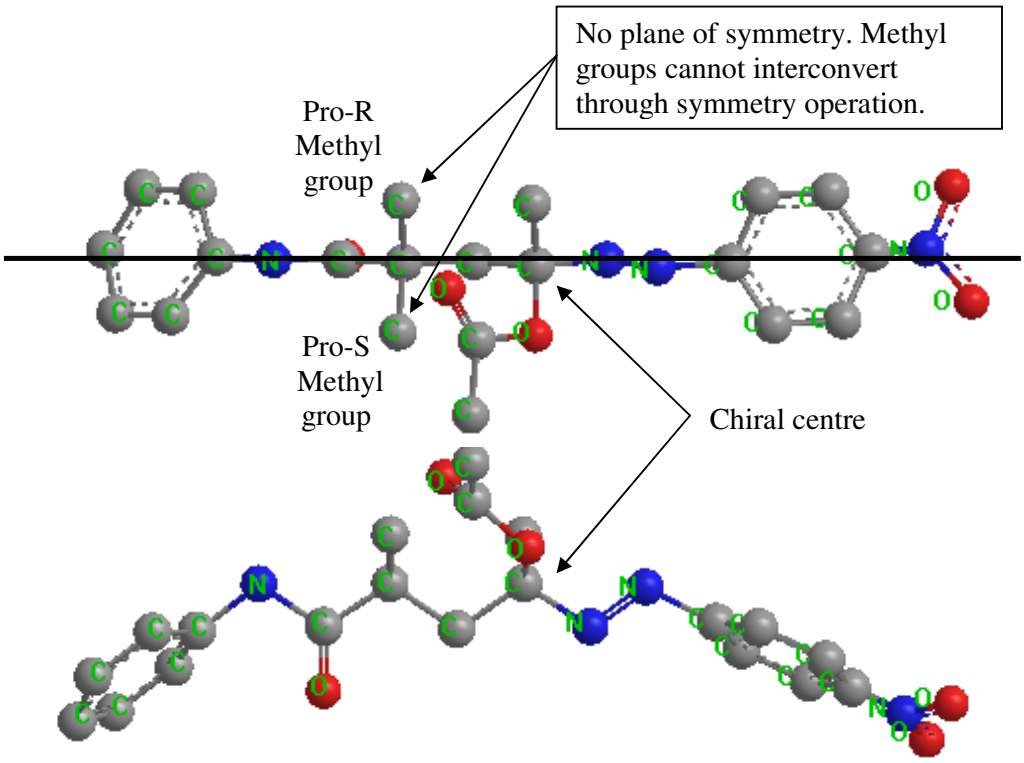


Figure 2.9 3D diagram of azoacetate **133f** without hydrogen's highlighting lack of symmetry plane.

2.2.3.2 IR studies of phenyl-azo β-lactams

The IR spectra of β-lactam molecules are one of the tools that aid in the identification of the compounds. Because of the inherent ring strain associated with the β-lactam ring due to the constrained nature of the system, the carbonyl stretch in these systems are seen at higher frequencies (wavenumbers) than similar acyclic amides such as the precursors to the β-lactams. Table 2.4 shows the carbonyl stretches of the β-lactam products **134a-j** and for comparison the carbonyl stretches of the acyclic precursors to the 4-bromophenyl substituted

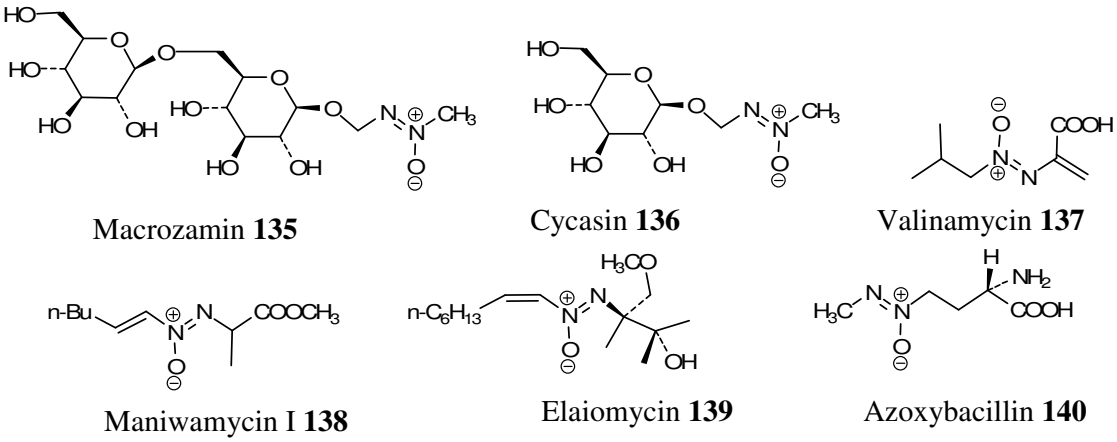
β-lactam (**130a**, **131a**, **132a**, **133a**). It can be clearly seen that the formation of the β-lactam ring can be confirmed by the change in the amide IR carbonyl stretch.

Table 2.4 Amide IR carbonyl stretching vibrations of β-lactams **134a-j** and precursors **130-133a**

Compound	**130a**	**131a**	**132a**	**133a**	**134a**	**134b**	**134c**
amide IR carbonyl stretch cm^{-1}	1661	1684	1671	1687	1755	1759	1757
Compound	**134d**	**134e**	**134f**	**134g**	**134h**	**134i**	**134j**
amide IR carbonyl stretch cm^{-1}	1751	1749	1759	1762	1761	1761	1753

2.3 Synthesis of Phenylazoxy β-lactam[84]

After the successful synthesis of a group of β-lactams bearing a phenylazo side chain it was decided to attempt a modification of the azo linkage. The most obvious target for this type of alteration was the oxidation to the azoxy group. Although the phenylazo moiety might be assumed to react in a comparable way to similar systems, giving the azoxy compound as product, due to the novel nature of the system it was judged to be worty of investigation. It was hoped that the synthesis of the azoxy version of the compound might also help overcome problems with the reduction of the phenylazo group to the corresponding hydrazine. Apart from a few notable exceptions (Figure 2.10) very little research has been reported on the synthesis or potential applications of these compounds. Macrozamin[85] discovered in 1951, was the first of a series of azoxy compounds found in nature. Many azoxy compounds have been found to be carcinogenic due to the generation of carbocations capable of DNA attack after metabolism.[86] However some of these compounds have found use as antibiotic agents. Valanimycin, produced by *Streptomyces viridifaciens* MG456-hF10, has been shown to exhibit both antibacterial activity and potent cytotoxic activity against *in vitro* cell cultures of mouse leukaemia L1210, P388/S (doxorubicin-sensitive), and P388/ADR (doxorubicin-resistant).[87]

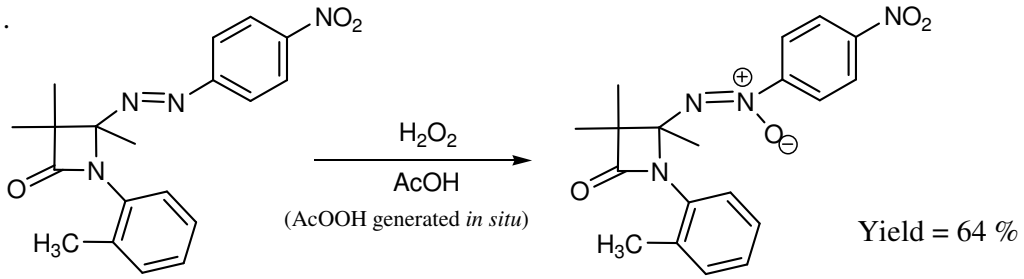


Macrozamin **135** Cycasin **136** Valinamycin **137**

Maniwamycin I **138** Elaiomycin **139** Azoxybacillin **140**

Figure 2.10 Naturally occurring azoxy compounds

The transformation of the azo group to the azoxy was successfully accomplished by employing a hydrogen peroxide and acetic acid oxidation system.[88] In this system the peracetic acid is generated in situ.[89] The substrate β-lactam **134h** was introduced to a hot solution (80 ^{o}C) of acetic acid to which hydrogen peroxide was added incrementally over 24 hours. After which time the product **141h** was precipitated out of solution by the introduction of ice water (Scheme 2.12).

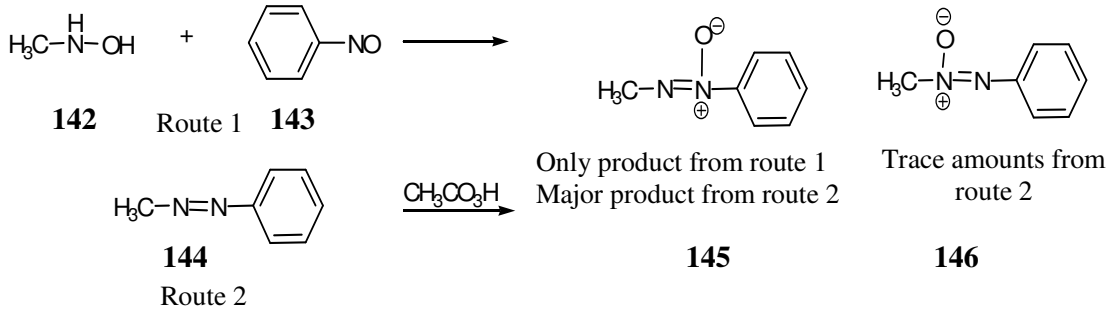


134h **141h**

Scheme 2.12 Synthesis of phenylazoxy β-lactam

The synthesis of the azoxy group in this way does allow some scope for error in the assignment of the absolute structure. Several strategies have been previously adopted for the regiospecific synthesis of azoxy compounds.[90] This can be done through the reaction of hydrazines or hydrazones with peroxy- acids (Peroxy acid = RCO_3H).[91] A reliable method for

the production of azoxybenzene derivatives has proven to be the reaction of some hyroxylamines **142** with nitrobenzene **143** which returns azoxy compound with the aromatic moiety in the proximal position to the azoxy oxygen (***Proximal***→R-N(O)==N-R'←***Distal***) (Scheme 2.13). Comparisons of results using this method have allowed some general observations to be made regarding the effect of the azoxy group on the spectra of the groups adjacent to it. This information has been used to accurately identify the configuration of the group in compounds where regiospecific synthetic procedures were not used. Of particular interest were the studies carried out by Freeman.[92] These studies catalogued the proton NMR shifts associated with unsymmetrical azoxy compounds and put forward a system for identification of the geometrical isomers of the compounds in question, based on the observed NMR chemical shifts of the attached groups. The main focus of the work was concerned with the assignment of the proton chemical shifts of alkyl protons attached directly to the proximal and distal carbons. In certain cases the azoxy group was attached to a phenyl ring for comparisons to be made between the proton in a proximal and distal positions. The synthesis of N-methyl-N'-phenyldiazine N'- oxide **145** led to an interesting observation. The compound was synthesised using two routes, one where the position of the azoxy oxygen could be controlled (Route 1, Scheme 2.13), and the other where selectivity was not assured **(Route 2 Scheme 2.13).**



Scheme 2.13 Selective and non-selective syntheses of azoxy compounds

However in both cases the same compound with the phenyl ring proximal to the azoxy oxygen was the dominant product. This observed selectivity for formation of the azoxy group proximal to the phenyl ring was unexpected and led the author to remark "The selectivity of these oxidations seems remarkable and no simple explanation suggests itself". In terms of the general trends observed in the spectra of these compounds it is suggested that the oxidation of the azo group results in a downfield shift of both groups attached to the newly formed azoxy group as a result of a reduction in the electron density at both nitrogen centres, with the larger shift associated with the proximal group. It is with this in mind that the assignment of the position of the azoxy group was undertaken.

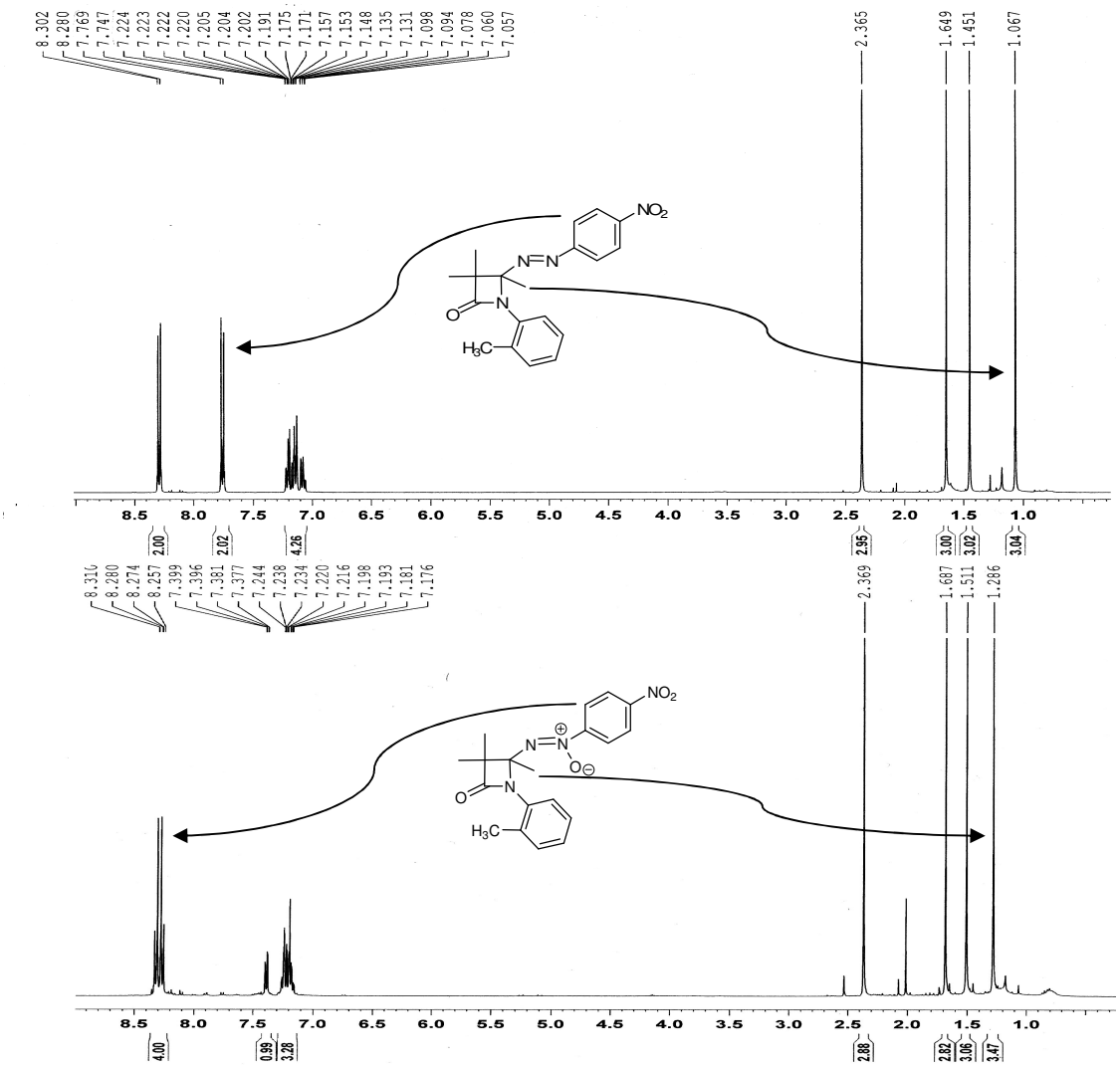
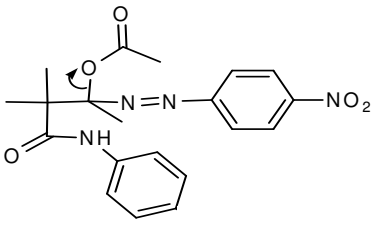


Figure 2.11 ^{1}H NMR spectra of phenylazo **134h** and phenylazoxy **141h** β-lactam

It can be clearly seen from the comparison of the ^{1}H spectra of the azo- **134h** and azoxy- **141h** bearing β-lactam molecules that the methyl protons nearest the azo- group have experienced a downfield shift after the oxidation (Figure 2.11). The shift from 1.07 to 1.29 represents a total downfield shift of 0.22 ppm. However the shift experienced by the aromatic nitrophenyl- protons can be seen to be more dramatic, with a shift from 7.76 to 8.27, a total shift of 0.51 ppm. These results are consistent with the results of the oxidations carried out by Freeman. The isolation of a single product without the necessity for chromatographic separation suggests that the procedure selectively produces a single isomeric form of the azoxy compound. Analysis of the spectral information further suggests that it is the isomer with the aromatic group in a proximal position to the azoxy oxygen that was isolated.

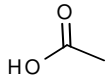
2.4 Alternative leaving groups

The ring closure of azoacetates to form β-lactams is an interesting process. Using the acetate moiety as leaving group provided an unexpectedly facile procedure. In general good leaving groups can be broadly described as "the conjugate bases of strong acids". Since leaving groups carry the bonding electrons after bond cleavage, the ease with which a leaving group leaves is related to its ability to stabilize the electrons after cleavage. In that regard the acetate leaving group could be expected to be quite a poor leaving group, since the acetate anion is the conjugate base of acetic acid, a relatively weak acid.



133f

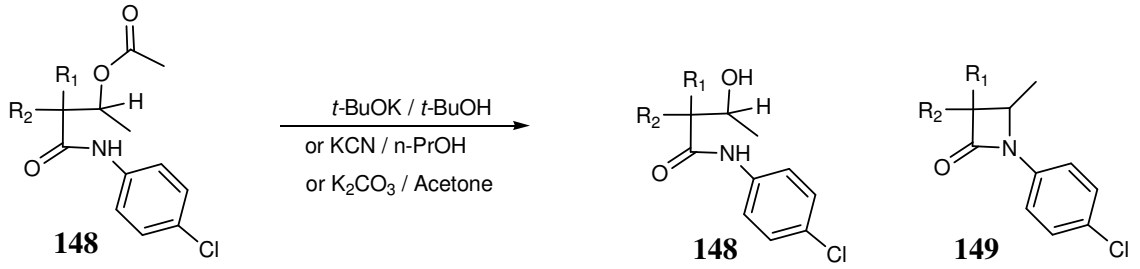
leaving group
acetate anion



Acetic acid
conjugate acid

Figure 2.12 The acetate leaving group in azoacetates

The ability and indeed propensity of the acetate group to act as leaving group in this way raises the question of why a seemingly chemically inert species would behave in this uncharacteristic manner. However if the presence of the neighbouring azo group is taken into consideration a plausible explanation presents itself. The concept of a neighbouring unsaturated group providing anchimeric assistance could be the reason for the enhanced leaving group potential of an otherwise relatively inert species. Some preliminary tests of this hypothesis were made by the original researchers.[43] These tests consisted of the synthesis and attempted cyclisation of a number of substrates containing the acetate group but without the presence of the phenylazo functionality **147 a** and **b** (Scheme 2.14). When treated with a variety of bases these substrates underwent deacetylation to give the alcohol **148 a** and **b** as opposed to the desired β-lactam product **149**. The failure of these model systems to furnish the β-lactam product lends credence to the theory that the neighbouring phenylazo group does provide anchimeric assistance in the cyclisation of the azoacetate species.

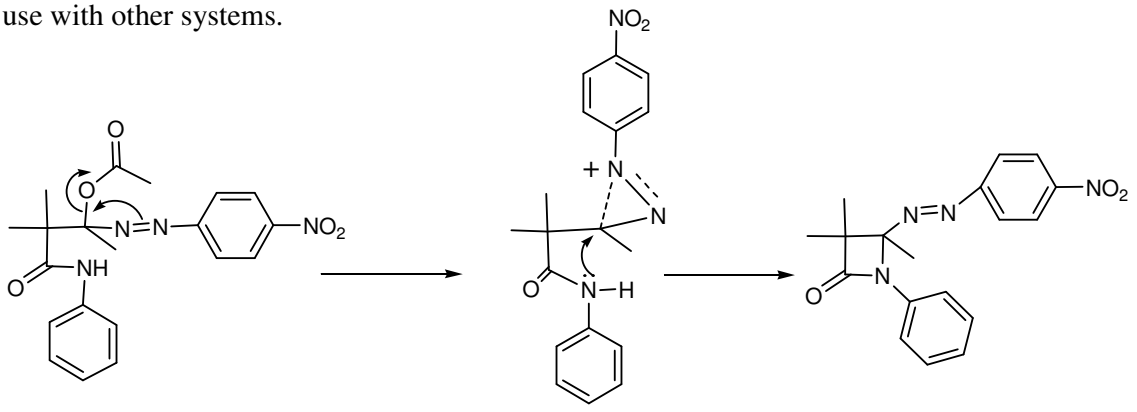


a $R_1=R_2=H$,
b $R_1=R_2=Me$,

Scheme 2.14 Failed β-lactam cyclisations using acetate as leaving group

Instances of this type of enhancement effect by a number of functionalities are well known, including assistance from neighbouring double bonds.[93] Although the exact nature of the assistance provided by the phenylazo group is unknown, systems similar to that shown in Scheme 2.15 have been proposed for other π-bonded systems. The discovery of this effect led to the investigation of alternative leaving groups at this position. It was hoped that the

observed enhancement in reactivity provided by the phenylazo group could be harnessed for use with other systems.

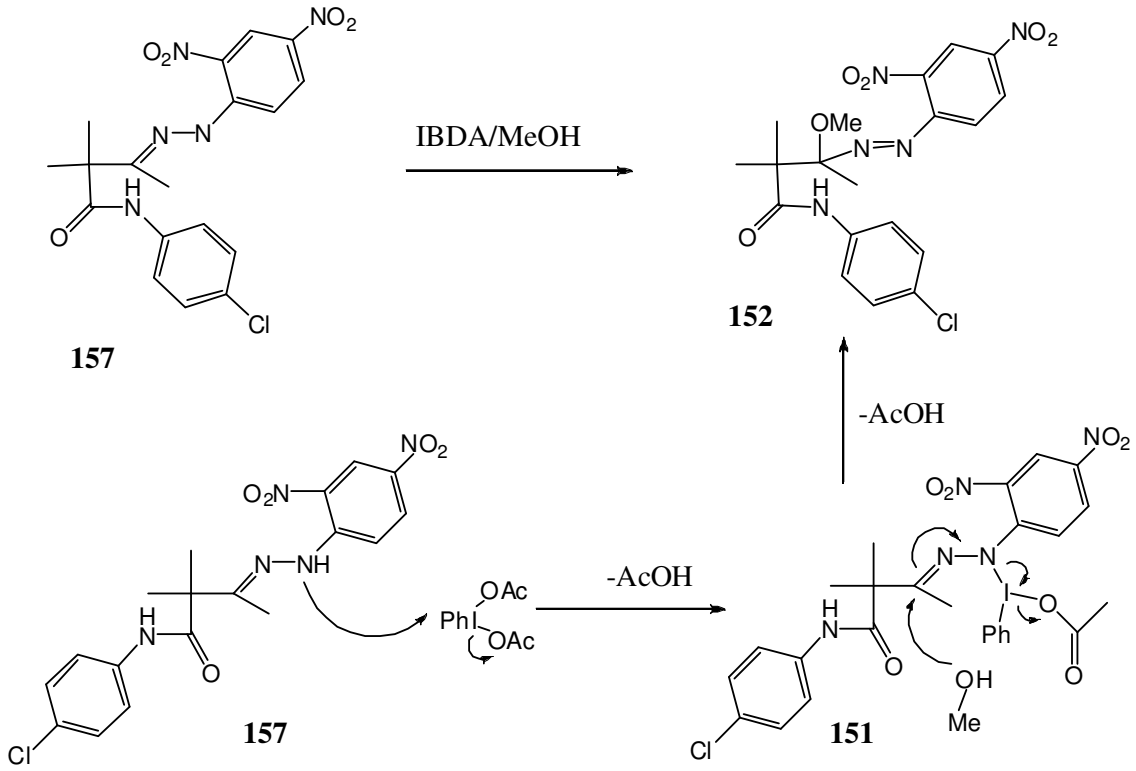


133f **150f** **134f**

Scheme 2.15 Proposed mechanism of anchimeric assistance

2.4.1 Alkoxy leaving groups

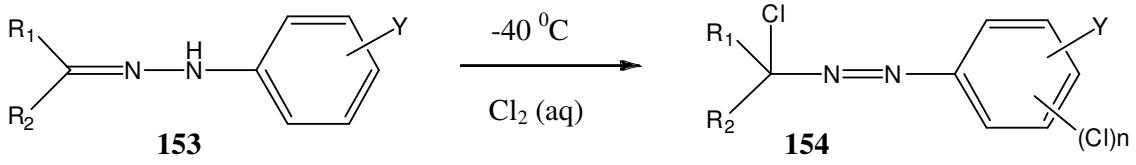
The first system to be examined was a slight variation on the azoacetate procedure. In Barton's paper concerning the oxidation of hydrazones using hypervalent organoiodine reagents, a straightforward procedure for the conversion of hydrazones to α-alkoxyazo compounds is presented.[45] The procedure utilises Iodobenzene diacetate for the addition of an alcohol to the hydrazone. The procedure described was adapted for use with our substrate hydrazones, a mechanism analogous to that proposed by the Barton group is shown (Scheme 2.17). Unfortunately, as expected the alcohol moiety introduced proved to be a poor leaving group. Although it was expected that this would be found to be the case, the ring closure had previously been achieved successfully with an acetate moiety, which would also have been expected to be a poor leaving group. The ring closure was attempted with a variety of bases and reaction conditions after which this group was ruled out as a means of achieving the desired ring closure.



Scheme 2.16 Barton type mechanism of formation of α-alkoxyphenylazo compounds

2.4.2 Chlorine as leaving group

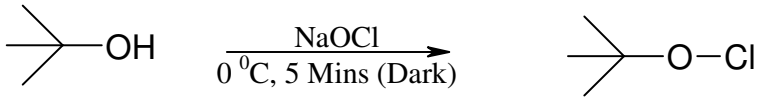
The next leaving group to be considered was chlorine. In contrast to the systems used so far chlorine is recognised as a good leaving group; however introducing chlorine into the system in the correct position to act as a leaving group was not trivial. The chlorination of hydrazones to form α-chloro phenylazo compounds had been achieved, but to date only on a limited scale. Chlorination of phenylhydrazones was investigated in the early 70's by M. W. Moon[94] of the Upjohn Company, although the origins of the procedure can be traced back to the late 50's. The chlorination was achieved using Cl_2 solutions in 3-4 molar excess at – 40 ^{0}C,



Scheme 2.17 Early hydrazone chlorination reported by Moon

However, it was found that using this system when phenyl groups were present resulted in chlorination of the ring in addition to the desired chlorination α- to the azo group (Figure 2.17).

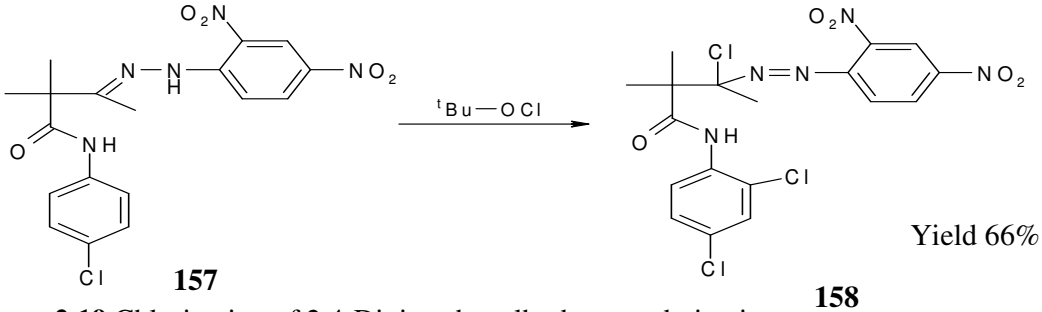
Unwanted over-chlorination is a commonly encountered problem associated with this reaction. The other commonly used system for the chlorination of hydrazones is using tert-Butyl hypochlorite, prepared by the chlorination of *t*-BuOH with sodium hypochlorite (Schme 2.18). The reagent is best prepared fresh just prior to use, as it is prone to degradation when exposed to light; this necessitates the entire procedure being carried out in the dark.



155 **156**

Scheme 2.18 Formation of *t*-butyl hypochlorite

Substituents R_1, R_2 and R_3 in the scheme are generally alkyl groups or highly substituted phenyl groups (usually 2,4,6-trichlorophenyl rings) as it also was found by these researchers that chlorination using *t*-butyl hypochlorite resulted in unwanted ring chlorination.



Scheme 2.19 Chlorination of 2,4-Dinitrophenylhydrazone derivative

As a precaution against this eventuality, in this case 2,4-dinitrophenylhydrazine was used in the generation of the hydrazones for these experiments. This did prevent unwanted chlorination on the nitro substituted ring; nevertheless in addition to the required chlorination rendering the α-chloro phenylazo compound, chlorination occurred at the 2- position on the

4-chloro substituted ring. Although this was unintentional it was not seen as a result that was detrimental to possible cyclisation of the product (Scheme 2.19).

2.4.3.1 NMR spectra of the chlorination product

The desired chlorination of hydrazone **157** to the α-chlorophenylazo derivative **158** was confirmed by NMR spectroscopy. In the ^{1}H NMR spectrum the presence of a single NH peak at 9.28 ppm due to the amide (confirmed as NH by HMQC and IR spectra) shows that the hydrazone has been converted to the desired product (Figure 2.13a and b). The integration and splitting patterns of the aromatic protons also show that the 4-chlorophenyl ring has been altered. 4-Chlorophenyl ring systems have a clearly defined splitting pattern not present in this proton NMR. This was further confirmed through analysis of the ^{13}C NMR spectrum.

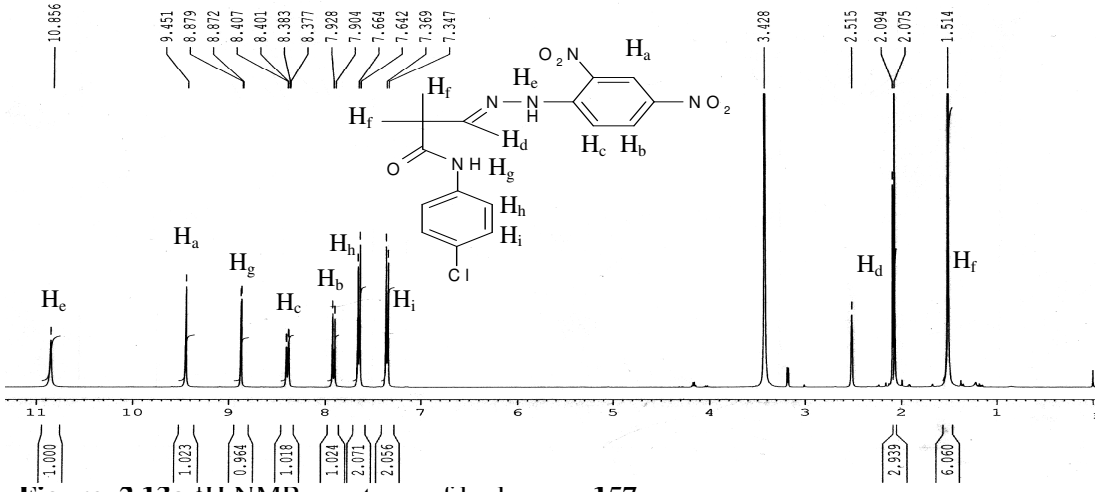


Figure 2.13a ^{1}H NMR spectrum of hydrazone **157**

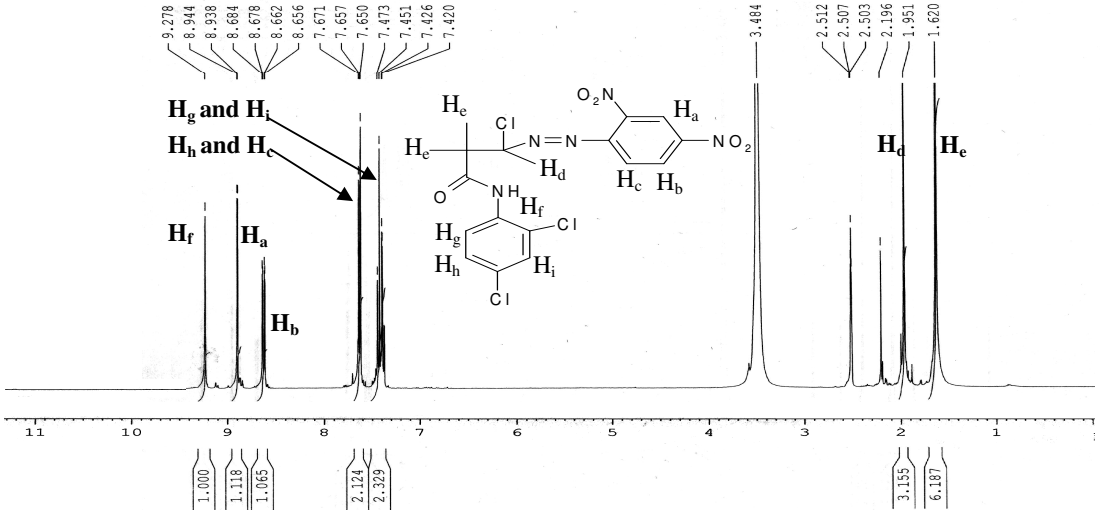


Figure 2.13b ^{1}H NMR spectrum of α-chlorophenylazo derivative **157**

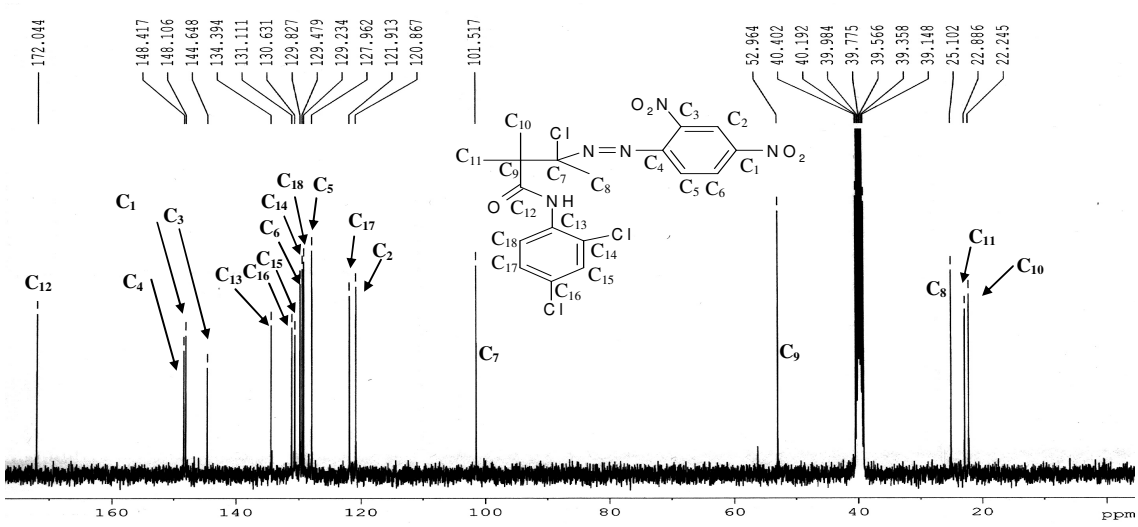


Figure 2.13c ^{13}C NMR spectrum of α-chlorophenylazo derivative **157**

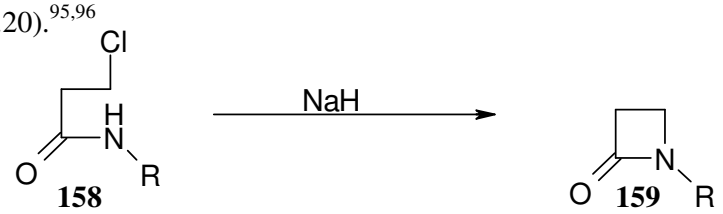
4-Chlorophenyl rings give rise to 4 aromatic carbon signals due to the equivalence of the carbons at the 2 and 3 ring positions, whereas the carbon spectrum for this compound shows 6 carbon signals due to the unsymmetrical 2,4-dichlorophenyl substituted ring (Figure 2.13c). The location of the chlorine at the 2 position on the ring was confirmed by combined analysis of all of the NMR spectra, though the HMBC spectrum proved particularly useful for this purpose. Chlorination at this position was expected due to the directing effects of the groups present before chlorination. Since the amide is a ring activating *ortho*/*para* directing group, and chlorine is a ring deactivating *ortho*/*para* directing group, the effect of the amide is more influential on the incoming group.

It is interesting to note that in the case of this product **157** the splitting of the α-methyl groups into separate signals can be seen in the ^{13}C NMR spectrum but not in the ^{1}H NMR spectrum. It might have been expected to see separate ^{1}H NMR signals due to the proximity of the newly formed chiral centre to the methyl groups as was seen to be the case with the azoacetates (section 2.2.4.1). This is possibly due to the relative size of the chlorine atom at the chiral centre having a less pronounced effect compared to the bulky acetate group. Since

the ^{13}C NMR spectrum shows that the methyl groups are distinct and distinguishable it is presumed that the ^{1}H NMR signals due to these groups are overlapping due to similar chemical shift.

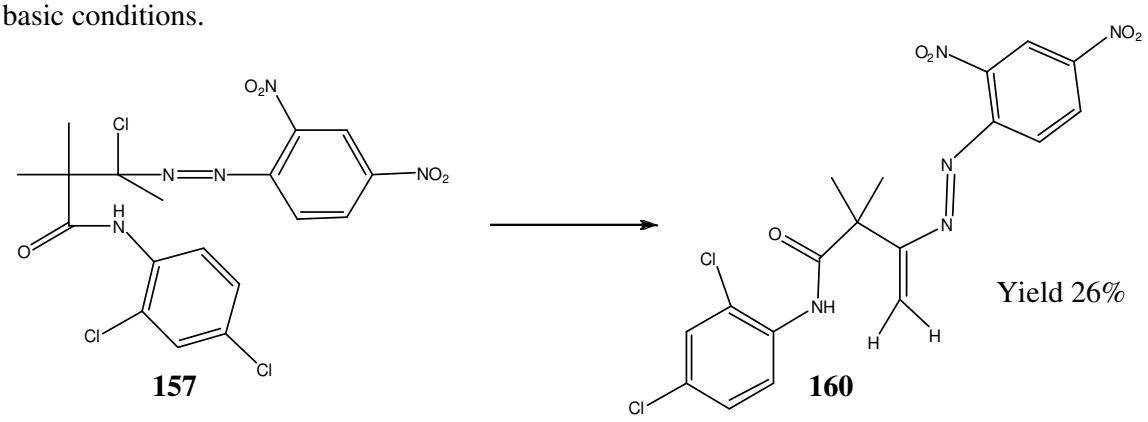
2.4.3.2 Cyclisation attempts with α-chloroazo group

Attempts to achieve the cyclisation of this species to a β-lactam proved unsuccessful. NaH was used in initial attempts to close the ring as this type of ring closure is relatively well known (Scheme 2.20).[95,96]



Scheme 2.20 General scheme showing chloro-propionamide ring closure to β-lactam

When these conditions were applied to our system results suggest that dehydrohalogenation had taken place. The formation of azoalkenes in this way have been previously reported. Eliminations of this kind have been observed by Rai *et al* where similar α-chloro-α-methylphenylazo compounds gave azoalkenes analogous to those reported here (Scheme 2.21).[97] The elimination products isolated in that instance resulted from simply stirring the α-chlorophenylazo derivative in triethylamine at room temperature for 15 minutes. This demonstrates the propensity of these compounds to undergo elimination under very mild basic conditions.



Scheme 2.21 Reaction of α-chlorophenylazo **157** with NaH

Attempts to close the ring using alternative base/solvent systems also failed. This may be due in part to the nature of the aromatic substituent attached to the amide. Many cyclisations reported using this type of system are reported, though it is known that when chlorine is employed as leaving group in this type of cyclisation it is preferable to have a hydroxamate present.[98] Though chlorine is a good leaving group, increasing the acidity of the NH group by employing the O-substituted hydroxamates was found to enhance the system towards cyclisation.[99] It was hoped that the highly substituted nature of the α-chlorophenylazo derivative used here would favour cyclisation over elimination however that was not found to be the case.

Conclusion

The original 1992 communication dealing with the synthesis of phenylazo substituted β-lactams reported the synthesis of two of these compounds, with different substituents attached to the N-aryl ring. Since the synthesis of the original members of this series of compounds was undertaken, little had been done to further assess the versatility of the compounds from a synthetic viewpoint.

It was found that some modifications to the series of reactions leading to the formation of the β-lactams were possible without significantly affecting the success of the cyclisation step. It was also found that two of the three targeted insertion points for new groups could be utilised to bring some diversity into the system.

The search for alternative leaving groups did not provide an alternative cyclisation process, but did show the synthesis of α-chloro and α-alkoxy phenylazo compounds from the hydrazones to be facile procedures. Although a complete alternative route proved elusive, several potential precursors to these β-lactams were successfully synthesised and are representative examples of novel classes of compounds containing the phenylazo moiety.

In addition investigations into the modification of the phenylazo group subsequent to cyclisation produced a β-lactam bearing the phenylazoxy moiety. Although the azo- to azoxy-transformation has been reported in many contexts, the direct attachment of this group to a β-lactam has not been previously reported.

Overall the work presented in this chapter aims to expand the understanding of both the limitations and synthetic potential of the procedures that originally led to the formation β-lactams from their azoacetate precursors. To that end, a good deal of useful information has been gathered concerning the practical aspects of the synthesis of these novel compounds.

2.5 Experimental

Ethyl acetoacetate, iodomethane, benzyl chloride and substituted acetoacetanilides were purchased from the Sigma Aldrich chemical company and were used as received. All solvents were dried or distilled prior to use. Melting points were determined using a Griffin melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 2000FT-IR spectrometer. NMR spectra were recorded using a Bruker AC 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. The ^{1}H and ^{13}C NMR chemical shifts (ppm) are relative to TMS and all coupling constants (*J*) are in Hertz (Hz).

***α-methyl-4-chloroacetoacetanilide* (120)**

4-Chloroacetoacetanilide **119** (10.8 g, 51.2 mmol) was dissolved in acetone (75 ml) and K_2CO_3 (7.2 g, 52.0 mmol) was added and the reaction brought to reflux. Iodomethane (10.66 g, 75.1 mmol) was added dropwise with stirring to the reaction vessel over 1 h and the reaction was heated under reflux for a further 2 h. The reaction mixture was cooled and poured into ice-water (150 ml) and allowed to stir for 30 min. A white precipitate **(120)** was collected and recrystallized several times from aqueous EtOH to remove any dimethylated product **(131j)**. Yield: (8.59 g, 74.4%); mp 125-129 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.23 (3H, d, CH_3, *J* = 6.8), 2.17 (3H, s, CH_3), 3.65 (1H, q, CH, *J* = 6.8), 7.37 (2H, d, ArH, *J* = 8.8), 7.50 (2H, d, ArH, *J* = 8.8), 10.34 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 13.0, 28.2, 54.5, 120.8, 127.1, 128.6, 137.8, 168.9, 204.3. IR (KBr) 3241, 1720, 1654, 1604 cm^{-1}.

***α-methyl-α-benzyl-4-chloroacetoacetanilide* (121)**

α-Methyl-4-chloroacetoacetanilide **120** (6.8 g, 30.2 mmol) was dissolved in acetone 50 ml and K_2CO_3 (7.2 g, 52.0 mmol) was added and the reaction brought to reflux. Benzyl chloride (6.58 g, 52.0 mmol) was added dropwise with stirring to the reaction vessel over 1 h and the reaction was heated under reflux overnight. The reaction mixture was cooled and poured into ice-water (150 ml) and allowed to stir for 30 min. A white precipitate **(121)** was collected and

recrystallized from an EtOAc:hexane 70:30 mixture. Yield: (8.53 g, 89.5%); mp 124-127 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.27 (3H, s, CH_3), 2.20 (3H, s, CH_3), 3.07 (1H, d, CH_2, J = 13.6), 3.36 (1H, d, CH_2, J = 13.6), 7.11-7.23 (5H, m, ArH), 7.77 (2H, d, ArH, J = 8.8), 7.43 (2H, d, ArH, J = 8.8), 9.68 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 18.7, 26.3, 39.7, 61.3, 122.3, 126.5, 127.6, 128.0, 128.1, 130.1, 136.7, 137.5, 170.2, 206.3; IR (KBr) 3328, 1720, 1654, 1604, 1543 cm^{-1}.

Preparation of acetoacetanilides

4-Bromoacetoacetanilide 130a

Ethyl acetoacetate **106** (65.1 g, 0.5 mol) was heated to 160 ^{o}C in a round bottomed flask with reflux condenser fitted. To this was added 4-bromoaniline (17.0 g, 0.1 mol). When the evolution of EtOH began the reaction was allowed to reflux for 2-3 h until the reaction was complete (TLC) before the EtOH was distilled off. The bulk of the remaining ethyl acetoacetate was removed by vacuum distillation at 90^{o} C, 50mbar. The resulting oily residue was scratched with a glass rod to promote precipitation. The precipitate was filtered and washed with hexane before being recrystallised from an EtOAc:hexane 30:70 mixture. Yield (22.8 g, 90.1 %); mp 132-135 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.21 (3H, s, CH_3), 3.56 (2H, s, CH_2), 7.48 (2H, ArH, J = 8.8), 7.55 (2H, d, ArH, J = 8.8) 10.21 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 30.2, 52.3, 114.9, 120.9, 131.5, 138.2, 165.2, 202.7; IR (KBr) 3292, 1718, 1661 cm^{-1}.

4-Methoxyacetoacetanilide 130b

Yield (18.2 g 88%); mp 131-134 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.21 (3H, s, CH_3), 3.51 (2H, s, CH_2), 3.72 (3H, s, CH_3), 6.88 (2H, d, ArH, J = 8.8), 7.49 (2H, d, ArH, J = 8.8), 9.79 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 30.1, 52.2, 55.1, 113.8, 120.6, 132.0, 155.3, 164.5, 202.9; IR (KBr) 3299, 1730, 1554, 1490 cm^{-1}.

2,3-Dimethylacetoacetanilide 130c

Yield (18.7 g, 91%); mp 142-146 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.10 (3H, s, CH_3), 2.23 (3H, s, CH_3), 2.25 (3H, s, CH_3), 3.58 (2H, s, CH_2), 7.07-7.01 (2H, m, ArH), 7.19-7.17 (2H, m, ArH), 9.40 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 13.9, 20.1, 30.1, 51.6, 123.3, 125.1, 127.0, 130.1, 135.8, 136.9, 165.0, 203.0; IR (KBr) 3296, 1720, 1665 cm^{-1}.

4-Methylacetoacetanilide 130d

Yield (16.2 g, 85%); mp 122-126 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.21 (3H, s, CH_3), 2.25 (3H, s, CH_3), 3.54 (2H, s, CH_2), 7.10 (2H, d, ArH, *J* = 8.0), 7.47 (2H, d, ArH, *J* = 8.0), 10.00 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 20.4, 30.1, 52.3, 119.6, 129.1, 132.3, 136.4, 164.7, 202.9; IR (KBr) 3293, 1711, 1666 cm^{-1}.

4-Ethylesteracetoacetanilide 130e

Yield (19.2 g, 77%); mp 118-121 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.30 (3H, t, CH_3, *J* = 6.8), 2.22 (3H, s, CH_3), 3.61 (2H, s, CH_2), 4.27 (2H, q, CH_2, *J* = 6.8), 6.88 (2H, d, ArH, *J* = 8.8), 7.49 (2H, d, ArH, *J* = 8.8), 10.41 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 14.13, 30.2, 52.4, 60.4, 118.4, 124.3, 130.2, 143.1, 165.2, 165.6, 202.6; IR (KBr) 3286, 1723, 1675 cm^{-1}.

Preparation of α,α-dimethylacetoacetanilides

α,α-dimethyl-4-bromoacetoacetanilide 131a

4-Bromoacetoacetanilide **130a** (6.62 g, 25.9 mmol) was dissolved in 150 ml of acetone, to this was added K_2CO_3 (7.8 g, 56.4 mmol) and the reaction was set to reflux. Once the reaction had reached reflux temperature iodomethane (22.45 g, 158.2 mmol) was added and the reaction was allowed to reflux overnight. The solvent was reduced to 1/5 of the original

volume by rotary evaporation and the entire contents of the reaction vessel were poured into a beaker containing 200 ml of ice-water. The product formed an oil and was extracted with CH_2Cl_2 (5 x 50 ml). The organic extracts were combined, dried over magnesium sulfate and evaporated to an oil under reduced pressure. A small portion of the oil was removed and precipitation was induced by scratching, this was then returned to the bulk in order to seed the crystallization of the remaining oil. The solid was filtered and washed with hexane, this was further recrystallized from an EtOAc:hexane 70:30 mixture. Yield (5.84 g, 79.4 %); mp 85-87 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.37 (6H, s, 2 x CH_3), 2.15 (3H, s, CH_3), 7.48 (2H, ArH, *J* = 9.2), 7.61 (2H, d, ArH, *J* = 8.8), 9.57 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 21.8, 25.7, 56.5, 115.3, 122.2, 131.3, 138.2, 171.8, 207.2; IR (KBr) 3361, 1684, 1531 cm^{-1}.

α,α-dimethyl-4-methoxyacetoacetanilide 131b

Yield (4.77 g, 78 %); mp 88-87 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.36 (6H, s, 2 x CH_3), 2.15 (3H, s, CH_3), 3.72 (3H, s, OCH_3), 6.88 (2H, d, ArH, *J* = 9.2), 7.50 (2H, d, ArH, *J* = 9.2), 9.33 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 21.9, 257, 55.1, 56.2, 113.6, 122.1, 131.8, 155.5, 171.3, 207.2; IR (KBr) 3365, 1677, 1529, 1450 cm^{-1}.

α,α-dimethyl-2,3-dimethylacetoacetanilide 131c

Yield (4.85 g, 80 %); mp 102-105 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.45 (6H, s, 2 x CH_3), 2.03 (3H, s, CH_3), 2.21 (3H, s, CH_3), 2.22 (3H, s, CH_3), 6.92 (1H, d, ArH, *J* = 7.2), 7.01 (1H, t, ArH, *J* = 7.6), 7.39 (1H, d, ArH, *J* = 8.0), 7.66 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 17.5, 20.5, 22.0, 25.8, 55.8, 126.4, 126.7, 130.7, 133.4, 133.7, 135.2, 171.6, 207.2; IR (KBr) 3366, 1688, 1530, 1495 cm^{-1}.

α,α-dimethyl-4-methylacetoacetanilide 131d

Yield (4.73 g, 83 %); mp 81-83 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.38 (6H, s, 2 x CH_3), 2.14 (3H, s, CH_3), 2.25 (3H, s, CH_3), 7.11 (2H, d, ArH, *J* = 9.2), 7.49 (2H, d, ArH, *J* = 9.2), 9.37 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 17.8, 22.1, 25.6, 56.2, 120.4, 130.1, 132.9, 135.3, 164.3, 203.7; IR (KBr) 3359, 1679, 1540, 1485 cm^{-1}.

α,α-dimethyl-4-ethylesteracetoacetanilide 131e

Yield (5.04 g, 70 %); mp 82-85 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.30 (3H, t, CH_3, *J* = 6.8), 1.39 (6H, s, 2 x CH_3), 2.16 (3H, s, CH_3), 4.27 (2H, q, CH_2, *J* = 6.8), 7.77 (2H, d, ArH, *J* = 8.8), 7.91 (2H, d, ArH, *J* = 8.8), 9.77 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 14.1, 21.9, 25.8, 56.6, 60.4, 119.5, 124.5, 129.9, 143.3, 165.3, 172.2, 207.4. IR (KBr) 3352, 1690, 1492 cm^{-1}.

α,α-dimethylacetoacetanilide 131f

Yield (4.26 g, 80 %); mp 67-69 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.38 (6H, s, 2 x CH_3), 2.16 (3H, s, CH_3), 7.08-7.05 (1H, m, ArH), 7.32-7.28 (2H, m, ArH), 7.64-7.62 (2H, m, ArH), 9.11 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 21.9, 25.8, 56.4, 120.4, 123.6, 128.4, 138.8, 171.6, 207.2; IR (KBr) 3364, 1687, 1240, 1120 cm^{-1}.

α,α-dimethyl-2,4-dimethylacetoacetanilide 131g

Yield (4.08 g, 67 %); mp 93-94 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.39 (6H, s, 2 x CH_3), 2.13 (3H, s, CH_3), 2.19 (3H, s, CH_3), 2.26 (3H, s, CH_3), 7.04-6.97 (3H, m, ArH), 9.11 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 16.5, 21.1, 23.3, 25.4, 59.0, 123.9, 126.5, 131.3, 133.7, 134.0, 134.8, 172.2, 208.0; IR (KBr) 3355, 1687, 1526, 1490 cm^{-1}.

α,α-dimethyl-2-methylacetoacetanilide 131h

Yield (4.27 g, 75 %); mp 93-95 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.41 (6H, s, 2 x CH_3), 2.18 (3H, s, CH_3), 2.20 (3H, s, CH_3), 7.25-7.14 (4H, m, ArH), 9.2 (1H, s, NH); ^{13}C

NMR (100MHz, DMSO-d_6) 17.6, 22.0, 25.8, 55.8, 125.9, 126.1, 126.7, 130.2, 133.9, 136.0, 171.6, 207.2; IR (KBr) 3361, 1684, 1531, 1488 cm^{-1}.

α,α-dimethyl-2-methoxyacetoacetanilide 131i

Yield (4.52 g, 74 %); mp 89-91 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.39 (6H, s, 2 x CH_3), 2.20 (3H, s, CH_3), 3.82 (3H, s, CH_3), 6.93 (1H, t, ArH, *J* = 7.6), 7.05 (1H, d, ArH, *J* = 8.0), 7.14 (1H, t, ArH, *J* = 8.0), 7.72 (1H, d, ArH, *J* = 8.0), 8.80 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 22.0, 25.9, 55.8, 56.1, 111.2, 120.2, 123.3, 125.5, 126.6, 150.9, 170.9, 207.8; IR (KBr) 3365, 1692, 1490, 1311 cm^{-1}.

α,α-dimethyl-4-chloroacetoacetanilide 131j

Yield (4.29 g, 69 %); mp 108-110 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.38 (6H, s, 2 x CH_3), 2.15 (3H, s, CH_3), 7.35 (2H, d, ArH, *J* = 8.8), 7.66 (2H, d, ArH, *J* = 8.8), 9.57 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 21.9, 25.7, 56.4, 121.8, 127.2, 128.4, 137.8, 171.8, 207.1; IR (KBr) 3365, 1688, 1528, 1488 cm^{-1}.

Preparation of 4-nitrophenylhydrazone derivatives

N-(4-bromophenyl)-2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)butanamide 132a

2,2-Dimethyl-4-bromoacetoacetanilide **131a** (4.0 g, 14.0 mmol) was dissolved in MeOH 75 ml, 4-nitrophenylhydrazine (2.6 g, 16.8 mmol) was dissolved in warm acetic acid (3.25 ml) and added to the stirring reaction vessel. After 1-2 min a yellow precipitate began to fall from solution, the reaction was allowed to stir overnight and the precipitate collected and dried. Yield (4.01 g, 68 %); mp 197-200 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.48 (6H, s, 2 x CH_3), 1.96 (3H, s, CH_3), 7.27 (2H, d, ArH, *J* = 8.8), 7.50 (2H, d, ArH, *J* = 9.2), 7.65 (2H, d, ArH, *J* = 8.8), 8.12 (2H, d, ArH, *J* = 8.8), 9.43 (1H, s, NH), 9.97 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 13.7, 23.5, 51.3, 111.6, 115.1, 122.5, 125.8, 131.2, 138.1, 138.5, 151.8, 151.9, 173.5; IR (KBr) 3322, 1671, 1596 cm^{-1}.

N-(4-methoxyphenyl)-2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)butanamide 132b

Yield (2.85 g, 55 %); mp 144-147 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.44 (6H, s, 2 x CH_3), 1.93 (3H, s, CH_3), 3.72 (3H, s, OCH_3), 6.86 (2H, d, ArH, *J* = 8.8), 7.24 (2H, d, ArH, *J* = 8.0), 7.49 (2H, d, ArH, *J* = 8.4), 8.10 (2H, d, ArH, *J* = 9.2), 9.14 (1H, s, NH), 9.92 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6): 13.8, 23.6, 51.1, 55.1, 111.6, 113.4, 122.4, 125.8, 132.0, 138.0, 151.8, 152.2, 155.4, 172.9; IR (KBr) 3325, 1677, 1599, 1493cm^{-1}.

N-(2,3-dimethylphenyl)-2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)butanamide 132c

Yield (3.66 g, 71 %); mp 161-165 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.47 (6H, s, 2 x CH_3), 1.99 (3H, s, CH_3), 2.02 (3H, s, CH_3), 2.24 (3H, s, CH_3), 7.07-6.97 (3H, m, ArH), 7.27 (2H, d, ArH, *J* = 9.2), 8.12 (2H, d, ArH, *J* = 9.2), 9.01 (1H, s, NH), 9.94 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6): 13.9, 14.0, 20.0, 23.7, 50.7, 111.7, 124.7, 125.1, 125.8, 127.4, 132.7, 136.3, 136.8, 138.0, 151.9, 152.3, 173.2; IR (KBr) 3325, 1677, 1599, 1488 cm^{-1}.

2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)-N-p-tolylbutanamide 132d

Yield (2.73 g, 55 %); mp 152-156 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.44 (6H, s, 2 x CH_3), 1.93 (3H, s, CH_3), 2.24 (3H, s, CH_3), 7.08 (2H, d, ArH, *J* = 8.4), 7.24 (2H, d, ArH, *J* = 8.4), 7.49 (2H, d, ArH, *J* = 8.8), 8.10 (2H, d, ArH, *J* = 9.6), 9.17 (1H, s, NH), 9.93 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6): 14.0, 17.7, 23.7, 50.8, 111.7, 125.8, 126.7, 130.1, 133.8, 136.4, 138.1, 151.9, 152.3, 173.1 IR (KBr) 3329, 1676, 1599, 1498 cm^{-1}.

Ethyl 4-(2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)butanamido)benzoate 132e

Yield (3.40 g, 59 %); mp 146-149 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.30 (3H, t, CH_3, *J* = 6.8), 1.46 (6H, s, 2 x CH_3), 1.94 (3H, s, CH_3), 4.27 (2H, q, CH_2, *J* = 6.8), 7.21 (2H, d, ArH, *J* = 9.2), 7.80 (2H, d, ArH, *J* = 8.8), 7.90 (2H, d, ArH, *J* = 8.8), 8.07 (2H, d, ArH, *J* = 9.2), 9.59 (1H, s, NH), 9.96 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6): 13.6, 14.1, 23.4, 51.5, 60.4, 111.6, 119.6, 124.3, 125.8, 129.8, 138.1, 143.5, 151.7, 151.8, 165.4, 173.9. IR (KBr) 3330, 1665, 1594, 1491 cm^{-1}.

2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)-N-phenylbutanamide 132f

Yield (2.90 g, 61 %); mp 136-138 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.46 (6H, s, 2 x CH_3), 1.94 (3H, s, CH_3), 7.04 (1H, t, ArH, *J* = 7.6), 7.31-7.24 (4H, m, ArH), 7.63 (2H, d, ArH, *J* = 8.0), 8.10 (2H, d, ArH, *J* = 9.2), 9.27 (1H, s, NH), 9.94 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6): 13.8, 23.6, 51.3, 111.6, 120.7, 123.4, 125.8, 128.3, 138.1, 139.0, 151.8, 152.1, 173.3. IR (KBr) 3324, 1669, 1600, 1501 cm^{-1}.

N-(2,4-dimethylphenyl)-2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)butanamide 132g

Yield (3.35 g, 65 %); mp 157-160 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.46 (6H, s, 2 x CH_3), 1.98 (3H, s, CH_3), 2.10 (3H, s, CH_3), 2.25 (3H, s, CH_3), 7.04-6.96 (3H, m, ArH), 7.27 (2H, d, ArH, *J* = 8.8), 8.12 (2H, d, ArH, *J* = 9.2), 8.90 (1H, s, NH), 9.94 (1H, s, NH); IR (KBr) 3322, 1667, 1594, 1493 cm^{-1}.

2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)-N-o-tolylbutanamide 132h

Yield (3.57 g, 72 %); mp 157-159 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.48 (6H, s, 2 x CH_3), 1.99 (3H, s, CH_3), 2.15 (3H, s, CH_3), 7.22-7.10 (4H, m, ArH), 7.27 (2H, d, ArH, *J* = 9.2), 8.12 (2H, d, ArH, *J* = 9.2), 8.96 (1H, s, NH), 9.94 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6): 13.8, 20.4, 23.6, 51.2, 111.6, 117.4, 120.7, 125.0, 125.8, 128.7, 132.4, 136.5, 138.0, 151.8, 152.1, 173.1 IR (KBr) 3323, 1668, 1592, 1490 cm^{-1}.

N-(2-methoxyphenyl)-2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)butanamide 132i

Yield (2.59 g, 50 %); mp 147-149 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.46 (6H, s, 2 x CH_3), 1.96 (3H, s, CH_3), 3.69 (3H, s, OCH_3), 6.91 (1H, t, ArH, *J* = 7.6), 6.98 (1H, d, ArH, *J* = 7.6), 7.07 (1H, t, ArH, *J* = 7.6), 7. 28 (2H, d, ArH, *J* = 8.8), 7.88 (1H, d, ArH, *J* = 7.6), 8.13 (2H, d, ArH, *J* = 9.2), 8.47 (1H, s, NH), 10.02 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6): 13.7, 23.6, 51.1, 55.7, 110.9, 111.6, 120.3, 121.3, 124.6, 125.8, 127.0, 138.3, 149.6, 151.7, 152.4, 172.3. IR (KBr) 3322, 1671, 1599, 1495 cm^{-1}.

N-(4-chlorophenyl)-2,2-dimethyl-3-(2-(4-nitrophenyl)hydrazono)butanamide 132j

Yield (2.98 g, 57 %); mp 181-183 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.44 (6H, s, 2 x CH_3), 1.92 (3H, s, CH_3), 7.23 (2H, d, ArH, *J* = 9.2), 7.34 (2H, d, ArH, *J* = 8.8), 7.66 (2H, d, ArH, *J* = 9.2), 8.09 (2H, d, ArH, *J* = 9.2), 9.40 (1H, s, NH), 9.94 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 13.7, 23.5, 51.3, 111.6, 122.1, 125.8, 127.0, 128.3, 138.0, 138.1, 151.8, 151.9, 173.5. IR (KBr) 3322, 1671, 1596, 1499 cm^{-1}.

2-benzyl-N-(4-chlorophenyl)-2-methyl-3-(2-(4-nitrophenyl)hydrazono)butanamide 122

Yield (2.46 g, 39 %); mp 220-224 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.31 (3H, s, CH_3), 1.97 (3H, s, CH_3), 3.19 (1H, d, ½ CH_2, J = 13.6), 3.44 (1H, d, ½ CH_2, J = 13.6), 7.23-7.12 (7H, m, ArH), 7.36 (2H, d, ArH, *J* = 8.8), 7.64 (2H, d, ArH, *J* = 9.2), 8.09 (2H, d, ArH, *J* = 9.2), 9.54 (1H, s, NH), 10.00 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 14.4, 20.5, 40.9, 55.7, 111.7, 122.4, 125.8, 126.4, 127.3, 127.8, 128.3, 130.3, 137.6, 137.8, 138.2, 150.7, 151.7, 172.1; IR (KBr) 3322, 1668, 1590, 1488 cm^{-1}.

Preparation of 2,4-dinitrophenylhydrazone derivatives

N-(4-chlorophenyl)-3-(2-(2,4-dinitrophenyl)hydrazono)-2,2-dimethylbutanamide 157

Yield (3.52 g, 60 %); mp 199-201 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.51 (6H, s, 2 x CH_3), 2.08 (3H, s, CH_3), 7.35 (2H, d, ArH, *J* = 8.8), 7.65 (2H, d, ArH, *J* = 8.8), 7.91 (1H, d, ArH, *J* = 9.6), 8.39 (1H, m, ArH), 8.09 (2H, d, ArH, *J* = 2.8), 9.45 (1H, s, NH), 10.86 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 14.1, 23.6, 52.1, 116.7, 122.6, 123.3, 127.7, 128.7, 129.6, 130.4, 137.4, 138.1, 145.1, 159.9, 173.1; IR (KBr) 3327, 1675, 1595, 1485 cm^{-1}.

Preparation of α-substituted Phenylazo derivatives

1. **Azoacetates 133a-j and 123**

4-(4-bromophenylamino)-3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 133a

The hydrazone substrate **132a** (3.0 g, 7.15 mmol) was dissolved in CH_2Cl_2 (100 ml). To this was added lead tetraacetate (3.8 g, 8.6 mmol) with stirring. 5 ml of glacial acetic acid was added to aid the dissolution of the lead tetraacetate and the solution was allowed to stir overnight. When the reaction was judged complete (TLC) the solvent volume was reduced by 75% and the remaining CH_2Cl_2 solution was washed alternately with 5 x 50 ml portions of water and 5 % aqueous sodium bicarbonate solution. The organic portion was dried over magnesium sulfate and evaporated to dryness and any persistent lead residues were removed by flash chromatography using a 25 : 75 EtOAc : hexane mixture as eluent. Yield (2.80 g, 82%); mp 202-205 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ =1.28 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.86 (3H, s, CH_3), 2.18 (3H, s, CH_3), 7.48 (2H, d, ArH, *J* = 8.8), 7.57 (2H, d, ArH, *J* = 9.2), 7.75 (2H, d, ArH, *J* = 8.8), 8.37 (2H, d, ArH, *J* = 8.8). 9.19 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 17.2, 20.5, 20.6, 21.5, 51.0, 103.6, 115.5, 123.0, 123.2, 125.0, 131.2, 138.0, 148.5, 153.9, 168.8, 171.1; IR (KBr) 3324, 1731, 1687, 1605, 1539 cm^{-1}.

4-(4-methoxyphenylamino)-3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 133b

Yield (2.16 g, 72%); mp 149-152 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.25 (3H, s, CH_3), 1.31 (3H, s, CH_3), 1.92 (3H, s, CH_3), 2.15 (3H, s, CH_3), 3.72 (3H, s, OCH_3), 6.79 (2H, d, ArH, *J* = 9.2), 7.28 (2H, d, ArH, *J* = 8.8), 7.67 (2H, d, ArH, *J* = 8.8), 7.81 (1H, s, NH), 8.23 (2H, d, ArH, *J* = 9.2), IR (KBr) 3326, 1688, 1600, 1536 cm^{-1}.

4-(2,3-dimethylphenylamino)-3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 133c

Yield (2.55 g, 85%); mp 168-172 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.31 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.93 (3H, s, CH_3), 2.07 (3H, s, CH_3), 2.22 (3H, s, CH_3), 2.25 (3H, s, CH_3), 7.07-6.95 (3H, m, ArH), 7.80 (2H, d, ArH, *J* = 8.8), 8.39 (2H, d, ArH, *J* = 8.8), 9.03 (1H, s, NH), ^{13}C NMR (100MHz, DMSO-d_6): 14.1, 17.3, 20.0, 20.8, 21.0, 21.6, 50.4, 103.8, 123.3, 125.0, 125.1, 127.5, 133.0, 136.5, 136.8, 148.4, 153.9, 168.9, 171.0; IR (KBr) 3329, 1683, 1605, 1545 cm^{-1}.

3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxo-4-(p-tolylamino)butan-2-yl acetate 133d

Yield (2.42 g, 84%); mp 153-157 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.30 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.88 (3H, s, CH_3), 2.19 (3H, s, CH_3), 2.25 (3H, s, CH_3), 6.87 (2H, d, ArH, *J* = 8.8), 7.44 (2H, d, ArH, *J* = 8.8), 7.76 (2H, d, ArH, *J* = 8.8), 8.37 (2H, d, ArH, *J* = 8.8), 9.00 (1H, s, NH), ^{13}C NMR (100MHz, DMSO-d_6): 17.3, 20.5, 20.6, 50.8, 55.1, 103.7, 113.5, 117.4, 123.0, 123.2, 124.9, 125.0, 131.6, 148.4, 153.9, 155.7, 168.8, 170.5; IR (KBr) 3330, 1735, 1687 cm^{-1}.

Ethyl 4-(3-acetoxy-2,2-dimethyl-3-((4-nitrophenyl)diazenyl)butanamido)benzoate 133e

Yield (2.30 g, 70%); mp 155-158 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.35-1.32 (6H, m, 2 x CH_3), 1.46 (3H, s, CH_3), 1.91 (3H, s, CH_3), 2.22 (3H, s, CH_3), 4.31 (2H, q, CH_2, *J* = 7.2), 7.83-7.77 (4H, m, ArH), 7.94 (2H, d, ArH, *J* = 8.8), 8.07 (2H, d, ArH, *J* = 8.4), 9.38 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6): 14.2, 17.1, 20.5, 20.6, 21.5, 51.2, 60.4, 103.6, 117.4, 120.2, 123.2, 125.0, 129.8, 143.0, 148.5, 153.8, 165.3, 168.7, 171.4. IR (KBr) 3331, 1735, 1688 cm^{-1}.

3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxo-4-(phenylamino)butan-2-yl acetate 133f

Yield (2.23 g, 80%); mp 136-138 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.30 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.88 (3H, s, CH_3), 2.19 (3H, s, CH_3), 7.07 (1H, t, ArH, *J* = 7.2), 7.30 (2H, t, ArH, *J* = 7.2), 7.63 (2H, d, ArH, *J* = 7.6), 7.63 (2H, d, ArH, *J* = 8.4), 8.10 (2H, d, ArH, *J* = 8.4), 9.09 (1H, s, NH), ^{13}C NMR (100MHz, DMSO-d_6): 17.2, 20.6, 20.7, 21.5, 50.9, 103.7,

121.2, 123.2, 123.8, 125.0, 128.4, 138.6, 148.5, 153.9, 168.8, 170.9. IR (KBr) 3320, 1687, 1530 cm^{-1}.

4-(2,4-dimethylphenylamino)-3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 133g

Yield (2.65 g, 89%); mp 161-165 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.30 (3H, s, CH_3), 1.37 (3H, s, CH_3), 1.92 (3H, s, CH_3), 2.14 (3H, s, CH_3), 2.21 (3H, s, CH_3), 2.25 (3H, s, CH_3), 7.02 (3H, m, ArH,), 7.80 (2H, d, ArH, *J* = 9.2), 8.40 (2H, d, ArH, *J* = 9.2), 9.09 (1H, s, NH), IR (KBr) 3324, 1690, 1610, 1545 cm^{-1}.

(E)-3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxo-4-(o-tolylamino)butan-2-yl acetate 133h

Yield (2.11 g, 73%); mp 159-161 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.31 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.92 (3H, s, CH_3), 2.19 (3H, s, CH_3), 2.22 (3H, s, CH_3), 7.22-7.11 (4H, m, ArH), 7.80 (2H, d, ArH, *J* = 9.2), 8.40 (2H, d, ArH, *J* = 9.2), 8.98 (1H, s, NH), ^{13}C NMR (100MHz, DMSO-d_6): 17.4, 17.8, 20.8, 21.0, 21.6, 50.5, 103.7, 123.3, 125.0, 126.0, 126.9, 130.1, 134.0, 136.6, 148.5, 153.9, 168.9, 170.9; IR (KBr) 3324, 1677, 1535 cm^{-1}.

4-(2-methoxyphenylamino)-3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 133i

Yield (2.64 g, 88%); mp 154-156 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.24 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.88 (3H, s, CH_3), 2.23 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 6.95-6.90 (1H, m, ArH), 7.07-7.06 (2H, m, ArH), 7.78 (2H, d, ArH, *J* = 8.8), 8.09 (1H, d, ArH, *J* = 8.0), 8.38 (2H, d, ArH, *J* = 9.6), 8.82 (1H, s, NH), ^{13}C NMR (100MHz, DMSO-d_6):17.1, 20.4, 21.0, 21.4, 51.1, 55.8, 103.8, 110.8, 119.9, 120.5, 123.2, 1240, 125.1, 127.3, 148.5, 148.6, 153.8, 168.5, 170.6; IR (KBr) 3324, 1679, 1611 cm^{-1}.

4-(4-chlorophenylamino)-3,3-dimethyl-2-((4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 133j

Yield (2.63 g, 87%); mp 173-175 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.28 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.86 (3H, s, CH_3), 2.17 (3H, s, CH_3), 7.36 (2H, d, ArH, *J* = 8.8), 7.61 (2H, d, ArH, *J* = 8.8), 7.75 (2H, d, ArH, *J* = 9.2), 8.38 (2H, d, ArH, *J* = 8.8), 9.21 (1H, s, NH), IR (KBr) 3324, 1688, 1616, 1525 cm^{-1}.

3-benzyl-4-(4-chlorophenylamino)-3-methyl-2-((E)-(4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 123

Yield (2.52 g, 71 %); mp 70-72 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.05 (3H, s, CH_3), 2.01 (3H, s, CH_3), 2.24 (3H, s, CH_3), 2.85-2.78 (1H, m, ½ CH_2), 3.90-3.75 (1H, m, ½ CH_2), 7.24-7.18 (5H, m, ArH), 7.38 (2H, d, ArH, *J* = 8.8), 7.62 (2H, d, ArH, *J* = 8.8), 7.78 (2H, d, ArH, *J* = 9.2), 8.39 (2H, d, ArH, *J* = 8.8), 9.36 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 16.7, 17.0, 38.0, 55.2, 104.2, 122.8, 123.3, 125.0, 126.5, 127.6, 128.0, 128.4, 130.4, 137.0, 148.6, 153.8, 168.8, 169.9; IR (KBr) 3300, 1752, 1686, 1492 cm^{-1}.

2. <u>α-methoxy phenylazo derivative 152</u>

N-(4-chlorophenyl)-3-((2,4-dinitrophenyl)diazenyl)-3-methoxy-2,2-dimethylbutanamide 152

IBDA (2.65 g, 8.25 mmol) was dissolved in methanol (100 mL) at room temperature under argon. The hydrazone **157** (3.14 g, 7.5 mmol) was added to the solution at room temperature and the reaction mixture was then stirred for three hours. The solution was evaporated and the residue was diluted with dichloromethane. The dichloromethane solution was then washed with an aqueous saturated sodium bicarbonate solution and water, dried, and evaporated. The residue was purified by column chromatography (hexane-Et0Ac, 80:20 to 20:80). Yield (2.42 g, 80 %). ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.32 (3H, s, CH_3), 1.37 (3H, s, CH_3), 1.52 (3H, s, CH_3), 3.31 (3H, s, OCH_3), 7.35 (2H, d, ArH, *J* = 8.8), 7.52 (1H, d, ArH, *J* = 8.4), 7.65 (2H, d, ArH, *J* = 8.8), 8.67-8.65 (1H, m, ArH), 8.93 (1H, s, ArH), 9.25 (1H, s, NH); ^{13}C NMR

(100MHz, DMSO-d_6) 14.7, 21.4, 21.7, 51.6, 52.1, 102.6, 120.8, 121.2, 122.6, 127.5, 128.6, 129.8, 138.1, 145.2, 148.0, 172.8

3. α-chloro phenylazo derivative 158

3-chloro-N-(2,4-dichlorophenyl)-3-((2,4-dinitrophenyl)diazenyl)-2,2-dimethylbutanamide 158

The substrate **157** (2.1g, 5 mmol) was dissolved in DCM 50 ml cooled to –50 ^{0}C and stirred in the dark. An excess of freshly prepared t-butyl hypochlorite (8 ml) was added to the solution and this was allowed to stir for a further 2 hours at -50 ^{0}C. The temperature was allowed to rise to 0 ^{0}C and the solution was placed in an ice-bath and left to stir overnight. The solvent was removed and the remaining yellow solid was separated from a small quantity of starting material by chromatography. Yield (1.61 g, 66%); ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.62 (6H, s, 2 x CH_3), 1.95 (3H, s, CH_3), 7.47-7.42 (2H, m, ArH), 7.76-7.65 (2H, m, ArH), 8.66 (1H, s, ArH), 8.94 (1H, s, ArH), 9.27 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 22.2, 22.9, 25.1, 53.0, 101.5, 120.9, 121.9, 128.0, 129.2, 129.5, 129.8, 130.6, 131.1, 134.4, 144.6, 148.1, 172.0

4. phenylazo alkene derivative 160

N-(2,4-dichlorophenyl)-3-((2,4-dinitrophenyl)diazenyl)-2,2-dimethylbut-3-enamide 160

NaH (60% oil dispersion, 160 mg, 4 mmol) was dissolved in 12 mls of DCM and 2 mls of DMF. The solution was allowed to stir under dry conditions (drying tube) until the evolution of hydrogen had ceased. A solution of the substrate **158** (4 mmol) was made up in 2 mls DMF and added dropwise to the NaH solution via septum over two hours and the reaction was allowed to progress with stirring overnight. TLC showed the consumption of the starting material and the components were separated via column chromatography (hexane-Et0Ac, 80:20 to 20:80)

160, Yield (0.47 g, 26%); ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.50 (6H, s, 2 x CH_3), 4.87 (2H, s, CH_2), 7.64-7.61 (2H, m, ArH), 7.69 (1H, s, ArH), 7.86 (1H, d, ArH, *J* = 8.8), 8.49-8.45 (1H, m, ArH), 8.89 (1H, s, ArH), 8.94 (1H, s, ArH), 10.70 (1H, s, NH);

Preparation of azetidinones derivatives (134f and **124) using K_2CO_3/Acetone**

Synthesis of azetidinone derivative (134f) using K_2CO_3/Acetone

Anhydrous potassium carbonate (0.5 g, 3.6 mmol) was added to azoacetate **133f** (1.2 g, 3 mmol) in 50 ml of refluxing acetone. The solution was allowed to reflux for 22 h after which time (TLC) showed the reaction to be complete. The insoluble material was filtered from the solution and the filtrate evaporated to dryness. The azetidinone product **(134f)** was isolated after silica gel column chromatography using $CHCl_3$ as eluent. Yield (0.223 g, 22 %); mp 87-89 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.08 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.92 (3H, s, CH_3), 7.15-7.11 (1H, m, ArH), 7.35-7.03 (2H, m, ArH), 7.43 (2H, d, ArH, *J* = 8.8), 7.88 (2H, d, ArH, *J* = 8.8), 8.37 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 17.3, 19.1, 19.2, 60.5, 90.2, 118.3, 123.4, 124.4, 124.9, 129.1, 137.2, 149.2, 154.1, 170.5; IR (KBr) 1759, 1599, 1527, 1495 cm^{-1}.

Synthesis of azetidinone derivative* (124) *using K_2CO_3/Acetone

(124a) Yield (0.12 g, 8.3%); mp 139-141 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 0.85 (3H, s, CH_3), 1.94 (3H, s, CH_3), 3.01 (1H, d, CH, *J* = 14.4), 3.32 (1H, d, CH, *J* = 14.4), 7.27-7.07 (9H, m, ArH,), 7.78 (2H, d, ArH, *J* = 8.8), 8.29 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 16.8, 17.8, 30.6, 64.6, 91.1, 120.0, 125.3, 124.9, 127.2, 128.5, 128.5, 129.1, 129.9 136.1, 136.6, 149.7, 154.2 170.0; IR (KBr) 1745, 1527, 1494 cm^{-1}. Anal. Calcd. for $C_{24}H_{21}ClN_4O_3$: C, 64.21; H, 4.72; N, 12.48. Found: C; 64.01, H; 4.84, N; 12.18.

(124b) Yield (0.17 g, 11.6%); mp 137-139 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.43 (3H, s, CH_3), 1.99 (3H, s, CH_3), 2.67 (1H, d, CH, *J* = 14.4), 2.90 (1H, d, CH, *J* = 14.8), 7.27-7.10

(9H, m, ArH,), 7.87 (2H, d, ArH, *J* = 8.8), 8.36 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 15.1, 15.9, 36.3, 62.7, 89.2, 118.0, 122.0, 123.3, 125.2, 126.7, 127.5, 127.9, 128.4, 134.2, 134.7,147.8, 152.3, 168.0; IR (KBr) 1757, 1528, 1494 cm^{-1}.

Preparation of azetidinones derivatives (134a-j) KCN/Propanol

Synthesis of azetidinone derivative* (134a) *using KCN/Propanol

The azoacetate derivative **133a** (1g, 2.1 mmol) was dissolved in hot propanol (40 ml) to which potassium cyanide (0.15 g, 2.3 mmol) was added. The solution was allowed to reflux for 30 min, after which TLC showed development of product spots and total consumption of the starting material, this was evaporated to dryness. Water (100 ml) was added to the reaction vessel and this was extracted with EtOAc (2 x 25 ml) followed by CH_2Cl_2 (2 x 25 ml). The combined extracts were dried over magnesium sulfate and upon evaporation to dryness an orange oil was collected. The product was isolated after column chromatography. Yield (0.14 g, 16 %); mp 155-158 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.19 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.62 (3H, s, CH_3), 7.18 (2H, d, ArH, *J* =8.8), 7.55 (2H, d, ArH, *J* =8.8), 7.76 (2H, d, ArH, *J* = 9.2), 8.28 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 13.3, 17.8, 24.4, 49.3, 83.0, 122.5, 123.4, 124.9, 127.7, 130.8, 132.4, 153.8, 174.0, 180.1; IR (KBr) 1755, 1520 cm^{-1}. Anal. Calcd. for $C_{18}H_{17}BrN_4O_3$: C, 51.81; H, 4.11; N, 13.43, Found: C; 52.10, H; 4.28, N; 13.21.

Synthesis of azetidinone derivative* (134b) *using KCN/Propanol

Yield (0.15 g, 20 %); mp 95-98 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 0.98 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.77 (3H, s, CH_3), 3.71 (3H, s, OCH_3), 6.77 (2H, d, ArH, *J =9.2*), 7.28 (2H, d, ArH, *J =9.2*), 7.79 (2H, d, ArH, *J* = 9.2), 8.28 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 17.2, 19.1, 19.2, 55.5, 60.4, 90.3, 114.3, 120.3 123.3, 124.8, 130.2, 149.2, 154.1, 156.5, 170.2; IR (KBr) 1759, 1513, 1392 cm^{-1}.

Synthesis of azetidinone derivative* (134c) *using KCN/Propanol

Yield (0.21 g, 25 %); mp 112-116 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.08 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.62 (3H, s, CH_3), 2.24 (3H, s, CH_3), 2.25 (3H, s, CH_3), 7.06-6.96 (3H, m, ArH), 7.76 (2H, d, ArH, *J* = 9.2), 8.29 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 15.2, 17.4, 19.3, 19.9, 20.7, 59.6, 91.8, 123.2, 124.3, 124.9, 125.8, 129.8, 132.9, 135.1, 138.5, 149.1, 154.2, 171.6; IR (KBr) 1757, 1526, 1471 cm^{-1}. Anal. Calcd. for $C_{20}H_{22}N_4O_3$: C, 65.56; H, 6.05; N, 15.29, Found: C; 65.61, H; 6.02, N; 15.20.

Synthesis of azetidinone derivative* (134d) *using KCN/Propanol

Yield (0.13 g, 15%); mp 103-107 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.07 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.89 (3H, s, CH_3), 2.33 (3H, s, CH_3), 7.13 (2H, d, ArH, *J* =8.0), 7.32 (2H, d, ArH, *J* =8.4), 7.88 (2H, d, ArH, *J* = 9.2), 8.37 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$)17.3, 19.1, 19.2, 21.0, 60.4, 90.1, 118.3, 123.4, 124.9, 129.6, 134.1, 134.6, 149.2, 154.1, 170.3; IR (KBr) 1751, 1523, 1513 cm^{-1}. Anal. Calcd. for $C_{19}H_{20}N_4O_3$: C, 64.76; H, 5.72; N, 15.90, Found: C; 64.81, H; 5.73, N; 15.85.

Synthesis of azetidinone derivative* (134e) *using KCN/Propanol

In the case of azetidinone derivative **(134e)** *it was the propanol transesterification product that was isolated*

Yield (0.10 g, 11 %); mp 97-100 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 0.94 (2H, t *J* =7.6), 1.00 (3H, s, CH_3), 1.29 (2H,m), 1.31 (3H, s, CH_3), 1.72-1.67 (1H, m), 1.88 (3H, s, CH_3), 4.18 (1H, t, *J* =6.4), 4.27 (1H, q, *J* =5.2), 7.38 (2H, d, ArH, *J* =8.8), 7.78 (2H, d, ArH, *J* =8.8), 7.91 (2H, d, ArH, *J* = 9.2), 8.27 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 9.5, 13.3, 16.5, 17.9, 18.1, 59.8, 65.5, 89.2, 116.3, 122.4, 123.9, 124.9, 129.7, 140.0, 148.3, 152.9, 165.0, 169.6; IR (KBr) 1749, 1709, 1509 cm^{-1}.

Synthesis of azetidinone derivative* (134f) *using KCN/Propanol

(134f) Yield (0.10 g, 12 %). Spectra identical to those reported using K_2CO_3/Acetone

Synthesis of azetidinone derivative* (134g) *using KCN/Propanol

Yield (0.21 g, 25%); orange oil; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.06 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.63 (3H, s, CH_3), 2.23 (3H, s, CH_3), 2.32 (3H, s, CH_3), 6.88 (1H, d, ArH, *J* =8.0), 7.02 (2H, d, ArH, *J* =8.0), 7.75 (2H, d, ArH, *J* = 9.2), 8.28 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 17.5, 18.9, 19.3, 19.8, 21.1, 59.5, 91.7, 123.1, 124.9, 126.3, 127.1, 130.5, 131.9, 136.2, 138.1, 149.1, 154.2, 171.5; IR (liquid film) 1762, 1527, 1504 cm^{-1}.

Synthesis of azetidinone derivative* (134h) *using KCN/Propanol

Yield (0.18 g, 22%); mp 108-110 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.06 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.65 (3H, s, CH_3), 2.37 (3H, s, CH_3), 7.22-7.06 (4H, m, ArH,), 7.76 (2H, d, ArH, *J* = 8.8), 8.29 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 17.6, 19.1, 19.3, 19.9, 59.6, 91.8, 128.2, 124.9, 126.2, 126.4, 128.2, 131.3, 133.3, 136.4, 149.1, 154.1, 171.3; IR (KBr) 1761, 1530, 1493 cm^{-1}. Anal. Calcd for $C_{19}H_{20}N_4O_3$: C, 64.76; H, 5.72; N, 15.90. Found: C; 64.66, H; 5.69, N; 15.83.

Synthesis of azetidinone derivative* (134i) *using KCN/Propanol

Yield (0.14 g, 16 %); mp 98-100 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.04 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.67 (3H, s, OCH_3), 6.89-6.85 (2H, m, ArH,), 7.19-7.15 (1H, m, ArH,), 7.60-7.58 (1H, m, ArH,), 7.79 (2H, d, ArH, *J* = 9.2), 8.29 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 16.9, 18.9, 19.9, 55.6, 59.8, 91.5, 112.0, 120.8, 123.1, 123.9, 124.9, 127.6, 128.4, 149.0, 153.9, 154.4, 172.4; IR (KBr) 1761, 1524, 1497 cm^{-1}

Synthesis of azetidinone derivative* (134j) *using KCN/Propanol

Yield (0.17 g, 20%); mp 132-134 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 0.98 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.82 (3H, s, CH_3), 7.18 (2H, d, ArH, *J* =8.8), 7.29 (2H, d, ArH, *J* =9.2), 7.78 (2H, d, ArH, *J* = 8.8), 8.28 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 17.3,

19.0, 19.1, 60.7, 90.3, 119.5, 123.4, 124.9, 129.1, 129.5, 135.8, 149.3, 154.0, 170.3; IR (KBr) 1753, 1534, 1346 cm^{-1}. Anal. Calcd for $C_{18}H_{17}ClN_4O_3$: C, 57.99; H, 4.60; N, 15.03. Found: C; 57.99, H; 4.73, N; 14.81.

Synthesis of azetidinone derivative (141h)

The azetidinone derivative **134h** (100 mg, 0.28 mmol) was dissolved in glacial acetic acid (25 ml) and heated to 80 ^oC, to this was added 0.5 ml of hydrogen peroxide solution (35% by weight) at 15 min intervals over 3 h. After 5 h the reaction mixture was treated with 2 ml of the hydrogen peroxide solution and allowed to stir at 80 ^oC overnight. A further 2 ml of hydrogen peroxide solution was added to the reaction mixture and the reaction allowed to stir for a further 3 h after which no starting material could be detected by TLC The reaction was cooled to room temperature and poured into 150 ml of ice-water which induced precipitation of a pale yellow solid that was filtered from solution. Yield (66 mg, 63.9 %); mp 132-134 ºC; ^1H NMR (400MHz, $CDCl_3$): δ = 1.29 (3H, s, CH_3), 1.51(3H, s, CH_3), 1.69 (3H, s, CH_3), 2.37 (3H, s, CH_3), 7.24-7.17 (3H, m, ArH,), 7.38 (1H, d, ArH, *J* = 8.8), 8.26 (2H, d, ArH, *J* = 9.6), 8.29 (2H, d, ArH, *J* = 10.0); ^{13}C NMR (100MHz, $CDCl_3$) 14.5, 17.7, 18.7, 19.0, 60.3, 86.3, 123.4, 124.6, 126.6, 127.2, 128.6, 131.3, 132.5, 136.7, 149.9, 150.4, 171.0; IR (KBr) 1752, 1535, 1485, 1465 cm^{-1}.

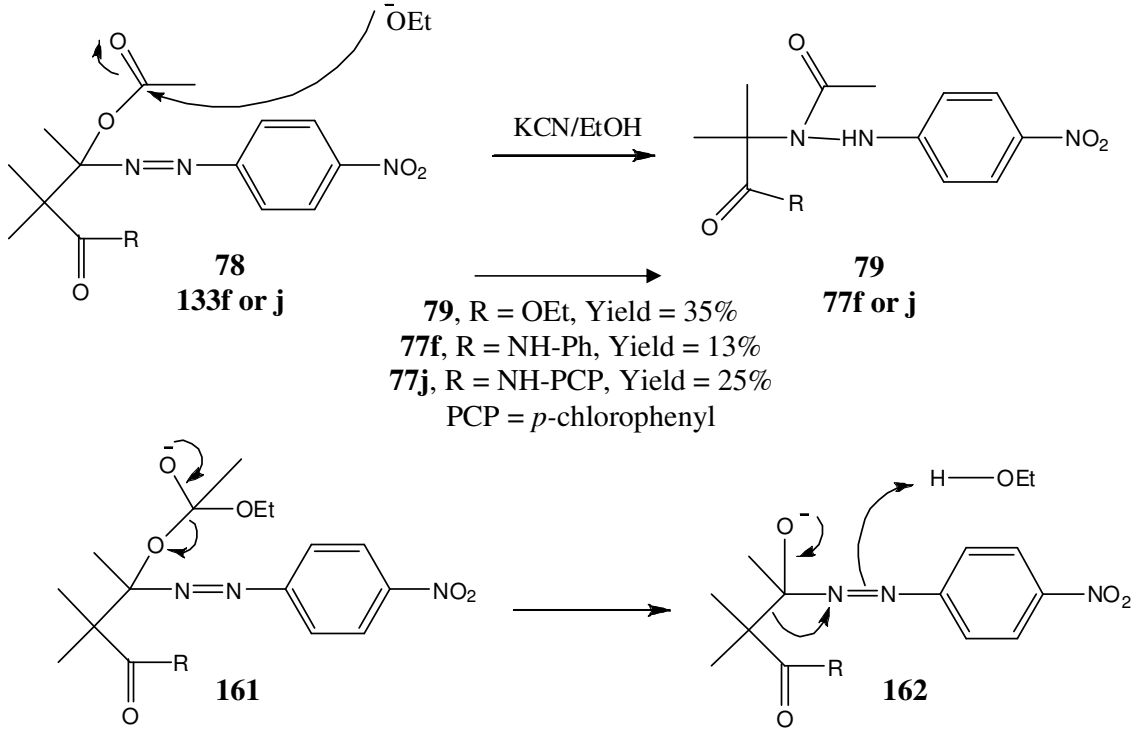
Chapter 3

Studies on the deacetylation reactions of azoacetates

ad astra per aspera

3.1 Introduction

Initial studies on the cyclisation of azoacetates to β-lactams carried out by this research group uncovered an interesting rearrangement involving the substrate azoacetates. It was found that both ester **78** and anilide **133** forms of these compounds underwent rearrangement to an acyl hydrazide (**79** or **77,** scheme 3.1).[78] The rearrangement was thought to follow deacetylation of the azoacetate and a mechanism was later proposed.



Scheme 3.1 Proposed azoacetate to N-acyl hydrazide rearrangement mechanism

The focus of this chapter is the further investigation of this deacetylation process with a variety of azoacetates. The first part of this chapter describes attempts at isolating the azocarbinol intermediates of the deacetylation. The azoacetate bearing the ester substituent (**78**, Scheme 3.1) was chosen for these studies over the corresponding anilide (**133**, scheme 13.1) form because the initial studies carried out within the group had found that this reaction was higher yielding with regard to the rearrangement product (35% for 79 compared to 13-25% for the anilides **77**). This reaction was also advantageous because it did not involve the

competing β-lactam cyclisation reaction. Previous reports of the successful isolation of azocarbinols from azoacetates by the Barton[45] and Warkentin[100,101,102] research groups encouraged this line of research. Azocarbinols are a notoriously unstable entity, with relatively few stable solid examples having been isolated to date. They are commonly observed as unstable oils that decompose over short periods of time.

The studies carried out by Barton were of particular interest due to the relative similarity between the substrates used by that group and the compounds observed to undergo rearrangement in our group (Figure 3.1).

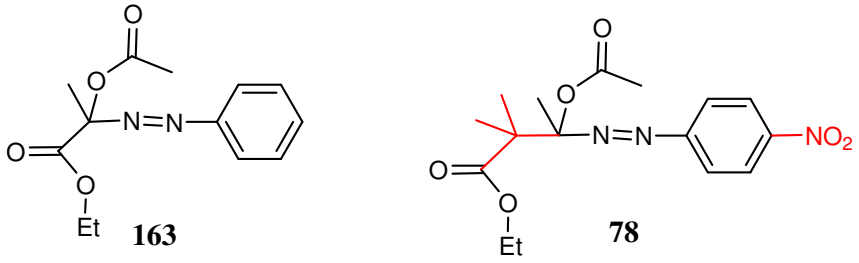


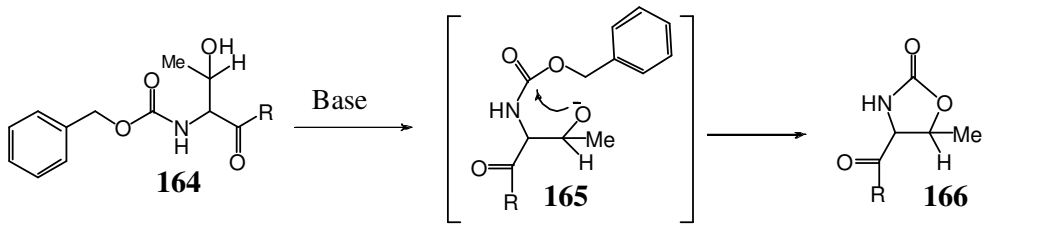
Figure 3.1 Similarities between Barton's substrate and that of the rearrangement substrate

The second part of this chapter deals with the synthesis and cyclisation of azoacetates derived from threonine to form hydantoins and oxazolidinones similar to the cyclisations commonly observed with threonyl derivatives.[103] The base catalysed generation of hydantoins is encountered throughout peptide chemistry regardless of the nature of the first residue in the peptide chain. The generation of oxazolidinones is however specific to the chemistry of N-protected threonyl and seryl compounds because the reaction takes place at the threonyl or seryl hydroxy group (Scheme 3.2a).

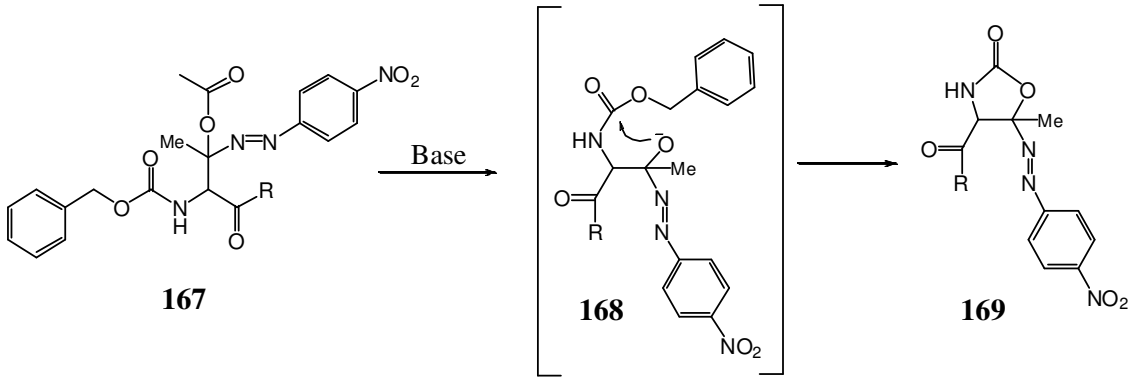
The synthesis of oxazolidinones from azoacetates (Scheme 3.2b) of this kind was of particular interest because it would most likely involve initial deacetylation through a process similar to that proposed in the formation of the N-acyl hydrazide rearrangement products (Scheme 3.1). The goal of this research was the further exploration of the deacetylation

reactions of azoacetates in an environment where cyclisation to oxazolidinones was the most likely outcome.

It was hoped that the propensity of these threonyl compounds to undergo cyclisation to oxazolidinones could be exploited in order to cyclise the azoacetate analogues of these compounds. This chemistry however depends on the initial base induced deacetylation of the acetate moiety as proposed in the rearrangement mechanism (Scheme 3.1). The acetate group is a more labile entity in basic conditions than the hydroxy hydrogen and this should favour the formation of the desired cyclisation products.



Scheme 3.2a Known cyclisation of threonyl derivative

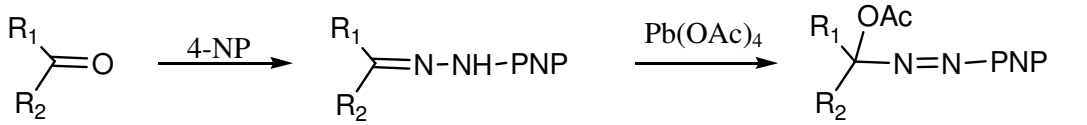


Scheme 3.2b Desired cyclisation of threonyl azoacetate

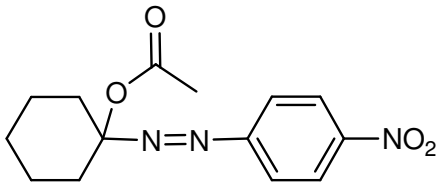
3.2 Results and Discussion

3.2.1 Isolation of azocarbinols

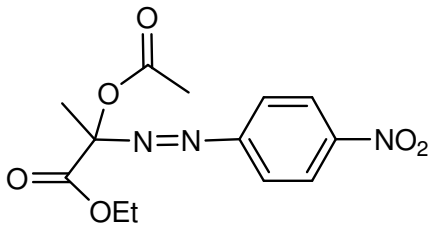
The reports of the instability of azocarbinols in general led to the decision to assess the viability of the various published procedures to synthesise these compounds. The immediate formation of the rearrangement product upon treatment with base observed previously by our group suggested that the isolation of the azocarbinol would not be trivial. It therefore seemed practical to carry out initial studies on substrates similar to those already shown to provide azocarbinol products. It was hoped that the contrasts and comparisons drawn from these tests would point towards the ideal conditions for investigation of the proposed azocarbinol intermediate. The synthesis of two simple azoacetates based on 4-nitrophenylhydrazone similar to those used by the Barton and Warkentin groups were undertaken as a reference (Scheme 3.3). This was seen as an ideal starting point, allowing the various published methods to be compared in terms of efficiency of completing the desired conversion.



170a, $R_1 + R_2$ = Cyclohexyl **171a,** Yield = 80% **172a,** Yield = 74%
170b, R_1 = Me, R_2 = CO_2Et **171b,** Yield = 58% **172b,** Yield = 69%



172a



172b

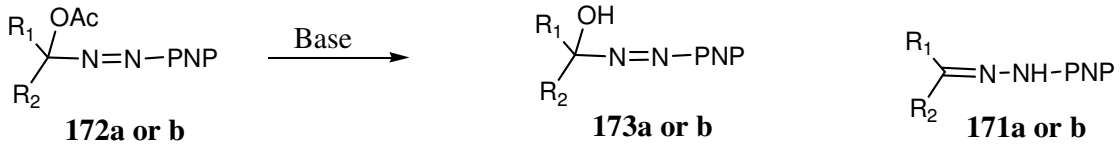
4-NP = 4-nitrophenylhydrazine

Scheme 3.3 Synthesis of model azoacetate systems.

This also allowed an assessment of the effect (if any) of the presence of the 4-nitrophenyl group on the stability of the synthesised compounds. The azoacetate derivative of cyclohexanone-4-nitrophenylhydrazone **172a** was chosen as the starting substrate for studies on the transformation from azoacetate to azocarbinol.

The various methods of transformation of azoacetates to azocarbinols were examined; in addition the basic conditions used for the cyclisation of azoacetates were assessed as means of achieving the transformation (Table 3.1). This set of experiments produced some interesting results. It was found that the published procedures for the isolation of azocarbinols did indeed achieve the desired transformation; however in the case of both substrates the transformation took place more efficiently using Barton's base.[104]

Table 3.1 Action of bases on azoacetates



172a or b **173a or b** **171a or b**

a, $R_1 + R_2$ = Cyclohexyl, **b,** R_1 = Me, $R_2 = CO_2Et$

Base/solvent	Substrate 172	173 % Yield	171 % Yield
MeLi/DCM	**a**	9	42
	b	11	38
Barton's base/EtOH	**a**	49	Trace on TLC
	b	58	Trace on TLC
KCN/*n*-EtOH	**a**	31	19
	b	42	21
K_2CO_3/Acetone	**a**	Not isolated	44
	b	Not isolated	50
t-BuOK/THF	**a**	Not isolated	30
	b	Not isolated	31

The MeLi procedure employed was found to give the desired azocarbinol in isolable quantities but the parent hydrazone was the major product. Strangely the isolation of the hydrazones upon treatment of azoacetates with base is rarely mentioned in the literature. [105]

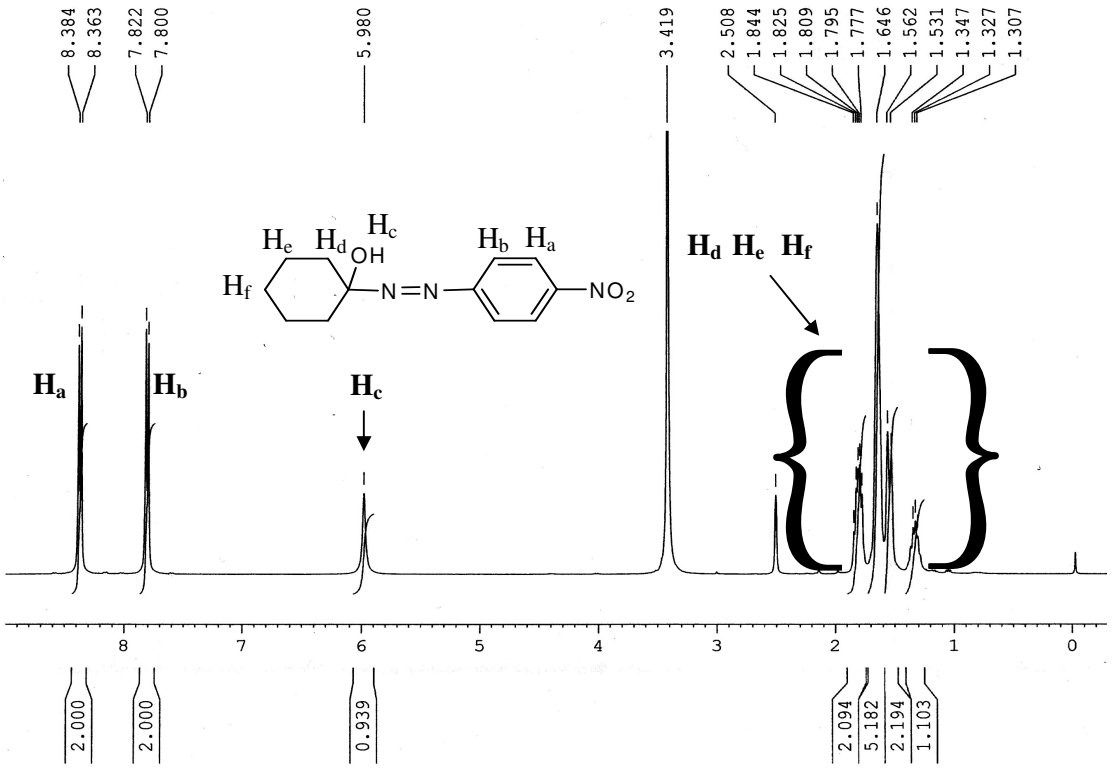


Figure 3.2a ^{1}H NMR spectrum of **173a**

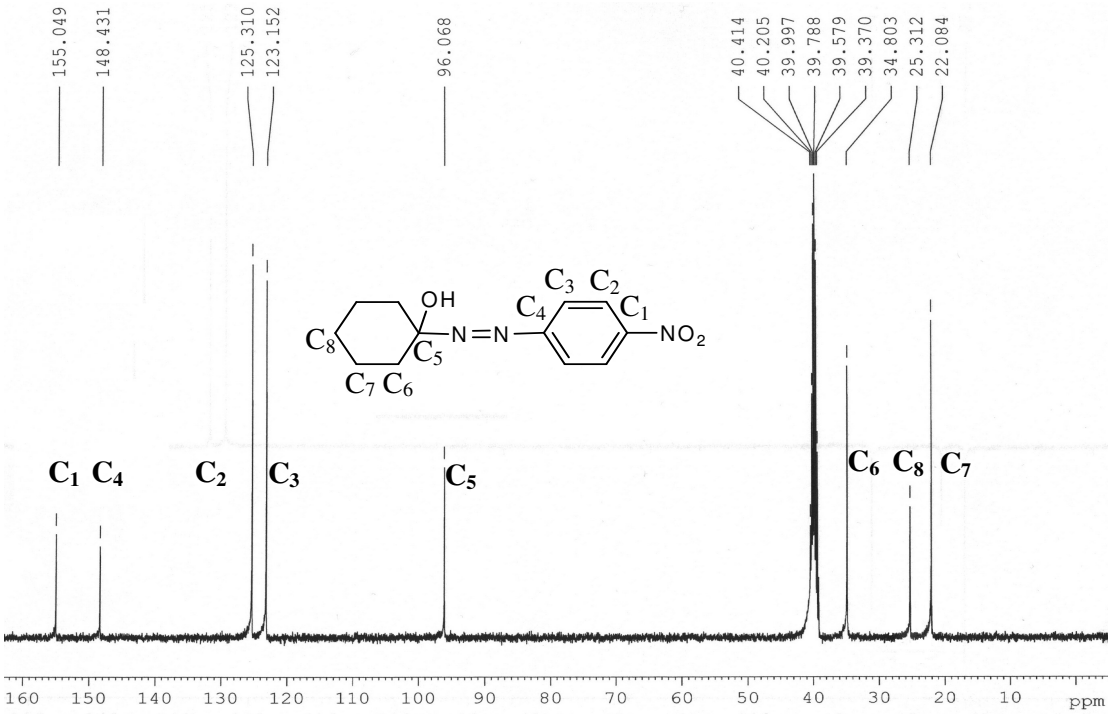
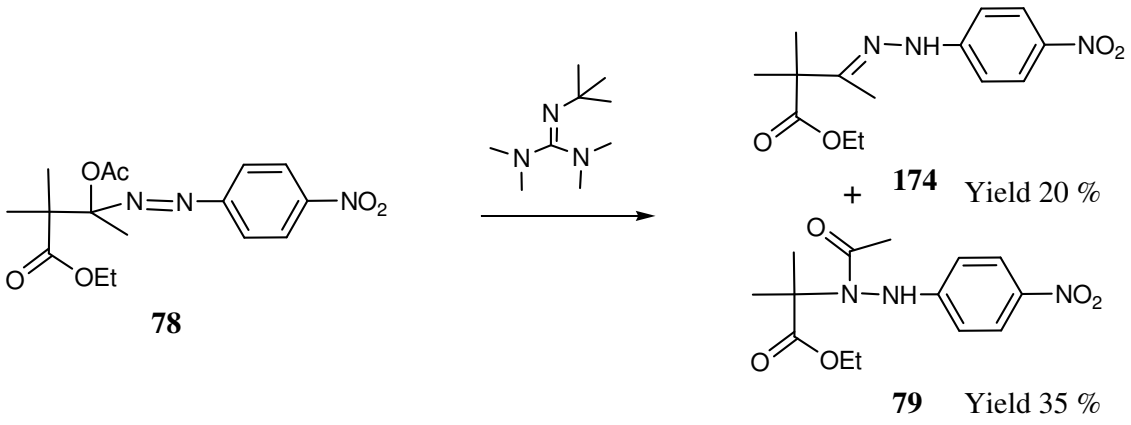


Figure 3.2b ^{13}C NMR spectrum of **173a**

It was also found that all of the bases used returned the parent hydrazone **171** (spectra identical to samples used to prepare azoacetates), either as the exclusive product, or as a by-product. Perhaps the most interesting results were achieved while using KCN as base. It was found that KCN could be used to convert azoacetates **172** to azocarbinols **173** (Table 3.1), though not as efficiently as the other reagents tested. As mentioned in chapter 2, KCN is known to be a reagent capable of catalysing the transesterification process. Since Barton suggests that the Barton's base transformation of azoacetates observed by his group were likely as a result of ethanolysis, it is conceivable that KCN acts in a similar manner.

Basic spectral comparisons between these compounds, (^{1}H and ^{13}C NMR, Figure 3.2 a, b and c) showed that it was possible to isolate the unstable azocarbinols **173** in reasonable yields in some cases and that the presence of the nitro group in the system was not detrimental to the successful isolation of simple azocarbinols. It was clear from the results of these tests that the most promising candidate for further study in the isolation of the azocarbinol as an intermediate in the rearrangement of interest would be Barton's base. The isolation of azocarbinols **173** using KCN was a positive result since this procedure has not been previously reported. In contrast to KCN, Barton's base is a strong, poorly-nucleophilic, neutral base that is often used in cases where electrophilic substitution of DBU or other amine bases is a problem. However, since it was suspected to have reacted *via* a similar mechanism to KCN, this result led to some reservations about the likelihood of success of using this reagent for the isolation of azocarbinols in the next set of experiments.

It was hoped that using Barton's base might favour the azocarbinol products analogous to **173a** and **b** under identical conditions. However using Barton's base resulted in the production of the rearrangement product **79** in a similar manner to the previously observed results with KCN, with the production of a small quantity of the parent hydrazone as by-product **174**. The azocarbinol intermediate of interest was not isolated (Scheme 3.4).



Scheme 3.4 Reaction of azoacetate **78** with Barton's base

The Warkentin lithiation procedure was also tested as a means of producing the desired azocarbinol.[106] However, as expected, the parent hydrazone was found to be the major product of the reaction with only trace amounts of the rearrangement product evident on TLC. Once again no azocarbinol was isolated. Since Barton's base was found to be the most reliable agent for the generation of azocarbinols but could not be used to isolate the desired compound; it seemed evident that the direct isolation of the suspected azocarbinol intermediate to the rearrangement product would not be possible.

3.2.2 Structural studies of N-acylhydrazide

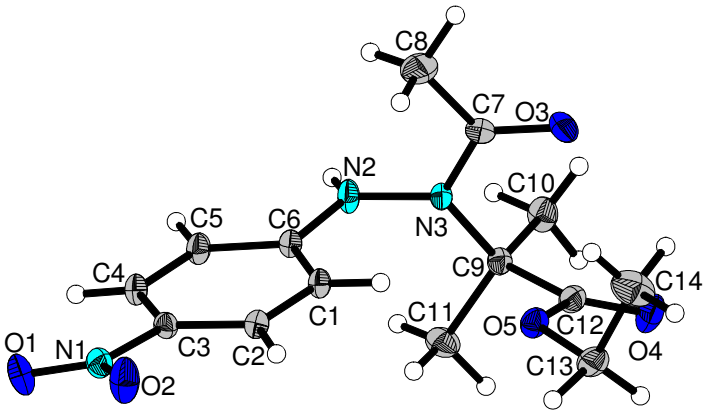


Figure 3.3 X-Ray crystal structure of N-acylhydrazide **79**

The N-acyl hydrazide **79** was previously isolated and a crystal structure obtained (Figure 3.3).[89] In order to obtain a complete set of data for the crystal suitable for publication, crystals of **79** were grown from the product of the reaction of Barton's base with azoacetate **78**

Crystals suitable for single crystal X-Ray crystallographic determinations of the N-acylhydrazide **79** were grown in a saturated solution of ethyl acetate : hexane 50:50. The substrate molecule was dissolved in the solvent mixture and allowed to stand for 5 days undisturbed; slow evaporation was controlled by covering the vessel (clean new test tube) with perforated foil. The resulting crystals were colourless blocks. The N-acyl hydrazide crystallizes in the orthorhombic system with $P2_12_12_1$ (#19) space group with 4 molecules per asymmetric unit (Table 3.3). It is this recently collected data that is reported here (appendix I) and appears in our current publication (appendix I).

Table 3.2 Selected bond distances (Å) and angles (^{O}) for N-acylhydrazide **79** with estimated standard deviations

Bond distances	(Å)
N(2)–N(3)	1.3838(14)
N(1)–O(1)	1.2301(15)
N(1)–O(2)	1.2359(15)
C(7)–O(3)	1.2284(16)
C(7)–C(8)	1.5069(19)
O(5)–C(13)	1.4539(16)
Bond angles	**(^{O})**
C(6)–N(2)–N(3)	119.73(10)
C(7)–N(3)–N(2)	120.04(11)
N(2)–N(3)–C(9)	119.79(10)
N(3)–C(7)–C(8)	118.13(12)
O(3)–C(7)–N(3)	119.40(12)

Table 3.3 Crystal data and structure refinement for N-acylhydrazide **79**.

Quantity	Measurement	
Empirical formula	$C_{14}H_{19}N_3O_5$	
Formula weight	309.32	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$ (#19)	
Unit cell dimensions	a = 10.6723(6) Å	α= 90°.
	b = 11.7713(7) Å	β= 90°.
	c = 12.3081(7) Å	γ = 90°.
Volume	1546.23(15) $Å^3$	
Z	4	
Density (calculated)	1.329 Mg/m^3	
Absorption coefficient	0.102 mm^{-1}	
F(000)	656	
Crystal size	1.00 x 1.00 x 0.90 mm^3	
Theta range for data	2.39 to 30.50°.	
Index ranges	–15<=h<=15, –16<=k<=16, –17<=l<=17	
Reflections collected	36459	
Independent reflections	2661 [R(int) = 0.0293]	
Completeness to theta =	99.7 %	
Absorption correction	Semi–empirical from equivalents	
Max. and min. transmission	0.9138 and 0.8407	
Refinement method	Full–matrix least–squares on F^2	
Data / restraints / parameters	2661 / 0 / 275	
Goodness–of–fit on F^2	1.048	
Final R indices [I>2sigma(I)]	R1 = 0.0350, wR2 = 0.0892	
R indices (all data)	R1 = 0.0360, wR2 = 0.0899	
Largest diff. peak and hole	0.443 and –0.150 e.$Å^{-3}$	

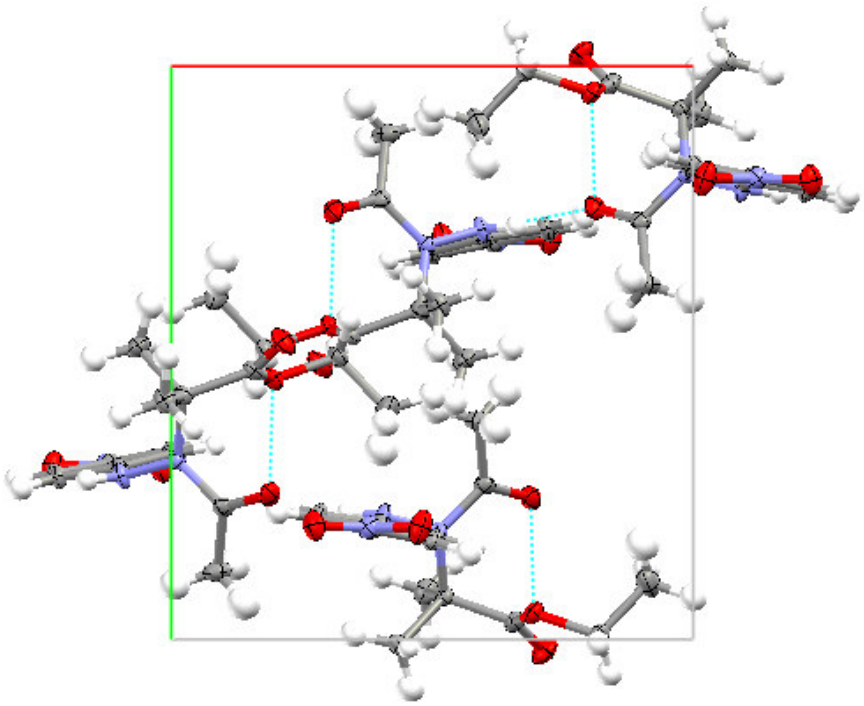


Figure 3.4 Mercury view of N-acylhydrazide **79** showing packing relative to the unit cell

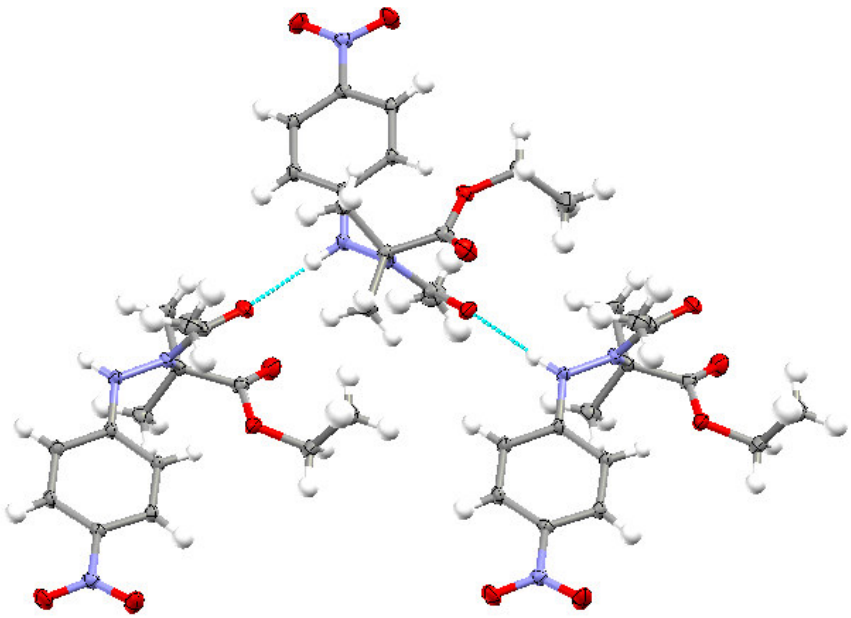


Figure 3.5 Mercury view of N-acylhydrazide **79** showing intermolecular hydrogen bonding interactions through the acyl carbonyl and hydrogen from the hydrazide

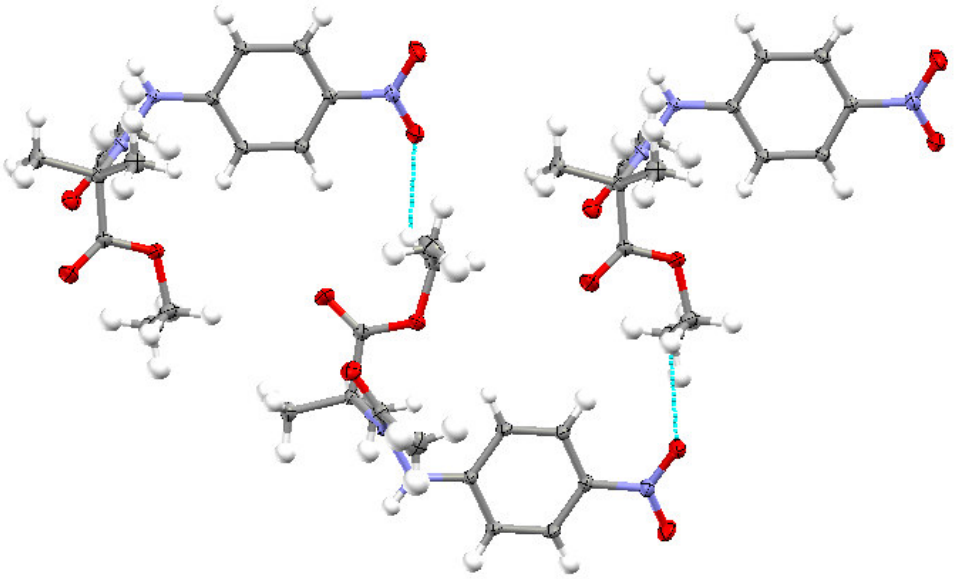
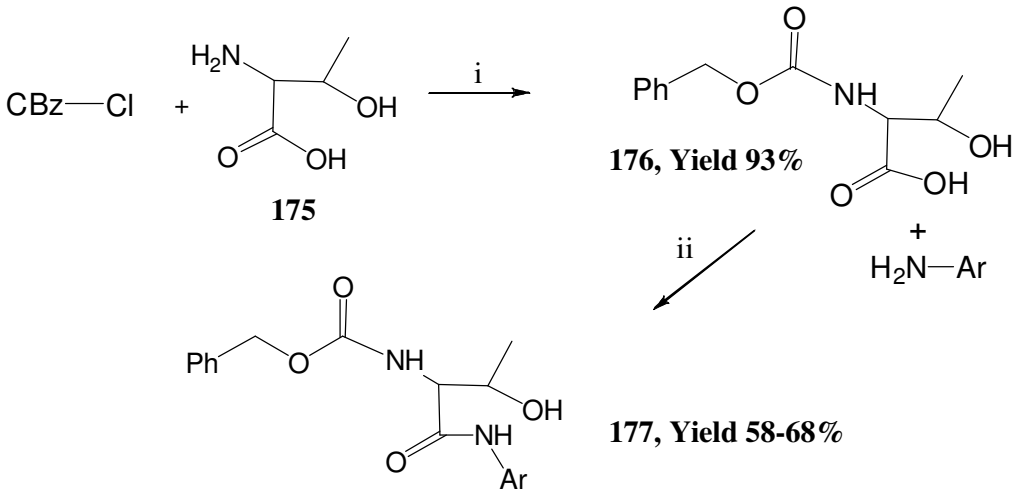


Figure 3.6 Mercury view of N-acylhydrazide **79** showing intermolecular hydrogen bonding interactions between the nitro group and ester

The hydrogen bonding through the ester carbonyl groups of the central N-acylhydrazide and nitro groups contribute most to the 3-dimensional packing within the structure of the crystal system (Figure 3.6). The interaction of these groups provides stability between the individual units within the lattice.

3.3 Cyclisation of threonyl azoacetates

The synthesis of azoacetates of N-protected threonyl anilides analogous to those used to generate the β-lactams in chapter 2 offered the opportunity to study these systems further. Suitable substrates (Cbz-threonyl anilides, **177**, Scheme 3.5) for these reactions were synthesised using standard peptide chemistry procedures.[107]

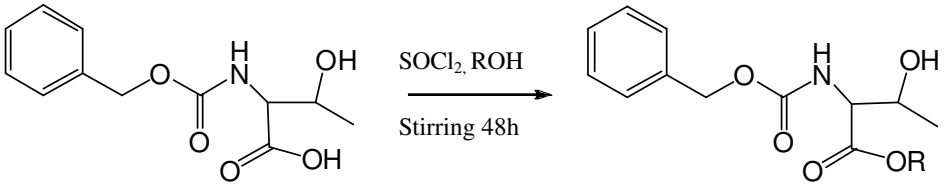


i a) 2N NaOH, Na_2CO_3, 0 ^{0}C 4h, b)HCl, yield 93%. ii, aniline, DCC, HOBt, CH_2Cl_2 yield range 58-68%

Ar = **177a** NH-4-methoxyphenyl, **177b** phenyl, **177c** 4-chlorophenyl, **177d** 3,4-dimethylphenyl.

Scheme 3.5 Synthesis of Cbz-threonyl anilides

The synthesis of a number of Z-threonyl alkyl esters was also undertaken. Several of the reported literature procedures were examined. After some experimentation it was found that the reaction of the amino acid in excess alcohol in the presence of thionyl chloride routinely returned the desired esters in good yield (**177**, Scheme 3.6).



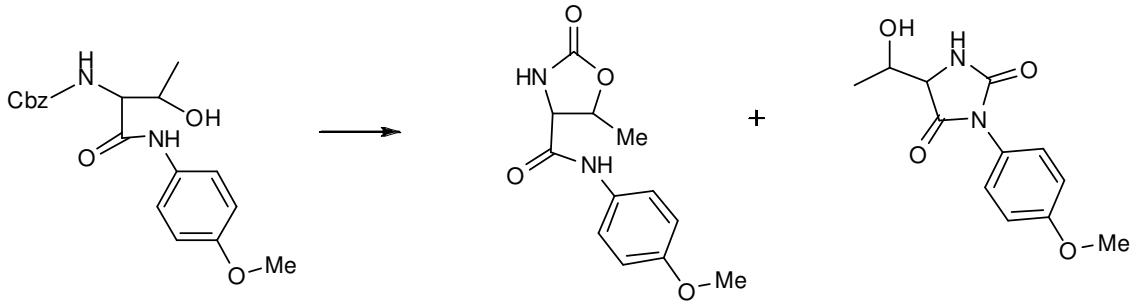
178 a-c Yield 38-72%

a R = Me, b R = Et, c R = *i*-Pr.

Scheme 3.6 Esterification of Z-threonine

3.3.1 Cyclisation of Z-threonyl anilides

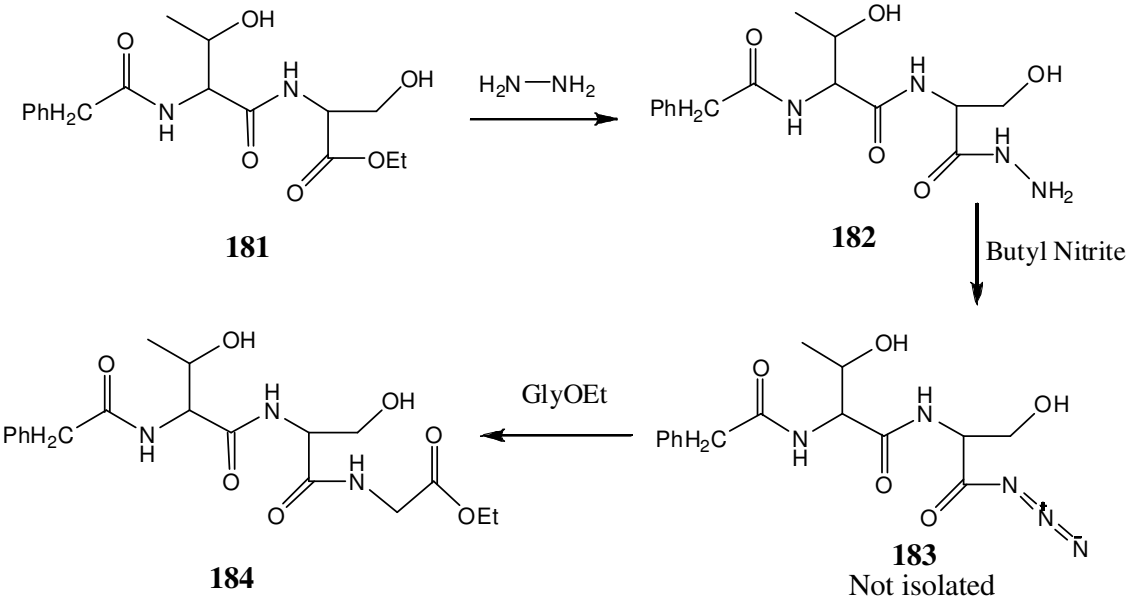
As mentioned at the beginning of this chapter the base induced cyclisation of N-protected threonyl compounds (esters, anilides, peptides and proteins) are known to give a mixture of hydantoins and oxazolidinones.



Scheme 3.7 Cyclisation of Z-Threonyl anilide to oxazolidinones **179a** and hydantoins **180a**

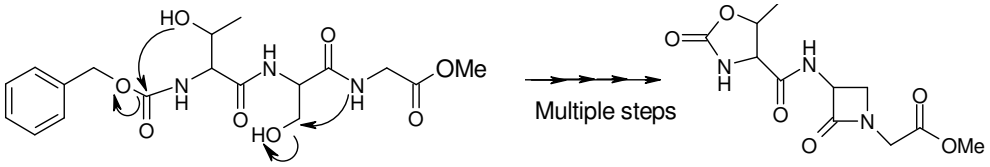
Although the compounds of interest to us were cyclic derivatives of threonyl based azoacetates, a simple Z-threonyl anilide was cyclised in order to have samples for spectral comparison to the desired azoacetate based systems (Scheme 3.7). The formation of both products is as a result of the base induced abstraction of an acidic proton.

Efforts were made to synthesise a larger peptide residue with the hope of forming a bicyclic system. To that end the Z-Thr-Ser-Gly-OEt tripeptide was synthesised using the azide coupling procedure to attach the terminal glycine ethyl ester residue.



Scheme 3.27 Synthesis of Z-Thr-Ser-Gly-OEt **184** tripeptide from Z-Thr-Ser-OEt **181**

It was hoped that, procedures similar to those that had been successfully employed to cyclise the threonyl anilides, could be used to isolate the oxazolidinone derivative of the tripeptide. Once this cyclisation had taken place it was planned to employ the Mitsunobu method of cyclisation to synthesise the β-lactam through the seryl residue, to give **185**.



184 **185**

Scheme 3.28 Planned synthesis of bicyclic β-lactam

However the desired oxazolidinone was not isolated after repeated attempts at the base induced ring closure. Because several cyclisation products were possible from the Mitsunobu cyclisation of the tripeptide without initial formation of the oxazolidinone ring, attempts to form the bicyclic ring were abandoned.

3.3.2 Oxidation of N-protected Threonyl Analogues

The objective of this step of the synthesis was the development of a method that allowed the oxidation of the hydroxy group in threonyl analogues to the corresponding carbonyl, with retention of chirality at the carbon adjacent to the newly formed carbonyl. This proved to be non-trivial. In a communication published in 1982 Andrew Stachulski of Beecham Pharmaceuticals research division remarked[108] "The amino acid literature contains remarkably little information on the oxidation of serine and threonine side chain to the ketonic level." References since that time to threonyl/seryl side chain oxidation could be described as sparse at best, and a comprehensive study on the oxidation in question has never been undertaken. Although the oxidations of alcohols to ketones are well known, the suitability of these reagents for oxidation of serine and threonine side chain to the carbonyl is not well understood. It would seem that the proximity of the amino group to the alcohol has an effect on the oxidation process. The literature available, dealt mainly with the oxidation of

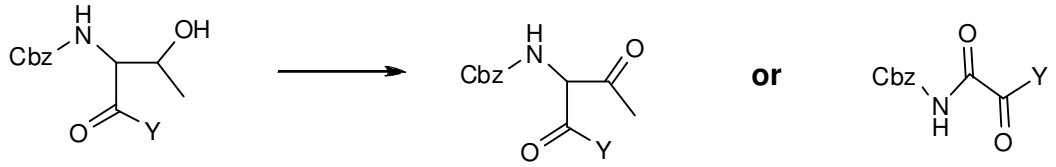
N-protected esters of threonine and serine. The following oxidation procedures were tested in an attempt to find the most suitable system.

1. Chromium based reagents (Jones and PCC procedures) [109]
2. DMSO mediated reagents and analogues (Pfitzner-Moffatt and Swern) [110]
3. Organo-iodine reagents (Dess-Martin[111,112] and IBX[113,114,115])
4. Dimethyl-dioxirane[116]

Table 3.4 is a summary of the results achieved using the various oxidation procedures, showing whether the procedure furnished the desired product, whether the product had retained chirality and the range of percentage yields achieved for the various products. It should be noted that optical activity was determined by specific rotation measurement. Although this technique does not give information on enantiomeric excess, good agreement was found between values obtained for products of different oxidation procedures (DMD and IBX products). The DMSO mediated Pfitzner-Moffatt procedure, where racemization was expected, gave values of $[\alpha]_D^{20} = 0$.

Jones reagent (a chromium based system) worked well for esters of threonine, but not so for the analogous anilide compounds, whereas PCC returned the over-oxidised oximate product in all cases. The formation of this product was shown to occur through initial formation of the desired ketonic threonyl derivative followed by further oxidation to give the oximate. If the desired ketonic product is synthesised independently using alternative oxidative procedures, and is afterward treated with chromic acid, it results in the production of an oximate identical to that produced directly from chromic acid.

Table 3.4 Oxidation of Z-threonyl esters and anilides



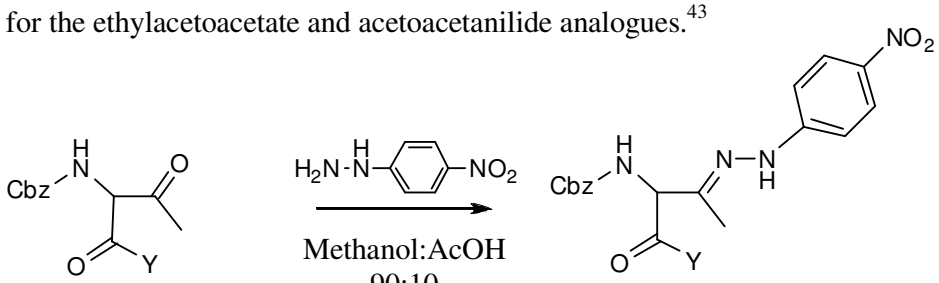
Esters[a] **178a-c** **186a-c** **188a-c**
Anilides[b] **177a-d** **187a-d** **189a-d**

Substrate		Oxidation systems				
		Chromium		Organo-iodine [c]	DMSO [d]	DMD
		Jones	PCC			
Ester [a] **178**	Product ⇨	**186**	**187**	**186**	**186**	
	Optically active ⇨	No	No	Yes	No	N/T[e]
	Yield % ⇨	80-95	59-65	59-81	88-95	
Anilide [b] **177**	Product ⇨	**189**	**189**	**187**	**187**	**187**
	Optically active ⇨	No	No	Yes	No	Yes
	Yield % ⇨	65-77	65-79	58-85	77-90	77-83

a, Esters, **178a** Y = OMe, **178b** OEt or **178c** O*i*-Pr; *b*, Anilides **177a** Y = NH-4-methoxyphenyl, **177b** NH-phenyl, **177c** NH-4-chlorophenyl, **177d** NH-3,4-dimethylphenyl; *c*, Organo-iodine = IBX or DMP; *d*, DMSO = Swern or Pfitzner-Moffatt procedure; *e*, N/T = not tested.

3.3.3 Synthesis and cyclisation of Z-threonyl azoacetate derivatives

186 and **187**, prepared from the above oxidation were further converted to 4-nitrophenylhydrazones (Scheme 3.8). This was achieved using the procedures utilized previously for the ethylacetoacetate and acetoacetanilide analogues.[43]



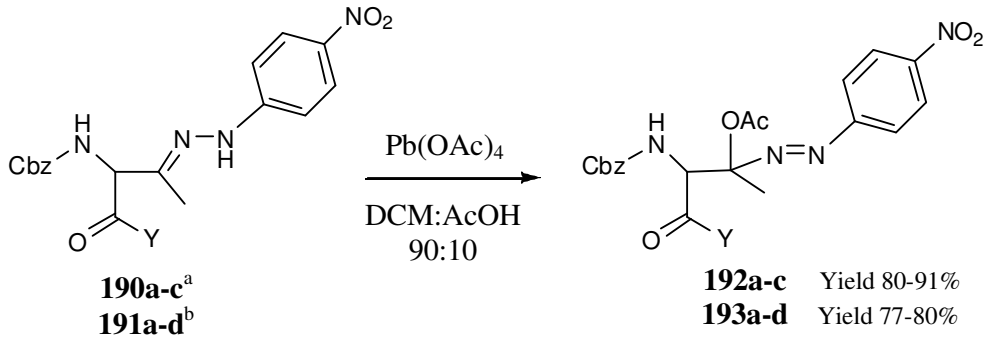
186a-c[a]
187a-d[b]

190a-c Yield 56-95%
191a-d Yield 60-75%

a, Esters, **186a** Y = OMe, **186b** OEt or **186c** O*i*-Pr; *b*, Anilides **187a** Y = NH-4-methoxyphenyl, **187b** NH-phenyl, **187c** NH-4-chlorophenyl, **187d** NH-3,4-dimethylphenyl.

Scheme 3.8 Synthesis of 4-nitrophenylhydrazones of ketonic threonyl derivatives

Similarly, the azoacetates were synthesised using the previously described procedures (Scheme 3.9).[43] The procedure gave azoacetates in good yields through reaction of the hydrazone with a slight excess of lead tetraacetate.



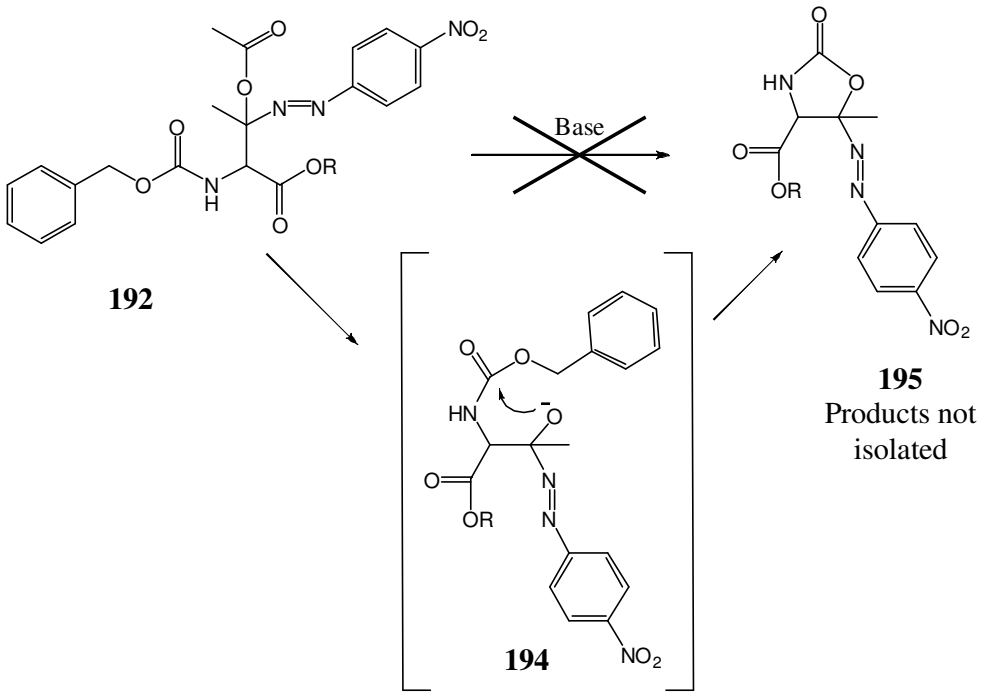
190a-c[a]
191a-d[b]

192a-c Yield 80-91%
193a-d Yield 77-80%

a, Esters, **190a** Y = OMe, **190b** OEt or **190c** O*i*-Pr; *b*, Anilides **191a** Y = NH-4-methoxyphenyl, **191b** NH-phenyl, **191c** NH-4-chlorophenyl, **191d** NH-3,4-dimethylphenyl.

Scheme 3.9 Synthesis of azoacetates of 4-nitrophenylhydrazone derivatives

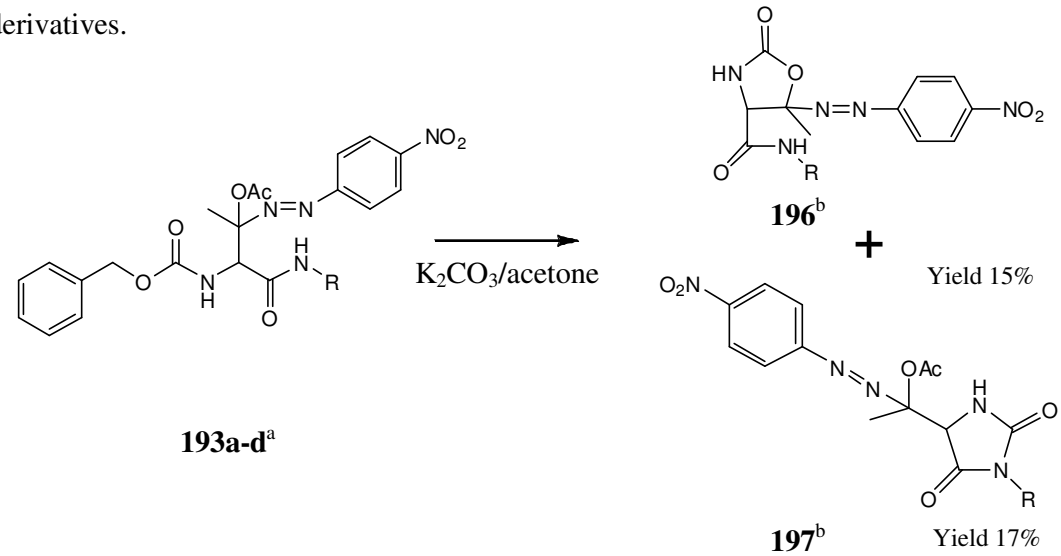
With regard to the cyclisation of the azoacetate derivatives of Z-threonine it was hoped that success would be achieved using the ester derivatives as starting materials. This was because the substance of most interest to us was the oxazolidinone derivative (**195**, scheme 3.10). Since the ester derivatives do not carry a second nitrogen bearing moiety, the possibility of hydantoin formation is excluded. A variety of base/solvent systems (K_2CO_3/Acetone, *t*-BuOK/THF, KCN/Propanol, NaOH/Ethanol) were examined as possible means of cyclising the azoacetates to the oxazolidinone, however none of these procedures were successful (Scheme 3.10). As was seen with the synthesis of the azocarbinols in some cases the parent hydrazone was the only compound that could be isolated in small quantities and identified.



Esters, **192a** R = Me, **192b** Et or **192c** *i*-Pr;

Scheme 3.10 Attempted synthesis of oxazolidinones from azoacetate derivatives

Fortunately efforts towards the desired cyclisation using the analogous anilide compounds as starting materials proved somewhat more successful (Scheme 3.11). The azoacetate derivatives of Z-threonyl anilides were subjected to similar conditions to the ester derivatives.



a, **193a** R = 4-methoxyphenyl, **193b** phenyl, **193c** 4-chlorophenyl, **193d** 3,4-dimethylphenyl; b, products **196a** and **197a** only isolated.

Scheme 3.11 Synthesis of oxazolidinone and hydantoin from azoacetate derivatives

It was found that in the case of the 4-methoxyphenyl azoacetate derivative **193** the desired oxazolidinone **196a** (Figure 3.6a) and hydantoin **197a** (Figure 3.6b) could be isolated when K_2CO_3/acetone conditions were employed.

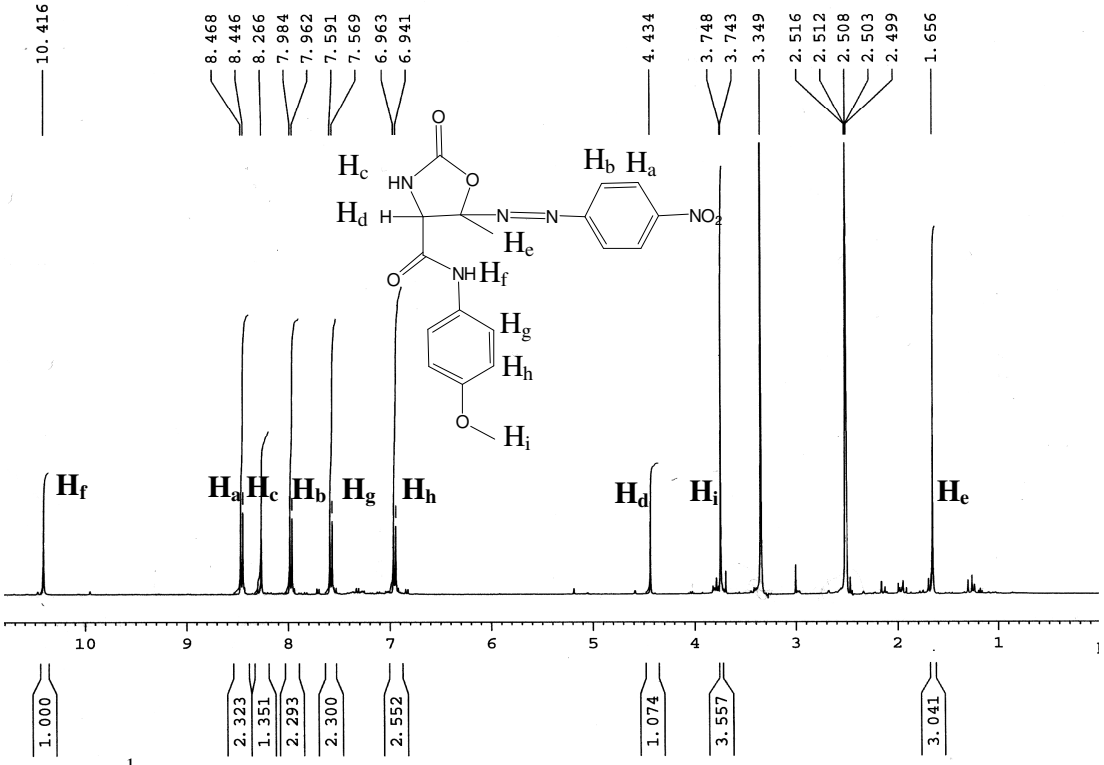


Figure 3.6a ^{1}H NMR spectrum of isolated oxazolidinone derivative **196a**

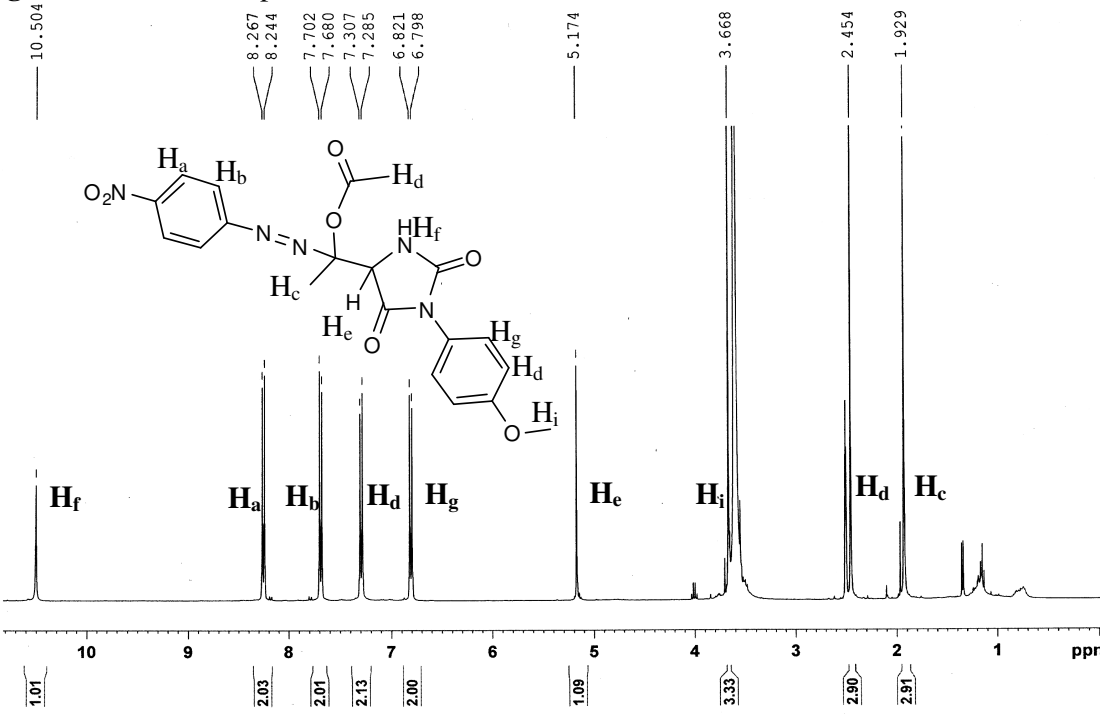
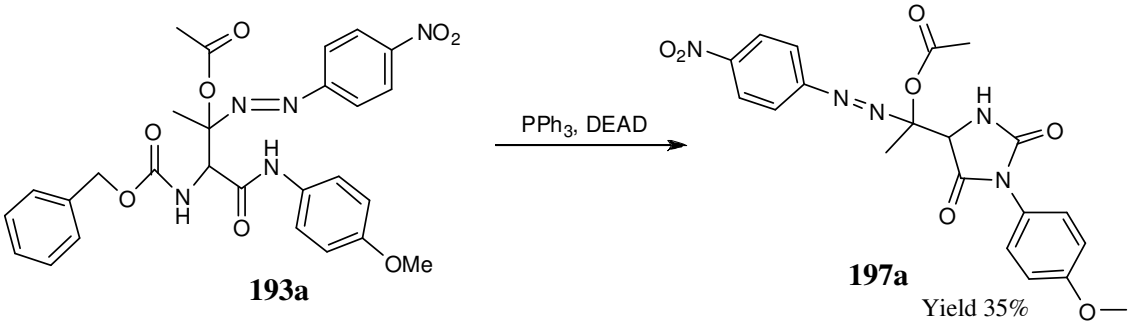


Figure 3.6b ^{1}H NMR spectrum of isolated hydantoin derivative **197a**

The products of this reaction were isolated in low yield, however similar oxazolidinone **196** and hydantoin **197** products were not isolated from the reactions of the other azoacetate derivatives **193b-d** in the series.

The azoacetate substrate **193a** was also subjected to Mitsunobu reaction conditions (PPh_3 and DEAD see chapter 1), a commonly utilised means of cyclisation of β-lactams . The substrate is somewhat similar structurally to the threonyl substrates often used in the mitsunobu cyclisation. It was hoped that this similarity, coupled with the tendency of the azoacetates to undergo deacetylation in basic conditions, might provide a route to a one pot synthesis of analogous β-lactams based on the azoacetate precursors. However, the spectra of the only product from the azoacetate under Mitsunobu conditions (PPh_3, Diethylazodicarboxylate a.k.a. DEAD) were identical to those of the hydantoin product **197a** isolated (scheme 3.12, see also scheme 3.11).

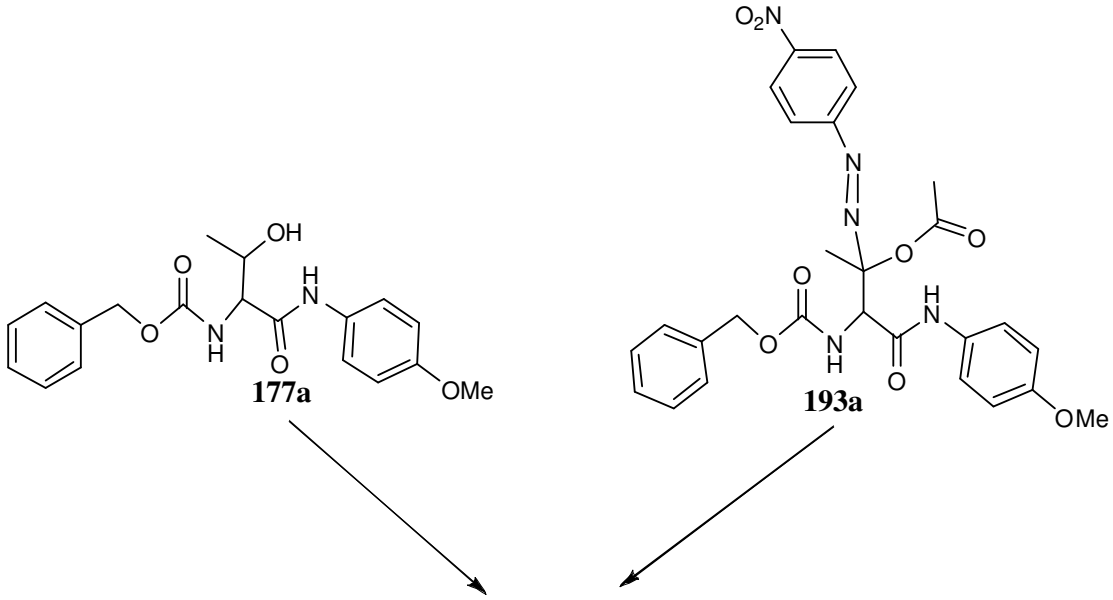


193a

Scheme 3.12 Formation of hydantoin product under Mitsunobu reaction conditions.

The formation of the oxazolidinone from the azoacetate does not conclusively prove the role of the deactylation process in the formation of the oxazolidinone. However, when all of the evidence is taken together it presents a convincing case for the mechanism involving the initial deacetylation of the acetate group. To recap, it has been shown that the KCN/propanol reaction conditions can be used to generate an azocarbinol from an azoacetate in a manner similar to known procedures from the literature (Barton or Warkentin procedures). It has also been shown that azoacetates based on N-protected threonyl anilides react to give products

analogous to those of simple N-protected threonyl anilides (Scheme 3.13), where reaction through the threonyl hydroxy group was established.



Scheme 3.13 Synthesis of the same ring systems from threonyl derivatives before and after azoacetate formation.

3.4 Conclusion:

The main focus of this chapter was the investigation into role of the deacetylation process in the formation of N-acyl hydrazides as rearrangement products from azoacetates. Although conclusive proof has not been established, good evidence to support the theory has been gathered and valuable insight into the chemistry of azoacetates has been achieved.

The deacetylation reaction was successfully adapted as a means of cyclising azoacetates derived from threonine. Simple Cbz protected threonyl and seryl peptides are known to readily undergo cyclisation through their hydroxy group in basic media to give oxazolidinones. The isolation of oxazolidinones products from azoacetates that are structurally similar to the basic peptides suggests that the initial step in the cyclisation process is the deacetylation of the acetate group. The cyclisation reactions of these compounds are novel processes. Over the course of these investigations further examples of heterocyclic systems bearing the phenylazo side chain have been synthesised. These novel compounds represent members of important biological families of compounds (Chapter 1) carrying previously unreported appendages. The syntheses presented in this chapter show these novel azoacetates to be valuable precursors in the synthesis of heterocycles.

The syntheses and chemistry of aryl hydrazones of protected threonyl anilides have been previously reported on a relatively limited scale. However, the reactions of these compounds with lead tetraacetate to give azoacetates, to date, have not been investigated.

3.5 Experimental

Amino acids were purchased from the Sigma Aldrich chemical company and were used as received. All solvents were dried or distilled prior to use. Melting points were determined using a Griffin melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 2000FT-IR spectrometer. NMR spectra were recorded using a Bruker AC 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. The ^{1}H and ^{13}C NMR chemical shifts (ppm) are relative to TMS and all coupling constants (*J*) are in Hertz (Hz).

1-cyclohexylidene-2-(4-nitrophenyl)hydrazone) 171a

Cyclohexanone (0.98 g, 10 mmol.) was dissolved in 10 ml of methanol. To this was added 4-nitrophenyl hydrazine (1.75 g, 11.5 mmol.) dissolved in 5 ml acetic acid with stirring over 30 minutes. The solution was allowed to stir overnight, and the solid precipitate was filtered off. An analytical sample was prepared by dissolving a small portion in ethyl acetate and passing it through a short column of silica, otherwise the dried product was used as collected. Orange-Brown product, (Yield 1.86 g, 80%). ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.62-1.58 (6H, m, 3 x CH_2), 2.29-2.27 (2H, m, CH_2), 2.46-2.43 (2H, m, CH_2), 7.13 (2H, d, ArH, *J* = 8.8 Hz), 8.05 (2H, d, ArH, *J* = 9.2 Hz), 9.96 (1H, s, NH). ^{13}C-NMR 100 MHz (DMSO-d_6) : 25.6, 25.9, 26.9, 27.3, 35.4, 111.3, 126.2, 137.8, 152.2, 155.4;

ethyl 2-(2-(4-nitrophenyl)hydrazono)propanoate 171b

Ethyl pyruvate (5 g, 43 mmol.) was dissolved in 70 ml of methanol. To this was added 4-nitrophenyl hydrazine (7.25 g, 5.5 mmol.) dissolved in 7 ml acetic acid with stirring over 30 minutes. The solution was allowed to stir overnight, and the solid precipitate was filtered off. the dried product was used as collected. Orange-Brown product, Yield (6.25 g, 58%) .^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.27 (3H, t, CH_3, *J* = 6.8 Hz), 2.11 (3H, s, CH_3), 4.21 (2H,

q CH_2, *J* = 6.8 Hz), 7.36 (2H, d, ArH, *J* = 9.2 Hz), 8.16 (2H, d, ArH, *J* = 9.2 Hz), 10.49 (1H, s).

α,α-Dimethylethylacetoacetate-4-nitrophenylhydrazone

α,α-Dimethylethylacetoacetate (1.58 g 10 mmol.) was dissolved 30 ml of methanol. To this was added 4-nitrophenyl hydrazine (1.68 g, 11 mmol.) dissolved in 3 ml acetic acid with stirring over 30 minutes. The solution was allowed to stir overnight, and the solid precipitate was filtered off. the dried product was used as collected. Orange-Brown product, (Yield 2.25 g, 77%) .^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.17 (3H, t, CH_3, *J* = 7.2 Hz), 1.36 (6H, s, 2 x CH_3), 1.89 (3H, s, CH_3), 4.11 (2H, q, CH_2, *J* = 7.2 Hz), 7.18 (2H, d, ArH, *J* = 9.2 Hz), 8.10 (2H, d, ArH, *J* = 9.2 Hz), 9.89 (1H, s, NH); ^{13}C-NMR 100 MHz (DMSO-d_6) :14.0, 14.3, 23.5, 50.5, 60.9, 111.9, 126.2, 138.6, 151.6, 152.1, 175.0;

Azoacetates

1-((4-nitrophenyl)diazenyl)cyclohexyl acetate 172a

The substrate hydrazone **171a** (1.28 g, 5.4 mmol.) was dissolved in 20 ml of DCM to which was added lead tetra acetate (2.63 g, 5.94 mmol., 1.1 molar excess), a few drops of acetic acid were added dropwise to the solution to help dissolve the lead salts, and the solution was allowed to stir overnight. The reaction mixture was monitored by TLC to ensure completion of the reaction and then washed with (1 x 20 ml) portion of water followed by (1 x 20 ml) portion of $NaHCO_3$ 5% solution followed by (3 x 20 ml) portion of water. The solution was dried and passed through a short silica column to remove any remaining lead salts. The column was eluted with 50:50 ethyl acetate:hexane. The solvent was removed and an orange oil remained. (Yield 1.12 g, 71%). ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.34-1.32 (1H, m, ½ CH_2), 1.84-1.56 (7H, m, 3½ CH_2), 2.17-2.14 (5H, m, CH_3 and CH_2), 7.78 (2H, d, ArH, *J* = 8.8 Hz), 8.36 (2H, d, ArH, *J* = 8.8 Hz); ^{13}C-NMR 100 MHz (DMSO-d_6): 21.7, 21.8, 24.7, 33.0, 102.5, 123.3, 125.3, 125.3, 148.8, 154.5, 169.3.

ethyl 2-acetoxy-2-((4-nitrophenyl)diazenyl)propanoate 172b

The azoacetate was synthesised using the procedure described for oxidation of cyclohexanol-4-nitrophenyl hydrazone

(Yield 1.15 g, 69%). ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.18 (3H, t, CH_3, *J* = 7.2 Hz), 1.73 (3H, s, CH_3), 2.18 (3H, s, CH_3), 4.21 (2H, q, CH_2, *J* = 7.2 Hz), 7.87 (2H, d, ArH, *J* = 8.8 Hz), 8.40 (2H, d, ArH, *J* = 8.8 Hz).

ethyl 3-acetoxy-2,2-dimethyl-3-((4-nitrophenyl)diazenyl)propanoate 78

The azoacetate was synthesised using the procedure described for oxidation of Cyclohexanol-4-nitrophenyl hydrazone

(Yield 2.63 g, 69%). ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.07 (3H, t, CH_3, *J* = 8.8 Hz), 1.26 (6H, s, 2 x CH_3), 1.89 (3H, s, CH_3), 2.11 (3H, s, CH_3), 4.03-4.00 (2H, m, CH_2), 7.87 (2H, d, ArH, *J* = 9.2 Hz), 8.40 (2H, d, ArH, *J* = 9.2 Hz).

Synthesis of 1-((4-nitrophenyl)diazenyl)cyclohexanol 173a (procedure 1, Barton's Base)

The substrate azoacetate **172a** (0.291 g, 1 mmol.) was dissolved in 3 ml of ethanol and stirred at room temperature under argon. To this was added Barton's base (0.171 g, 1 mmol.) and the reaction mixture was allowed to stir for overnight under an argon atmosphere. The solution was diluted with 20 ml of DCM, this was washed with (2 x 20 ml) portions of cold 5% HCl followed by (2 x 20 ml) portions of cold water. The organic extracts were dried over $MgSO_4$ and the solvent was removed under vacuum at room temperature. TLC testing of the remaining oil showed the presence of some starting material and a new compound. The oil was separated by careful chromatography using a gradient of solvents (Ethyl acetate:Hexane, 10:90 -> 90:10). The product was recovered as an orange solid. (Yield 0.1 g, 40%, 25% starting material also recovered).

^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.35- 1.31 (1H, m, ½ CH_2), 1.56-1.53 (2H, m, CH_2), 1.64-1.56 (5H, m, 2½ CH_2), 1.77-1.84 (2H, m, CH_2), 5.98 (1H, br s, OH), 7.81 (2H, d, ArH, *J*

= 8.4), 8.37 (2H, d, ArH, *J* = 8.8);); ^{13}C-NMR 100 MHz (DMSO-d_6): 22.1, 25.3, 34.8, 96.1, 123.2, 125.3, 148.4, 155.0.

Ethyl-2-hydroxy-2-((4-nitrophenyl)diazenyl)propanoate 173b (procedure 1)

2-Acetoxy(4-nitro-phenylazo)- propionic acid ethyl ester (1 g, 3.2 mmol.) was dissolved in 9 ml of ethanol and stirred at room temperature under argon. To this was added Barton's base (0.513 g, 3 mmol.) and the reaction mixture was allowed to stir for overnight under an argon atmosphere. The solution was diluted with 50 ml of DCM, this was washed with (2 x 25 ml) portions of cold 5% HCl followed by (2 x 25 ml) portions of cold water. The organic extracts were dried over $MgSO_4$ and the solvent was removed under vacuum at room temperature. TLC testing of the remaining oil showed the presence of some starting material and a new compound. The oil was separated by careful chromatography using a gradient of solvents (Ethyl acetate:Hexane, 10:90 -> 90:10). The product was recovered as an unstable orange oil. (Yield 0.50 g, 58%, 17% starting material also recovered).

^{1}H-NMR 400 MHz (DMSO) 1.19 (3H, t, CH_3, *J* = 7.2), 1.62 (3H, s, CH_3), 4.17 (2H, q, CH_2, J = 5.2), 7.38 (1H, br s, OH), 7.86 (2H, d, ArH, *J* = 9.2), 8.41 (2H, d, ArH, *J* = 9.2); ^{13}C-NMR 100 MHz (DMSO-d_6): 14.4, 23.1, 61.6, 97.2, 123.5, 125.5, 149.1, 154.3, 170.1.

Synthesis of 1-((4-nitrophenyl)diazenyl)cyclohexanol 173a (procedure 2, Methyl lithium)

The substrate azoacetate **172a** (0.873 g, 3 mmol.) was dissolved in 30 ml of anhydrous ether cooled to -10 ^{0}C using a bath of ice and salt and stirred under argon. To this was added CH_3Li (6.6 mmol., 3.6 ml of a 1.84M solution in hexane) over 10 minutes. The solution was allowed to stir for a further 30 minutes and cold saturated ammonium chloride (30 ml) was added slowly. The organic layer was separated and the aqueous layer extracted with (2 x 20 ml) portions of ether. The combined organic extracts were dried over $MgSO_4$ and evaporated without heating. TLC of the remaining oil showed the presence of two compounds and a

small amount of starting material. The components were separated using careful chromatography (Yield 0.07 g, 9%).

Spectral data of the azocarbinol identical to that acquired from Barton procedure.

ethyl 2-hydroxy-2-((4-nitrophenyl)diazenyl)propanoate 173b (procedure 3, KCN)

2-acetoxy(4-Nitro-phenylazo)-propionic acid ethyl ester (1 g, 3.2 mmol.) was dissolved in 30 ml of ethanol and stirred at room temperature under argon. To this was added KCN (0.27 g, 4.1 mmol.) and the reaction was monitored by TLC. A new spot was seen to develop over the course of several hours, after 72 hours some starting material remained. The solution was diluted with 50 ml of DCM, this was washed with (5 x 25 ml) portions of cold water. The aqueous phase was poured directly into a ready prepared solution of sodium hypochlorite along with all of the glassware. The solvent was slowly blown off of the solution in the fume cupboard using nitrogen to give an orange oil. The oil was separated into its component parts by careful chromatography using a gradient of solvents (Ethyl acetate:Hexane, 10:90 -> 90:10). The product was recovered as an unstable orange oil. (Yield 0.36 g, 42%)

Spectral data identical to that of sample prepared using Barton Base procedure.

N-acyl hydrazide derivative as rearrangement product using KCN/Ethanol 79

Synthesis of ethyl 2-(1-acetyl-2-(4-nitrophenyl)hydrazinyl)-2-methylpropanoate 79

The azoacetate derivative **78** (1 g, 2.8 mmol) was dissolved in hot EtOH (40 ml) to which of potassium cyanide (0.24 g, 3.7 mmol) was added. The solution was allowed to reflux overnight, before being evaporated to dryness. Water (100 ml) was added to the reaction vessel and this was extracted with EtOAc : EtO_2 (15:85). The combined extracts were dried over magnesium sulfate and upon evaporation to a reduced volume a pale brown solid precipitated. The precipitate was filtered off and washed with petroleum ether and dried. (Yield 0.31 g, 35 %); mp 172-174 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.23 (3H, t, CH_3,

J = 6.8), 1.27 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.88 (3H, s, CH_3), 4.16-4.06 (2H, m, CH_2), 6.90 (1H, d, ArH , *J* = 7.6), 7.15 (1H, d, ArH, *J* = 7.6), 8.11 (1H, d, ArH, *J* = 8.0), 8.17 (1H, d, ArH, *J* = 7.6), 9.58 (1H, s, NH); anal. Calcd. for $C_{13}H_{19}N_3O_5$ requires C, 54.36; H, 6.14; N, 13.58; found C, 54.53; H, 6.29; N, 13.51.

Azocarbinols

Synthesis of Barton's Base, 2-t-butyl-1,1,3,3-tetramethyl guanidine

Barton's original procedure was followed, although triphosgene was substituted for phosgene for safety reasons, also the quantity of triphosgene was reduced to 1/3.

Triphosgene (16.576 g, 0.056 mol) was dissolved in 50 ml of cold dry toluene. To this was added dropwise with stirring N,N,N',N'-tetra methyl urea (10 g, 0.086 Mole) dissolved in 40 ml of dry benzene. The solution was allowed to stir for 1 hour after which the solvent was removed by distillation between 30-35 ^{0}C at reduced pressure. The white crystalline Vilsmeier salt that remained was taken up in 30 ml of acetonitrile and cooled to 0^{0}C while 30 ml of *t*-butylamine was being added. Next the mixture was refluxed for 18 hours cooled and the solvent removed at 30 ^{0}C under reduced pressure. The gummy residue that remained was triturated repeatedly with ether (4 x 50 ml) the remaining solid was treated slowly and carefully with 80 ml of 25% NaOH solution, this was then extracted with ether and the combined extracts dried over K_2CO_3. The solvent was removed slowly at 35 ^{0} C under house vacuum. The yellow oil that remained was distilled under vacuum on the Kughlerohr instrument at 75 ^{0} C to give a transparent oil which was stored under argon and refrigerated. (Yield 0.53 g, 55 %);^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 0.45 (9H, s, 3x CH_3), 1.88 (12H, s, 4 x CH_3)

N-acyl hydrazide derivative as rearrangement product using Barton's base 78

Synthesis of ethyl 2-(1-acetyl-2-(4-nitrophenyl)hydrazinyl)-2-methylpropanoate 78

The substrate azoacetate 215 (702 mg, 2 mmol.) was dissolved in 10 ml of ethanol and the reaction vessel flushed with nitrogen. To which was added of Barton's base (342 mg, 2 mMol) by syringe through a rubber septum. The reaction was stirred for 1 hour before TLC testing showed the consumption of all starting material and the emergence of a new spot. After 2 hours 50 ml of DCM was used to dilute the reaction mixture. The entire solution was washed with 20 ml of cold 5% HCl and 20 ml of cold water and the organic phase dried and the solvent removed under vacuum to give an orange solid. (Yield 0.23 g, 37 %);

Spectra identical to those from KCN/ethanol procedure

N-acyl hydrazide derivative as rearrangement product of anilide azoacetate 70f

Yield (0.14 g, 16 %); mp 195-197 ºC ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.49 (3H, s, CH_3), 1.53 (3H, s, CH_3), 1.92 (3H, s, CH_3), 6.96 (1H, s br, ArH),7.07 (1H, t, ArH, *J* = 7.2), 7.33-7.26 (3H, m, ArH), 7.61 (2H, d, ArH, *J* = 7.6), 8.15 (2H, s br, ArH), 9.06 (1H, s br, NH), 9.48 (1H, s br, NH); ^{13}C NMR (100 MHz, DMSO-d_6) 21.7, 22.9, 24.3, 65.6, 110.5 (d, *J* = 222), 120.8, 123.5, 126.2 (d, *J* = 69), 128.3, 138.7, 138.9, 154.2, 171.8, 172.8. IR (KBr) 3338, 3288, 1647, 1592, 1522, 1329, 1272, 1108, 750, 695 cm^{-1}.

Benzyloxycarbonyl-threonine (Cbz-Threonine) 176

To a solution of L-threonine **175** (11.91g, 0.1 mol) in 50 mls of 2N NaOH was added alternately benzyl chloroformate (14.5 mls. 17.32 g, 0.101 mol) and Na_2CO_3 (65 mls, 0.13 mol) in 10 roughly equal aliquots over 30 minutes. The solution was allowed to stir for 4 hours and then extracted twice with 20 ml portions of ether. The aqueous phase was cooled to 0^{o} C and conc. HCl was added dropwise to the solution to pH 3-4. The oil that separated was transferred to ethyl acetate (50 ml) and the aqueous phase was then saturated with NaCl and extracted with a further 50 ml of ethyl acetate. The organic extracts were combined and dried over $MgSO_4$. The solvent was removed and a small potion of the oil that remained was

removed, cooled and scratched. The solid collected was used to seed the remaining oil, which crashed out overnight. (Yield 23.53 g, 93%). M.p. 101-103

Spectra consistent with sample purchased from sigmaaldrich®

Z-Threonyl-4-methoxy-anilide, (benzyl-3-hydroxy-1-(4-methoxyphenylamino)-1-oxobutan-2-ylcarbamate) 177a

Cbz-L-Threonine **176** (10 g, 0.040 mol), 4-methoxy aniline (4.92 g, 0.040 mol) and 1-hydroxy-benzotriazole monohydrate (6.12 g, 0.040 mol) were finely ground and dissolved in 150 ml of dichloromethane. The solution was cooled to 0^{o} C in an ice bath and finely ground dicyclohexylcarbodiimide (8.2 g, 0.040 mol) was added. The solution was allowed to stir overnight at room temperature and the N,N'-dicyclohexylurea that precipitated was filtered from the solution and washed with 50 ml of dichloromethane. The combined organic extracts were washed with 50 ml of saturated $NaHCO_3$ solution followed by 50 ml of 10% citric acid solution, another 50 ml of saturated $NaHCO_3$ solution, and finally 50 ml of water. The organic extracts were dried and passed through a 4 cm silica plug to remove a baseline impurity (TLC) and eluted using 70:30 Ethyl acetate: Hexane. The solvent was removed *in vacuo*, and the residue collected. (Yield 9.74 g, 68%) .^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.12 (3H, d, CH_3, J = 6.0 Hz), 3.72 (3H, s, OCH_3), 4.04-4.00 (1H, m, CH), 4.11- 4.09 (1H, m, CH), 4.95 (1H, d, OH, J = 6.0 Hz), 5.09 (2H, s, CH_2), 6.89 (2H, d, ArH, J = 8.8 Hz), 7.08 (1H, d, NH, J = 8.4 Hz), 7.32 (5H, m, ArH), 7.54 (2H, d, ArH, J = 8.8 Hz), 9.82 (1H, s, NH). ^{13}C-NMR 100 MHz (DMSO-d_6) : 20.6, 55.5, 61.7, 65.9, 67.2, 114.2, 121.2, 128.0, 128.2, 128.7, 132.4, 137.3, 155.6, 156.5, 169.1. IR (KBr) 3361, 1683, 1588, 1510, 1239, 1071 cm^{-1}.

Z-Threonyl-4-chloro anilide, (benzyl-1-(4-chlorophenylamino)-3-hydroxy-1-oxobutan-2-ylcarbamate) 177c

This coupling was carried out using the same procedure as that used for Z-Thr-N-Ph-4-OMe, except that the aniline used was 4-Chloroaniline

(Yield 8.69 g, 60%).

^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.14 (3H, d, CH_3, *J* = 6.0 Hz), 4.04-4.03 (1H, m, CH), 4.14-4.11 (1H, m, CH), 4.94 (1H, d, OH, *J* = 6.0 Hz), 5.07 (2H, s, CH_2), 7.14 (1H, d, NH, *J* = 8.4 Hz), 7.38-7.33 (7H, m, ArH), 7.66 (2H, d, ArH, *J* = 8.4 Hz), 10.12 (1H, s, NH), ^{13}C-NMR 100 MHz (DMSO-d_6) : 20.6, 61.8, 66.0, 67.1, 121.2, 127.3, 128.0, 128.2, 128.7, 129.0, 137.3, 138.2, 156.6, 169.8. IR (KBr) 3280 2360, 1692, 1649, 1536, 1515, 1242, 1037, 696 cm^{-1}.

Z-Threonyl-3,4-dimethyl anilide, (benzyl-1-(3,4-dimethylphenylamino)-3-hydroxy-1-oxobutan-2-ylcarbamate) 177d

This coupling was carried out using the same procedure as that used for Z-Thr-N-Ph-4-OMe, except that the aniline used was 3,4-Dimethylaniline.

(Yield 8.26 g, 58%). ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.12 (3H, d, CH_3, *J* = 5.6 Hz), 2.16 (3H, s, CH_3), 2.18 (3H, s, CH_3), 4.02 (1H, s, CH), 4.10-4.08 (1H, m, CH), 4.94 (1H, s br, OH), 5.07 (2H, s, CH_2), 7.05 (1H, d, ArH, *J* = 8.0 Hz), 7.10 (1H, d, NH, *J* = 8.4 Hz), 7.35 (7H, m, ArH), 9.80 (1H, s, NH). ^{13}C-NMR 100 MHz (DMSO-d_6) : 19.1, 20.0, 20.6, 61.7, 65.9, 67.2, 117.2, 120.9, 128.0, 128.2, 128.7, 129.9, 131.4, 136.6, 137.0, 137.4, 156.5, 169.3. IR (KBr) 3281, 2360, 1696, 1654, 1537, 1243, 1040, 696 cm^{-1}.

Z-Threonyl-anilide (benzyl-3-hydroxy-1-oxo-1-(phenylamino)butan-2-ylcarbamate) 177b

This coupling was carried out using the same procedure as that used for Z-Thr-N-Ph-4-OMe, except that aniline was used.

(Yield 7.87 g, 60%); ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.13 (3H, d, CH_3, *J* = 6.4 Hz), 4.05-4.01 (1H, m, CH), 4.14-4.11 (1H, m, CH), 4.92 (1H, d, OH, *J* = 6.0 Hz), 5.08 (2H, s, CH_2), 7.06-7.04 (1H, m, ArH), 7.10 (1H, d, NH, *J* = 8.8 Hz), 7.39-7.30 (7H, m, ArH), 7.62 (2H, d, ArH, *J* = 7.6 Hz), 9.96 (1H, s, NH); ^{13}C-NMR 100 MHz (DMSO-d_6) : 20.2, 61.4,

65.6, 66.8, 119.2, 123.3, 127.6, 127.8, 128.3, 128.7, 136.9, 138.8, 156.7, 169.2; IR (KBr) 3291, 1688, 1650, 1537, 1520, 1240, 1045, 698 cm^{-1}.

Esters of threonine

Methyl-2-(benzyloxycarbonylamino)-3-hydroxybutanoate 178a (Z-Thr-OMe)

To a solution of Z-threonine **176** (5.83 g, 23 mmol) in methanol (100 cm^3), was added thionyl chloride (2.1 cm^3, 28.8 mmol) dropwise over 30 min and the mixture was allowed to stir for 20 h. After TLC showed consumption of the starting material, the solvent was removed and the remaining oil taken up in 30 cm^3 of $CHCl_3$. The organic solution was washed alternately with saturated $NaHCO_3$ and brine (3 x 10 cm^3 each). The solution was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The white solid product was dried and collected. (Yield 4.43 g 72%); mp 77-79 ^0C; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.25 (3H, d, CH_3, *J* = 6.4 Hz), 2.72 (1H, s, OH), 3.75 (3H, s, CH), 4.34-4.32 (2H, m, 2 x CH), 5.13 (2H, s, CH_2), 5.87-5.77 (1H, m, NH), 7.37-7.32 (5H, m, ArH); IR (KBr) 3450, 3314, 1715 cm^{-1}.

Ethyl-2-(benzyloxycarbonylamino)-3-hydroxybutanoate 178b (Z-Thr-OEt)

(Yield 3.33 g 51%); mp 29-31 ^0C; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.19-1.13 (6H, m, 2 x CH_3), 2.70 (1H, s, OH), 4.11 (2H, q, CH_2, *J* = 7.2), 4.23-4.20 (2H, m, 2 x CH), 5.03 (2H, s, CH_2), 5.75-5.72 (1H, m, NH), 7.27-7.19 (5H, m, ArH); IR (KBr) 3401, 1721 cm^{-1}.

Isopropyl-2-(benzyloxycarbonylamino)-3-hydroxybutanoate 178b (Z-Thr-O*i*-Pr)

(Yield 6.82 g 38%); mp 37-39 ^0C; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.21-1.07 (9H, m, 3 x CH_3), 4.01-3.98 (1H, m, CH), 4.08-4.03 (1H, m, CH), 4.75 (1H, d, OH, J = 6.8), 4.95-4.88 (1H, m, CH), 5.06 (2H, s, CH_2), 7.15 (1H, d, NH, *J* = 8.4), 7.43-7.31 (5H, m, ArH); IR (KBr) 3345, 1721 cm^{-1}.

Oxidation of Z-Threonyl anilides and esters

Synthesis of 1-hydroxy-1,2-benziodoxal-3(1H)-one-1-oxide (IBX)

2-iodobenzoic acid (50g, 0.2 moles) was added to a solution of oxone (181 g, 0.29 moles) in 650 ml of distilled water. The reaction mixture was carefully and slowly heated to 73 ^{0}C (at no time was the reaction temperature allowed to leave the 70-75 ^{0}C range) and the reaction was stirred for 3 hours at this temperature. The solution was allowed to settle and cooled to 0 ^{0}C for 4-5 h; with occasional agitation (No agitation for the last 90 minutes) the solution was filtered through a sintered glass funnel and rinsed with (6 x 100 ml) portions of water followed by (2 x 100 ml) portions of acetone. The white crystalline product was dried under vacuum over three days Yield 85 %. The mother liquor was allowed to sit and filtered for solids several times before disposal. All oxidising materials collected were treated with $NaSO_3$ and NaOH before disposal. (Yield 47.6 g 85%);

IBX oxidation of (Z-Thr-N-Ph-OMe 177b)

Synthesis of benzyl-1-(4-methoxyphenylamino)-1,3-dioxobutan-2-ylcarbamate 187a

The substrate **177a** (0.716 g, 2.0 mmol.) was dissolved in 20 ml of ethyl acetate containing 4 ml of DMSO, to this was added IBX(1.67 g, 6.0 mmol.). The mixture was refluxed for 3 hours after which TLC testing showed no remaining starting material. The reaction mixture was allowed to cool and the Excess IBX and IBA by-product were filtered off. The filtrate was washed with (3 x 20 ml) portions of water and the solvent was removed under vacuum to leave a brown/yellow oily residue. The residue was passed through a short column of silica eluted with 50:50 ethyl acetate:hexane and the product **187a** was recovered as a white solid. (Yield 0.43 g, 61%); mp 130-133 ºC; ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 2.23 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 5.06 (1H, d, CH, *J* = 8.0 Hz) 5.10 (2H, s, CH_2) 6.91 (1H, d, ArH, *J* = 8.8 Hz) 7.37 (5H, m, ArH) 7.51 (2H, d, ArH, *J* = 9.2 Hz) 7.96 (1H, d, NH, *J* = 8.4 Hz) 10.28 (1H, s, NH) ^{13}C-NMR 100 MHz (DMSO-d_6) :27.3, 55.5, 66.1, 66.3, 114.3, 121.3, 128.1, 128.2,

128.7, 131.9, 137.1, 156.0, 156.3, 164.6, 202.1; IR (KBr): 3311, 3219, 1701, 1685, 1640, 1529, 1499, 1238, 1055, 697 cm^{-1}. $[\alpha]_D^{20}$ -6.6.

Dess-Martin Periodinane oxidation of (Z-Thr-N-Ph-OMe 177a) Method 1

Synthesis of benzyl-1-(4-methoxyphenylamino)-1,3-dioxobutan-2-ylcarbamate 187a

The substrate **177a** (0.79 g, 2.23 mmol.) in 10 mls of DCM was added to a stirred solution of Dess-Martin Periodinane (1.05g, 2.47 mmol.) in 10 mls of DCM and 1 ml of pyridine under argon. The solution was allowed to stir for 72 hours after which TLC showed the formation of some product. The solution was diluted with 50 mls of diethyl ether, filtered to remove any solid and washed with (5 x 10 ml) portions of 5% NaOH. 10 ml $NaHCO_3$ and 10 ml water. The ether layer was dried and evaporated to a small volume, and then separated by chromatography. (Yield 0.38 g, 48%) product **187a** and 29% starting material.

Product had identical spectra to that prepared using IBX

Dess-Martin Periodinane oxidation of (Z-Thr-N-Ph-OMe 177a) Method 2

Synthesis of benzyl-1-(4-methoxyphenylamino)-1,3-dioxobutan-2-ylcarbamate 187a

The substrate **177a** (0.79 g, 2.23 mmol.) in DCM (10 mls) was added to a stirred solution of Dess-Martin Periodinane (1.05 g, 2.47 mmol.) in DCM (10 mls). The solution was allowed to stir for 6 hours while DCM (10 ml pre-shaken with water) was added dropwise. TLC monitoring showed the formation of product and consumption of the starting material. The solution was diluted with of diethyl ether (50 mls), filtered to remove any solid and washed with a (5 x 10 ml portions) of 5% NaOH. 10 ml $NaHCO_3$ and 10 ml water. The ether layer was dried and evaporated to a small volume, (Yield 0.51 g, 66%) product 291a recovered.

Product had identical spectra to that prepared using IBX

Generation of dimethyldioxirane

Into a 500 ml three necked round bottomed flask fitted with a powder addition funnel and connected a cooled receiving vessel (Acetone and Liquid N_2) with two additional cold traps (Acetone and Liquid N_2 / Liquid N_2) was added water (35 ml), acetone (27.5ml) and $NaHCO_3$ (24 g). The system was cooled to 0 ^{0}C and placed under vacuum before the addition over 30 min of Oxone ($2KHSO_5.KHSO_4.K_2SO_4$) (45g) was commenced. When the addition of the Oxone was complete the system was placed under a gentle stream of nitrogen for 1 hour. The fractions of yellow volatile liquid collected in the receiving vessel and the first cold trap were combined and used immediately.

Dimethyldioxirane oxidation of (Z-Thr-N-Ph-4-OMe 177a)

Synthesis of benzyl-1-(4-methoxyphenylamino)-1,3-dioxobutan-2-ylcarbamate 187a

The substrate **177a** (0.5 g, 1.4 mMol) were dissolved in the acetone and stirred. The freshly prepared dimethyldioxirane solution was added dropwise over 1 hour with the progress of the reaction monitored by TLC every 10 minutes. When no more traces of starting material were detected the addition of the DMD solution was ceased (17 mls of solution required). The reaction was allowed to stir for a further hour before 1 ml of isopropyl alcohol was added to the solution to quench any excess DMD (left over DMD solution also quenched with excess DMD). The solvent was removed under vacuum and the solid **187a** remaining was collected (Yield 0.36 g, 73%).

Product had identical spectra to that prepared using IBX

Moffatt oxidation of (Z-Thr-N-Ph-4-OMe 177a)

Synthesis of benzyl-1-(4-methoxyphenylamino)-1,3-dioxobutan-2-ylcarbamate 187a

The substrate **177a** (0.358 g, 1 mmol.) was dissolved under nitrogen in 8 ml of anhydrous ethyl acetate containing 0.7 ml anhydrous DMSO. To this was added DCC (0.447 g, 2.17

mmol.) and H_3PO_4 (0.078 g, 0.8 mmol., (260µL of a 3.12M solution in ether)). After 2.5 hours the reaction mixture was treated with 1 ml of water containing two drops of acetic acid in order to hydrate the excess DCC. The reaction was allowed to stir overnight under a slow stream of nitrogen. The solids (DCU and DCC) were filtered off and the filtrate diluted with water and extracted twice with ethyl acetate (10 ml). The extracts were washed with water to remove the DMSO, dried over $MgSO_4$, and concentrated to dryness. The product **187a** was a white solid. (Yield 0.30 g, 88 %).

Product had identical spectra to that prepared using IBX except $[\alpha]_D^{20} = 0.1$.

Preparation of Pyridinium chlorochromate (PCC)

Concentrated HCl (1.84 ml, 0.022 mole, 12 N) was added to cold pyridine (1.6 ml) with constant stirring. The solution was cooled to 0 ^{0}C in an ice-bath and added dropwise to Chromium trioxide (2 g, 0.02 mol.) with vigorous stirring. The orange Pyridinium chlorochromate formed immediately and was filtered from the solution and placed between filter papers and dried in a desiccator under vacuum. (Yield 3.65 g, 89%) mp 204-205 ^{0}C.

Pyridinium chlorochromate oxidation of (Z-Thr-N-Ph-4-OMe 177a)

Synthesis of benzyl-2-(4-methoxyphenylamino)-2-oxoacetylcarbamate 189a

The substrate (0.716 g, 2 mmol.) was added to a stirred solution of Pyridinium chlorochromate (0.43 g, 2 mmol.) in 10 mls of chloroform. The progress of the reaction was monitored by TLC, when no starting material remained 10 mls of ether was added to the reaction vessel and the entire mixture was passed through a previously prepared short column (10 cm x 2 cm) and eluted with 50:50 ethyl acetate:hexane. If no chromium baseline spot persisted on TLC the solution was evaporated and the product collected. Occasionally a passing through a second short column was necessary. (Yield 0.34, g 55%); ^{1}H-NMR 400

MHz ($CDCl_3$): δ = 3.59 (3H, s, CH_3), 5.07 (2H, s, CH_2), 6.71 (2H, d, ArH, *J* = 8.8 Hz), 7.25-7.18 (5H, m, ArH), 7.35 (2H, d, ArH, *J* = 8.8 Hz), 8.82 (1H, s, NH), 9.35 (1H, s, NH).

Preparation of Jones reagent

Jones reagent was prepared by dissolving chromium trioxide (CrO_3) (26.72 g, 0.26 Mol) in 23 ml of concentrated sulphuric acid, and then diluting the mixture to 100 ml with water.

Jones oxidation of (Z-Thr-N-Ph-4-OMe 177a)

Synthesis of benzyl-2-(4-methoxyphenylamino)-2-oxoacetylcarbamate 189a

The substrate (1.5 g, 4.1 mMol) was dissolved in 50 ml of acetone, Celite 545 filtering aid (diatomaceous earth) was added in order to aid with removal of the reduced Chromium salts upon formation. Jones reagent (2.0 ml, 1.1 molar excess) was added dropwise with stirring at room temperature. The mixture was allowed to stir overnight after which time the now green solution was treated with one ml of isopropyl alcohol, the solution was allowed to stir for a further hour. The solution was then gravity filtered through a fresh portion of Celite, this process was repeated with the filtrate before the solvent was removed under vacuum. The residue was taken up in 20 ml of ethyl acetate and washed with consecutive 20 ml portions of 10% $NaHCO_3$, and water. The organic portion was dried over $MgSO_4$, and passed through a previously prepared short column (10 cm x 2 cm) and eluted with 50:50 ethyl acetate:hexane. After the removal of the solvent a waxy solid remained. (Yield 0.63, g 50%)

Product had identical spectra to that prepared using PCC

Jones oxidation of (Z-Thr-OMe 178a)

Methyl-2-(benzyloxycarbonylamino)-3-oxobutanoate 186a

Z-Thr-OMe **178a** (1.02 g, 3.8 mmol) was dissolved in acetone to which was added Celite 545 filtering agent (0.5 g), and the suspension was cooled to 0 ^{0}C. 1 cm^3 of Jones reagent (prepared as previously described) was added dropwise over 10 min. After 30 min stirring a further 2 cm^3 portion of Jones reagent was added all at once to the reaction vessel. After 4 h

stirring TLC monitoring showed the total consumption of the starting material and the excess oxidant was quenched by stirring with excess *i*-PrOH for 30 min. The solution was then gravity filtered through a fresh portion of Celite, this process was repeated with the filtrate before the solvent was removed under vacuum. The residue was taken up in 20 ml of ethyl acetate and washed consecutive 20 ml portions of 10% $NaHCO_3$, and water. The organic portion was dried over $MgSO_4$, and passed through a previously prepared short column (10 cm x 2 cm) and eluted with 50:50 ethyl acetate:hexane. (Yield 0.78 g 74%); transparent yellow oil; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 2.37 (3H, s, CH_3), 3.81 (3H, s, OCH_3), 5.16-5.12 (3H, m, CH and CH_2), 6.12 (1H, d, NH, *J* = 6.4), 7.38-7.32 (5H, m, ArH);

Ethyl-2-(benzyloxycarbonylamino)-3-oxobutanoate 186b

(Yield 0.9 g 84%); transparent pale yellow oil; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.29 (3H, m, CH_3), 2.35 (3H, s, CH_3), 4.26 (2H, m, CH_2), 5.16-5.11 (3H, m, CH and CH_2), 6.14 (1H, d, NH, *J* = 6.8), 7.37-7.31 (5H, m, ArH);

Isopropyl-2-(benzyloxycarbonylamino)-3-oxobutanoate 186c

(Yield 0.9 g 90%); transparent yellow oil; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.29-1.24 (6H, m, 2 x CH_3), 2.36 (3H, s, CH_3) 4.01-3.98 (1H, m, CH), 5.11-5.06 (4H, m, 2 x CH and CH_2), 6.09 (1H, d, NH, *J* = 6.8), 7.36-7.31 (5H, m, ArH);

Hydrazones of oxidised Z-threonyl anilides and esters

Benzyl-1-(4-methoxyphenylamino)-3-(2-(4-nitrophenyl)hydrazono)-1-oxobutan-2-ylcarbamate 191a

The substrate (3.56 g, 10 mmol.) was dissolved 30 ml of methanol. To this was added 4-nitrophenyl hydrazine (1.68 g, 11 mmol.) dissolved in 5 ml acetic acid with stirring over 30 minutes. The solution was allowed to stir overnight, and the solid precipitate was filtered off. The dried product was used as collected. Yellow solid product, (Yield 2.75 g, 60%). ^{1}H-NMR

400 MHz (DMSO-d_6) : δ = 1.99 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 5.01 (1H, d, CH, *J* = 8.4 Hz), 5.09 (2H, s, CH_2), 6.90 (2H, d, ArH, *J* = 9.2 Hz), 7.18 (2H, d, ArH, *J* = 9.2 Hz), 7.40-7.31 (5H, m, ArH), 7.51 (2H, d, ArH, *J* = 8.8 Hz), 7.90 (1H, d, NH, *J* = 8.4 Hz), 8.05 (2H, d, ArH, *J* = 9.2 Hz), 10.06 (1H, s, NH), 10.09 (1H, s, NH); ^{13}C-NMR 100 MHz (DMSO-d_6): 14.0, 55.1, 61.4, 65.7 111.7, 113.8, 120.9, 125.7, 127.7, 127.8, 128.3, 131.8, 136.9, 138.5, 146.2, 151.3, 155.4, 156.0, 166.6;

Benzyl-3-(2-(4-nitrophenyl)hydrazono)-1-oxo-1-(phenylamino)butan-2-ylcarbamate 191b

(Yield 2.96 g, 65%). ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.99 (3H, s, CH_3), 5.04 (1H, d, CH, *J* = 8.0 Hz), 5.09 (2H, s, CH_2), 7.07 (1H, t, ArH, *J* = 7.2 Hz), 7.16 (2H, d, ArH, *J* = 9.2 Hz), 7.37-7.31 (7H, m, ArH), 7.60 (2H, d, ArH, *J* = 8.0 Hz), 7.93 (1H, d, NH, *J* = 8.4 Hz), 8.03 (2H, d, ArH, *J* = 8.8 Hz), 10.11 (1H, s, NH), 10.20 (1H, s, NH); ^{13}C-NMR 100 MHz (DMSO-d_6): 14.1, 61.4, 65.7, 111.7, 119.3, 123.6, 125.7, 127.7, 127.8, 128.3, 128.8, 136.8, 138.5, 138.6, 146.0, 151.2, 156.1, 167.2.

Benzyl-1-(3,4-dimethylphenylamino)-3-(2-(4-nitrophenyl)hydrazono)-1-oxobutan-2-ylcarbamate 191d

(Yield 3.67 g, 75%). ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.99 (3H, s, CH_3), 2.17 (3H, s, CH_3), 2.19 (3H, s, CH_3), 5.07 (1H, d, CH, *J* = 8.0 Hz), 5.11 (2H, s, CH_2), 7.08 (1H, t, ArH, *J* = 8.0 Hz), 7.22 (2H, d, ArH, *J* = 9.2 Hz), 7.39-7.31 (7H, m, ArH), 7.91 (1H, d, NH, *J* = 8.0 Hz), 8.06 (2H, d, ArH, *J* = 8.8 Hz), 10.05 (1H, s, NH), 10.10 (1H, s, NH); ^{13}C-NMR 100 MHz (DMSO-d_6): 14.4, 19.1, 19.9, 61.8, 66.1, 112.1, 117.4, 121.1, 126.1, 128.1, 128.2, 128.7, 130.0, 131.7, 136.7, 136.8, 137.2, 138.9, 146.5, 151.7, 156.4, 167.2.

Methyl-2-(benzyloxycarbonylamino)-3-(2-(4-nitrophenyl)hydrazono)butanoate 190a

Hydrazones 307a-c were prepared using procedures identical to those used for the corresponding compounds 308a-d already described.

(Yield 3.56 g, 95.3 %); mp 163-165; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.98 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 4.99 (1H, d, CH, *J* = 8.4), 5.10 (2H, s, CH_2), 7.17 (2H, d, ArH, *J* = 9.2), 7.38-7.31 (5H, m, ArH), 8.12 (2H, d, ArH, *J* = 9.2), 8.20 (1H, d, NH, *J* = 8.4), 10.11(1H, s, NH). IR (KBr) 3363, 1752, 1499 cm^{-1}.

Ethyl-2-(benzyloxycarbonylamino)-3-(2-(4-nitrophenyl)hydrazono)butanoate 190b

(Yield 3.13 g, 84 %); mp 138-140; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.21 (3H, t, CH_3, *J* = 6.8), 1.97 (3H, s, CH_3), 4.18 (2H, m, CH_2), 4.92 (1H, d, CH, *J* = 8.0), 5.09 (2H, s, CH_2), 7.17 (2H, d, ArH, *J* = 9.2), 7.38-7.33 (5H, m, ArH), 8.11 (2H, d, ArH, *J* = 9.2), 8.16 (1H, d, NH, *J* = 8.4), 10.11 (1H, s, NH). IR (KBr) 3326, 1721, 1597 cm^{-1}.

Isopropyl 2-(benzyloxycarbonylamino)-3-(2-(4-nitrophenyl)hydrazono)butanoate 190c

(Yield 2.14 g, 56 %); mp 120-122; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.21 (6H, m, CH_3), 1.98 (3H, s, CH_3), 4.88 (2H, m, CH_2), 4.92 (1H, d, CH, *J* = 8.0), 5.02 (3H, s, CH and CH_2), 7.20 (2H, d, ArH, *J* = 8.4), 7.37-7.34 (5H, m, ArH), 8.11 (3H, m, NH and ArH), 10.00 (1H, s, NH). IR (KBr) 3413, 1715, 1579 cm^{-1}.

Azoacetates: LTA oxidation of Hydrazones of Z-threonyl anilides and esters

Azoacetates **193 a-d and 192 a-c** were prepared using procedures identical to those used for the corresponding compounds **265a** already described 0.005 moles of substrate was used in each case.

Methyl-3-acetoxy-2-(benzyloxycarbonylamino)-3-((4-nitrophenyl)diazenyl)butanoate 192a

(Yield 2.13 g, 94 %); red oil; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.84 (3H, s, CH_3), 2.10 (3H, s, CH_3), 3.58 (3H, s, OCH_3), 4.94-4.92 (1H, m, CH), 5.07-5.04 (2H, m, CH_2), 5.65-5.60 (1H,

m, NH), 7.30-7.27 (5H, m, ArH), 7.72 (2H, d, ArH, *J* = 8.8), 8.26 (2H, d, ArH, *J* = 8.8). IR (KBr) 3372, 1741, 1543 cm^{-1}.

Ethyl-3-acetoxy-2-(benzyloxycarbonylamino)-3-((4-nitrophenyl)diazenyl)butanoate 192b

(Yield 2.15 g, 91 %); red oil; ^{1}H-NMR 400 MHz ($CDCl_3$) : δ = 1.01 (3H, t, CH_3, *J* =6.8), 1.84 (3H, s, CH_3), 2.08 (3H, s, CH_3) 4.02 (3H, q, CH_2, *J* = 6.8), 4.94-4.90 (1H, m, CH), 5.07-5.02 (2H, m, CH_2), 7.29-7.27 (5H, m, ArH), 7.73 (2H, d, ArH, *J* = 8.8), 8.26 (2H, d, ArH, *J* = 8.8); IR (KBr) 3364, 1721, 1523 cm^{-1}.

3-(benzyloxycarbonylamino)-4-(4-methoxyphenylamino)-2-((4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 193a

The azoacetate was synthesised using the procedure described for oxidation of Cyclohexanol-4-nitrophenyl hydrazone.

(Yield 2.20 g, 80 %); ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.85 (3H, s, CH_3), 2.13 (3H, s, CH_3), 3.76 (3H, s, OCH_3), 4.83 (1H, d, CH, *J* = 8.4 Hz), 4.95 (2H, s, CH_2), 6.91 (2H, d, ArH, *J* = 9.2 Hz), 7.31-7.28 (5H, m, ArH), 7.56 (2H, d, ArH, *J* = 9.2 Hz), 7.73 (2H, d, ArH, *J* = 9.2 Hz), 7.97 (1H, d, NH, *J* = 8.4 Hz), 8.42 (2H, d, ArH, *J* = 9.2 Hz), 10.28 (1H, s, NH).

3-(benzyloxycarbonylamino)-2-((4-nitrophenyl)diazenyl)-4-oxo-4-(phenylamino)butan-2-yl acetate 193b

The azoacetate was synthesised using the procedure described for oxidation of Cyclohexanol-4-nitrophenyl hydrazone

(Yield 2.02 g, 78 %); ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.85 (3H, s, CH_3), 2.11 (3H, s, CH_3), 5.04-4.85 (3H, m, CH and CH_2), 7.11-7.07 (1H, m, ArH), 7.35-7.25 (7H, m, ArH), 7.63 (2H, d, ArH, *J* = 7.6 Hz), 7.70 (2H, d, ArH, *J* = 8.8 Hz), 7.93 (1H, d, NH, *J* = 10.4 Hz), 8.37 (2H, d, ArH, *J* = 8.8 Hz), 10.42 (1H, s, NH); ^{13}C-NMR 100 MHz (DMSO-d_6): 16.9, 21.4, 58.2, 65.7, 102.1, 119.5, 123.1, 123.8, 124.9, 127.5, 127.7, 128.2, 128.8, 136.7, 138.4, 148.4, 154.3, 155.7, 165.3, 168.5.

3-(benzyloxycarbonylamino)-4-(3,4-dimethylphenylamino)-2-((4-nitrophenyl)diazenyl)-4-oxobutan-2-yl acetate 193d

The azoacetate was synthesised using the procedure described for oxidation of Cyclohexanol-4-nitrophenyl hydrazone

(Yield 2.11 g, 77 %); ^{1}H-NMR 400 MHz (DMSO-d_6) : δ = 1.88 (3H, s, CH_3), 2.12 (3H, s, CH_3), 2.18 (3H, s, CH_3), 2.26 (3H, s, CH_3), 5.01-4.91 (3H, m, CH and CH_2), 7.08-7.03 (3H, m, ArH), 7.31-7.26 (5H, m, ArH), 7.71 (2H, d, ArH, *J* = 8.8 Hz), 7.93 (1H, d, NH, *J* = 9.6 Hz), 8.36 (2H, d, ArH, *J* = 8.8 Hz), 9.93 (1H, s, NH); ^{13}C-NMR 100 MHz (DMSO-d_6): 14.1, 17.3, 20.1, 21.1, 57.9, 65.6, 102.2, 123.1, 123.8, 124.9, 125.3, 127.5, 127.7, 128.2, 131.8, 135.5, 136.7, 137.1, 148.4, 154.3, 155.6, 165.5, 168.7.

Cyclisation of Z-threonyl anilides

Isolation of N-(4-methoxyphenyl)-5-methyl-2-oxooxazolidine-4-carboxamide 179a and 5-(1-hydroxyethyl)-3-(4-methoxyphenyl)imidazolidine-2,4-dione 180a

The substrate (0.716 g, 2 mmol.) was dissolved in acetone and to this was added K_2CO_3 (1.2 molar equivalents). The reaction was then refluxed for 5 hours after which TLC showed the consumption of the starting material. The reaction mixture was filtered to remove the remaining potassium salts, the solvent was reduced to a small volume and taken up in 20 mls of ethyl acetate. The organic phase was washed repeatedly with water until a baseline spot on TLC no longer persisted. The organic extracts were dried and the solvent removed under vacuum to give a white powder that was separated into its component parts by careful chromatography.

N-(4-methoxyphenyl)-5-methyl-2-oxooxazolidine-4-carboxamide 179a (Yield 0.15 g, 30 %) ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.40 (3H, d, CH_3, *J* = 6.4), 3.79 (3H, s, OCH_3), 3.99 (1H, d, CH, *J* = 4.8), 4.58-4.56 (1H, m, CH), 6.91 (2H, d, ArH, *J* = 4.8), 7.53 (2H, d, ArH, *J* = 4.8), 7.97 (1H, s, NH), 10.05 (1H, s, NH), ^{13}C NMR (100 MHz, DMSO-d_6) 21.1, 55.5,

61.6, 75.9, 114.3, 121.5, 131.9, 156.0, 158.5, 168.6; Mass Spectrum: $C_{12}H_{14}N_2O_4Na$ requires 273.09 [M+Na $^+$] found 273.1.; $[\alpha]_D^{20}$ +46.8

5-(1-hydroxyethyl)-3-(4-methoxyphenyl)imidazolidine-2,4-dione 180a

(Diasteromeric mixture showing duplicate peaks (Bold italic) with roughly 3:2 ratio)

(Yield 0.14 g, 27%) ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.16 (3H, d, CH_3, *J* = 6.4) ***1.21 (2H, d,* 0.7 *CH_3, J = 6.4)***, 3.78 (5H, s, 1.7 CH_3), 4.04 (1H, s, CH), ***4.18 (0.7H, s, 0.7 CH***), 5.08, (1H, d, CH, *J* = 6.0), ***5.24, (0.7H, d CH, J = 6.0),*** 7.00 (3H, m, ArH), 8.20 (3H, m, ArH), ***8.37 (0.7H, s, NH),*** 8.44 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) 17.9, **20.6**, 55.7, 62.3, **62.8**, 65.8, **66.8**, 114.2, **114.3**, 125.1, **125.3**, 128.3, **128.4**, 156.8, **157.0**, 158.7, **158.8**, 171.8, **171.7**. Mass Spectrum: $C_{12}H_{14}N_2O_4Na$ requires 273.09 [M+Na $^+$] found 273.1.

Cyclisation of azoacetate 193a to Oxazolidinone 196a and Hydantoin 197a

The azoacetate **193a** (0.549 g, 1 mMol) was dissolved in acetone and the reaction vessel flushed with nitrogen. To this *via* a powder addition funnel was added K_2CO_3 (1.2 molar equivalents). The reaction was allowed to stir for 24 hours and TLC monitoring showed the formation of two new spots, and a significant quantity of starting material. The reaction was then refluxed for 3 further hours after which TLC showed the consumption of the starting material. The reaction mixture was filtered to remove the remaining potassium salts, the solvent was reduced to a small volume and taken up in 20 mls of ethyl acetate. The organic phase was washed repeatedly with water until a baseline spot on TLC no longer persisted. The organic extracts were dried and the solvent removed under vacuum to give an orange oil that was separated into its component parts by careful chromatography.

N-(4-methoxyphenyl)-5-methyl-5-((4-nitrophenyl)diazenyl)-2-oxooxazolidine-4-carboxamide **196a**

(Yield 0.06 g, 15%) ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.65 (3H, s, CH_3), 3.74 (3H, s, OCH_3), 4.43 (1H, s, CH), 6.69 (2H, d, ArH, *J* = 8.8), 7.59 (2H, d, ArH, *J* = 8.8), 7.97 (2H, d, ArH, *J* = 8.8), 8.26 (1H, s, NH), 8.45 (2H, d, ArH, *J* = 8.8), 10.41 (1H, s, NH),

1-(1-(4-methoxyphenyl)-2,5-dioxoimidazolidin-4-yl)-1-((4-nitrophenyl)diazenyl)ethyl acetate **197a**

(Yield 0.07 g, 17%) ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.75 (3H, s, CH_3), 2.44 (3H, s, CH_3), 3.74 (3H, s, OCH_3), 5.04 (1H, s, CH), 6.95 (2H, d, ArH, *J* = 9.2), 7.55 (2H, d, ArH, *J* = 8.8), 8.00 (2H, d, ArH, *J* = 9.2), 8.45 (2H, d, ArH, *J* = 9.2), 10.70 (1H, s, NH) ^{13}C NMR (100 MHz, DMSO-d_6) 23.2, 23.3, 55.1, 65.4, 98.6, 113.9, 120.8, 123.3, 124.8, 130.9, 148.9, 151.4, 153.7, 155.6, 162.1, 169.6.

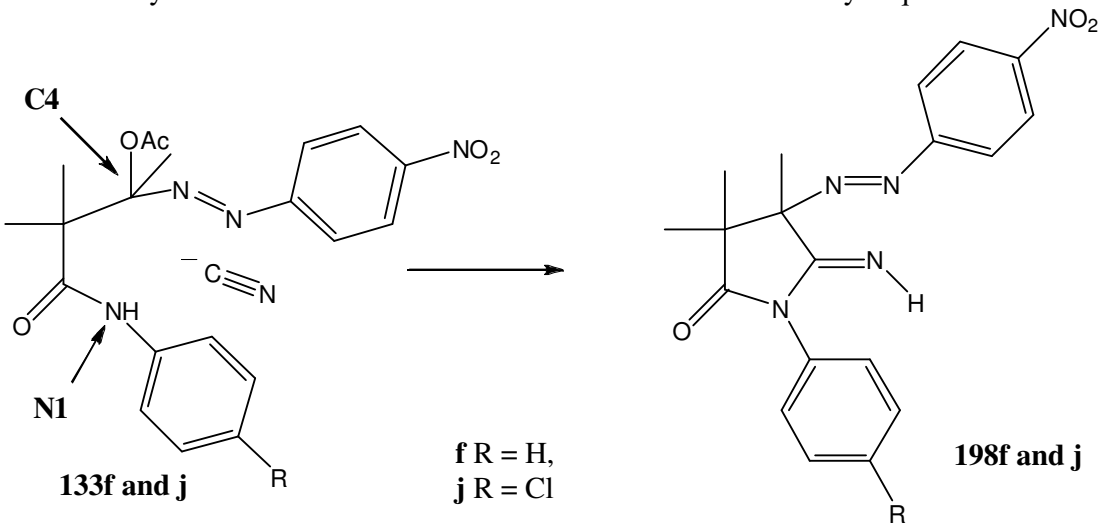
Chapter 4

Isolation and characterisation of azoacetate intermolecular cyclisation products

Consummatum est

4.1 Introduction:

The base induced cyclisations of azoacetates to azetidinones investigated in chapter 2 also led to the isolation of a previously unreported intermolecular cyclisation product involving the incorporation of the cyanide ion. Previous researchers investigating the ring closure of the azoacetate to the azetidinone had reported the discovery of a rearrangement product formed when potassium cyanide was employed in an alcoholic medium. However when alterations were made to the reaction stoichiometry, a 2-iminopyrrolidin-5-one (**198,** scheme 4.2) was isolated as the major product. Examples of the cyclisation of 3-cyanopropionamide derivatives to form 2-iminopyrrolidin-5-one can be found in the literature (see chapter 1), however the cyclisation of our azoacetate substrates **133** in a similar way is quite novel.



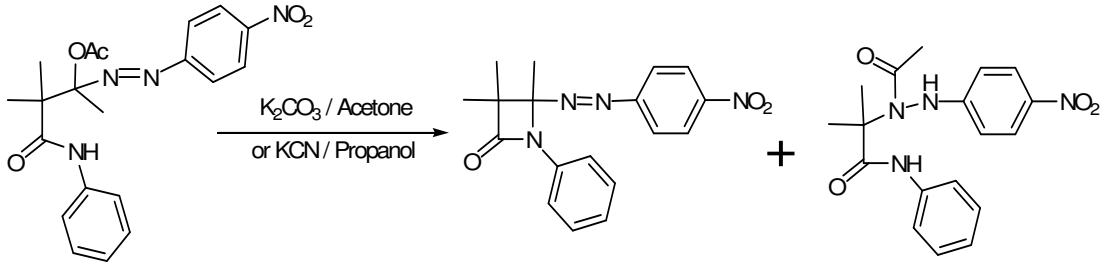
Scheme 4.1 Cyanide unit bridging the N1-C4 gap in cyclisation

As with the β-lactams in chapter 2 it is essentially an N1-C4 ring closure, however in this case a unit of cyanide is bridging this N1-C4 gap. In this case, as with the various other syntheses involving azoacetates presented in the previous chapters, we believe that the similarity between the reactivity of our azoacetate substrates and more simple propionamides is in general a result of their structural similarity.

4.2 Results and Discussion

4.2.1 Isolation of 2-iminopyrrolidin-5-ones

Isolation of the 2-iminopyrrolidin-5-ones bearing the 4-nitrophenylazo functionality was achieved as an extension of work carried out by this research group towards the isolation of azetidinones from azoacetate precursors **133**. It was found at that time that reaction of the azoacetate with potassium carbonate in acetone gave the azetidinone **134f**. However, when the reaction conditions were changed and potassium cyanide was used with propanol, rearrangement product **77** could be isolated in addition to the azetidinone (**134f**, Scheme 4.2).

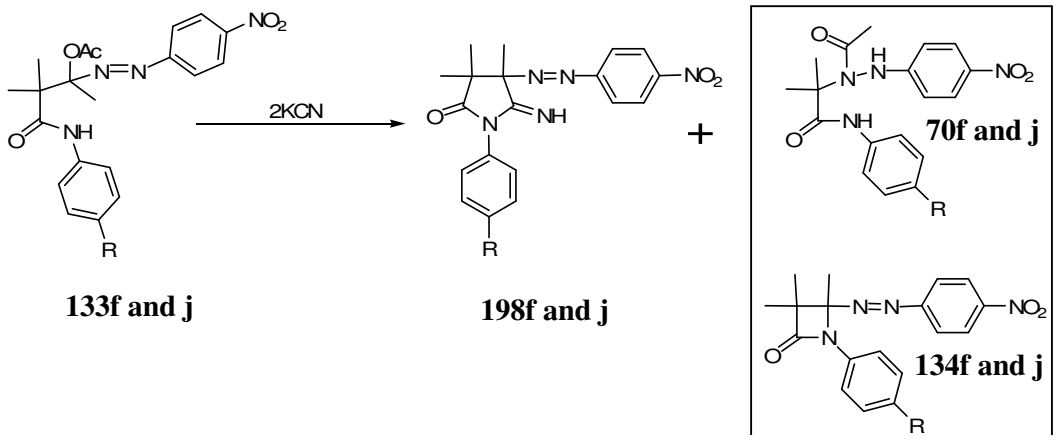


133f **134f** **77f**

Scheme 4.2 Previously isolated products from reaction of azoacetate with KCN

However after several repetitions of the reaction using various azoacetates (**133,** see chapter 2) it was noted that in some cases small quantities of a further reaction product could be seen on TLC. After adjustment of the reaction conditions it was found that an excess of KCN could be used in order to increase the ratio of the new material compared to the β-lactam. It was found that a twofold excess of base could increase the ratio of the new compound. Any further excess of KCN was found to have no further enhancement effect on the product ratio. Two substrates in particular showed an affinity for intermolecular reaction and these were chosen for further study, these were the azoacetates derived from aryl hydrazones of acetoacetanilide **133f** and 4-chloro substituted acetoacetanilide **133j**.

In both cases when the ratio of cyanide added was increased twofold, it was found the trace compound, subsequently identified as 2-iminopyrrolidin-5-one, (a cyclised intermolecular reaction product), and could be isolated as the major product. It was also noted that the increase in the proportion of this product was accompanied by a marked decrease in the proportion of β-lactam isolated, whereas the proportion of the rearrangement product found remained essentially unchanged (Scheme 4.2). This may be as a consequence of the differing reaction mechanisms involved in the production of these compounds. The formation of the β-lactam, as has been previously discussed, is presumed to occur as a result of the base induced abstraction of the relatively acidic amido proton from the substrate azoacetate, followed by cyclisation with the acetate group acting as a leaving group.



133f and j **198f and j**

198f R = H, yield 18% **70f**, yield 16% **134f** yield 12%.
198j R = Cl yield 23%, **70j** yield 17%. **134j,** yield 20%

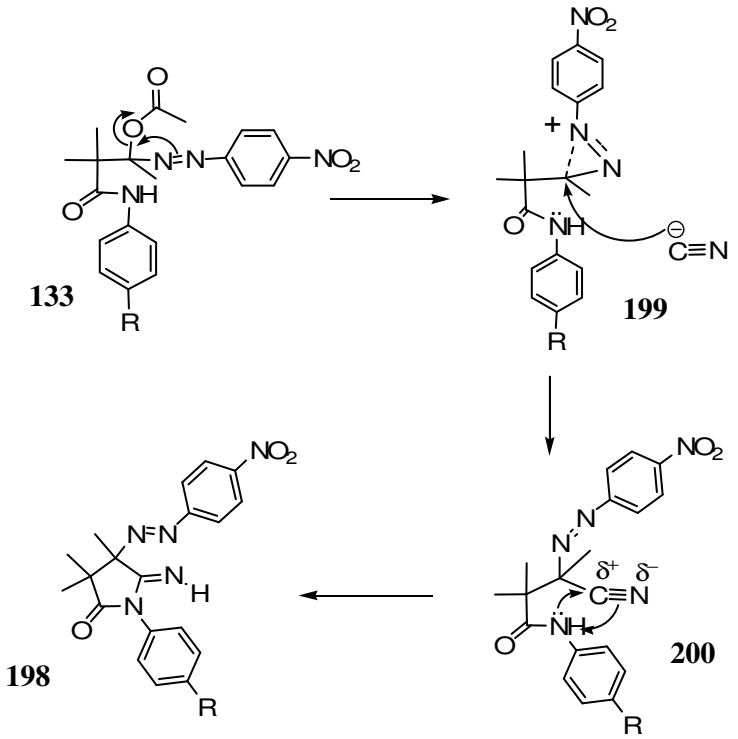
Scheme 4.3 Isolated 2-iminopyrrolidin-5-one **198** from reaction of azoacetate with KCN

The reason for the increase in 2-iminopyrrolidin-5-one is presumably due to the increased concentration of cyanide ions available in solution. The solvent system employed for the β-lactam cyclisation while adequate for reactions where the cyanide acts as base, does not lend itself well to the nucleophilic substitution process. This is due to the polar alcohol providing a source of hydrogen bond formation thus retarding the action of the cyanide ion as a nucleophile. Nucleophilic substitution reactions are usually performed in aprotic solvents

where the lone pairs of the solvent can complex the positive ion and enhance the nucleophilicity of the negative ion. Therefore it is presumably due to the increased concentration of cyanide ions present that allows the cyanide to act as a nucleophile rather than as a base, since nucleophilicity is a kinetic property of the ion. Therefore increased numbers of interactions lead to a decrease in the effect on the system of solvent polarity.

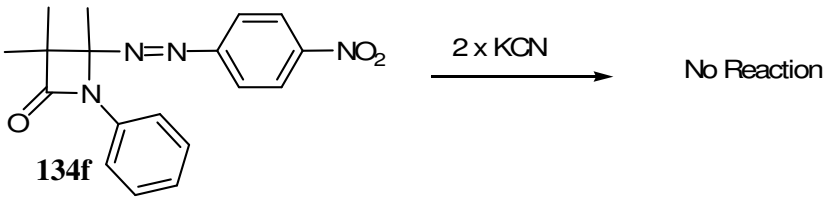
4.2.2 Mechanism of formation of 2-iminopyrrolidin-5-ones

It has already been shown that the acetate group can act as a leaving group although not fulfilling the classic criteria for a good leaving group (conjugate base of a strong acid) (Chapter 2). Presumably, the mechanism of formation of the intermolecular incorporation of the cyanide unit is simply as a result of the competitive reaction between the two nucleophiles in solution. If this is the case the mechanism should involve the initial formation of a nitrile through nucleophilic displacement of the acetate group by the cyano nucleophile. Once again it can be postulated that some anchimeric assistance is provided by the neighbouring phenylazo functionality. However in this case it is the cyanide ion that attacks at the reaction centre (Scheme 4.3). The subsequent cyclisation through the newly formed nitrile is expected due to the proximity of the nucleophilic amide to the polar nitrile in basic media. In contrast to what is generally reported about the stability of imines carrying a hydrogen on the nitrogen (primary imine),[117] this cyclic imino group proved to be stable enough to allow for isolation and consequently sent for X-Ray crystal structure determination.



Scheme 4.4 Proposed mechanism of anchimeric assistance allowing the formation of the 2-iminopyrrolidin-5-one derivative.

To discount the possibility of a ring expansion of the newly formed β-lactam by attack of the cyanide ion had taken place, an isolated sample of β-lactam was reintroduced to refluxing propanol containing a two-fold excess of KCN (Scheme 4.4).



Scheme 4.5 Reaction of β-lactam with KCN

After heating under reflux for 48 hours no change was observed in the starting material. Since the isolated 2-iminopyrrolidin-5-ones had formed within 24 hours, it seems clear that this reaction pathway was not involved in their formation.

4.2.3 ^{1}H NMR studies of 2-iminopyrrolidin-5-ones

Initial attempts to characterize the isolated compound by NMR proved difficult due to the similarity between the spectra of the 2-iminopyrrolidin-5-one (**198j**, figure 4.2b) and the β-lactam (**134j**, figure 4.2a). $CDCl_3$ had been used as the NMR solvent for all of the β-lactam molecules synthesized to this point. The new compounds isolated after chromatography from the reaction of the azoacetates with excess cyanide were found to dissolve well in this solvent and it was therefore initially chosen so that comparisons could be made between the spectra of the new compounds and the formerly synthesized β-lactams.

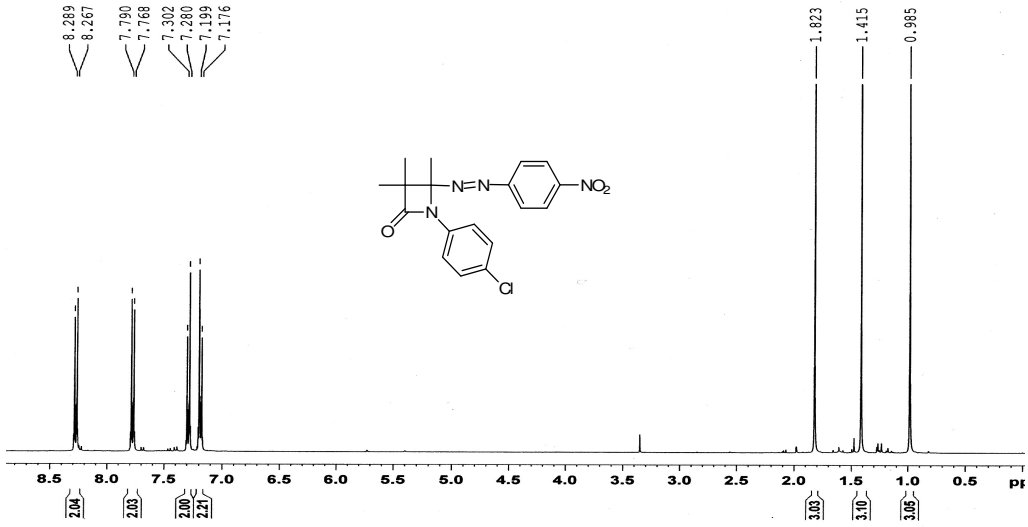


Figure 4.2a ^{1}H NMR spectrum of β-lactam derivative **134j** in $CDCl_3$

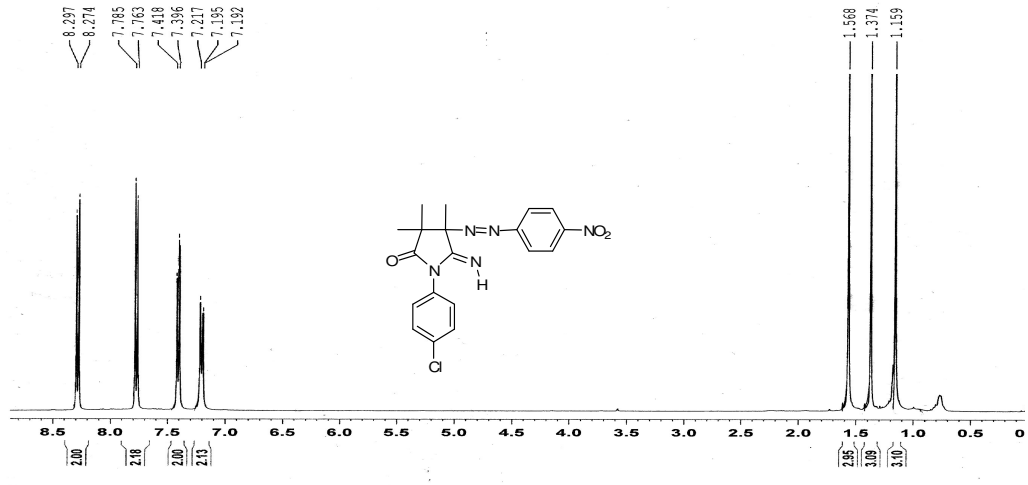
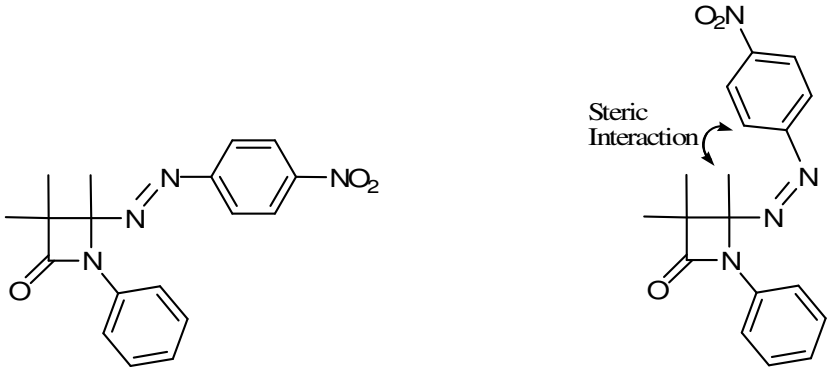


Figure 4.2b ^{1}H NMR spectrum of 2-iminopyrolidin-5-one derivative **198j** in $CDCl_3$

Initial results showed the spectra of the two compounds to be remarkably similar, with only a slight difference between the chemical shift of the methyl protons of either compound. This led to the early assumption that the compound isolated may have been an isomer of the β-lactam molecule. It was thought that since the ^{1}H NMR spectra were so similar that such minor differences could be attributed to the isolation of the β-lactam in the *cis* configuration (Figure 4.3). This was deemed plausible since in theory the compounds would be separable by chromatography and isolation of both configurations of this type of azo-compound is not uncommon.



Trans configuration *Cis* configuration

Figure 4.3 Possible isomers of β-lactam **134f**

The *trans* isomer in most cases, tends to be the predominantly observed isomer due to the stability of the system imparted by the lower levels of steric interaction with neighbouring groups. The dominance of the *trans* isomer over the *cis* was supported by the identification by previous researchers of several isolated β-lactams of this type as the *trans*-isomer using X-Ray crystallography studies. The hypothesis that the *cis* isomer had been isolated was soon abandoned as further spectral evidence was gathered. This data suggested that the isolated compound did not show the characteristic spectral features common to β-lactams. For instance the IR spectrum of the compound did not show the characteristic 4-membered lactam strong carbonyl stretching vibration common to the other β-lactams (~1750 cm^{-1}).

However when the NMR spectra were obtained using DMSO-d_6 as solvent, the appearance of a broad singlet in the proton spectrum integrating as a one proton could be seen at low-field. This indicated the possible presence of a proton attached directly to a heteroatom. This was also confirmed using the IR spectrum where the presence of a characteristic -NH- stretching vibration was observed. This raised several questions with regard to the NMR spectra of the compound in question. Not only was the identification of the compound now an issue but the presence and absence of a peak in different NMR solvents must be considered.

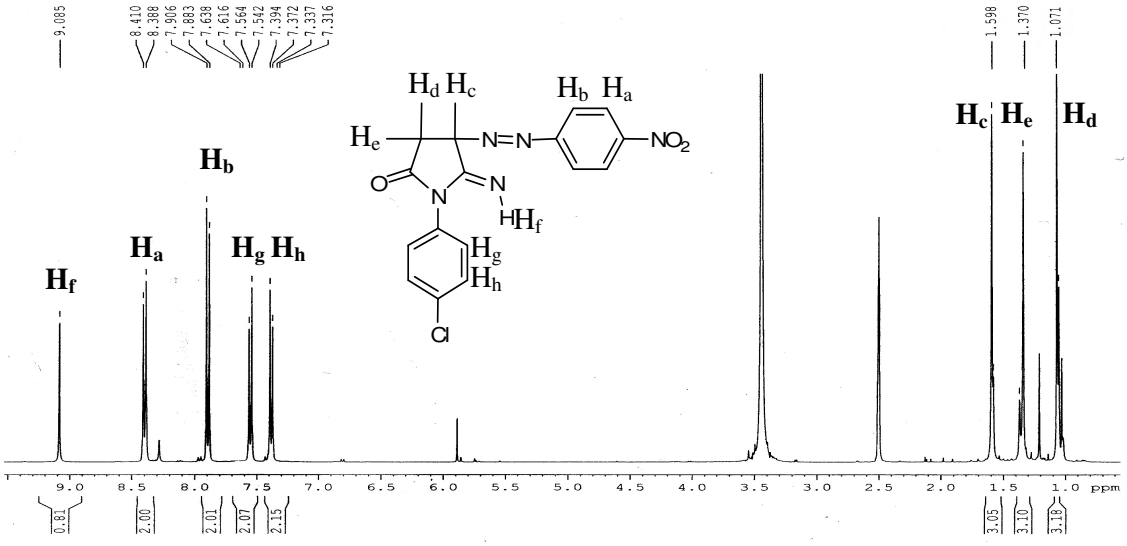


Figure 4.4 ^{1}H NMR spectrum of 2-iminopyrrolidin-5-one **198j** in DMSO-d_6

There are several potential causes for this type of signal dampening. A commonly observed example of this phenomenon in ^{1}H NMR spectra is chemical exchange. In cases where a molecule can convert between two configurations in solution, a dynamic equilibrium can exist which allows the appearance of a spectrum to change relative to the rate of exchange between the two configurations. In this case the flipping of the proton about the imino bond would be considered an unsymmetrical two-site exchange. Although the imino moiety is, in general terms, a rigid moiety,[118] this type of flipping between configurations is conceivable because of the unique positioning of the imino group adjacent to the nitrogen lone pair of the

amide (Figure 4.5). Resonance stabilization provided by the iminium ion intermediate allows flipping from one conformer to the other. [119]

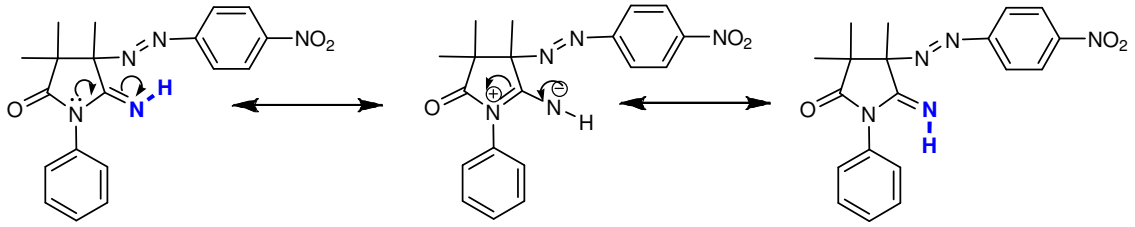
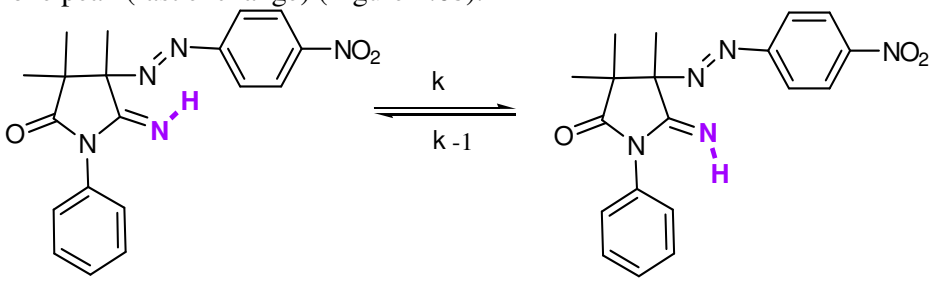


Figure 4.5 Mechanism of flipping between configurations of 2-iminopyrrolidin-5-one **198f**

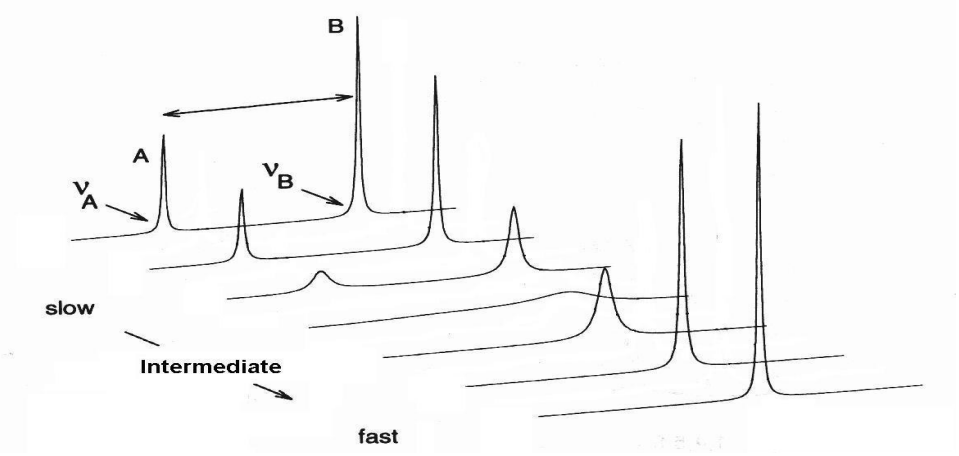
Depending on the rate of population exchange between the two configurations (Figure 4.6a), the signals can appear in the spectrum as two peaks (slow exchange), no peak (intermediate exchange) or one peak (fast exchange) (Figure 4.6b).



198f Configuration A

198f Configuration B

Figure 4.6a Configurations of 2-iminopyrrolidin-5-one **198f**



Adapted from Figure 4.7 in "Nuclear Magnetic Resonance", Oxford chemistry primers. [120]

Figure 4.6b Graphical representation of line broadening and coalescence related to rate of exchange between configurations

Essentially what is seen with respect to the rate of exchange between the configurations is broadening, coalescence and narrowing of the signal. The rate of exchange of the configurations can be affected by a number of factors including temperature, solvent viscosity and solvent polarity. It is therefore feasible that the absence of the imino proton in spectrum as observed in $CDCl_3$ is as a result of an intermediate rate of transition between the two configurations of the molecule. Likewise it is possible that the more polar DMSO-d_6 allows a faster rate of transition through stabilization of the iminium ion, therefore resulting in the appearance of the proton signal as a single peak.

Another possible reason for the absence of the imino- proton in the $CDCl_3$ spectrum could be line broadening as a result of the spin-lattice relaxation process. However this type of line broadening tends to affect an entire spectrum rather than exerting a localized effect on a single nucleus or group of equivalent nuclei. This phenomenon was explored somewhat further in the case of anomalies observed in the carbon spectrum of this compound and is discussed in more detail in the next section.

4.2.3 ^{13}C NMR studies of 2-iminopyrrolidin-5-ones

The ^{13}C NMR spectrum of the 2-iminopyrrolidin-5-ones when obtained using $CDCl_3$ as solvent also had some interesting features. The carbon peaks associated with the three methyl groups were of particular interest (Figure 4.7). Their appearance showed significant line broadening making them barely distinguishable from the baseline. This unusual phenomenon was investigated further, three possibilities for the cause of the line broadening were considered:

an effect caused by the instrument.

an effect inherent to the solvent.

an effect inherent to the compound.

Initially it was suspected that the appearance of the peaks may have been due to an effect caused by a problem with the decoupling of the carbon signals. The experiment was rerun with the decoupler turned off in order to assess whether the decoupled spectrum would be affected. Although the signal strength would be expected to diminish significantly due to the lack of Nuclear Overhauser Enhancement (NOE) it was hoped that a weaker but clearly defined spectrum would point to a problem experienced during the decoupling process. However when the decoupler was turned off the reduction in the signal strength meant that the broadened peaks were diminished to the point where they were indistinguishable from the baseline noise. Increasing the number of scans was briefly investigated but as expected the signal definition improved but this had no effect on the intensity of the signal. The next approach was an investigation of the effect of the relaxation time on the signal. A series of experiments with gradually increased relaxation time were set up with relaxation times ranging from 1 second to 60 seconds, run with 512 scans. This technique did produce the desired enhancement of the line broadened peaks, with an improvement in both the signal definition and signal strength for all peaks. However the signal enhancement of the peaks of interest was found to be relatively low.

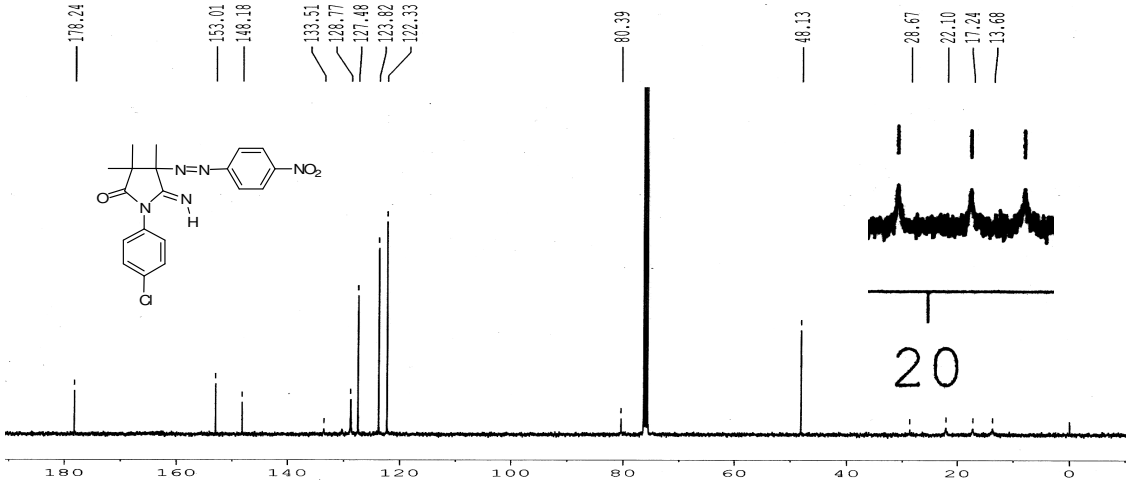


Figure 4.7 ^{13}C NMR spectrum of 2-iminopyrrolidinone **198j** in $CDCl_3$ using standard NMR settings (1024 scans, 1 second relaxation delay)

The enhancement of the signal observed by extending the relaxation delay as expected, was greatest at the longest delay time (60 seconds, Figure 4.8a). The enhancement of the signal in this manner is a function of the spin-lattice relaxation time. Spin-lattice relaxation is caused by variable local fields which stimulate nuclei to flip amongst their accessible spin states. As the nuclei approach equilibrium the energy released is dispersed to the surroundings.

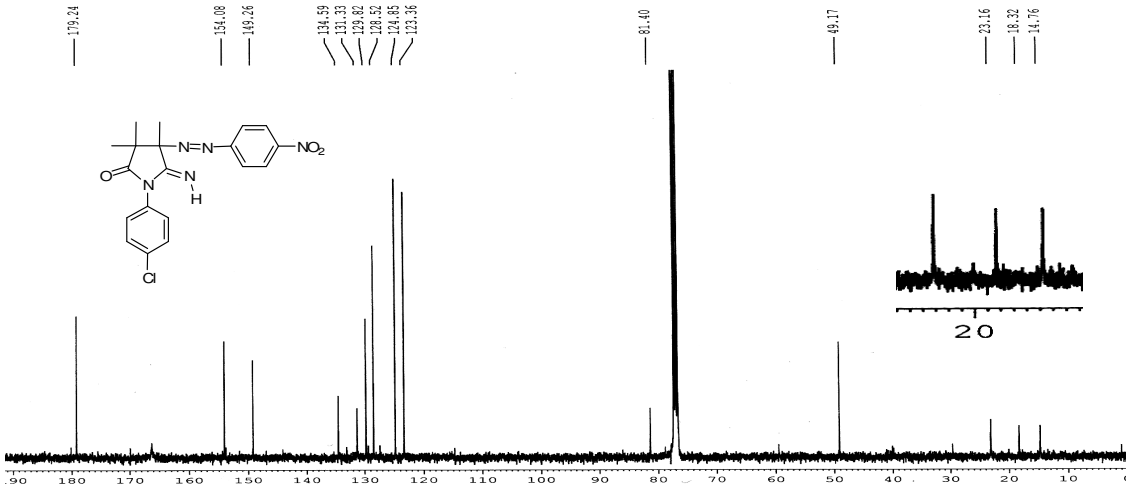


Figure 4.8a ^{13}C NMR spectrum of 2-iminopyrrolidinone derivative **198j** in $CDCl_3$ 512 scans with a relaxation delay of 60 seconds

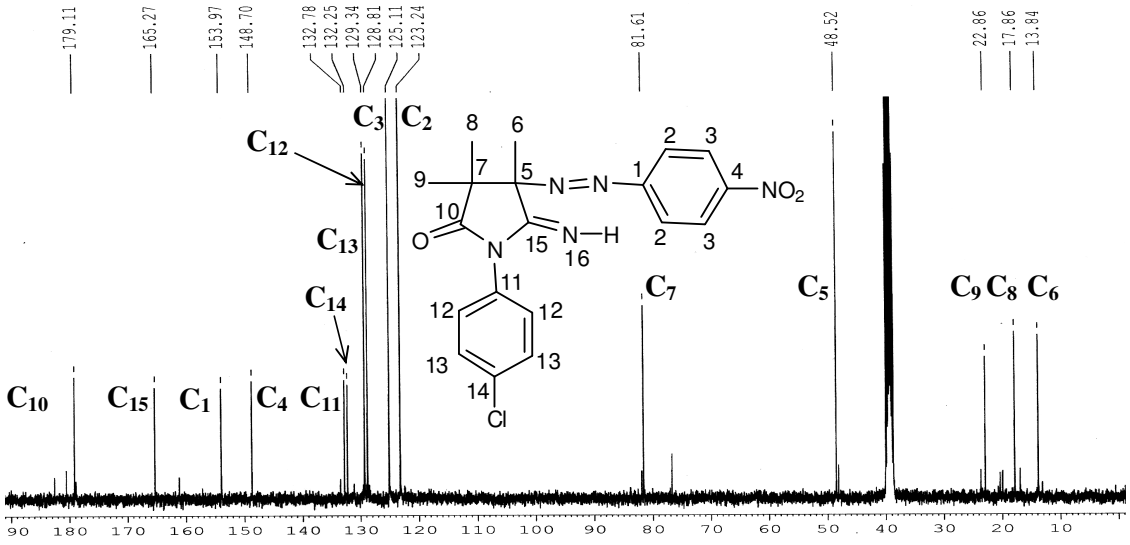


Figure 4.8b ^{13}C NMR spectrum of 2-iminopyrrolidin-5-one **198j** in DMSO-d_6 1028 scans with a relaxation delay of 1 second.

These variances in populations are typified by T_1 the spin-lattice relaxation time. For a collection of spin -½ nuclei, assuming exponential relaxation, there is a disparity in the number of m = + ½ and m = - ½ spins. For this reason it is favourable to wait a long time after T_1 has elapsed before recording a spectrum. The extended relaxation time in this case can be seen to have an enhancement effect on the signals in the entire spectrum as compared to the spectrum run with standard 1 second relaxation delay (Figure 4.8a). This increased enhancement did allow the positive identification of the product however it remains unclear why the localized line broadening effect is experienced by the carbon nuclei of the methyl groups in such a dramatic way. Also, as with the proton spectrum these effects were solvent dependant, the spectrum obtained in DMSO-d_6 showed a clearly defined spectrum without line broadening (Figure 4.8b).

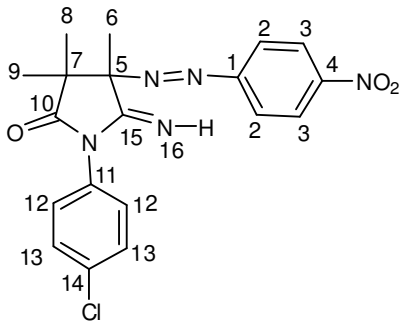
The overall outcome of these results suggests that the observed line broadening, is not likely to be a result of a localized effect caused by the spin-lattice relaxation process. There are many relaxation processes that can have an effect on NMR line broadening. (Spin-spin relaxation, modulation of the anisotropic chemical shift interaction by molecular tumbling, modulation of scalar coupling internal motion, and interaction with the strong fields generated by unpaired electrons in a paramagnetic molecule). Unfortunately it is difficult to pinpoint with any degree of certainty which of the many possible mechanisms results in the anomalous line broadening observed in the spectra presented here. It is most likely a combination of effects involving the molecule and the solvent in a similar manner to that described for the proton spectrum. Further evidence to suggest that the presence of the imino group adjacent to the amine in the ring plays a role was investigated by synthesising a similar molecule (pyrrolidine-2,5-dione derivative) whereby flipping was not a possibility. The results of this are discussed in section 4.3.

4.2.4 HMQC and HMBC studies of 2-iminopyrrolidin-5-ones

Full characterization of the isolated product required collaborative analysis of all of the all available spectra. The presence of the -NH- moiety was confirmed from the ^{1}H NMR and IR spectra, and the presence of a previously unaccounted for quaternary carbon from the ^{13}C and DEPT spectra. However the factor that proved crucial to the eventual full characterization of the 2-iminopyrrolidin-5-ones was the simultaneous analysis of the HMQC and HMBC spectra of these compounds. HMQC (Heteronuclear Multiple Quantum Correlation) is a 2D NMR spectroscopic technique which correlates the carbon and proton spectra. The technique shows through bond interactions of the proton with the carbon nucleus immediately attached to that proton. This allows the identification of the carbon atom to which a particular proton or group of equivalent protons are directly attached (i.e. a carbon-hydrogen correlation over 1 bond length). HMBC (Heteronuclear Multiple Bond Correlation) on the other hand shows interaction of the proton with neighbouring carbon nuclei and excludes information on the carbon nucleus immediately attached to the proton. (i.e. a carbon-hydrogen correlation over 2 to 3 bond lengths).

The information provided by these two spectra removes the ambiguity surrounding the identification of specific sites within the molecule. HMBC is particularly useful in the assignment of quaternary carbon signals. For instance in the case of quaternary carbon sites 5 and 7 (Table 4.1), examination of the ^{13}C, DEPT and HMQC spectra allow a reasonable evaluation to be made of the position of each signal on the carbon spectrum. Estimations can be made on the expected chemical shift experienced by the nuclei based on the effects of the surrounding groups. This process of site identification is satisfactory when the structure of the compound has already been elucidated. However when the structure is unknown, as in this case, the additional information provided by the HMBC allows the structure of the compound to be identified conclusively.

The correlation between the proton attached to nitrogen site 16 and carbons 15 and 5 in conjunction with the correlation between the protons attached to carbon 6 and carbons 5 and 15 showed the position of the newly incorporated cyanide unit. Simply put, because the protons at sites 16 and 6 can both "see" the quaternary carbon sites 5 and 15 they must be beside each other.



Site	^{1}H	^{13}C	HMQC	HMBC	HMBC	HMBC	HMBC
1		154.0					
2	7.88-7.91		123.2	125.1	154.0		
3	8.38-8.41		125.1	123.2	148.7		
4		148.7					
5		48.5					
6	1.60		13.8		48.5	81.6	165.3
7		81.6					
8[a]	1.07		17.9	22.9	48.5	81.6	179.1
9[a]	1.34		22.9	17.9	48.5	81.6	179.1
10		179.1					
11		132.8					
12	7.37-7.39		128.8	129.3	132.8		
13	7.31-7.33		129.3	128.8	132.3		
14		132.3					
15		165.3					
16	9.09				48.5		165.3

Table 4.1 Correlation table of ^{1}H, ^{13}C, HMQC and HMBC data for 2-iminopyrrolidin-5-one **198j.**

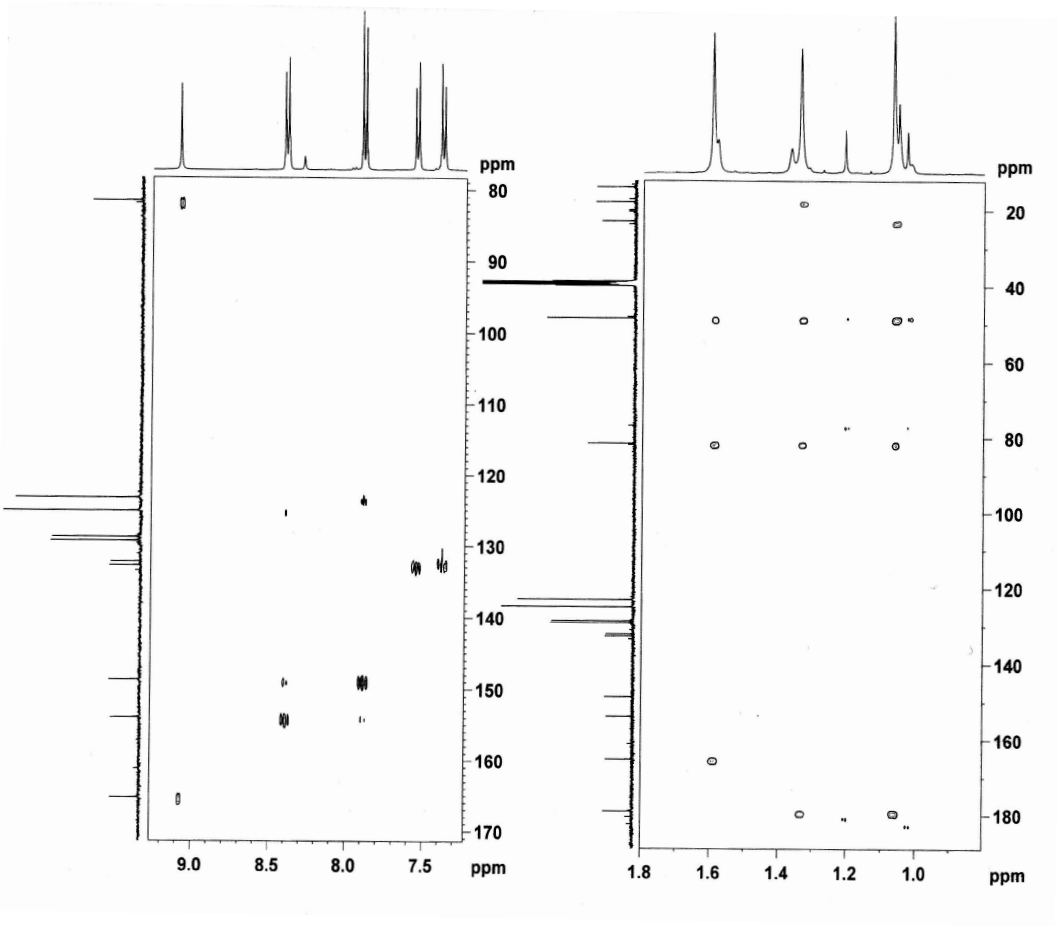


Figure 4.9 Relevant portions of HMBC spectrum of 2-iminopyrrolidin-5-one **198j**

It should be noted that the examination of the HMBC (Figure 4.9) established that a unit comprising of a quaternary carbon, attached to a heteroatom bearing a single hydrogen, was incorporated into the system adjacent to carbon site 5. However, with this knowledge, it was possible to go back and examine the reaction conditions for the source of this new moiety and postulate the likely structure of the molecule. Considering the entities present in the reaction solution the structure including the incorporation of the cyanide unit resulting in the formation of the 2-iminopyrrolidin-5-one seemed the only viable candidate. At this point comparison of the proposed structure with the available spectra confirmed that the structure was correct.

In the case of this molecule it was possible not only to identify the structure but accurately assign the position of every carbon within the system. It should be noted that positions 8 and 9 (Table 4.1) have been assigned arbitrarily, although it is possible to distinguish the signals from each other, identification of the precise position of the carbon centers in relation to the adjacent chiral centre is not possible using the spectral techniques used here. It is possible to hypothesize the most likely position of either group on the ^{1}H and ^{13}C spectra based on the electronic effects experienced by the groups when in a position *cis* or *trans* to the large adjacent phenylazo group. However the information available from the spectra does not allow the same level of unambiguous assignment as with all of the other carbons within the spectrum.

4.2.5 IR studies of 2-iminopyrrolidin-5-one

As has been previously mentioned the IR spectrum proved a useful tool in the identification of the 2-iminopyrrolidin-5-one

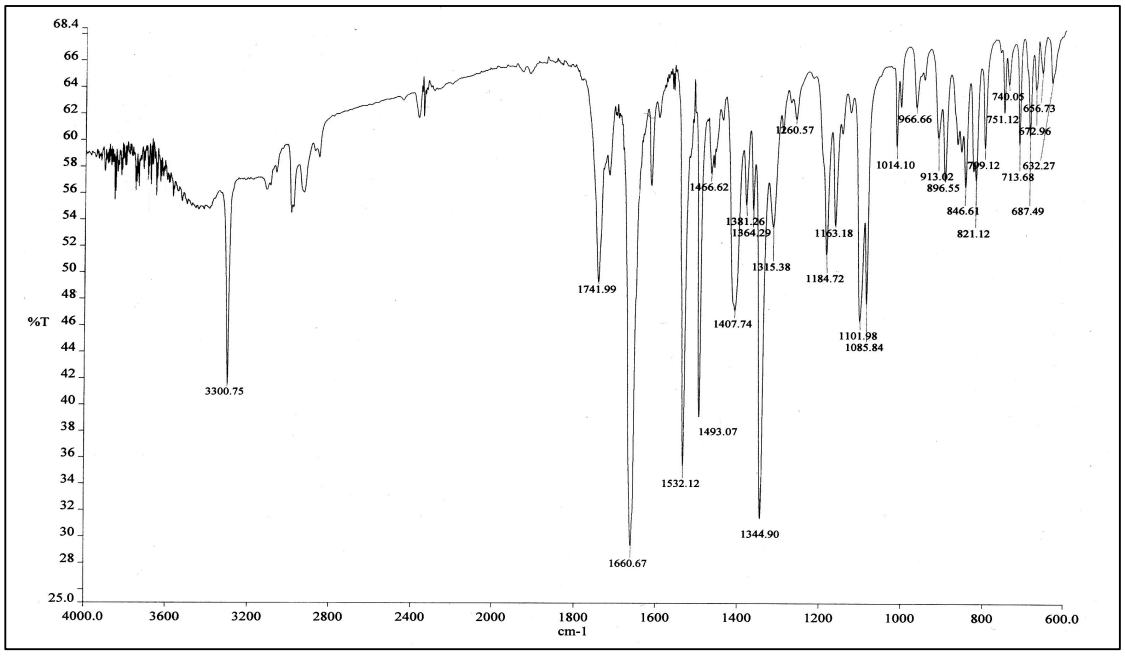


Figure 4.11 IR spectrum of 2-iminopyrrolidin-5-one **198j**

The presence of a strong stretching vibration at 3300 cm^{-1} is indicative of the presence of a secondary amine roughly in the region where it might be expected to find the imino =N-H stretch (3350–3320). Although the vibration falls outside the quoted literature rangethis is not uncommon in IR spectroscopy and can be attributed to several factors including the influences of other functional groups within a molecule,[121] the impact of preferred spatial orientations, and environmental effects (chemical and physical interactions) on the molecule. The other feature of the IR that proved most useful in characterizing of these compound was the carbonyl stretch, it was this feature that ruled out the possibility that a β-lactam may have been isolated. Lactams have a characteristic range of carbonyl stretches, the greater the ring stress, the higher is the carbonyl absorption frequency. In the case of β-lactams the high level of ring stress imparted by the highly constrained ring results in the appearance of the carbonyl stretch in the 1750 cm^{-1} region of the spectrum making it easily identifiable. The carbonyl band seen in the spectrum appears at 1660 cm^{-1} and is indicative of an amide.

4.2.6 Structural studies of 2-iminopyrrolidin-5-one

Crystals suitable for single crystal X-ray crystallographic determination of the 2-iminopyrrolidin-5-one **198f** were grown from a saturated solution of ethyl acetate : hexane 85 : 15. The substrate molecule was dissolved in the solvent mixture and allowed to stand for 20 days undisturbed; slow evaporation was controlled by covering the vessel (clean new test tube) with perforated foil. The resulting crystals were orange block-like clusters. The 2-iminopyrrolidin-5-one **198f** crystallized in the triclinic system with P1 space group with a single molecule per asymmetric unit. Some of the main features of interest within this molecule include the imine, N-H bond distance is 0.88(3) Å. The imine C=N bond distance is 1.267(2) Å. The bond angle across these two bonds is C(9)-N(4)-H(1N4) 108.6(18). These values compare well to the values for this moiety as quoted in the mainstream literature.[122]

The bond lengths and angles associated with the phenylazo group were also as expected. The slight disparity between the values for C(7)-N(3) and C(6)-N(2) bond distances is a reflection of the proximity of N(2) to the aromatic ring. The double bond character of this system would be expected to result in a slight reduction of the C(6)-N(2) bond length due to the sp^2 hybridized C(6) electrons being more tightly bound than the sp hybridized electrons associated with C(7). Similarly inconsistency between the bond angles through these bonds can be seen, however in the case of the bond angles other factors, not as easily quantifiable may have an effect. Steric effects from neighbouring groups as well as intermolecular interactions within the crystal lattice all have contributory effects on the orientation of these groups. The carbonyl C=O distance for this molecule is 1.211(2) Å, which is consistent with literature values. The O-N-O nitro bond distances 1.220(2) and 1.223(2) fall between the characteristic N-O single bond value (1.36 Å) and characteristic N=O double bond value (1.18 Å). The resonance possibilities allowed by this group results in both bonds having some single bond and double bond character.

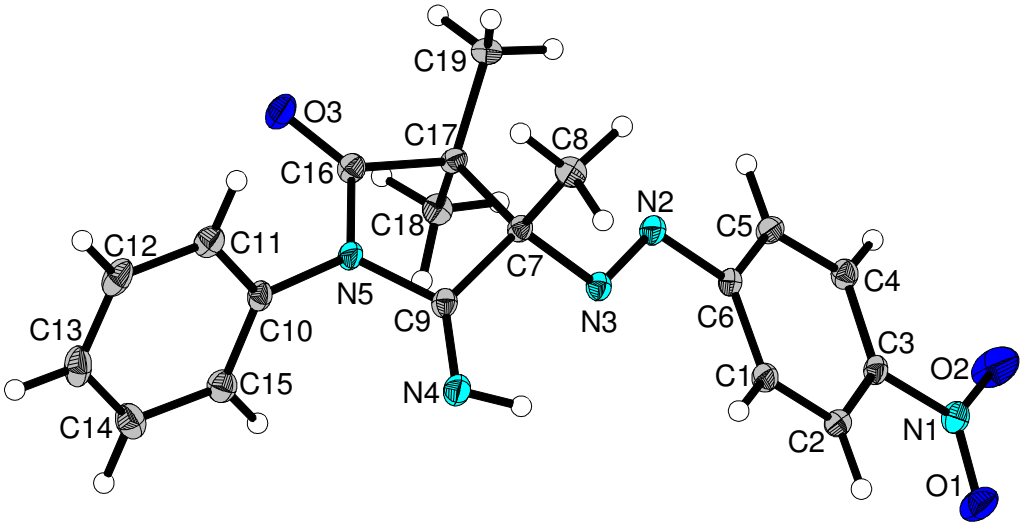


Figure 4.12 X-Ray crystal structure of 2-iminopyrrolidin-5-one **198f**

Table 4.2 Selected bond distances (Å) and angles (^{O}) for 2-iminopyrrolidin-5-one **198f** with estimated standard deviations

Bond distances	(Å)
N(5) - C(10)	1.435(2)
N(3) - C(7)	1.478(2)
C(9) - N(4)	1.402(2)
N(2) - N(3)	1.251(2)
C(3) - N(1)	1.470(2)
N(1) - O(1)	1.220(2)
N(1) - O(2)	1.223(2)
Bond angles	**(^{O})**
C(9)-N(4)-H(1N4)	108.6(18)
N(2)-N(3)-C(7)	110.96(13)
N(3)-N(2)-C(6)	113.78(14)
C(16)-N(5)-C(10)	122.55(13)
O(1)-N(1)-C(3)	118.68(14)

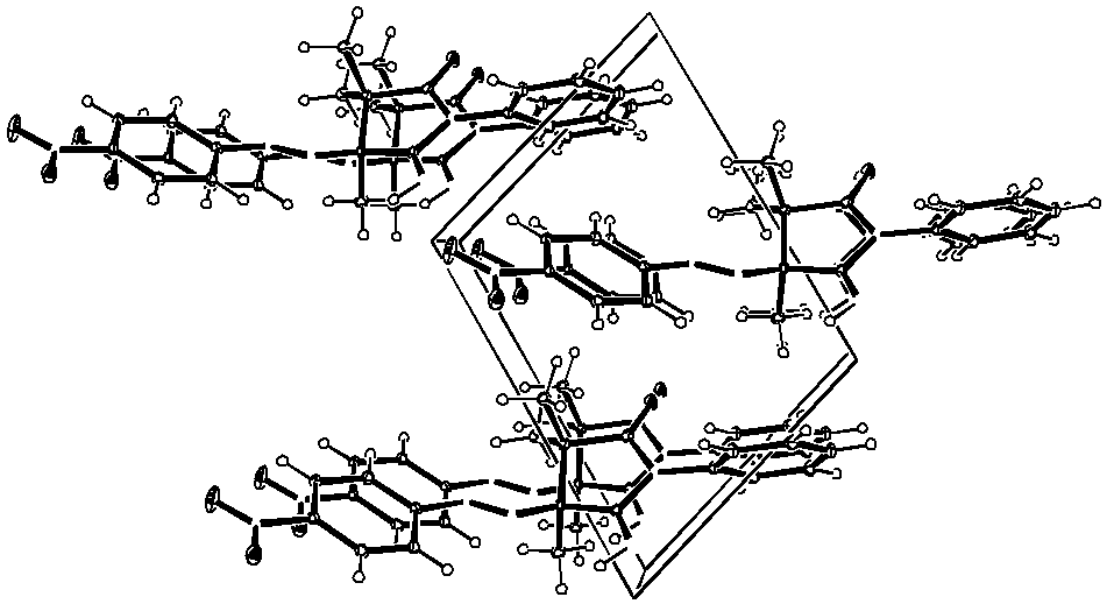


Figure 4.13 ORTEP view of 2-iminopyrrolidin-5-one **198f** showing packing relative to the unit cell

Table 4.3 Crystal data and structure refinement for 2-iminopyrrolidin-5-one **198f**

Quantity	Measurement
Empirical formula	C_{19} H_{19} N_5 O_3
Formula weight	365.39
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P1 (#1)
Unit cell dimensions	a = 6.1179(9) Å α= 72.868(3)°.
	b = 7.7340(12) Å β= 79.475(3)°.
	c = 9.9532(15) Å γ = 81.828(3)°.
Volume	440.52(12) Å^3
Z	1
Density (calculated)	1.377 Mg/m^3
Absorption coefficient	0.097 mm^{-1}
F(000)	192
Crystal size	1.00 x 0.40 x 0.30 mm^3
Theta range for data	2.17 to 30.50°.
Index ranges	-8<=h<=8, -11<=k<=11, -14<=l<=14
Reflections collected	10436
Independent reflections	2668 [R(int) = 0.0378]
Completeness to theta =	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9716 and 0.8046
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2668 / 3 / 251
Goodness-of-fit on F^2	1.054
Final R indices [I>2sigma(I)]	R1 = 0.0375, wR2 = 0.0955
R indices (all data)	R1 = 0.0394, wR2 = 0.0972
Largest diff. peak and hole	0.374 and -0.229 e.Å^{-3}

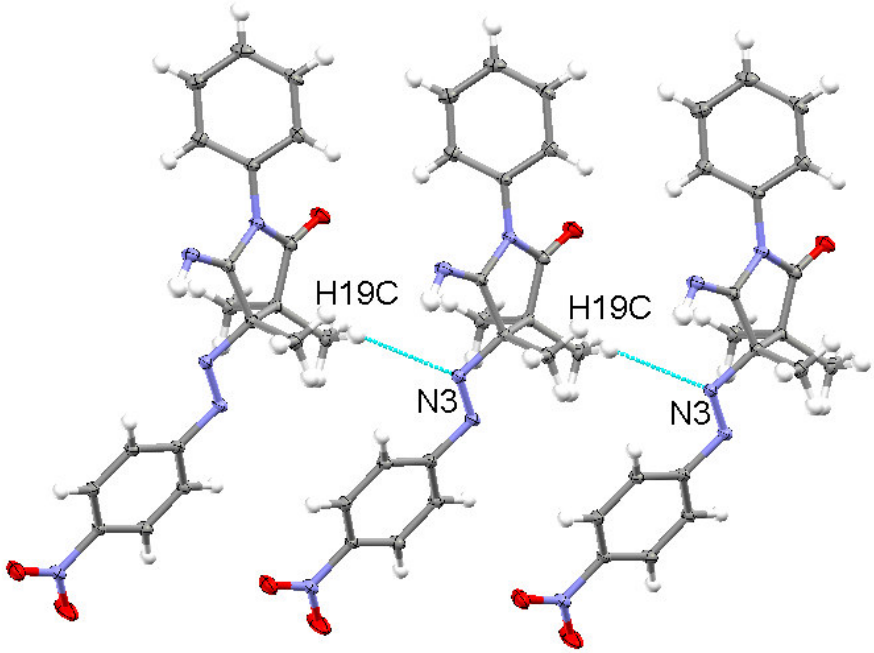


Figure 4.14 Mercury view of 2-iminopyrrolidin-5-one **198f** showing intermolecular hydrogen bonding interactions through the phenylazo group.

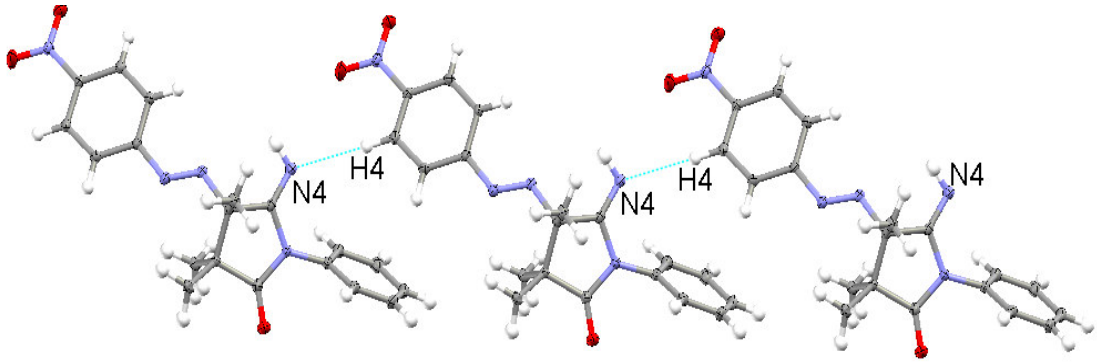


Figure 4.15 Mercury view of 2-iminopyrrolidin-5-one **198f** showing intermolecular hydrogen bonding interactions through the imine group.

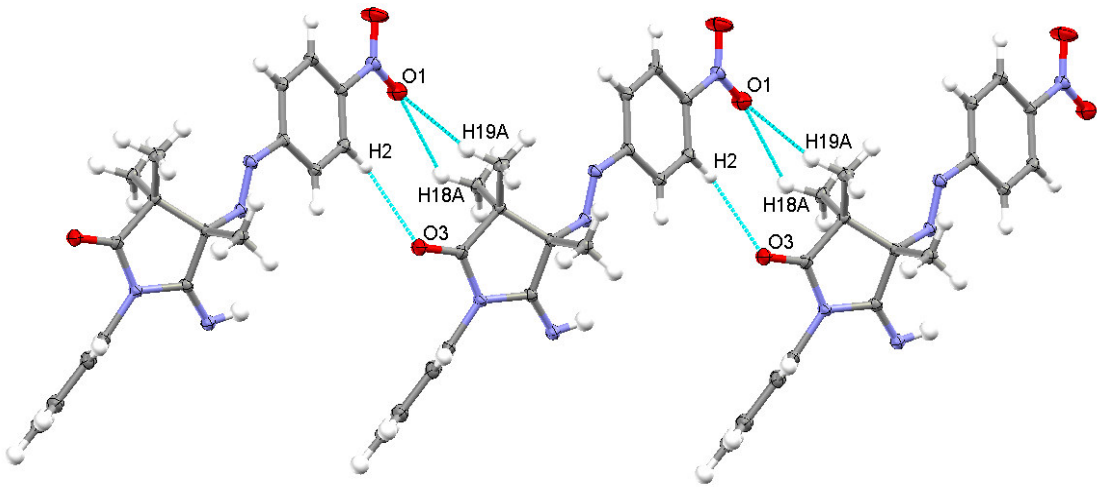
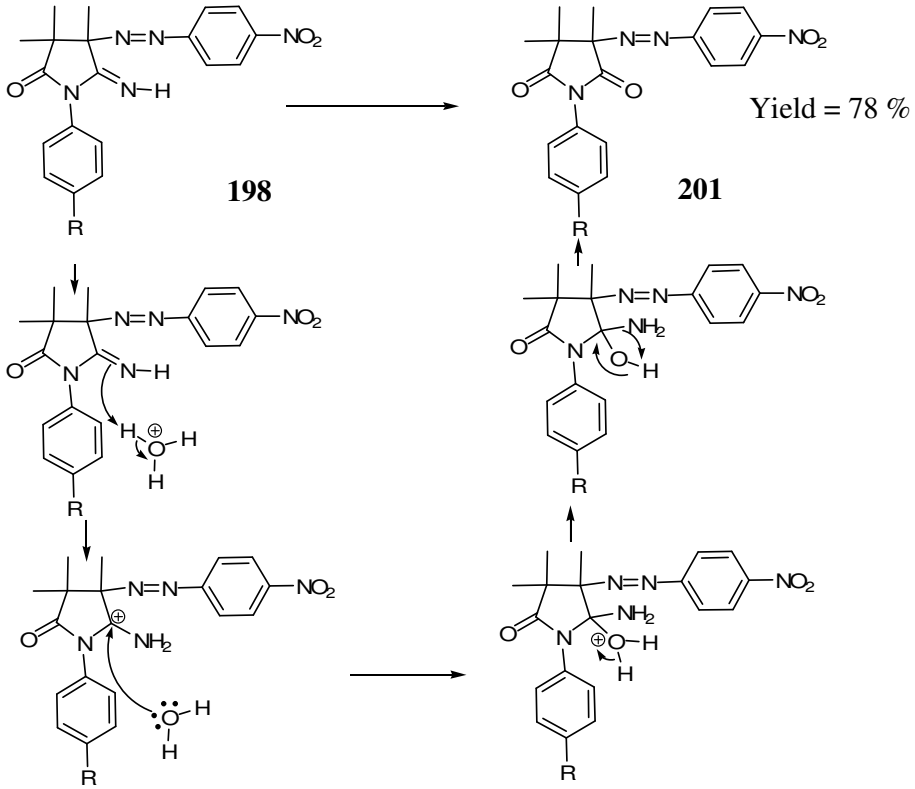


Figure 4.16 Mercury view of 2-iminopyrrolidin-5-one **198f** showing intermolecular hydrogen bonding interactions through the nitro group and the carbonyl.

Solid crystals are made up of a rigid lattice of molecules, atoms or ions, the locations of which are characteristic of the substance. The homogeny of the interior of this solid body results in the crystal having a distinctive form; a regular shape to the facade develops as a crystal grows. The molecules and indeed the groups within the molecules, become organized into regular repeating patterns and this determines the possible nonbonding interactions between functional groups. The hydrogen bonding interactions shown in Figures 4.14 - 4.16 have been generated from calculated values. The "Mercury 1.4.2" crystal viewing package has the ability to identify all contacts between atoms of any type that are shorter than a specified van der Waals corrected distance. In this way it can be used to predict likely intermolecular hydrogen bond interactions and insert representations of these predicted bonds into the crystal structure diagrams. The visualization of these interactions is helpful to the understanding of the spacial arrangement of the molecules in the solid phase.

4.3 Synthesis and characterization of pyrrolidine-2,5-dione (succinimide) derivative.

Many syntheses of pyrrolidine-2,5-diones (succinimide) molecules have been reported, the molecules are not only a valuable medicinal structures but a valuable synthetic intermediate. The synthesis of the pyrrolidine-2,5-dione derivatives **201** was completed by refluxing the substrate 2-iminopyrrolidin-5-ones **198** in aqueous acetic acid for 4 hours.

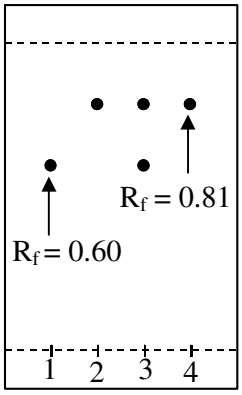


201f R = H or **201j** R = Cl

Scheme 4.6 Mechanism of formation of pyrrolidine-2,5-dione derivatives

It was decided to use the 2-iminopyrrolidin-5-one **198f** discussed in the previous section in the synthesis of the pyrrolidine-2,5-dione (**201**, Scheme 4.6). It was hoped that a successful synthesis would not only produce another novel molecule but also perhaps shed some further light on the anomalous spectral issues arising from the synthesis of the parent compound. The line broadening observed in the ^{13}C spectrum of the 2-iminopyrrolidin-5-one was of particular interest. The synthesis was successfully achieved using acetic acid to effect the desired transformation.

It had previously been noticed that when a pure crystalline sample of the 2-iminopyrrolidin-5-one **198f** was left standing in air for a period of several days the TLC showed the development of trace amounts of a new spot. TLC comparison of the pyrrolidine-2,5-dione with the decomposing sample of 2-iminopyrrolidin-5-one showed the pyrrolidine-2,5-dione to have an identical R_f value to the unknown compound. Attempts to isolate enough of this compound for further analysis by chromatography failed. However when the decomposing sample was reacted with aqueous acetic acid TLC monitoring of the reaction showed development of a single product corresponding to the unknown compound and this was confirmed to be the pyrrolidine-2,5-dione derivative. This suggests that the substrate 2-iminopyrrolidin-5-one is prone to hydrolysis on contact with atmospheric moisture over time.



The spot in lane 1 represents a pure sample of 2-iminopyrrolidin-5-one

The spot in lane 2 represents a pure sample of pyrrolidine-2,5-dione

The spots in lane 3 represent a partially decomposed sample of 2-iminopyrrolidin-5-one left open to the atmosphere for 4 days.

The spot in lane 4 represents the reaction product (also pyrrolidine-2,5-dione) of the degraded sample after reflux with acetic acid for 4 hours

Figure 4.17 Representation of TLC (EtOAc:Hex 30:70) showing transformation of degraded 2-iminopyrrolidin-5-one **198f** to pyrrolidine-2,5-dione **201f** using acetic acid

4.3.1 NMR studies of pyrrolidine-2,5-dione derivative.

In the proton spectrum of the 2-iminopyrrolidin-5-one observed broadening could be rationalized in terms of the chemical exchange experienced by the cyclic imino functionality. However in the ^{13}C spectrum the line broadening not only affected the imino carbon but also had a drastic effect on the three methyl carbons attached to two different ring positions within the molecule. The synthesis of the pyrrolidine-2,5-dione offered an opportunity to alter a single functionality within the molecule and observe what the effect of that change would

have on the NMR spectra of the new compound. Since it was suspected that the line broadening was somehow caused by the presence of the imino group, the synthesis of the pyrrolidine-2,5-dione allowed this to be investgated without altering any other moiety within the system. When the ^{13}C spectrum of the pyrrolidine-2,5-dione was obtained using $CDCl_3$ as solvent, no line broadening akin to what had been observed for the 2-iminopyrrolidin-5-one could be detected (Figure 4.18). Again this evidence lends credence to the assertion that it is the presence of the imino group that causes this drastic effect on the spectra run in $CDCl_3$.

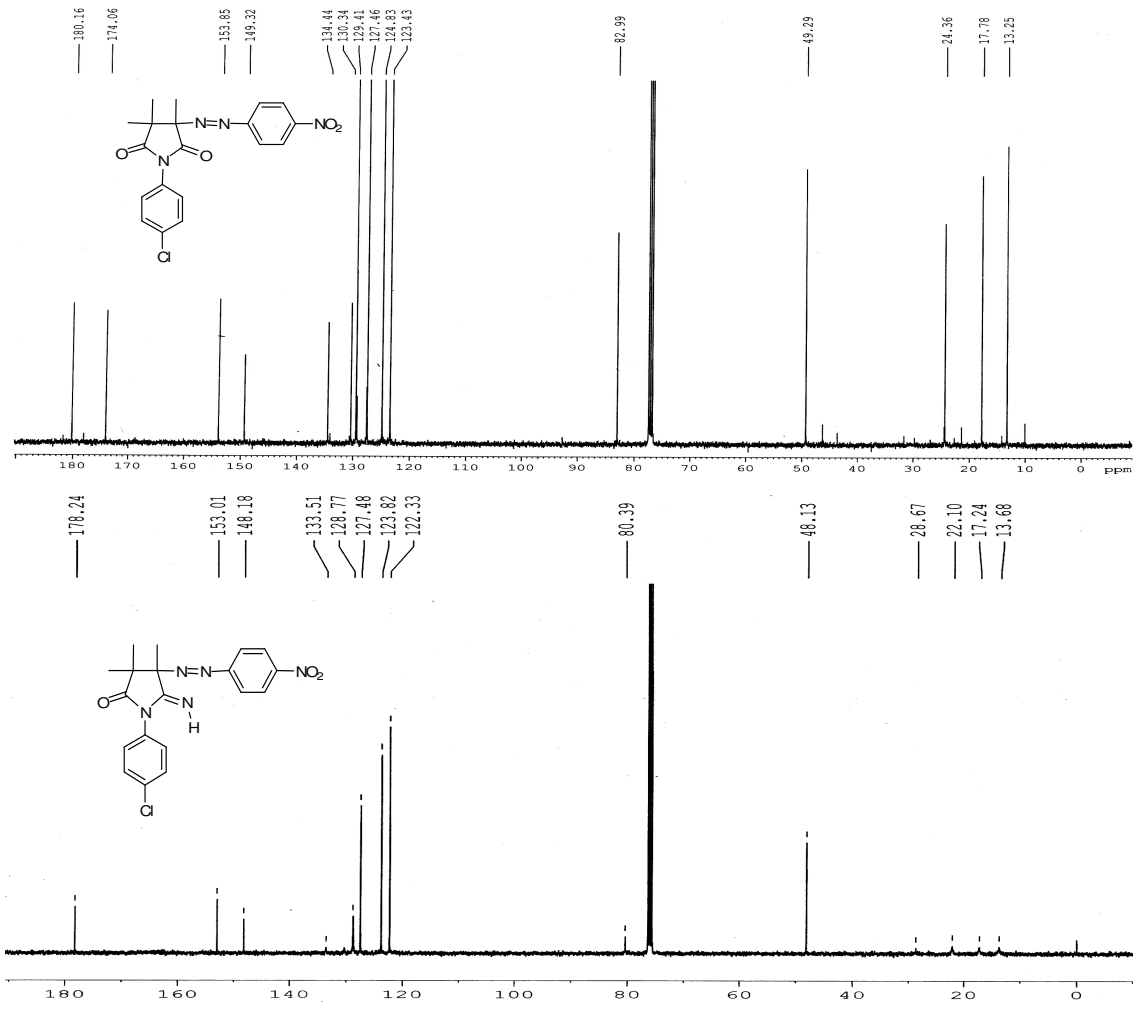
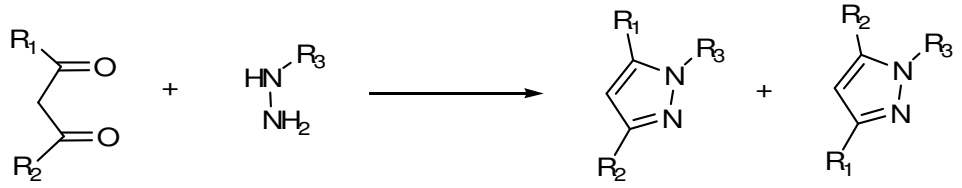


Figure 4.18 Comparison of the ^{13}C NMR spectra of the 2-iminopyrrolidin-5-one **198j** and the structurally similar pyrrolidine-2,5-dione derivative **201j** both in $CDCl_3$.

4.4 Pyrazole formation

Due to their wide ranging applications in medicinal and agrochemical roles new synthetic routes to pyrazoles have received much attention. Thus far, the full potential of these compounds has not been exploited due to the limited number of synthetic procedures available for their synthesis.

Currently the most common method of obtaining pyrazoles is through the reaction of 1,3-diketones **202** with hydrazine or its derivatives **203**.[123] However a commonly encountered problem with this route is to maintain regioselectivity in the product. To date this problem remains the greatest challenge within the field. The method often produces a mixture of regioisomers (e.g. **204** and **205** Scheme 4.7) if the reactivity of the two carbonyl groups is not significantly different, a problem that has obvious repercussions on the scope of the procedure

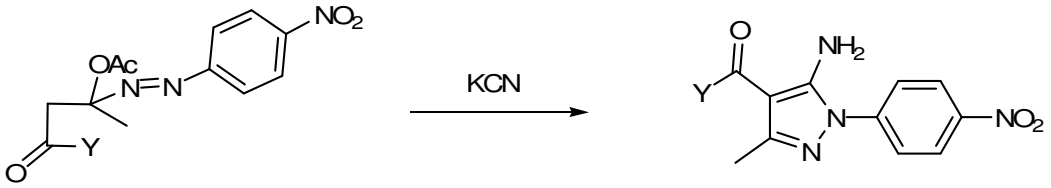


202 **203** **204** **205**

Two Products

Scheme 4.7 General scheme for the formation of pyrazoles *via* diketones and hydrazines

This problem is compounded by the difficulties arising from the synthesis of pure 1,3-diketones **202** as starting material. Methods for producing this entity often return complex mixtures of condensation products not easily separated from the desired product. In light of these issues, the formation of the pyrazole through incorporation of the cyanide ion into a parent azoacetate is an interesting example. This setup ensures regioselectivity in the finished product, and has the added advantage of introducing exocyclic functional groups that provide the opportunity for further fuctionalization and diversification within the product.



206a and b **207a and b**

a,Y = OEt, b, Y = NH-phenyl,

Yield, **206a** = 15%
206b = 15%

Scheme 4.8 Transformation of α,α-unsubstituted azoacetate to pyrazole.

The presence of an extra nitrogen atom in the product suggests that a cyanide unit has also been incorporated into the molecule. The initial formation of a nitrile seems the most likely route towards the cyclisation to the pyrazole, however a direct substitution seems unlikely. This seems counterintuitive from several standpoints. Firstly, attack of a nucleophile such as the cyanide ion at an unsubstituted α- carbon at first glance seems an unlikely reaction; and secondly it has just been shown in the previous sections that in the case of the di-methylated analogue under similar conditions the cyanide ion reacts readily to displace the acetate moiety followed by cyclisation to the 2-iminopyrrolidin-5-one ring. There is some theoretical support for this direct attack at the α-carbon. In order to best rationalize how these groups might interact to give the product in question the HASB (Hard Acid Soft Base) approach to the reactive species can be applied. This is not a scientific theory in the true sense of the term, but a set of principles based on experimental observations used to explain atypical reaction results. The system, introduced by Pearson and Songstad, is based on a proposed affinity of similar groups for reaction with each other[124]. Although empirical in nature, the principles have been used to predict the behaviour of many organic reactions that are not easily explained by other means. The principles work by sorting organic functional groups based on the similarities in their experimental properties. The properties attributed to the groups under the HASB principles is given in Table 4.4.[125]

Table 4.4 Summary table of properties of acids and bases under the HASB system

	Hard Acid	Hard Base	Soft Acid	Soft Base
Reaction Role	electrophiles	nucleophiles	electrophiles	nucleophiles
Bonding Orbital	high LUMO	low HOMO	low LUMO	high HOMO
Polarizability	low	low	high	high
Valence electrons	None unshared	Tightly held	unshared	Loosely held
Examples	H^+, Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cr^{2+}, Fe^{3+}	H_2O, ^-OH, F^-, AcO^-, SO_4^{2-}, Cl^-, CO_3^{2-}, NO_3, ROH	Cu^+, Ag^+, Pd^{2+}, I_2 Pt^{2+}, Hg^{2+}, BH_3, **$\underline{CH_2}$**, carbenes, Br_2	RS, I^-, **$\underline{CN^-}$**, CO, $(RO)_3P$, RCN, C_2H_4, C_6H_6, H^-, R^-
Bond Type	Ionic		Covalent	

Both reaction centers can be thought of as ambident in nature, and as such, can react either through the carbon or the heteroatom; however the position of the carbon and nitrogen in the final isolated product suggests that a nitrile was formed at some point. This would strongly suggest that the initial step is that of carbon-carbon bond formation.

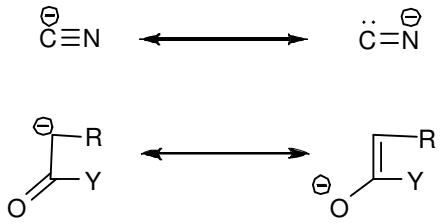
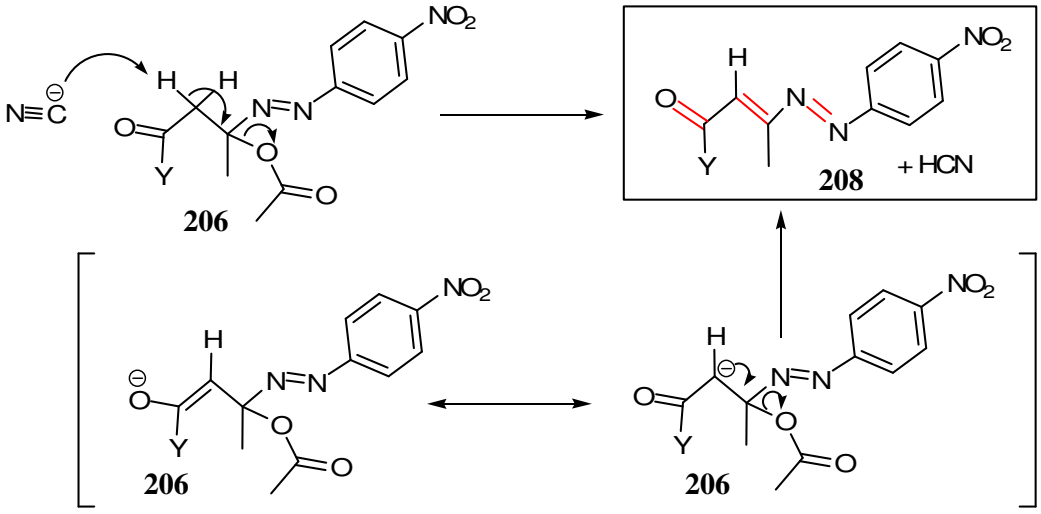


Figure 4.19 Ambident cyanide ion and β-keto compound shown in both forms

Since the cyanide ion falls under the category of a soft base and a $-CH_2-$ can be categorized as a soft acid (highlighted in Table 4.4), direct reaction between the two groups can be rationalized. However the $-CH_2-$ unit in question is part of a β-keto system and must be considered as such.

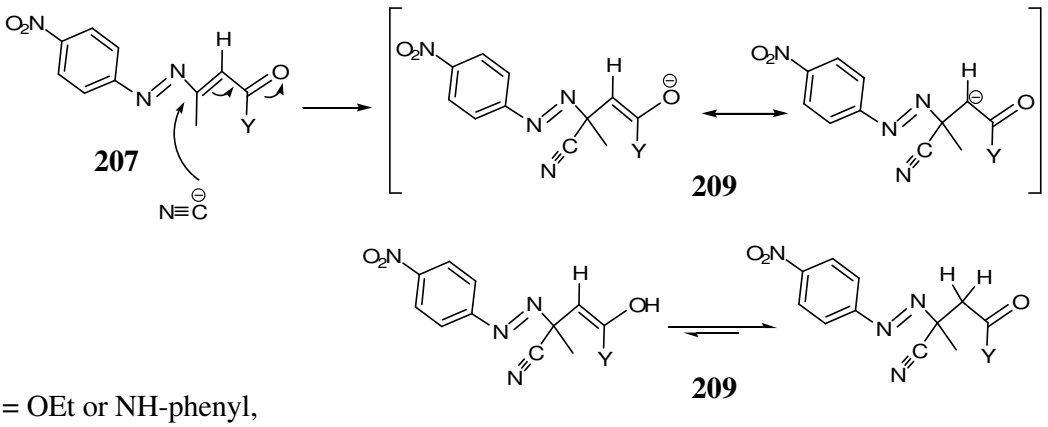
The most likely scenario is an initial base induced abstraction of one of the highly acidic alpha-hydrogen atoms followed by elimination. This type of competing elimination reaction is commonly found in systems where the substrate has a hydrogen atom that can be easily removed and the base chosen can act as a base or a nucleophile. Often in such cases the base induced enolate formation is the result of resonance stabilization offered by this conformation. However, in this case, due to the presence of the azoacetate group, it is feasible that loss of the acetate moiety produces a more favorable conformation due to the stabilization provided by conjugation within the system (Scheme 4.9). If the formation of the enolate is assumed to take place the subsequent degradation to the more conjugated system with loss of the acetate group is also a likely outcome.



Y = OEt or NH-phenyl,

Scheme 4.9 Base induced elimination of acetate group from azoacetate

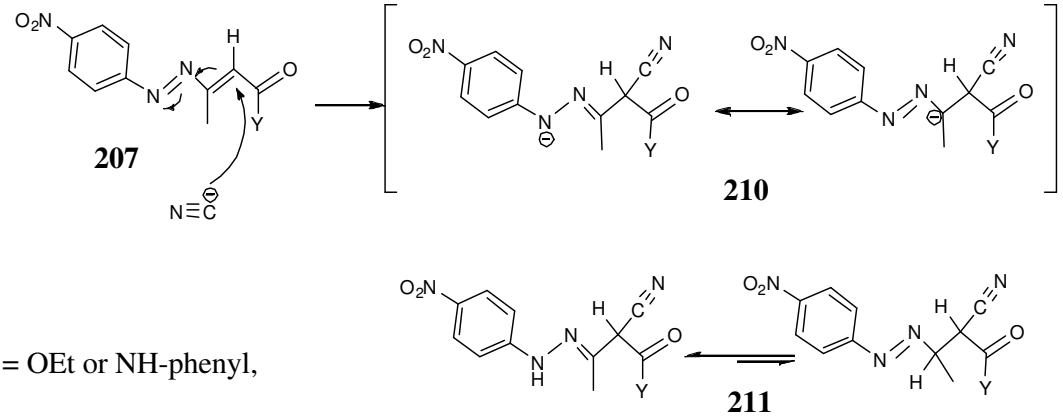
If this hypothesis is accepted then reasonable assumptions can be made as to the mechanism of formation of the pyrazole. Firstly the configuration of this proposed intermediate is such that it is a typical substrate for nucleophilic addition. The additions across the carbon-carbon double bonds of these enone type substrates are quite well known. However in these cases the attacking nucleophile almost exclusively adds to the carbon furthest from the carbonyl (Scheme 4.10).



Y = OEt or NH-phenyl,

Scheme 4.10 Mechanism for nucleophilic attack by cyanide ion at a position β to the carbonyl.

The reason that this configuration is usually preferred is once again the resonance stabilization provided by the formation of the enolate intermediate (**209,** Scheme 4.10). Protonation of the more negative oxygen usually occurs to give the enol which tautomerizes to give the carbonyl. But, in the case of the proposed intermediate there is an alternative to the enolate formation through azo/hydrazone tautomerisation that offers equal if not greater stability through attack α to the carbonyl.

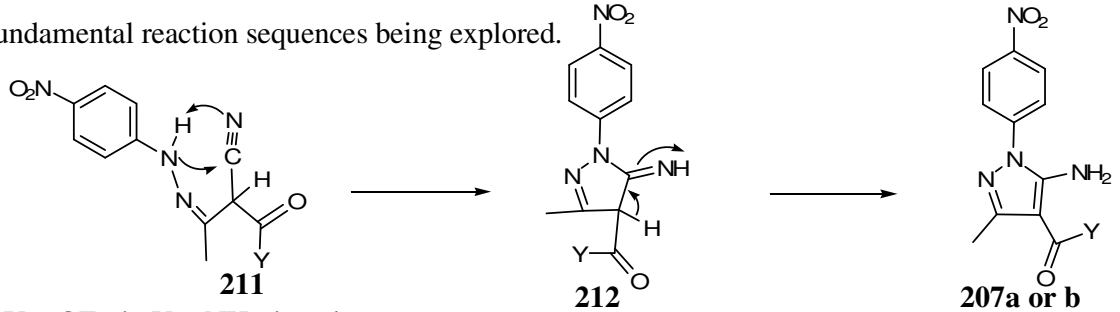


Y = OEt or NH-phenyl,

Scheme 4.11 Mechanism for nucleophilic attack by cyanide ion at a position α to the carbonyl.

The formation of, and subsequent cyclisation of this compound **211** has been previously reported.[67] In that case the reaction between hydrazidic halides with cyanoacetic acid

derivatives were investigated (Scheme 1.17). This is essentially condensation of the two molecules to the hydrazone with the cyano group already in place followed by cyclisation to the pyrazole in a manner identical to that proposed here (See chapter 1). Although the products are the same there are some differences in the experimental procedures and fundamental reaction sequences being explored.



a,Y = OEt, b, Y = NH-phenyl,

Scheme 4.12 Mechanism of cyclisation to form the pyrazole.

Structural study of Pyrazole

Crystals suitable for single crystal X-Ray crystallographic determination of the pyrazole were grown in acetonitrile/methanol using the slow cooling technique. This involved dissolving the substrate molecule in hot acetonitrile/methanol and cooling the solution to room temperature over 10 days. Evaporation was minimized by covering the cooling vessel (clean new test tube) with foil. The resulting crystals were colourless and needle-like in appearance.

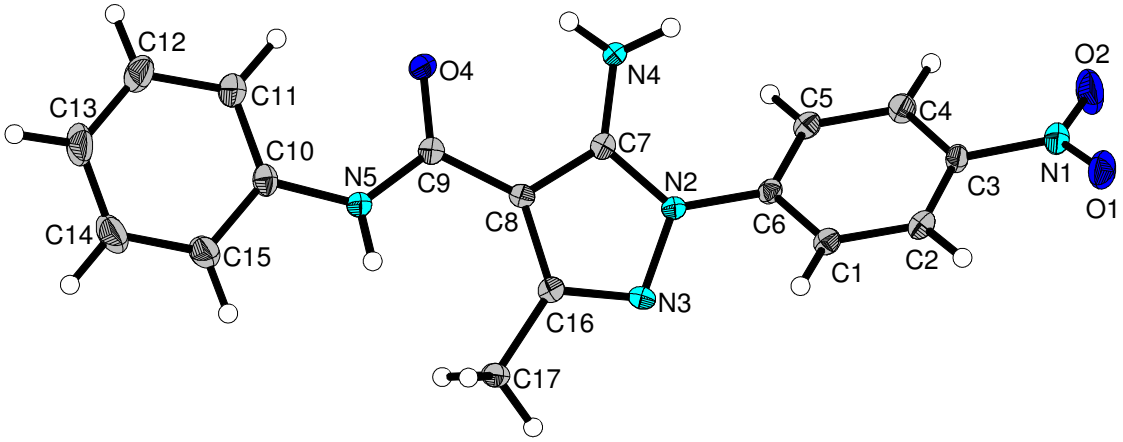


Figure 4.20 Crystal structure of pyrazole **207b**

The pyrazole **207b** crystallizes in the monoclinic space group P21/n (#14) with four molecules per asymmetric unit. Some of the main features of interest within this molecule include the free amine, C-N bond distance is 1.350(2) Å. The observed bond angles and internal torsional angles within the 5 membered ring are indicative of a planar system. This would be expected for a small aromatic system.

Table 4.5 Selected bond distances (Å) and angles (^{O}) for pyrazole **207b** with estimated standard deviations

Bond distances	(Å)
C(7)–N(4)	1.350(2)
N(2)–C(7)	1.363(2)
N(3)–C(16)	1.320(2)
N(2)–N(3)	1.393(2)
C(9)–O(4)	1.243(2)
N(1)–O(2)	1.227(2)
N(1)–O(1)	1.229(2)
Bond angles	(^{O})
C(7)–N(2)–N(3)	111.61(15)
N(2)-N(3)-C(7)	110.96(13)
N(3)-N(2)-C(6)	113.78(14)
C(16)-N(5)-C(10)	122.55(13)
O(1)-N(1)-C(3)	118.68(14)
Torsional angles	(^{O})
C(7)–N(2)–N(3)–C(16)	1.6(2)
N(3)–N(2)–C(7)–C(8)	–1.7(2)
N(2)–C(7)–C(8)–C(16)	1.0(2)

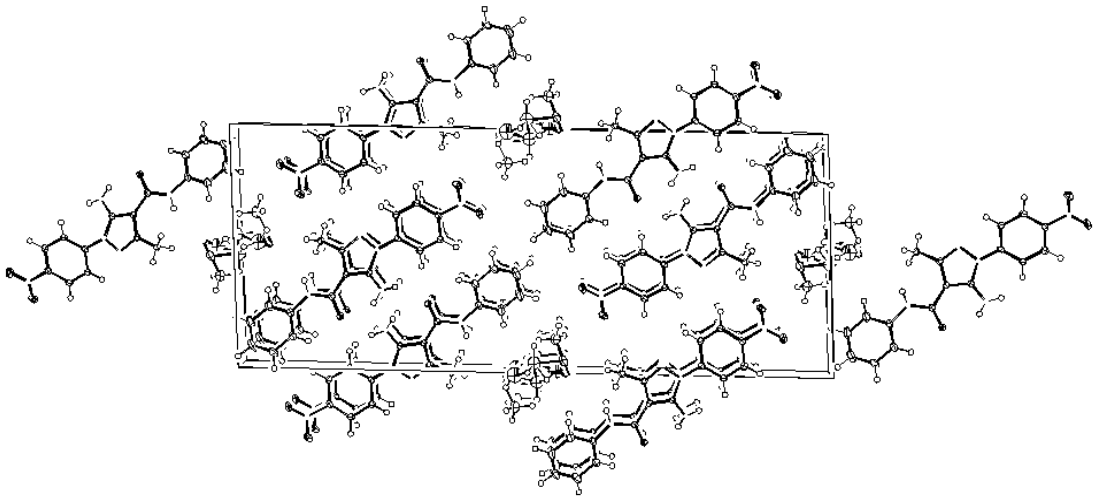


Figure 4.21 ORTEP view of pyrazole **207b** showing packing relative to the unit cell

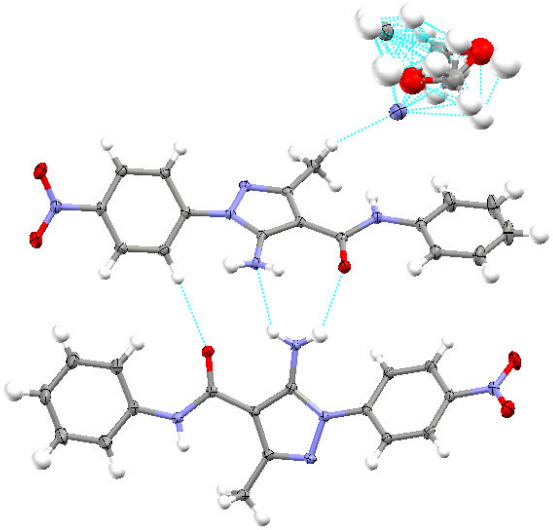


Figure 4.22 Mercury view of pyrazole **207b** depicting the hydrogen bonding interactions, including interactions with acetonitrile solvent molecule.

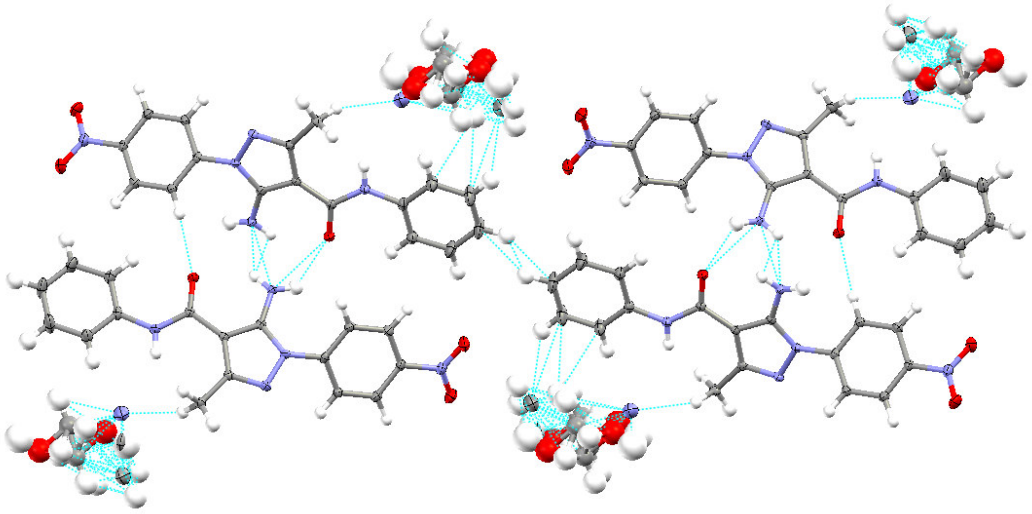


Figure 4.23 Mercury view of pyrazole **207b** depicting all intermolecular interactions, including interactions with solvent molecules (methanol and acetonitrile).

Table 4.4 Crystal data and structure refinement for pyrazole **207b**

Quantity	Measurement
Empirical formula	$C_{18.625}\ H_{17.75}\ N_{5.75}\ O_{3.125}$
Molecular formula	$C_{17}\ H_{15}\ N_5\ O_3$ x 0.75 ($C_2\ H_3\ N$) x 0.125 ($C\ H_4$
Formula weight	372.29
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/n$ (#14)
Unit cell dimensions	a = 13.394(2) Å α= 90°.
	b = 3.8267(6) Å β= 92.688(3)°.
	c = 33.470(5) Å γ = 90°.
Volume	1713.6(5) $Å^3$
Z	4
Density (calculated)	1.443 Mg/m^3
Absorption coefficient	0.103 mm^{-1}
F(000)	779
Crystal size	1.00 x 0.10 x 0.05 mm^3
Theta range for data	1.67 to 28.35°.
Index ranges	–17<=h<=17, –5<=k<=5, –44<=l<=44
Reflections collected	16121
Independent reflections	4230 [R(int) = 0.0280]
Completeness to theta =	99.1 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9948 and 0.8070
Refinement method	Full–matrix least–squares on F^2
Data / restraints / parameters	4230 / 0 / 323
Goodness–of–fit on F^2	1.124
Final R indices [I>2sigma(I)]	R1 = 0.0653, wR2 = 0.1581
R indices (all data)	R1 = 0.0716, wR2 = 0.1617
Largest diff. peak and hole	0.712 and –0.389 e.$Å^{-3}$

4.5 Conclusion:

It has been shown that the substrate azoacetates discussed in this chapter provide a facile route to a selection of heterocyclic systems through incorporation of a cyanide unit. It has also been shown that the azo moiety can be assimilated in an endocyclic (pyrazole) or exocyclic (iminopyrrolidin-5-one) position depending on the substitution of these azoacetates. The isolation of the 2-iminopyrrolidin-5-one derivatives had not been reported by the original researchers, However once it had been isolated, it was found that with minor modifications to the reaction stoichiometry the 2-iminopyrrolidin-5-one can be isolated as the major product.

Additionally this structure was easily modified to produce a pyrrolidine-2,5-dione derivative upon reaction with aqueous acetic acid. Pyrrolidine-2,5-dione derivatives have been widely employed in a range of roles within the medicinal and synthetic chemistry fields. Initial characterization of the 2-iminopyrrolidin-5-ones proved difficult due to some unusual effects observed in the 1H and 13C spectra of these compounds when CDCl3 was employed as solvent. The high level of structural similarity between the pyrrolidine-2,5-dione and the 2-iminopyrrolidin-5-one (different only by a cyclic C==O versus C==NH unit) provided a unique opportunity to study an unusual NMR phenomenon. The solvent specific line broadening of 2-iminopyrrolidin-5-one investigated here is not often observed. The details of these syntheses have recently been accepted for publication as a full paper in "Heterocycles" (see Appendix IV).

Overall, it has been shown that the azoacetates chosen for study in this thesis provide access to a variety of phenylazo substituted heterocycles and important information has been gathered concerning the construction of heterocycles directly attached to the phenylazo group via azoacetates.

4.6 Experimental:

Ethyl acetoacetate and substituted acetoacetanilides were purchased from the Sigma Aldrich chemical company and were used as received. All solvents were dried or distilled prior to use. Melting points were determined using a Griffin melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 2000FT-IR spectrometer. NMR spectra were recorded using a Bruker AC 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. The ^{1}H and ^{13}C NMR chemical shifts (ppm) are relative to TMS and all coupling constants (*J*) are in Hertz (Hz).

Synthesis of 2-iminopyrrolidin-5-ones (198f and 198j)

5-imino-3,3,4-trimethyl-4-((4-nitrophenyl)diazenyl)-1-phenylpyrrolidin-2-one 198f

The azoacetate **(133f)** (1g, 2.1 mmol) was dissolved in hot propanol (40 ml) to which potassium cyanide (0.30 g, 4.6 mmol) was added. The solution was allowed to reflux for 30 min, after which TLC showed development of product spots and total consumption of the starting material, this was evaporated to dryness. Water (100 ml) was added to the reaction vessel and this was extracted with EtOAc (2 x 25 ml) followed by CH_2Cl_2 (2 x 25 ml). The combined extracts were dried over magnesium sulfate and upon evaporation to dryness an orange oil was collected. The product was isolated after column chromatography.

198f, Yield (0.17 g, 18 %); mp 142-145 ºC ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.19 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.61 (3H, s, CH_3), 7.34-7.28 (2H, m, ArH), 7.41-7.39 (1H, m, ArH), 7.52-7.47 (2H, m, ArH,), 7.76 (2H, d, ArH, *J* = 8.8), 8.26 (2H, d, ArH, *J* = 9.2); 9.00 (1H, s NH); 13C NMR (100 MHz, DMSO-d6) 13.3, 17.9, 24.4, 49.3, 83.1, 123.4, 124.8, 126.3, 128.7, 129.2, 131.9, 149.3, 153.9, 174.3, 180.5; IR (KBr) 1661, 1528, 1377 cm-1

(134f) Yield (0.10 g, 12 %). Spectra identical to those reported in chapter 2

(77f) Yield (0.14 g, 16 %); mp 195-197 ºC ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.49 (3H, s, CH_3), 1.53 (3H, s, CH_3), 1.92 (3H, s, CH_3), 6.96 (1H, s br, ArH),7.07 (1H, t, ArH, *J* = 7.2),

7.33-7.26 (3H, m, ArH), 7.61 (2H, d, ArH, *J* = 7.6), 8.15 (2H, s br, ArH), 9.06 (1H, s br, NH), 9.48 (1H, s br, NH); ^{13}C NMR (100MHz, DMSO-d_6) 21.7, 22.9, 24.3, 65.6, 110.5 (d, *J* = 222), 120.8, 123.5, 126.2 (d, *J* = 69), 128.3, 138.7, 138.9, 154.2, 171.8, 172.8. IR (KBr) 3338, 3288, 1647, 1592, 1522, 1329, 1272, 1108, 750, 695 cm^{-1}.

1-(4-chlorophenyl)-5-imino-3,3,4-trimethyl-4-((4-nitrophenyl)diazenyl)pyrrolidin-2-one 198j

Yield (0.21 g, 23%); mp 201-203 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.07 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.60 (3H, s, CH_3), 7.32 (2H, d, ArH, *J* =8.4), 7.38 (2H, d, ArH, *J* =8.4), 7.89 (2H, d, ArH, *J* = 9.2), 8.40 (2H, d, ArH, *J* = 9.2), 9.08 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO-d_6) 13.8, 17.9, 22.9, 48.5, 81.6, 123.2, 125.1, 128.8, 129.3, 132.3, 132.8, 148.7, 154.0, 165.3, 179.1. IR (KBr) 3300, 1742, 1661, 1492 cm^{-1}.

Synthesis of succinimide (201f)

Synthesis of 3,3,4-trimethyl-4-((4-nitrophenyl)diazenyl)-1-phenylpyrrolidine-2,5-dione (201f)

The 2-iminopyrrolidin-5-one **198f** (200 mg, 0.54 mmol) was dissolved in 10 ml of aqueous acetic acid. The solution was heated to 50 ^{o}C for 24 h, 100 ^{o}C for a further 24 h and finally allowed to reflux for 4 h after which TLC showed total consumption of the starting material. The solution was allowed to cool and added to ice-water (100 ml) which induced precipitation of a pale yellow solid that was filtered from solution. Yield (153 mg, 78 %); product decomposed in air over 24 h; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.61 (3H, s, CH_3), 7.30-7.25 (2H, m, ArH), 7.35-7.32 (1H, m, ArH), 7.43-7.37 (2H, m, ArH), 7.76 (2H, d, ArH, *J* = 8.8), 8.26 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100 MHz, $CDCl_3$) 13.3, 17.9, 24.4, 49.3, 83.1, 123.4, 124.8, 126.3, 128.7, 129.2, 131.9, 149.3, 153.9, 174.3, 180.5; IR (KBr) 1719, 1529, 1396, 1346 cm^{-1}.

Synthesis of pyrazoles (207a and **207b)**

Synthesis of 5-amino-3-methyl-1-(4-nitrophenyl)-N-phenyl-1H-pyrazole-4-carboxamide (207a)

The substrate azoacetate **206a** (1 g, 3.1 mmol) was dissolved in hot n-PrOH (40 ml) to which potassium cyanide (0.3 g, 4.6 mmol) was added. The solution was allowed to reflux for 4 h, before being evaporated to dryness. Water (100 ml) was added to the reaction vessel and this was extracted with CH_2Cl_2 (15:85). The combined extracts were dried over magnesium sulfate and upon evaporation to a reduced volume a pale brown solid precipitated. The precipitate was filtered off and washed with petroleum ether and the product isolated after flash chromatography. Yield (0.13 g, 15 %) mp 210-212 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.27 (3H, t, CH_3, *J* = 7.2), 2.26 (3H, s, CH_3), 4.20 (2H, q, CH_2, *J* = 7.2), 6.64 (2H, s br, CH_2), 7.81 (2H, d, ArH , *J* = 7.2), 8.32 (2H, d, ArH, *J* = 7.2); ^{13}C NMR (100 MHz, DMSO-d_6) 14.3, 14.4, 59.1, 93.9, 123.0, 124.9, 143.1, 144.9, 150.6, 151.2, 164.0; IR (KBr) 3349, 1676, 1548, 1344 cm^{-1}. Mass Spectrum: $[M+H]^+$ found 291.5, $C_{13}H_{15}N_4O_4$ requires 291.1.

Ethyl 5-amino-3-methyl-1-(4-nitrophenyl)-1H-pyrazole-4-carboxylate 207b

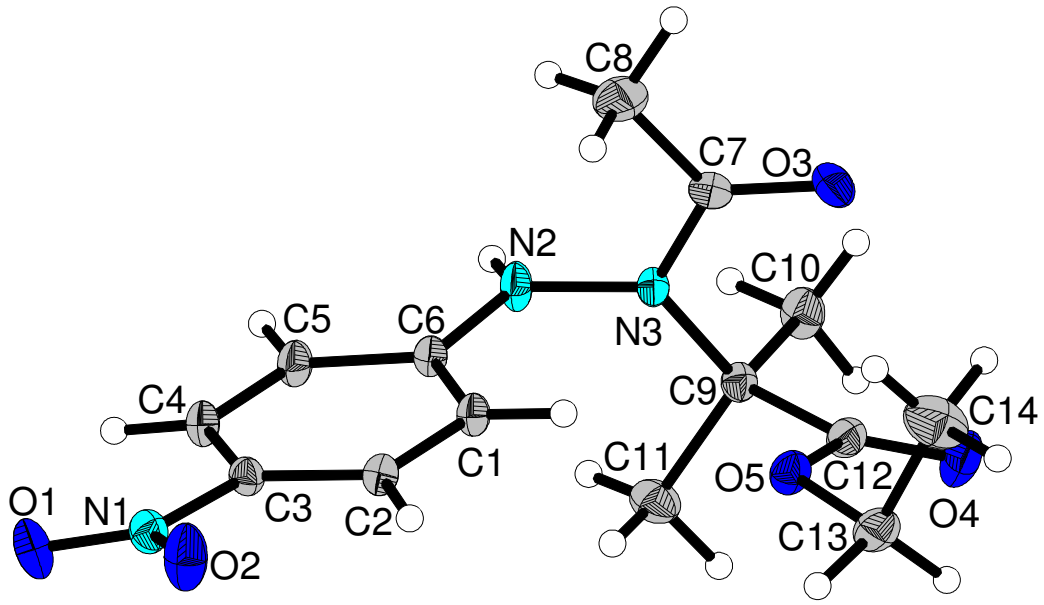
207b synthesized using an identical procedure to **207b** using **206a** as substrate

Yield (0.12 g, 15 %); mp 181-183 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.94 (3H, s, CH_3), 6.60 (2H, s br, CH_2), 7.06 (1H, t, ArH , *J* = 7.6), 7.32 (2H, t, ArH, *J* = 7.6), 7.63 (2H, d, ArH , *J* = 7.6), 7.88 (2H, d, ArH , *J* = 9.2), 8.35 (2H, d, ArH, *J* = 9.2), 8.85 (1H, s, NH,); ^{13}C NMR (100 MHz, DMSO-d_6) 14.2, 98.4, 120.4, 122.6, 123.3, 124.9, 128.5, 138.8, 143.3, 144.7, 148.2, 150.7, 163.3; IR (KBr) 3455, 3429, 3313, 1650, 1599, 1543, 1495 cm^{-1}. Mass Spectrum: $[M+Na]^+$ found 360.4, $C_{17}H_{15}N_5NaO_3$ requires 360.1.

Appendix I

(Crystal structure and supporting data for N-acyl hydrazide derivative **79**)

Crystal structure and supporting data for N-acyl hydrazide derivative **72**



72 molecule; thermal ellipsoids are drawn on the 50% probability level

Table 1. Crystal data and structure refinement for **72**.

Identification code	**72**	
Empirical formula	$C_{14}\ H_{19}\ N_3\ O_5$	
Formula weight	309.32	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$ (#19)	
Unit cell dimensions	a = 10.6723(6) Å	α= 90°.
	b = 11.7713(7) Å	β= 90°.
	c = 12.3081(7) Å	γ = 90°.
Volume	1546.23(15) $Å^3$	
Z	4	
Density (calculated)	1.329 Mg/m^3	
Absorption coefficient	0.102 mm^{-1}	
F(000)	656	
Crystal size	1.00 x 1.00 x 0.90 mm^3	
Theta range for data collection	2.39 to 30.50°.	

Index ranges	–15<=h<=15, –16<=k<=16, –17<=l<=17
Reflections collected	36459
Independent reflections	2661 [R(int) = 0.0293]
Completeness to theta = 30.50°	99.7 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9138 and 0.8407
Refinement method	Full–matrix least–squares on F^2
Data / restraints / parameters	2661 / 0 / 275
Goodness–of–fit on F^2	1.048
Final R indices [I>2sigma(I)]	R1 = 0.0350, wR2 = 0.0892
R indices (all data)	R1 = 0.0360, wR2 = 0.0899
Largest diff. peak and hole	0.443 and –0.150 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for **72**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	x	y	z	U(eq)
C(1)	4928(1)	3245(1)	8056(1)	16(1)
C(2)	5010(1)	3283(1)	9182(1)	16(1)
C(3)	6152(1)	3078(1)	9680(1)	15(1)
N(1)	6218(1)	3047(1)	10862(1)	20(1)
O(1)	7256(1)	3016(1)	11296(1)	28(1)
O(2)	5223(1)	3039(1)	11374(1)	31(1)
C(4)	7234(1)	2875(1)	9080(1)	18(1)
C(5)	7157(1)	2831(1)	7956(1)	18(1)
C(6)	5997(1)	2991(1)	7438(1)	15(1)
N(2)	5945(1)	2853(1)	6330(1)	19(1)
N(3)	4880(1)	3121(1)	5742(1)	15(1)
C(7)	3999(1)	2309(1)	5545(1)	19(1)
O(3)	3113(1)	2529(1)	4943(1)	24(1)
C(8)	4117(2)	1188(1)	6127(1)	30(1)
C(9)	4772(1)	4252(1)	5210(1)	16(1)
C(10)	5124(1)	4166(1)	4010(1)	23(1)
C(11)	5630(1)	5103(1)	5785(1)	24(1)
C(12)	3426(1)	4693(1)	5356(1)	18(1)
O(4)	2849(1)	5201(1)	4668(1)	28(1)
O(5)	3055(1)	4539(1)	6384(1)	19(1)

C(13)	1807(1)	4925(1)	6680(1)	22(1)
C(14)	863(2)	4008(2)	6483(2)	35(1)

Table 3. Bond lengths [Å] and angles [°] for **72**.

C(1)–C(2)	1.3897(17)
C(1)–C(6)	1.4033(16)
C(1)–H(1)	0.930(18)
C(2)–C(3)	1.3850(16)
C(2)–H(2)	0.95(2)
C(3)–C(4)	1.3912(17)
C(3)–N(1)	1.4572(15)
N(1)–O(1)	1.2301(15)
N(1)–O(2)	1.2359(15)
C(4)–C(5)	1.3872(17)
C(4)–H(4)	0.91(2)
C(5)–C(6)	1.4050(16)
C(5)–H(5)	0.93(2)
C(6)–N(2)	1.3750(15)
N(2)–N(3)	1.3838(14)
N(2)–H(1N2)	0.867(19)
N(3)–C(7)	1.3627(16)
N(3)–C(9)	1.4879(16)
C(7)–O(3)	1.2284(16)
C(7)–C(8)	1.5069(19)
C(8)–H(8A)	0.91(3)
C(8)–H(8B)	0.98(3)
C(8)–H(8C)	0.98(3)
C(9)–C(10)	1.5280(17)
C(9)–C(11)	1.5305(18)
C(9)–C(12)	1.5386(17)
C(10)–H(10A)	0.97(2)
C(10)–H(10B)	0.92(2)
C(10)–H(10C)	1.00(2)
C(11)–H(11A)	0.937(19)
C(11)–H(11B)	0.97(2)
C(11)–H(11C)	0.97(2)

C(12)–O(4)	1.2050(16)
C(12)–O(5)	1.3381(16)
O(5)–C(13)	1.4539(16)
C(13)–C(14)	1.497(2)
C(13)–H(13A)	0.96(2)
C(13)–H(13B)	1.00(2)
C(14)–H(14A)	0.96(2)
C(14)–H(14B)	0.99(2)
C(14)–H(14C)	0.94(3)

C(2)–C(1)–C(6)	119.77(11)
C(2)–C(1)–H(1)	119.3(11)
C(6)–C(1)–H(1)	120.9(11)
C(3)–C(2)–C(1)	119.37(11)
C(3)–C(2)–H(2)	120.5(13)
C(1)–C(2)–H(2)	120.0(13)
C(2)–C(3)–C(4)	121.76(10)
C(2)–C(3)–N(1)	119.22(11)
C(4)–C(3)–N(1)	119.01(11)
O(1)–N(1)–O(2)	123.49(10)
O(1)–N(1)–C(3)	118.59(11)
O(2)–N(1)–C(3)	117.92(11)
C(5)–C(4)–C(3)	119.05(11)
C(5)–C(4)–H(4)	122.9(13)
C(3)–C(4)–H(4)	118.0(13)
C(4)–C(5)–C(6)	120.02(11)
C(4)–C(5)–H(5)	117.2(13)
C(6)–C(5)–H(5)	122.6(13)
N(2)–C(6)–C(1)	122.06(11)
N(2)–C(6)–C(5)	118.00(11)
C(1)–C(6)–C(5)	119.91(10)
C(6)–N(2)–N(3)	121.60(10)
C(6)–N(2)–H(1N2)	121.6(12)
N(3)–N(2)–H(1N2)	114.6(12)
C(7)–N(3)–N(2)	120.04(11)
C(7)–N(3)–C(9)	119.73(10)
N(2)–N(3)–C(9)	119.79(10)

O(3)–C(7)–N(3)	119.40(12)
O(3)–C(7)–C(8)	122.39(13)
N(3)–C(7)–C(8)	118.13(12)
C(7)–C(8)–H(8A)	111.0(17)
C(7)–C(8)–H(8B)	106.3(15)
H(8A)–C(8)–H(8B)	111(2)
C(7)–C(8)–H(8C)	106.6(17)
H(8A)–C(8)–H(8C)	110(2)
H(8B)–C(8)–H(8C)	112(2)
N(3)–C(9)–C(10)	110.33(10)
N(3)–C(9)–C(11)	109.66(10)
C(10)–C(9)–C(11)	110.10(11)
N(3)–C(9)–C(12)	108.82(10)
C(10)–C(9)–C(12)	111.34(10)
C(11)–C(9)–C(12)	106.50(10)
C(9)–C(10)–H(10A)	107.3(13)
C(9)–C(10)–H(10B)	111.7(13)
H(10A)–C(10)–H(10B)	109.9(19)
C(9)–C(10)–H(10C)	108.9(13)
H(10A)–C(10)–H(10C)	108.4(19)
H(10B)–C(10)–H(10C)	110.6(18)
C(9)–C(11)–H(11A)	111.9(12)
C(9)–C(11)–H(11B)	105.4(15)
H(11A)–C(11)–H(11B)	105.6(18)
C(9)–C(11)–H(11C)	110.8(14)
H(11A)–C(11)–H(11C)	108.8(19)
H(11B)–C(11)–H(11C)	114(2)
O(4)–C(12)–O(5)	125.48(12)
O(4)–C(12)–C(9)	124.24(12)
O(5)–C(12)–C(9)	109.90(10)
C(12)–O(5)–C(13)	117.76(10)
O(5)–C(13)–C(14)	110.51(12)
O(5)–C(13)–H(13A)	108.8(13)
C(14)–C(13)–H(13A)	110.2(13)
O(5)–C(13)–H(13B)	106.5(12)
C(14)–C(13)–H(13B)	107.6(12)
H(13A)–C(13)–H(13B)	113.1(18)

C(13)–C(14)–H(14A)	109.6(14)
C(13)–C(14)–H(14B)	112.2(14)
H(14A)–C(14)–H(14B)	108.8(19)
C(13)–C(14)–H(14C)	116.4(17)
H(14A)–C(14)–H(14C)	105(2)
H(14B)–C(14)–H(14C)	105(2)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **72**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	12(1)	19(1)	16(1)	1(1)	–2(1)	1(1)
C(2)	14(1)	17(1)	16(1)	2(1)	0(1)	1(1)
C(3)	17(1)	16(1)	13(1)	1(1)	–2(1)	0(1)
N(1)	22(1)	22(1)	15(1)	0(1)	–2(1)	2(1)
O(1)	23(1)	41(1)	20(1)	2(1)	–8(1)	–3(1)
O(2)	24(1)	53(1)	17(1)	3(1)	2(1)	11(1)
C(4)	13(1)	22(1)	18(1)	2(1)	–3(1)	2(1)
C(5)	13(1)	24(1)	17(1)	1(1)	–1(1)	3(1)
C(6)	13(1)	17(1)	14(1)	1(1)	–1(1)	2(1)
N(2)	13(1)	29(1)	13(1)	0(1)	–1(1)	6(1)
N(3)	14(1)	17(1)	14(1)	0(1)	–3(1)	2(1)
C(7)	21(1)	17(1)	18(1)	–4(1)	0(1)	0(1)
O(3)	21(1)	26(1)	25(1)	–6(1)	–7(1)	–2(1)
C(8)	39(1)	17(1)	34(1)	2(1)	–3(1)	–2(1)
C(9)	17(1)	17(1)	14(1)	1(1)	–1(1)	1(1)
C(10)	25(1)	29(1)	14(1)	3(1)	2(1)	4(1)
C(11)	25(1)	21(1)	26(1)	–2(1)	–1(1)	–6(1)
C(12)	20(1)	18(1)	17(1)	–1(1)	–1(1)	4(1)
O(4)	30(1)	32(1)	23(1)	6(1)	–1(1)	13(1)
O(5)	18(1)	22(1)	18(1)	–1(1)	2(1)	5(1)
C(13)	19(1)	22(1)	25(1)	–3(1)	3(1)	5(1)
C(14)	27(1)	39(1)	39(1)	–11(1)	6(1)	–7(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **72**.

Atom	x	y	z	U(eq)
H(1)	4160(17)	3380(16)	7723(15)	18(4)
H(2)	4300(20)	3491(18)	9602(18)	31(5)
H(4)	7965(19)	2775(18)	9449(16)	28(5)
H(5)	7884(19)	2642(19)	7577(17)	27(5)
H(1N2)	6619(17)	2782(17)	5942(16)	21(4)
H(8A)	4930(20)	960(20)	6150(20)	44(6)
H(8B)	3790(20)	1310(20)	6860(20)	47(7)
H(8C)	3610(30)	640(20)	5720(20)	53(7)
H(10A)	5980(20)	3893(19)	3973(17)	28(5)
H(10B)	4600(20)	3675(18)	3641(17)	25(5)
H(10C)	5090(20)	4941(19)	3680(18)	33(6)
H(11A)	5456(18)	5153(16)	6529(16)	19(4)
H(11B)	5410(20)	5840(20)	5486(19)	37(6)
H(11C)	6500(20)	4900(20)	5692(18)	33(5)
H(13A)	1600(20)	5586(19)	6257(17)	30(5)
H(13B)	1826(19)	5078(17)	7480(17)	27(5)
H(14A)	900(20)	3780(20)	5730(20)	35(6)
H(14B)	0(20)	4260(20)	6655(19)	35(6)
H(14C)	970(30)	3330(30)	6880(20)	54(7)

Table 6. Torsion angles [°] for **72**.

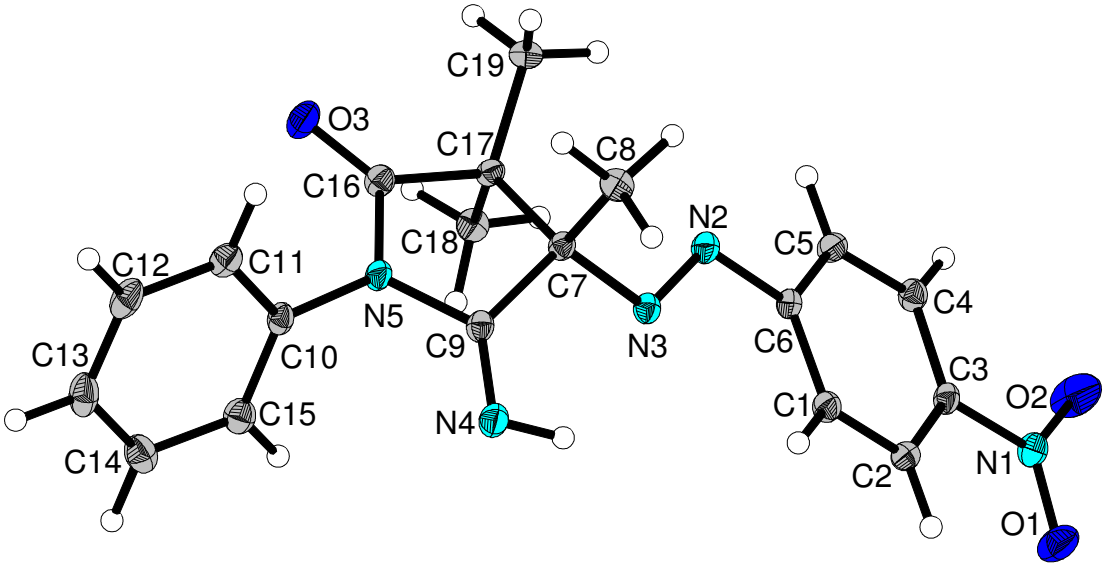
C(6)–C(1)–C(2)–C(3)	0.41(19)
C(1)–C(2)–C(3)–C(4)	2.48(19)
C(1)–C(2)–C(3)–N(1)	–176.10(11)
C(2)–C(3)–N(1)–O(1)	–170.44(12)
C(4)–C(3)–N(1)–O(1)	10.93(19)
C(2)–C(3)–N(1)–O(2)	10.50(18)
C(4)–C(3)–N(1)–O(2)	–168.12(12)
C(2)–C(3)–C(4)–C(5)	–2.7(2)
N(1)–C(3)–C(4)–C(5)	175.88(12)
C(3)–C(4)–C(5)–C(6)	0.0(2)

C(2)–C(1)–C(6)–N(2)	174.71(12)
C(2)–C(1)–C(6)–C(5)	–3.01(19)
C(4)–C(5)–C(6)–N(2)	–175.03(12)
C(4)–C(5)–C(6)–C(1)	2.78(19)
C(1)–C(6)–N(2)–N(3)	9.78(19)
C(5)–C(6)–N(2)–N(3)	–172.46(11)
C(6)–N(2)–N(3)–C(7)	–91.96(15)
C(6)–N(2)–N(3)–C(9)	95.72(14)
N(2)–N(3)–C(7)–O(3)	–173.61(11)
C(9)–N(3)–C(7)–O(3)	–1.28(18)
N(2)–N(3)–C(7)–C(8)	9.64(18)
C(9)–N(3)–C(7)–C(8)	–178.03(12)
C(7)–N(3)–C(9)–C(10)	–74.17(14)
N(2)–N(3)–C(9)–C(10)	98.18(13)
C(7)–N(3)–C(9)–C(11)	164.40(12)
N(2)–N(3)–C(9)–C(11)	–23.25(15)
C(7)–N(3)–C(9)–C(12)	48.27(14)
N(2)–N(3)–C(9)–C(12)	–139.39(10)
N(3)–C(9)–C(12)–O(4)	–141.30(14)
C(10)–C(9)–C(12)–O(4)	–19.48(19)
C(11)–C(9)–C(12)–O(4)	100.56(15)
N(3)–C(9)–C(12)–O(5)	45.46(13)
C(10)–C(9)–C(12)–O(5)	167.28(11)
C(11)–C(9)–C(12)–O(5)	–72.69(13)
O(4)–C(12)–O(5)–C(13)	5.8(2)
C(9)–C(12)–O(5)–C(13)	178.94(10)
C(12)–O(5)–C(13)–C(14)	88.59(16)

Appendix II

(Crystal structure and supporting data for 2-iminopyrrolidin-5-one derivative **198f**)

Crystal structure and supporting data for 2-iminopyrrolidin-5-one derivative **198f**



jam01, molecule; thermal ellipsoids are drawn on the 50% probability level

Table 1. Crystal data and structure refinement for **198f**.

Identification code	**198f**
Empirical formula	C_{19} H_{19} N_5 O_3
Formula weight	365.39
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P1 (#1)
Unit cell dimensions	a = 6.1179(9) Å α= 72.868(3)°.
	b = 7.7340(12) Å β= 79.475(3)°.
	c = 9.9532(15) Å γ = 81.828(3)°.
Volume	440.52(12) $Å^3$
Z	1
Density (calculated)	1.377 Mg/m^3
Absorption coefficient	0.097 mm^{-1}
F(000)	192
Crystal size	1.00 x 0.40 x 0.30 mm^3
Theta range for data collection	2.17 to 30.50°.
Index ranges	-8<=h<=8, -11<=k<=11, -14<=l<=14
Reflections collected	10436

Independent reflections	2668 [R(int) = 0.0378]
Completeness to theta = 30.50°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9716 and 0.8046
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2668 / 3 / 251
Goodness-of-fit on F^2	1.054
Final R indices [I>2sigma(I)]	R1 = 0.0375, wR2 = 0.0955
R indices (all data)	R1 = 0.0394, wR2 = 0.0972
Largest diff. peak and hole	0.374 and -0.229 e.$Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **198f**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	x	y	z	U(eq)
C(1)	4850(3)	4705(2)	5165(2)	15(1)
C(2)	6312(3)	5961(2)	4357(2)	15(1)
C(3)	6023(3)	6809(2)	2948(2)	13(1)
N(1)	7535(2)	8183(2)	2104(2)	16(1)
O(1)	9093(2)	8411(2)	2625(2)	26(1)
O(2)	7152(3)	9049(2)	915(2)	38(1)
C(4)	4354(3)	6465(2)	2317(2)	15(1)
C(5)	2919(3)	5179(2)	3138(2)	15(1)
C(6)	3190(3)	4298(2)	4546(2)	14(1)
N(2)	1641(2)	2977(2)	5288(2)	15(1)
N(3)	2280(2)	1855(2)	6366(2)	13(1)
C(7)	625(3)	525(2)	7068(2)	12(1)
C(8)	-1350(3)	1494(2)	7845(2)	16(1)
C(9)	1713(3)	-1023(2)	8168(2)	14(1)
N(4)	3102(3)	-1005(2)	8964(2)	18(1)
N(5)	807(2)	-2610(2)	8214(2)	15(1)
C(10)	1227(3)	-4349(2)	9204(2)	14(1)
C(11)	-558(3)	-5196(2)	10109(2)	18(1)
C(12)	-170(3)	-6941(2)	10999(2)	21(1)
C(13)	1972(3)	-7811(2)	10984(2)	22(1)
C(14)	3747(3)	-6928(2)	10093(2)	20(1)

C(15)	3392(3)	-5190(2)	9190(2)	18(1)
C(16)	-404(3)	-2367(2)	7112(2)	15(1)
O(3)	-1428(2)	-3512(2)	6965(1)	22(1)
C(17)	-73(3)	-479(2)	6089(2)	13(1)
C(18)	1902(3)	-799(2)	4953(2)	16(1)
C(19)	-2155(3)	348(2)	5404(2)	17(1)

Table 3. Bond lengths [Å] and angles [°] for **198f**.

C(1)-C(2)	1.384(2)
C(1)-C(6)	1.395(2)
C(1)-H(1)	0.9500
C(2)-C(3)	1.394(2)
C(2)-H(2)	0.9500
C(3)-C(4)	1.386(2)
C(3)-N(1)	1.470(2)
N(1)-O(1)	1.220(2)
N(1)-O(2)	1.223(2)
C(4)-C(5)	1.391(2)
C(4)-H(4)	0.9500
C(5)-C(6)	1.394(2)
C(5)-H(5)	0.9500
C(6)-N(2)	1.436(2)
N(2)-N(3)	1.251(2)
N(3)-C(7)	1.478(2)
C(7)-C(9)	1.531(2)
C(7)-C(8)	1.537(2)
C(7)-C(17)	1.557(2)
C(8)-H(8A)	0.9800
C(8)-H(8B)	0.9800
C(8)-H(8C)	0.9800
C(9)-N(4)	1.267(2)
C(9)-N(5)	1.402(2)
N(4)-H(1N4)	0.88(3)
N(5)-C(16)	1.386(2)
N(5)-C(10)	1.435(2)
C(10)-C(11)	1.389(2)

C(10)-C(15)	1.390(2)
C(11)-C(12)	1.394(2)
C(11)-H(11)	0.9500
C(12)-C(13)	1.385(3)
C(12)-H(12)	0.9500
C(13)-C(14)	1.391(3)
C(13)-H(13)	0.9500
C(14)-C(15)	1.392(2)
C(14)-H(14)	0.9500
C(15)-H(15)	0.9500
C(16)-O(3)	1.211(2)
C(16)-C(17)	1.530(2)
C(17)-C(19)	1.525(2)
C(17)-C(18)	1.543(2)
C(18)-H(18A)	0.9800
C(18)-H(18B)	0.9800
C(18)-H(18C)	0.9800
C(19)-H(19A)	0.9800
C(19)-H(19B)	0.9800
C(19)-H(19C)	0.9800

C(2)-C(1)-C(6)	119.54(15)
C(2)-C(1)-H(1)	120.2
C(6)-C(1)-H(1)	120.2
C(1)-C(2)-C(3)	118.02(15)
C(1)-C(2)-H(2)	121.0
C(3)-C(2)-H(2)	121.0
C(4)-C(3)-C(2)	123.62(15)
C(4)-C(3)-N(1)	118.43(14)
C(2)-C(3)-N(1)	117.92(14)
O(1)-N(1)-O(2)	123.40(16)
O(1)-N(1)-C(3)	118.68(14)
O(2)-N(1)-C(3)	117.91(15)
C(3)-C(4)-C(5)	117.60(14)
C(3)-C(4)-H(4)	121.2
C(5)-C(4)-H(4)	121.2
C(4)-C(5)-C(6)	119.80(15)

C(4)-C(5)-H(5)	120.1
C(6)-C(5)-H(5)	120.1
C(5)-C(6)-C(1)	121.36(15)
C(5)-C(6)-N(2)	115.15(14)
C(1)-C(6)-N(2)	123.48(14)
N(3)-N(2)-C(6)	113.78(14)
N(2)-N(3)-C(7)	110.96(13)
N(3)-C(7)-C(9)	108.05(13)
N(3)-C(7)-C(8)	107.52(13)
C(9)-C(7)-C(8)	108.76(13)
N(3)-C(7)-C(17)	115.37(13)
C(9)-C(7)-C(17)	102.90(13)
C(8)-C(7)-C(17)	113.88(13)
C(7)-C(8)-H(8A)	109.5
C(7)-C(8)-H(8B)	109.5
H(8A)-C(8)-H(8B)	109.5
C(7)-C(8)-H(8C)	109.5
H(8A)-C(8)-H(8C)	109.5
H(8B)-C(8)-H(8C)	109.5
N(4)-C(9)-N(5)	122.70(15)
N(4)-C(9)-C(7)	130.71(15)
N(5)-C(9)-C(7)	106.55(13)
C(9)-N(4)-H(1N4)	108.6(18)
C(16)-N(5)-C(9)	112.61(13)
C(16)-N(5)-C(10)	122.55(13)
C(9)-N(5)-C(10)	124.62(14)
C(11)-C(10)-C(15)	121.36(15)
C(11)-C(10)-N(5)	119.17(14)
C(15)-C(10)-N(5)	119.40(14)
C(10)-C(11)-C(12)	119.14(16)
C(10)-C(11)-H(11)	120.4
C(12)-C(11)-H(11)	120.4
C(13)-C(12)-C(11)	120.28(16)
C(13)-C(12)-H(12)	119.9
C(11)-C(12)-H(12)	119.9
C(12)-C(13)-C(14)	119.82(16)
C(12)-C(13)-H(13)	120.1

C(14)-C(13)-H(13)	120.1
C(13)-C(14)-C(15)	120.75(17)
C(13)-C(14)-H(14)	119.6
C(15)-C(14)-H(14)	119.6
C(10)-C(15)-C(14)	118.63(16)
C(10)-C(15)-H(15)	120.7
C(14)-C(15)-H(15)	120.7
O(3)-C(16)-N(5)	124.76(15)
O(3)-C(16)-C(17)	126.92(15)
N(5)-C(16)-C(17)	108.19(13)
C(19)-C(17)-C(16)	111.40(13)
C(19)-C(17)-C(18)	110.44(13)
C(16)-C(17)-C(18)	104.73(13)
C(19)-C(17)-C(7)	116.64(13)
C(16)-C(17)-C(7)	101.68(13)
C(18)-C(17)-C(7)	111.02(13)
C(17)-C(18)-H(18A)	109.5
C(17)-C(18)-H(18B)	109.5
H(18A)-C(18)-H(18B)	109.5
C(17)-C(18)-H(18C)	109.5
H(18A)-C(18)-H(18C)	109.5
H(18B)-C(18)-H(18C)	109.5
C(17)-C(19)-H(19A)	109.5
C(17)-C(19)-H(19B)	109.5
H(19A)-C(19)-H(19B)	109.5
C(17)-C(19)-H(19C)	109.5
H(19A)-C(19)-H(19C)	109.5
H(19B)-C(19)-H(19C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for jam01. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	17(1)	13(1)	13(1)	-1(1)	-3(1)	-3(1)
C(2)	15(1)	14(1)	16(1)	-3(1)	-4(1)	-2(1)
C(3)	14(1)	11(1)	14(1)	-2(1)	0(1)	-3(1)
N(1)	17(1)	15(1)	15(1)	-2(1)	0(1)	-5(1)
O(1)	24(1)	28(1)	24(1)	-1(1)	-5(1)	-13(1)
O(2)	42(1)	42(1)	24(1)	14(1)	-13(1)	-25(1)
C(4)	19(1)	14(1)	13(1)	-2(1)	-3(1)	-3(1)
C(5)	16(1)	15(1)	15(1)	-3(1)	-4(1)	-4(1)
C(6)	15(1)	10(1)	15(1)	-2(1)	-3(1)	-3(1)
N(2)	16(1)	13(1)	15(1)	-1(1)	-2(1)	-4(1)
N(3)	14(1)	12(1)	14(1)	-3(1)	0(1)	-2(1)
C(7)	13(1)	12(1)	11(1)	-2(1)	-1(1)	-3(1)
C(8)	15(1)	18(1)	15(1)	-6(1)	0(1)	0(1)
C(9)	14(1)	12(1)	13(1)	-2(1)	0(1)	-3(1)
N(4)	22(1)	14(1)	19(1)	-2(1)	-6(1)	-4(1)
N(5)	17(1)	11(1)	15(1)	0(1)	-5(1)	-5(1)
C(10)	19(1)	11(1)	12(1)	-1(1)	-3(1)	-4(1)
C(11)	19(1)	18(1)	16(1)	-4(1)	-2(1)	-6(1)
C(12)	29(1)	19(1)	16(1)	0(1)	-2(1)	-13(1)
C(13)	36(1)	14(1)	15(1)	-1(1)	-6(1)	-5(1)
C(14)	26(1)	17(1)	17(1)	-5(1)	-6(1)	1(1)
C(15)	20(1)	17(1)	16(1)	-3(1)	-2(1)	-3(1)
C(16)	17(1)	15(1)	13(1)	-3(1)	-2(1)	-3(1)
O(3)	28(1)	19(1)	21(1)	-2(1)	-8(1)	-10(1)
C(17)	13(1)	14(1)	11(1)	-3(1)	-3(1)	-3(1)
C(18)	17(1)	17(1)	16(1)	-6(1)	-1(1)	-1(1)
C(19)	14(1)	18(1)	18(1)	-5(1)	-6(1)	-1(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **198f**.

Atom	x	y	z	U(eq)
H(1)	4976	4125	6136	18
H(2)	7479	6237	4750	18
H(4)	4194	7085	1357	18
H(5)	1757	4901	2742	18
H(8A)	-820	1934	8545	25
H(8B)	-2473	642	8329	25
H(8C)	-2015	2526	7155	25
H(1N4)	3460(50)	120(40)	8770(30)	28(7)
H(11)	-2025	-4594	10121	21
H(12)	-1379	-7536	11619	26
H(13)	2228	-9008	11580	26
H(14)	5219	-7517	10100	24
H(15)	4604	-4592	8577	21
H(18A)	1570	-1711	4532	25
H(18B)	3256	-1231	5396	25
H(18C)	2133	343	4209	25
H(19A)	-2471	-423	4852	25
H(19B)	-1905	1565	4774	25
H(19C)	-3427	435	6146	25

Table 6. Torsion angles [°] for **198f**.

C(6)-C(1)-C(2)-C(3)	1.6(2)
C(1)-C(2)-C(3)-C(4)	0.1(2)
C(1)-C(2)-C(3)-N(1)	178.16(14)
C(4)-C(3)-N(1)-O(1)	-175.49(16)
C(2)-C(3)-N(1)-O(1)	6.3(2)
C(4)-C(3)-N(1)-O(2)	5.0(2)
C(2)-C(3)-N(1)-O(2)	-173.17(18)
C(2)-C(3)-C(4)-C(5)	-1.0(2)
N(1)-C(3)-C(4)-C(5)	-179.10(14)

C(3)-C(4)-C(5)-C(6)	0.3(2)
C(4)-C(5)-C(6)-C(1)	1.4(2)
C(4)-C(5)-C(6)-N(2)	-179.06(14)
C(2)-C(1)-C(6)-C(5)	-2.3(2)
C(2)-C(1)-C(6)-N(2)	178.14(14)
C(5)-C(6)-N(2)-N(3)	160.32(14)
C(1)-C(6)-N(2)-N(3)	-20.1(2)
C(6)-N(2)-N(3)-C(7)	-179.31(12)
N(2)-N(3)-C(7)-C(9)	168.07(13)
N(2)-N(3)-C(7)-C(8)	-74.70(16)
N(2)-N(3)-C(7)-C(17)	53.59(17)
N(3)-C(7)-C(9)-N(4)	35.4(2)
C(8)-C(7)-C(9)-N(4)	-81.1(2)
C(17)-C(7)-C(9)-N(4)	157.83(17)
N(3)-C(7)-C(9)-N(5)	-147.02(13)
C(8)-C(7)-C(9)-N(5)	96.56(15)
C(17)-C(7)-C(9)-N(5)	-24.54(16)
N(4)-C(9)-N(5)-C(16)	-170.87(16)
C(7)-C(9)-N(5)-C(16)	11.26(18)
N(4)-C(9)-N(5)-C(10)	3.8(2)
C(7)-C(9)-N(5)-C(10)	-174.05(14)
C(16)-N(5)-C(10)-C(11)	-64.9(2)
C(9)-N(5)-C(10)-C(11)	120.88(18)
C(16)-N(5)-C(10)-C(15)	111.97(18)
C(9)-N(5)-C(10)-C(15)	-62.2(2)
C(15)-C(10)-C(11)-C(12)	-1.4(3)
N(5)-C(10)-C(11)-C(12)	175.49(16)
C(10)-C(11)-C(12)-C(13)	0.3(3)
C(11)-C(12)-C(13)-C(14)	1.0(3)
C(12)-C(13)-C(14)-C(15)	-1.4(3)
C(11)-C(10)-C(15)-C(14)	1.0(3)
N(5)-C(10)-C(15)-C(14)	-175.88(16)
C(13)-C(14)-C(15)-C(10)	0.5(3)
C(9)-N(5)-C(16)-O(3)	-176.36(16)
C(10)-N(5)-C(16)-O(3)	8.8(3)
C(9)-N(5)-C(16)-C(17)	7.50(18)
C(10)-N(5)-C(16)-C(17)	-167.31(14)

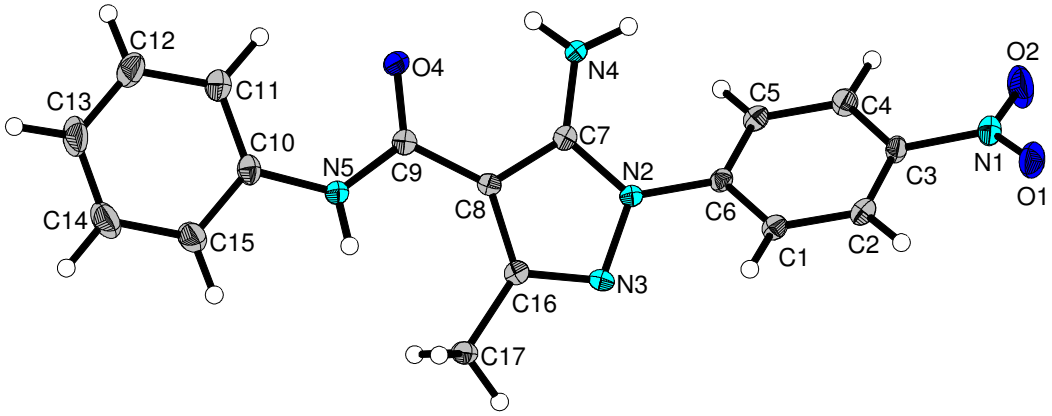
O(3)-C(16)-C(17)-C(19)	36.8(2)
N(5)-C(16)-C(17)-C(19)	-147.21(14)
O(3)-C(16)-C(17)-C(18)	-82.6(2)
N(5)-C(16)-C(17)-C(18)	93.39(15)
O(3)-C(16)-C(17)-C(7)	161.70(17)
N(5)-C(16)-C(17)-C(7)	-22.27(16)
N(3)-C(7)-C(17)-C(19)	-93.72(17)
C(9)-C(7)-C(17)-C(19)	148.86(13)
C(8)-C(7)-C(17)-C(19)	31.3(2)
N(3)-C(7)-C(17)-C(16)	144.91(13)
C(9)-C(7)-C(17)-C(16)	27.49(15)
C(8)-C(7)-C(17)-C(16)	-90.04(16)
N(3)-C(7)-C(17)-C(18)	33.96(18)
C(9)-C(7)-C(17)-C(18)	-83.46(15)
C(8)-C(7)-C(17)-C(18)	159.01(13)

Symmetry transformations used to generate equivalent atoms:

Appendix III

(Crystal structure and supporting data for pyrazole derivative **207b**)

Crystal structure and supporting data for pyrazole derivative **207b**



jam02, molecule; thermal ellipsoids are drawn on the 50% probability level

Table 1. Crystal data and structure refinement for **207b**.

Identification code	**207b**	
Empirical formula	$C_{18.625}\ H_{17.75}\ N_{5.75}\ O_{3.125}$	
Molecular formula	$C_{17}\ H_{15}\ N_5\ O_3$ x 0.75 ($C_2\ H_3\ N$) x 0.125 ($C\ H_4\ O$)	
Formula weight	372.29	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/n$ (#14)	
Unit cell dimensions	a = 13.394(2) Å	α= 90°.
	b = 3.8267(6) Å	β= 92.688(3)°.
	c = 33.470(5) Å	γ = 90°.
Volume	1713.6(5) $Å^3$	
Z	4	
Density (calculated)	1.443 Mg/m^3	
Absorption coefficient	0.103 mm^{-1}	
F(000)	779	
Crystal size	1.00 x 0.10 x 0.05 mm^3	
Theta range for data collection	1.67 to 28.35°.	
Index ranges	–17<=h<=17, –5<=k<=5, –44<=l<=44	
Reflections collected	16121	
Independent reflections	4230 [R(int) = 0.0280]	
Completeness to theta = 28.35°	99.1 %	

Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9948 and 0.8070
Refinement method	Full–matrix least–squares on F^2
Data / restraints / parameters	4230 / 0 / 323
Goodness–of–fit on F^2	1.124
Final R indices [I>2sigma(I)]	R1 = 0.0653, wR2 = 0.1581
R indices (all data)	R1 = 0.0716, wR2 = 0.1617
Largest diff. peak and hole	0.712 and –0.389 e.$Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **207b**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	x	y	z	U(eq)
C(1)	6474(1)	6031(5)	2778(1)	15(1)
C(2)	6972(1)	4984(5)	3130(1)	17(1)
C(3)	6467(1)	5161(5)	3482(1)	16(1)
N(1)	6980(1)	3973(5)	3855(1)	22(1)
O(1)	7763(1)	2324(5)	3832(1)	28(1)
O(2)	6601(1)	4682(6)	4172(1)	37(1)
C(4)	5493(1)	6357(6)	3496(1)	18(1)
C(5)	4998(1)	7407(5)	3142(1)	16(1)
C(6)	5488(1)	7209(5)	2785(1)	14(1)
N(2)	4991(1)	8199(4)	2421(1)	13(1)
N(3)	5529(1)	9842(4)	2128(1)	15(1)
C(7)	4050(1)	7338(5)	2282(1)	13(1)
N(4)	3377(1)	5671(4)	2501(1)	14(1)
C(8)	3951(1)	8532(5)	1888(1)	13(1)
C(9)	3036(1)	7973(5)	1639(1)	14(1)
O(4)	2272(1)	6763(4)	1784(1)	18(1)
N(5)	3080(1)	8782(5)	1244(1)	17(1)
C(10)	2333(2)	8435(5)	933(1)	18(1)
C(11)	1413(2)	6846(6)	977(1)	23(1)
C(12)	745(2)	6558(7)	647(1)	29(1)
C(13)	977(2)	7824(7)	281(1)	34(1)
C(14)	1878(2)	9490(7)	240(1)	36(1)
C(15)	2558(2)	9791(7)	563(1)	27(1)

C(16)	4900(1)	10054(5)	1814(1)	14(1)
C(17)	5246(2)	11775(5)	1445(1)	17(1)
N(6) [a)]	4802(2)	6425(10)	590(1)	41(1)
C(19) [a)]	5518(3)	5013(13)	480(1)	41(1)
C(20) [a)]	6369(3)	3298(14)	354(1)	48(1)
O(5) [b)]	5110(20)	4770(80)	351(8)	76(7)
C(21) [b)]	5403(19)	5370(80)	–18(8)	50(6)

[a)] s.o.f. fixed to 0.75 [b)] s.o.f. fixed to 0.125; only isotropic refinement possible (s.o.f.: **s**ite **o**ccupation **f**actor)

Table 3. Bond lengths [Å] and angles [°] for **207b**.

C(1)–C(2)	1.385(3)
C(1)–C(6)	1.397(3)
C(1)–H(1)	0.94(3)
C(2)–C(3)	1.387(3)
C(2)–H(2)	0.96(2)
C(3)–C(4)	1.386(3)
C(3)–N(1)	1.468(2)
N(1)–O(2)	1.227(2)
N(1)–O(1)	1.229(2)
C(4)–C(5)	1.390(3)
C(4)–H(4)	0.97(2)
C(5)–C(6)	1.394(3)
C(5)–H(5)	0.95(3)
C(6)–N(2)	1.411(2)
N(2)–C(7)	1.363(2)
N(2)–N(3)	1.393(2)
N(3)–C(16)	1.320(2)
C(7)–N(4)	1.350(2)
C(7)–C(8)	1.395(2)
N(4)–H(1N4)	0.84(3)
N(4)–H(2N4)	0.90(3)
C(8)–C(16)	1.430(2)
C(8)–C(9)	1.465(2)
C(9)–O(4)	1.243(2)
C(9)–N(5)	1.361(2)

N(5)–C(10)	1.417(2)
N(5)–H(1N5)	0.86(3)
C(10)–C(15)	1.389(3)
C(10)–C(11)	1.389(3)
C(11)–C(12)	1.393(3)
C(11)–H(11)	1.00(3)
C(12)–C(13)	1.368(4)
C(12)–H(12)	0.94(3)
C(13)–C(14)	1.377(4)
C(13)–H(13)	0.98(3)
C(14)–C(15)	1.384(3)
C(14)–H(14)	0.99(3)
C(15)–H(15)	0.95(3)
C(16)–C(17)	1.491(3)
C(17)–H(17A)	0.99(3)
C(17)–H(17B)	0.97(3)
C(17)–H(17C)	0.94(3)
N(6)–C(19)	1.175(5)
C(19)–C(20)	1.397(6)
C(20)–H(20A)	0.9800
C(20)–H(20B)	0.9800
C(20)–H(20C)	0.9800
O(5)–C(21)	1.33(4)
O(5)–H(1O5)	0.8400
C(21)–H(21A)	0.9800
C(21)–H(21B)	0.9800
C(21)–H(21C)	0.9800

C(2)–C(1)–C(6)	119.88(17)
C(2)–C(1)–H(1)	120.8(16)
C(6)–C(1)–H(1)	119.3(16)
C(1)–C(2)–C(3)	118.28(17)
C(1)–C(2)–H(2)	121.8(14)
C(3)–C(2)–H(2)	119.9(14)
C(4)–C(3)–C(2)	122.85(18)
C(4)–C(3)–N(1)	118.47(17)
C(2)–C(3)–N(1)	118.67(17)

O(2)–N(1)–O(1)	123.85(18)
O(2)–N(1)–C(3)	118.04(17)
O(1)–N(1)–C(3)	118.12(17)
C(3)–C(4)–C(5)	118.62(18)
C(3)–C(4)–H(4)	118.7(14)
C(5)–C(4)–H(4)	122.7(15)
C(4)–C(5)–C(6)	119.41(17)
C(4)–C(5)–H(5)	119.1(15)
C(6)–C(5)–H(5)	121.4(15)
C(5)–C(6)–C(1)	120.95(17)
C(5)–C(6)–N(2)	120.25(16)
C(1)–C(6)–N(2)	118.79(16)
C(7)–N(2)–N(3)	111.61(15)
C(7)–N(2)–C(6)	128.42(16)
N(3)–N(2)–C(6)	119.27(15)
C(16)–N(3)–N(2)	104.93(15)
N(4)–C(7)–N(2)	123.93(17)
N(4)–C(7)–C(8)	129.24(17)
N(2)–C(7)–C(8)	106.82(16)
C(7)–N(4)–H(1N4)	115.2(17)
C(7)–N(4)–H(2N4)	121.8(18)
H(1N4)–N(4)–H(2N4)	116(3)
C(7)–C(8)–C(16)	104.61(16)
C(7)–C(8)–C(9)	121.93(16)
C(16)–C(8)–C(9)	133.39(17)
O(4)–C(9)–N(5)	122.46(17)
O(4)–C(9)–C(8)	121.05(17)
N(5)–C(9)–C(8)	116.48(16)
C(9)–N(5)–C(10)	129.25(17)
C(9)–N(5)–H(1N5)	115.6(17)
C(10)–N(5)–H(1N5)	115.1(17)
C(15)–C(10)–C(11)	119.39(19)
C(15)–C(10)–N(5)	116.51(18)
C(11)–C(10)–N(5)	124.10(18)
C(10)–C(11)–C(12)	119.3(2)
C(10)–C(11)–H(11)	119.1(15)
C(12)–C(11)–H(11)	121.6(15)

C(13)–C(12)–C(11)	121.2(2)
C(13)–C(12)–H(12)	117.8(18)
C(11)–C(12)–H(12)	121.0(18)
C(12)–C(13)–C(14)	119.3(2)
C(12)–C(13)–H(13)	122.4(19)
C(14)–C(13)–H(13)	118.3(18)
C(13)–C(14)–C(15)	120.6(2)
C(13)–C(14)–H(14)	119.9(18)
C(15)–C(14)–H(14)	119.4(18)
C(14)–C(15)–C(10)	120.1(2)
C(14)–C(15)–H(15)	121.5(17)
C(10)–C(15)–H(15)	118.4(17)
N(3)–C(16)–C(8)	111.99(16)
N(3)–C(16)–C(17)	118.43(16)
C(8)–C(16)–C(17)	129.58(17)
C(16)–C(17)–H(17A)	112.1(15)
C(16)–C(17)–H(17B)	111.5(15)
H(17A)–C(17)–H(17B)	104(2)
C(16)–C(17)–H(17C)	109.2(17)
H(17A)–C(17)–H(17C)	110(2)
H(17B)–C(17)–H(17C)	110(2)
N(6)–C(19)–C(20)	179.1(5)
C(19)–C(20)–H(20A)	109.5
C(19)–C(20)–H(20B)	109.5
H(20A)–C(20)–H(20B)	109.5
C(19)–C(20)–H(20C)	109.5
H(20A)–C(20)–H(20C)	109.5
H(20B)–C(20)–H(20C)	109.5
C(21)–O(5)–H(1O5)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **207b**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	14(1)	17(1)	15(1)	–3(1)	1(1)	–1(1)
C(2)	13(1)	18(1)	20(1)	–2(1)	–1(1)	1(1)
C(3)	16(1)	18(1)	14(1)	1(1)	–4(1)	–1(1)
N(1)	18(1)	29(1)	17(1)	2(1)	–3(1)	–1(1)
O(1)	21(1)	34(1)	27(1)	5(1)	–6(1)	7(1)
O(2)	31(1)	63(1)	15(1)	4(1)	–2(1)	9(1)
C(4)	17(1)	21(1)	15(1)	–2(1)	2(1)	1(1)
C(5)	13(1)	17(1)	18(1)	–2(1)	1(1)	0(1)
C(6)	14(1)	12(1)	15(1)	–1(1)	–2(1)	–2(1)
N(2)	12(1)	16(1)	13(1)	1(1)	1(1)	–1(1)
N(3)	15(1)	16(1)	14(1)	2(1)	3(1)	–2(1)
C(7)	13(1)	10(1)	14(1)	–3(1)	0(1)	2(1)
N(4)	11(1)	17(1)	14(1)	2(1)	0(1)	–2(1)
C(8)	14(1)	12(1)	13(1)	–1(1)	2(1)	0(1)
C(9)	15(1)	12(1)	14(1)	–2(1)	1(1)	1(1)
O(4)	15(1)	24(1)	16(1)	0(1)	1(1)	–4(1)
N(5)	14(1)	23(1)	14(1)	2(1)	0(1)	–3(1)
C(10)	21(1)	17(1)	14(1)	–2(1)	–3(1)	2(1)
C(11)	22(1)	26(1)	18(1)	0(1)	–3(1)	–2(1)
C(12)	27(1)	30(1)	28(1)	–1(1)	–10(1)	–7(1)
C(13)	45(1)	31(1)	25(1)	3(1)	–19(1)	–10(1)
C(14)	51(2)	39(1)	18(1)	7(1)	–8(1)	–13(1)
C(15)	33(1)	30(1)	18(1)	4(1)	–3(1)	–10(1)
C(16)	14(1)	13(1)	14(1)	–3(1)	2(1)	0(1)
C(17)	17(1)	18(1)	15(1)	1(1)	2(1)	–4(1)
N(6) [a)]	29(1)	61(2)	32(2)	2(2)	3(1)	8(2)
C(19) [a)]	35(2)	63(3)	22(2)	–6(2)	–10(1)	2(2)
C(20) [a)]	39(2)	76(3)	30(2)	–11(2)	2(2)	–1(2)

[a)] s.o.f. fixed to 0.75 (s.o.f.: **s**ite **o**ccupation **f**actor)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **207b**.

Atom	x	y	z	U(eq)
H(1)	6791(19)	5940(70)	2533(8)	21(6)
H(2)	7645(18)	4140(70)	3135(7)	19(6)
H(4)	5186(18)	6430(70)	3753(7)	18(6)
H(5)	4347(19)	8370(70)	3152(7)	20(6)
H(1N4)	2884(19)	4830(70)	2370(7)	20(6)
H(2N4)	3560(20)	4550(80)	2730(9)	30(7)
H(1N5)	3648(19)	9510(70)	1171(7)	20(6)
H(11)	1253(19)	5870(80)	1245(8)	26(7)
H(12)	120(20)	5500(80)	669(8)	36(8)
H(13)	530(20)	7550(90)	43(9)	43(8)
H(14)	2060(20)	10320(90)	–26(10)	44(9)
H(15)	3190(20)	10900(80)	538(8)	33(7)
H(17A)	5320(18)	10090(70)	1225(7)	21(6)
H(17B)	4756(19)	13440(70)	1337(8)	24(6)
H(17C)	5860(20)	12900(80)	1504(8)	31(7)
H(20A) [a)]	6959	4754	413	72
H(20B) [a)]	6298	2876	65	72
H(20C) [a)]	6446	1062	495	72
H(1O5) [b)]	5611	4362	503	114
H(21A) [b)]	5069	7468	–127	74
H(21B) [b)]	5228	3363	–189	74
H(21C) [b)]	6129	5721	–10	74

[a)] s.o.f. fixed to 0.75 [b)] s.o.f. fixed to 0.125 (s.o.f.: **s**ite **o**ccupation **f**actor)

Table 6. Torsion angles [°] for **207b**.

C(6)–C(1)–C(2)–C(3)	–0.3(3)
C(1)–C(2)–C(3)–C(4)	–0.4(3)
C(1)–C(2)–C(3)–N(1)	178.48(18)
C(4)–C(3)–N(1)–O(2)	–13.3(3)
C(2)–C(3)–N(1)–O(2)	167.8(2)

C(4)–C(3)–N(1)–O(1)	166.62(19)
C(2)–C(3)–N(1)–O(1)	–12.3(3)
C(2)–C(3)–C(4)–C(5)	0.4(3)
N(1)–C(3)–C(4)–C(5)	–178.53(18)
C(3)–C(4)–C(5)–C(6)	0.4(3)
C(4)–C(5)–C(6)–C(1)	–1.1(3)
C(4)–C(5)–C(6)–N(2)	178.77(18)
C(2)–C(1)–C(6)–C(5)	1.1(3)
C(2)–C(1)–C(6)–N(2)	–178.82(18)
C(5)–C(6)–N(2)–C(7)	–45.9(3)
C(1)–C(6)–N(2)–C(7)	134.0(2)
C(5)–C(6)–N(2)–N(3)	144.46(18)
C(1)–C(6)–N(2)–N(3)	–35.6(3)
C(7)–N(2)–N(3)–C(16)	1.6(2)
C(6)–N(2)–N(3)–C(16)	172.83(16)
N(3)–N(2)–C(7)–N(4)	178.86(17)
C(6)–N(2)–C(7)–N(4)	8.6(3)
N(3)–N(2)–C(7)–C(8)	–1.7(2)
C(6)–N(2)–C(7)–C(8)	–171.91(17)
N(4)–C(7)–C(8)–C(16)	–179.51(19)
N(2)–C(7)–C(8)–C(16)	1.0(2)
N(4)–C(7)–C(8)–C(9)	–2.2(3)
N(2)–C(7)–C(8)–C(9)	178.38(16)
C(7)–C(8)–C(9)–O(4)	8.3(3)
C(16)–C(8)–C(9)–O(4)	–175.3(2)
C(7)–C(8)–C(9)–N(5)	–170.48(17)
C(16)–C(8)–C(9)–N(5)	6.0(3)
O(4)–C(9)–N(5)–C(10)	–0.6(3)
C(8)–C(9)–N(5)–C(10)	178.15(19)
C(9)–N(5)–C(10)–C(15)	174.0(2)
C(9)–N(5)–C(10)–C(11)	–6.1(3)
C(15)–C(10)–C(11)–C(12)	1.7(3)
N(5)–C(10)–C(11)–C(12)	–178.1(2)
C(10)–C(11)–C(12)–C(13)	–0.3(4)
C(11)–C(12)–C(13)–C(14)	–1.6(4)
C(12)–C(13)–C(14)–C(15)	2.1(4)
C(13)–C(14)–C(15)–C(10)	–0.6(4)

C(11)–C(10)–C(15)–C(14)	–1.3(4)
N(5)–C(10)–C(15)–C(14)	178.6(2)
N(2)–N(3)–C(16)–C(8)	–0.9(2)
N(2)–N(3)–C(16)–C(17)	178.80(16)
C(7)–C(8)–C(16)–N(3)	–0.1(2)
C(9)–C(8)–C(16)–N(3)	–176.98(19)
C(7)–C(8)–C(16)–C(17)	–179.71(19)
C(9)–C(8)–C(16)–C(17)	3.4(4)

Symmetry transformations used to generate equivalent atoms:

Appendix IV

(Heterocycles Paper)

HETEROCYCLES, Vol. 75, No. 11, 2008, pp. -. © The Japan Institute of Heterocyclic Chemistry
Received, 8th May 2008 , Accepted, 27th June 2008 , Published online, 2nd July 2008. COM-06-11433

INTER- AND INTRA- MOLECULAR CYCLISATION REACTIONS OF AZOACETATES DERIVED FROM ARYL HYDRAZONES OF ETHYLACETOACETATE AND ACETOACETANILIDES

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Abstract – The base induced cyclisations of azoacetates to azetidinones were investigated. In addition to the isolation of the desired products, it was found that 2-iminopyrrolidine-5-one derivatives as well as N-acyl hydrazides could be isolated when metal cyanides were employed as base. These entities were further modified to form pyrrolidine-2,5-diones and diazetidinones respectively.

INTRODUCTION

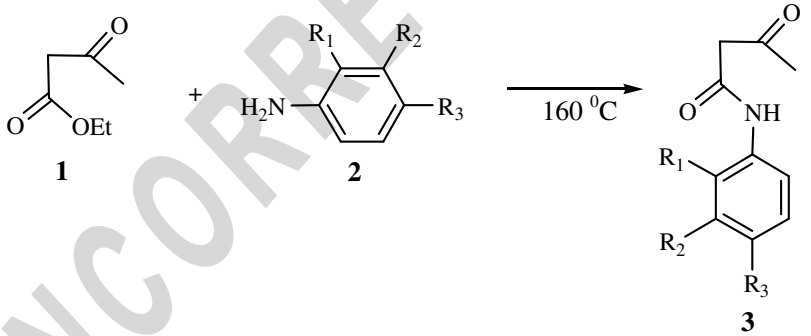
The search for new β-lactam species as antibacterial agents has been in decline in recent times due to problems encountered with the rapid development of resistant strains of bacteria. However due to the low levels of toxicity inherent to these compounds there has been a simultaneous surge in interest in any newly generated species in ancillary roles. Monocyclic β-lactams with non-classical features, bearing simple aryl and alkyl moieties have been of particular interest recently in a variety of roles ranging from anti-cancer agents,[1] and HIV-1 protease inhibitors,[2] to anti-fungal agents.[3] The synthesis of some azetidinones and diazetidinones through the reaction of the appropriate azoacetates with various bases has been previously reported by this group.[4] Herein we report in full the experimental details of these procedures and the related spectral data. In addition to these published intramolecular ring closures it has been discovered that products of intermolecular reactions between the azoacetate and the cyanide ion can be isolated in reasonable yields in some cases. The generation of the 2-iminopyrrolidine-5-one derivatives is presumably due to a nucleophilic displacement of the acetate group by the cyanide ion to form the nitrile entity followed by spontaneous cyclisation. Relatively few examples of synthetic routes to these

molecules can be found in the mainstream literature.[5] However it will be shown here that these compounds provide a valuable route to the synthetically and medicinally versatile pyrrolidine-2,5-dione (succinimide) molecules.[6] The azoacetate derived from α,α-dimethyl ethylacetoacetate was found to form an N-acyl hydrazide rearrangement product that could be isolated and subsequently, after hydrolysis of the ester moiety, cyclised to form a diazetidinone derivative. It was found that azo functionality in the azetidinones reported here could be easily oxidized to give the corresponding azoxy species. The scope of this reaction to furnish further compounds is currently under investigation. Also discussed is the unexpected cyclisation of the α,α-unsubstituted azoacetate derivatives to form pyrazole ring derivatives.

RESULTS AND DISCUSSION

The azoacetates in question were synthesized in several steps from simple inexpensive starting materials using facile procedures. Where possible commercially available acetoacetanilides were used as starting materials, the remainder were generated by simply heating the appropriately substituted aniline **(2a-e)** (1 molar equivalents) with ethyl acetoacetate (**1**) (5 molar equivalents) to 160 °C until the evolution of ethanol had ceased, after removing the bulk of the remaining ethyl acetoacetate by vacuum distillation the desired acetoacetanilides could be easily crystallized by scratching the reaction vessel.[7]

Table 1. Synthesis of acetoacetanilides from ethyl acetoacetate

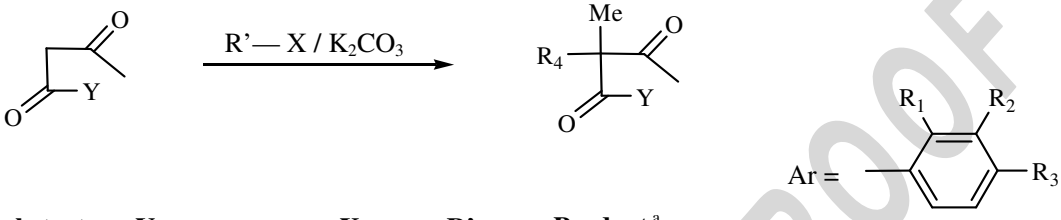


Entry	Substrate	R_1	R_2	R_3	Product	Yield (%)
1	**2a**	H	H	Br	**3a**	90
2	**2b**	H	H	OMe	**3b**	88
3	**2c**	Me	Me	H	**3c**	91
4	**2d**	H	H	Me	**3d**	85
5	**2e**	H	H	CO_2Et	**3e**	77

These were further purified by recrystallization from EtOAc/hexane before being used in the next stage. Dimethylation of the acetoacetanilides (**3a-j**) was achieved by refluxing overnight with an excess of methyl iodide (6 molar equivalents) and potassium carbonate (3 molar equivalents). In the case of the methyl, benzyl substituted example **7** the substrate acetoacetanilide was initially methylated using methyl iodide in a 1:1:1.5 ratio of substrate : base : methyl iodide. This resulted in the production of the mono-methylated species **6** as the major product but also resulted in the production of some of the di-methylated

product **5j**. It was found that the desired mono-methylated compound could be isolated in pure form after several recrystallizations from ethanol. The subsequent benzylation was carried out under similar reaction conditions using an excess of benzyl chloride as alkylating agent.

Table 2. Alkylation of ester and anilides



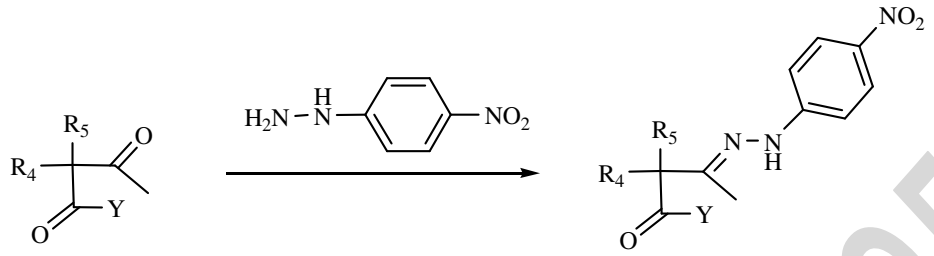
Substrate	Y	X	R'	Product [a]
1	OEt	I	Me	**4**
3	NH-Ar	I	Me	**5**
3	NH-Ar	I	Me	**6**
6	NH-Ar	Cl	Bn	**7**

Entry	Substrate	Y = OEt or NH-Ar			Product [a]	Yield (%)
		R_1	R_2	R_3		
1	**1**	-	-	-	**4**	80
2	**3a**	H	H	Br	**5a**	79
3	**3b**	H	H	OMe	**5b**	78
4	**3c**	Me	Me	H	**5c**	80
5	**3d**	H	H	Me	**5d**	83
6	**3e**	H	H	COOEt	**5e**	70
7[b]	**3f**	H	H	H	**5f**	80
8[b]	**3g**	Me	H	Me	**5g**	67
9[b]	**3h**	Me	H	H	**5h**	75
10[b]	**3i**	OMe	H	H	**5i**	74
11[b]	**3j**	H	H	Cl	**5j**	69
12[b]	**3j**	H	H	Cl	**6**	74
13	**6**	H	H	Cl	**7**	90

[a] In all entries $R_4 = CH_3$, except in entry 12 (Product **6)** where, R_4 = H, and entry 13 (Product **7**) where, R_4 = Bn, [b]Starting materials were commercially available acetoacetanilides.

4-Nitrophenylhydrazone derivatives were prepared of the starting materials ethyl acetoacetate (**1)** and the acetoacetanilide (**3f)** along with α,α-disubstituted ethyl acetoacetate (**4)** and all of the α,α-disubstituted acetoacetanilides (**5a-j** and **7,** Table 3) by stirring the substrates in a slight excess (1.1 molar equivalents) of 4-nitrophenylhydrazine in MeOH under mild acidic conditions (~5% acetic acid).[8] This procedure routinely furnished the desired hydrazones in reasonable yields (50-72%).

Table 3. Synthesis of 4-nitrophenylhydrazone derivatives

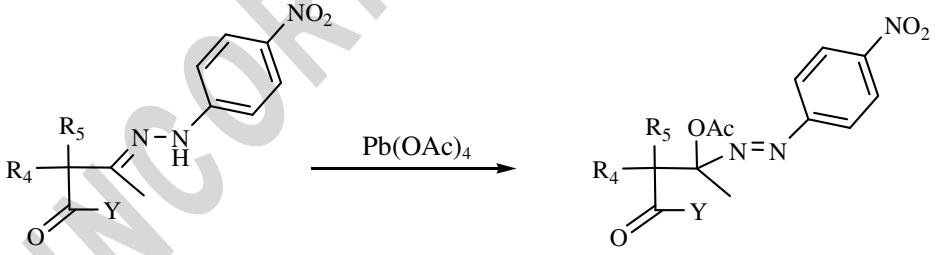


Substrate	Y	R_4	R_5	Product	Yield(%)
1	OEt	H	H	**8**	55
3f [a]	NH-Ar	H	H	**9f**	59
4	OEt	Me	Me	**10**	65
5a-j [a]	NH-Ar	Me	Me	**11a-j**	50-72
7 [a]	NH-Ar	Me	Bn	**12**	39

[a] With reference to aromatic groups Ar, substitutions at R_1, R_2 and R_3 are as shown in Table 2

The synthesis of the azoacetates of these hydrazones was found to be equally facile (Table 4), once again it was found that merely stirring the substrate hydrazones overnight in a mildly acidic CH_2Cl_2 solution (10% acetic acid) containing a slight excess (1.1 molar equivalents) of $Pb(OAc)_4$ was sufficient to effect the desired transformation.[9] After a standard work-up, flash chromatography was used to separate the azoacetates from any persistent lead salt residues

Table 4. Synthesis of azoacetate derivatives

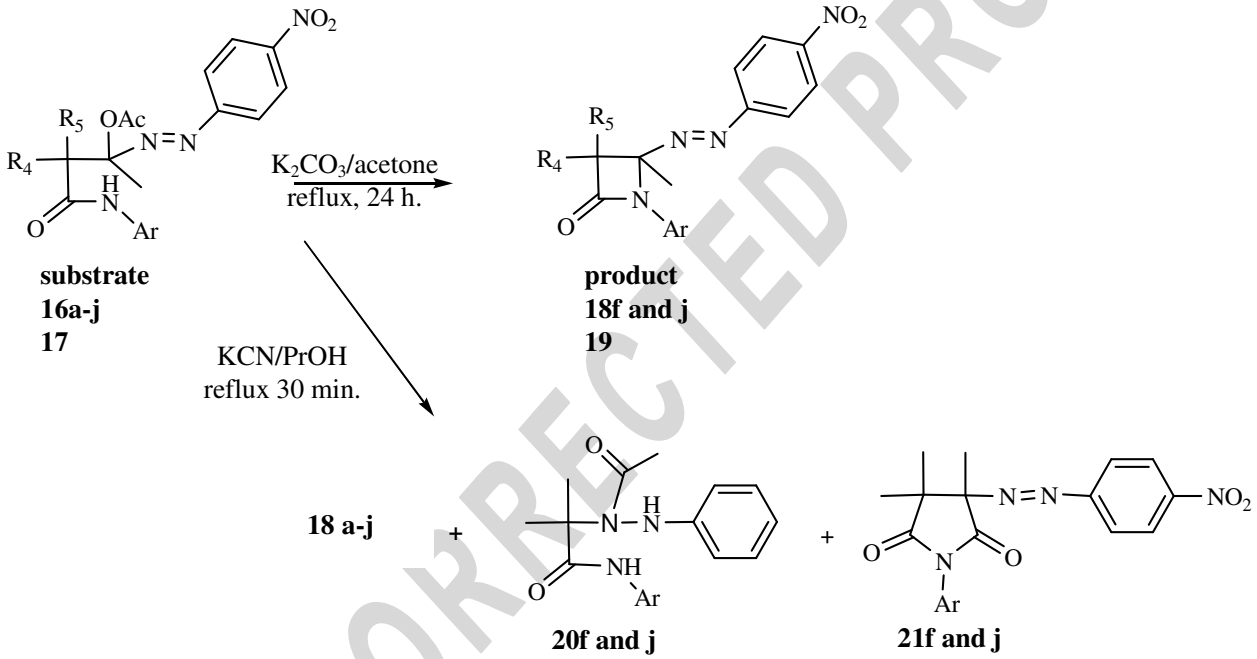


Substrate	Y	R_4	R_5	Product	Yield(%)
8	OEt	H	H	**13**	65
9f [a]	NH-Ar	H	H	**14f**	68
10	OEt	Me	Me	**15**	77
11a-j [a]	NH-Ar	Me	Me	**16a-j**	70-89
12 [a]	NH-Ar	Me	Bn	**17**	56

[a] With reference to aromatic groups Ar, substitutions at R_1, R_2 and R_3 are as shown in Table 2

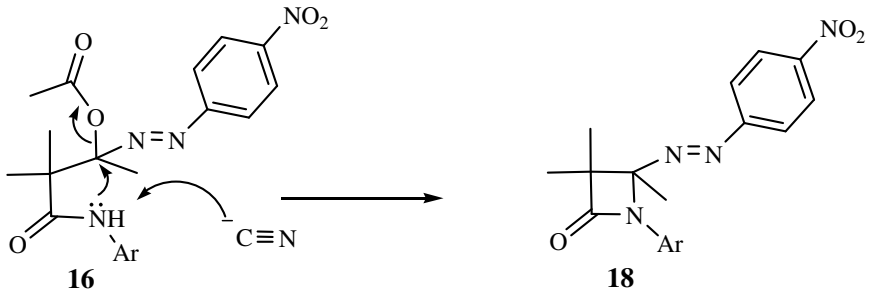
The reactions of the azoacetates with various bases produced the most interesting results (Scheme 1). It was found that the azoacetates derived from α,α-dimethylated acetoacetanilide or α-methyl-α-benzyl acetoacetanilide could be cyclised to azetidinones **(18f, 18j** and **19)** upon treatment with K_2CO_3 under reflux in acetone. It was also found that the cyclisation could be carried out in other conditions;

azetidinones **(18a-j)** were also isolated when the reaction was carried out in *n*-propanol using KCN as base. When the reactions of previously reported azoacetates **(16f** and **16j)** were more closely examined it was found that in addition to the reported rearrangement products **20f** and **20j**, an additional cyclised compound could be isolated in both cases. The isolation of these 2-iminopyrrolidine-5-one products (**21f** and **21j)** is a relatively rare example of the incorporation of cyanide moiety into a heterocyclic system in a one step process. It would seem that both the basic and nucleophilic properties of the cyanide species have an effect on the formation of reaction products in this case. The formation of these products has been confirmed by spectral analysis (NMR, IR, MS) and in the case of azetidinone (**18j)** and 2-iminopyrrolidine-5-one derivative (**21f)** by X-ray crystal structure determination (Figures 1a and b.)

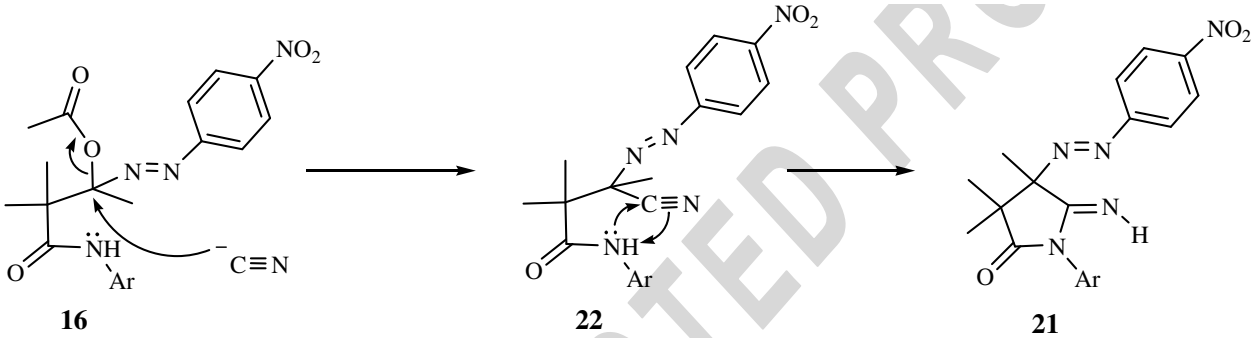


Scheme 1

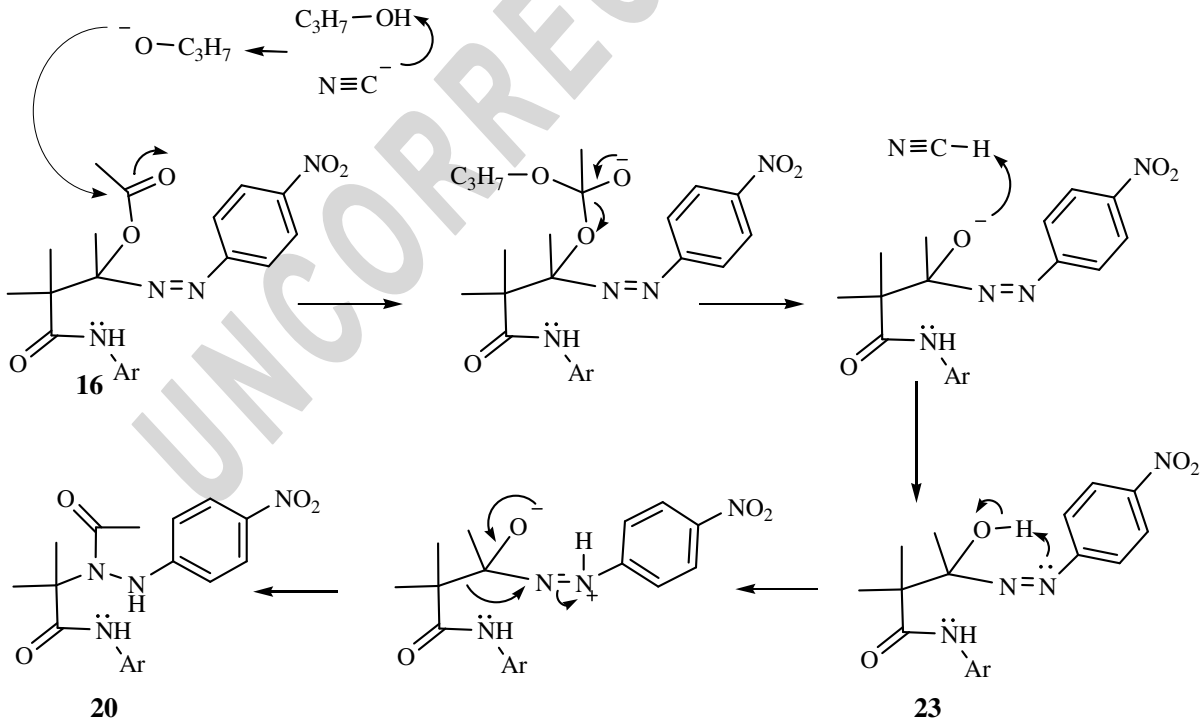
The formation of these three products are clearly as a result of different reaction mechanisms, they do however all conform to previously postulated reaction pathways. Instances of the base induced abstraction of the relatively acidic amido-hydrogen, followed by cyclisation through loss of a leaving group (Scheme 2a) can be commonly found in the literature related to β-lactam formation.[10] However the acetate moiety acting as leaving group in this manner under such mild conditions has, to our knowledge, not been previously reported. Of note amongst the azetidinone products isolated was compound **18e**, it was found that the transesterification product of the azetidinone was isolated. The action of the base/propanol media in this case resulted in the isolation of the 4-propyl ester derivative as sole azetidinone product in this case. Also two diastereomeric forms of azetidinone derivative (**19)** were isolated and could be differentiated on the basis of differing chemical shift values for key NMR spectral values (e.g. benzyl CH_2 shift).



Scheme 2a: Proposed mechanism of formation of azetidinone **18**



Scheme 2b: Proposed mechanism of formation of **21**



Scheme 2c: Proposed mechanism of formation of **20**

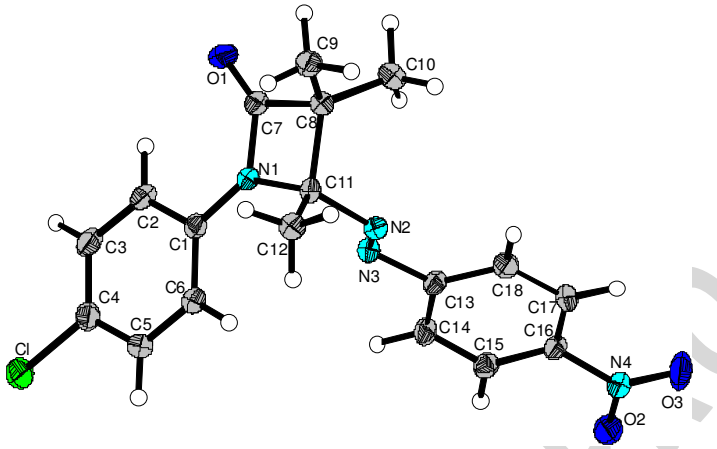


Figure 1a: X-Ray crystal structure of azetidinone **18j**

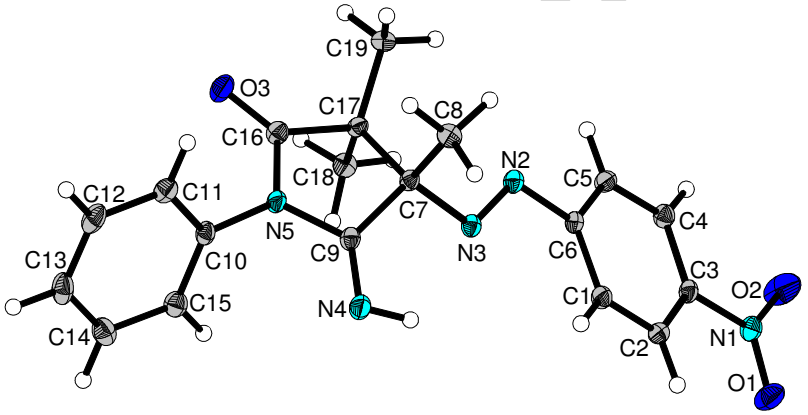


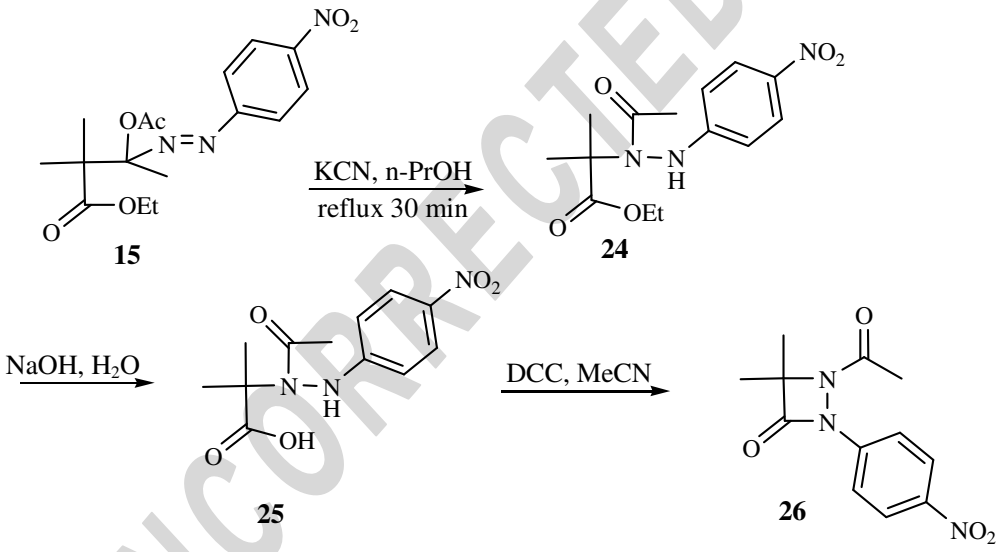
Figure 1b: X-Ray crystal structure of 2-iminopyrrolidine-5-one derivative **21f**

The α-azo-α-nitrile functionality is a known species and it has been demonstrated that in certain cases, the nitrile group can be introduced at a position α to an azo-group by reaction of a hydrazone with metal cyanides.[11] The intermolecular nucleophilic displacement of a leaving group by the cyanide species to form a nitrile followed by cyclisation, as in the case of the formation 2-iminopyrrolidine-5-ones (**21f** and **21j**) has also previously been studied by Nisole et al.[12] The isolation 2-iminopyrrolidine-5-ones directly upon treatment of carbamoylated chloroenamines with metal cyanides in alcohol have similarly been observed by Vilsmaier et al.[13] In that case, the cyclisation took place spontaneously under the action of the basic reaction media with a similar mechanism proposed; some parallels can be drawn to the observations made here, in so far as the isolation of the nitrile intermediate was not achieved. Since the formation of the cyclised product through incorporation of the cyanide ion is evident, it is reasonable to assume that the intermediate **22** in **Scheme 2b**, plays a role.

It is proposed that the rearrangement of azoacetates (**16f** and **16j**) to N-acyl hydrazides (**20f** and **20j**) takes place via an alcoholysis mechanism involving the formation of an azocarbinol species (a.k.a. α-hydroxy phenylazo compounds) such as intermediate **23** in **Scheme 2c**. It seems most likely that it is this species

that undergoes the rearrangement. The transformation of azoacetates to azocarbinols in this way has previously been shown to be possible.[14]Barton et al utilized 2-t-butyl-1,1,3,3-tetramethyl guanidine base (Barton's base) to effect this type of transformation in ethanol, however efforts to isolate intermediate **23** in a similar manner failed. The azocarbinol species in general is notoriously unstable, and it would seem that the formation of the rearrangement product is the favored process in this case.

In order to further investigate the versatility of the compounds synthesized, some supplementary reactions were carried out on the products mentioned thus far and it was found that a further array of interesting compounds could be created. In the case of the N-acyl hydrazide derivative (**24)** isolated from the reaction of azoacetate (**15)** with potassium cyanide it was found that the ester moiety could be easily hydrolyzed to the carboxylic acid **25** and this could then be cyclised to give a diazetidinone product (**26**, Scheme 3). The structure of the rearrangement product **24** was confirmed by X-Ray crystallography.



Scheme 3

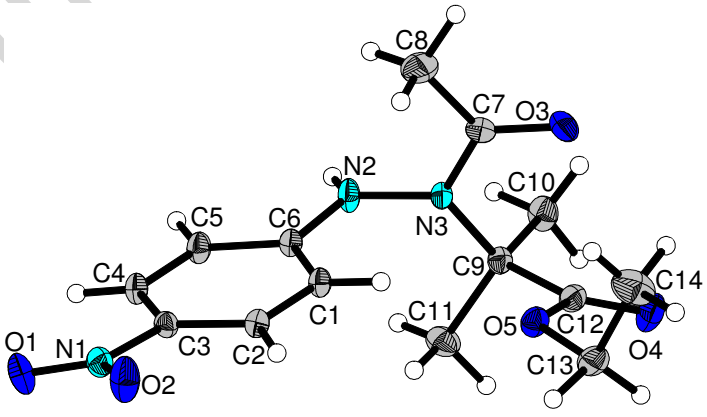
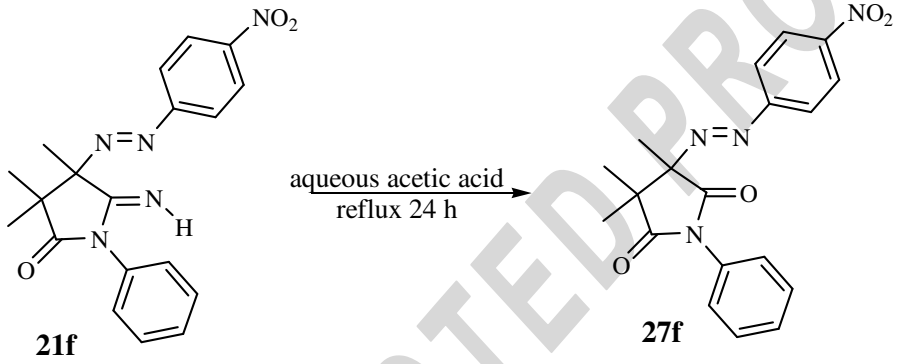


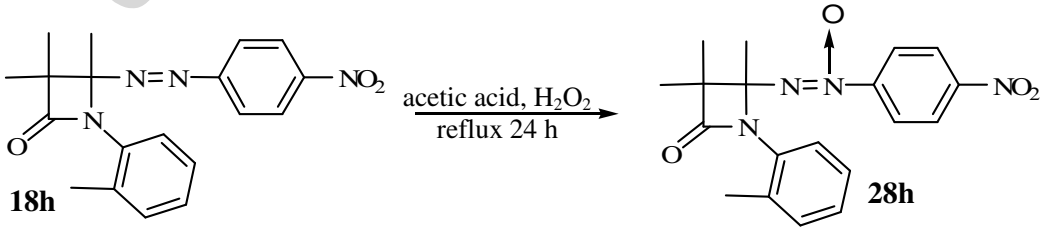
Figure 2: X-Ray crystal structure of rearrangement product **24**

Among the many published synthetic routes to the versatile succinimide compounds perhaps the simplest is the acid catalyzed hydrolysis of 2-iminopyrrolidine-5-ones.[15] The appearances of several versions of the procedure in the literature show it to be a robust high-yielding technique that can be used when a variety of functional groups are present adjacent to the imino functionality. In the example presented here (Scheme 4) a smooth transition with high yield was achieved. 2-iminopyrrolidine-5-one (**21f)** was converted to the corresponding succinimide (**27f)**. The substrate **21f** was introduced to a refluxing solution of aqueous acetic acid and allowed to reflux for a further 24 h after which the desired succinimide was easily extracted into organic solvent (CH_2Cl_2) and purified by flash chromatography.



Scheme 4

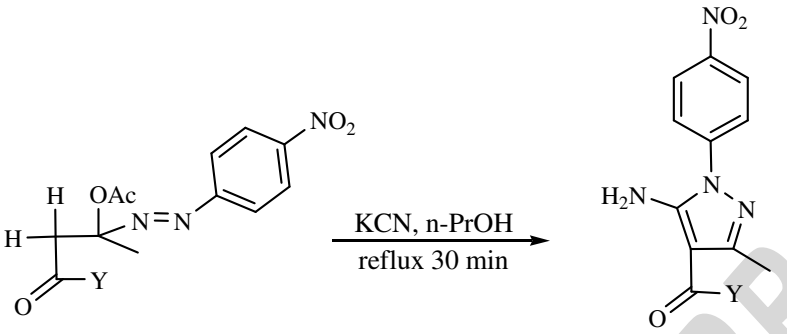
It was found that the azoxy derivative **28h** of azetidinone (**18h)** could be generated through reaction with hydrogen peroxide in the presence of acetic acid. Although all spectral data supports the formation of the azoxy functionality at a position adjacent to the phenyl group, since an X-Ray crystal structure of the product was not obtained in this case this characterization is somewhat tentatively assigned. The formation of the azoxy functionality is in little doubt; however guidelines to the assignment of the position of the azoxy oxygen found in the literature were at times contradictory and at best speculative. Some consensus seems to have been reached on the issue of the increase in the deshielding effect on groups adjacent to the newly formed azoxy group by comparison to the parent azo compound. The creation of the azoxy group presented here was indeed accompanied by a large downfield shift by the aromatic proton signals adjacent to the proposed site of the azoxy oxygen.



Scheme 5

The production of the 2-iminopyrrolidine-5-one derivative (**21f)** raised the question of what might happen under similar conditions with an α,α-unsubstitued azoacetate. The investigations of this question led to an unexpected cyclisation. It was found that when these substrates **13** and **14f** were subjected to identical

conditions to those used previously with the α,α-dimethylated azoacetate derivatives, that once again a cyano unit was incorporated into the cyclic product. However, in this case the absence of alkyl groups at positions α to the carbonyl allows for the formation of the pyrazole derivatives **(29** and **30f)** as the intermolecular reaction products.[16]



Substrate		**Product**
13	Y=OEt	**29**
14f	Y=NH-Ar	**30f**

Scheme 6

Confirmation of the structure has been established by X-Ray crystal structure analysis in the case of compound **30f**. Although the isolation of these compounds has been previously reported, different methodologies were employed in the synthesis. Furthermore, the crystal structure of this species has not been published to date.

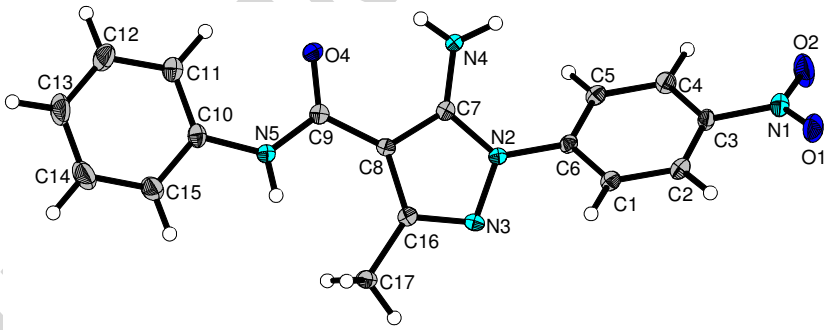
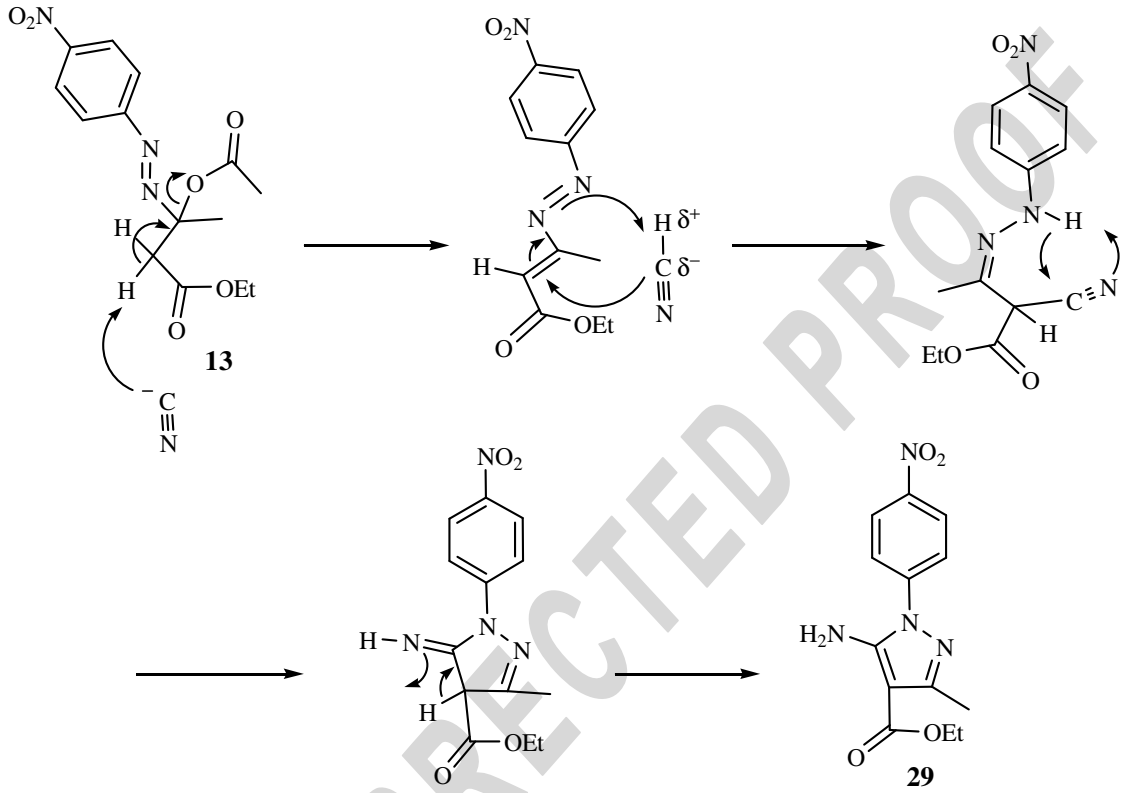


Figure 3. X-Ray crystal structure of pyrazole derivative **30f**

Scheme 7 depicts a proposed mechanism for the formation of pyrazole derivative (**29)** from the corresponding azoacetate derivative (**13**). In light of the basic reaction conditions, the formation of the pyrazole is presumably due to initial elimination of a unit of acetic acid aided by stabilization of the product provided by the neighboring phenylazo group. Nucleophilic addition at this newly formed double bond provides a cyano substituted hydrazone substrate for cyclisation to the pyrazole ring, as has been

previously proposed by Shawali et al.[17] In that case the proposed formation of the hydrazone species was as a result of the condensation of a hydrazidic halide and ethyl cyanoacetate.



Scheme 7

In conclusion the results presented here, demonstrate the synthetic versatility of these azoacetates derived from simple, inexpensive starting material. The examples shown here of both endo- and exocyclic incorporation of the azo functionality into heterocyclic systems demonstrate the value of a previously unexplored resource in the synthetic sphere. A range of products from established medicinally important families of compounds have been synthesized through simple modifications using facile procedures. It is deemed noteworthy to point out that the synthesis of products based solely on 4-nitrophenyl hydrazone derivatives was due to their inherent stability to the acidic conditions required for condensation. Some arylhydrazone derivatives of β-keto esters are prone to spontaneous cyclisation giving, pyrazoles and pyrazolones.[18] Our research has not been based exclusively on arylhydrazones of this type, however these compounds were chosen for presentation at this time due to the reliability and reproducibility of the results obtained.

EXPERIMENTAL

Ethyl acetoacetate, iodomethane, benzyl chloride and substituted acetoacetanilides were purchased from the Sigma Aldrich chemical company and were used as received. All solvents were dried or distilled prior to use. Melting points were determined using a Griffin melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 2000FT-IR spectrometer. NMR spectra were recorded using a Bruker AC 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. The ^{1}H and ^{13}C NMR chemical shifts (ppm) are relative to TMS and all coupling constants (*J*) are in Hertz (Hz).

X-Ray crystallographic data

*"CCDC 272234 **(18j)**, CCDC 686905 **(21f)**, CCDC 686907 **(24)**, and CCDC 686906 **(30f)**, contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033."*

Preparation of acetoacetanilides (3a-e)

These compounds were prepared according to a general procedure a representative example is given.

***Synthesis of 4-bromoacetoacetanilide* (3a)**

Ethyl acetoacetate (65.1 g, 0.5 mol) was heated to 160 ^{o}C in a round bottomed flask with reflux condenser fitted. To this was added 4-bromoaniline (17.0 g, 0.1 mol). When the evolution of EtOH began the reaction was allowed to reflux for 2-3 h until the reaction was complete (TLC) before the EtOH was distilled off. The bulk of the remaining ethyl acetoacetate was removed by vacuum distillation at 90^{o} C, 50mbar. The resulting oily residue was scratched with a glass rod to promote precipitation. The precipitate was filtered and washed with hexane before being recrystallised from an EtOAc:hexane 30:70 mixture. Yield (22.8 g, 90.1 %); mp 132-135 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ =, 2.21 (3H, s, CH_3), 3.56 (2H, s, CH_2), 7.48 (2H, ArH, *J* = 8.8), 7.55 (2H, d, ArH, *J* = 8.8) 10.21 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 30.2, 52.3, 114.9, 120.9, 131.5, 138.2, 165.2, 202.7; IR (KBr) 3292, 1718, 1661, 1607, 1554, 1491, 1396, 1162, 1075, 832, 505 cm^{-1}.

Preparation of α,α-dimethylethylacetoacetate (4) and α,α-dimethylacetoacetanilides (5a-j)

These compounds were prepared according to a general procedure a representative example is given.

***Synthesis of* α,α-*dimethyl-4-bromoacetoacetanilide* (5a)**

(6.62 g, 25.9 mmol) of 4-bromoacetoacetanilide was dissolved in 150 ml of acetone, to this was added K_2CO_3 (7.8 g, 56.4 mmol) and the reaction was set to reflux. Once the reaction had reached reflux

temperature iodomethane (22.45 g, 158.2 mmol) was added and the reaction was allowed to reflux overnight. The solvent was reduced to 1/5 of the original volume by rotary evaporation and the entire contents of the reaction vessel were poured into a beaker containing 200 ml of ice-water. The product formed an oil and was extracted with CH_2Cl_2 (5 x 50 ml). The organic extracts were combined, dried over magnesium sulfate and evaporated to an oil under reduced pressure. A small portion of the oil was removed and precipitation was induced by scratching, this was then returned to the bulk in order to seed the crystallization of the remaining oil. The solid was filtered and washed with hexane, this was further recrystallized from an EtOAc:hexane 70:30 mixture. Yield (5.84 g, 79.4 %); mp 85-87 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ =, 1.37 (6H, s, 2 x CH_3), 2.15 (3H, s, CH_3), 7.48 (2H, ArH, *J* = 9.2), 7.61 (2H, d, ArH, *J* = 8.8), 9.57 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 21.8, 25.7, 56.5, 115.3, 122.2, 131.3, 138.2, 171.8, 207.2; IR (KBr) 3361, 1684, 1531, 1490, 1394, 1310, 1239, 1240, 1117, 1071, 818 cm^{-1}.

Preparation of α-methyl-4-chloroacetoacetanilide (6) and α-methyl-α-benzyl- 4-chloroacetoacetanilide (7)

***Synthesis of α-methyl-4-chloroacetoacetanilide* (6)**

(10.8 g, 51.2 mmol) of 4-chloroacetoacetanilide **(3j)** was dissolved in acetone (75 ml) and) K_2CO_3 (7.2 g, 52.0 mmol) was added and the reaction brought to reflux. Iodomethane (10.66 g, 75.1 mmol) was added dropwise with stirring to the reaction vessel over 1 h and the reaction was allowed to reflux for a further 2 h. The reaction mixture was cooled and poured into ice-water (150 ml) and allowed to stir for 30 min, a white precipitate **(6)** was collected and recrystallized several times from aqueous EtOH to remove any dimethylated product **(5j)**. Yield: (8.59 g, 74.4%); mp 125-129 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ =, 1.23 (3H, d, CH_3, *J* = 6.8), 2.17 (3H, s, CH_3), 3.65 (1H, q, CH, *J* = 6.8), 7.37 (2H, d, ArH, *J* = 8.8), 7.50 (2H, d, ArH, *J* = 8.8), 10.34 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 13.0, 28.2, 54.5, 120.8, 127.1, 128.6, 137.8, 168.9, 204.3. IR (KBr) 3241, 1720, 1654, 1604, 1543, 1494, 1403, 1081, 826 cm^{-1}.

***Synthesis of α-methyl-α-benzyl-4-chloroacetoacetanilide* (7)**

(6.8 g, 30.2 mmol) of α-methyl-4-chloroacetoacetanilide **(6)** was dissolved in acetone 50 ml and (7.2 g, 52.0 mmol) of K_2CO_3 was added and the reaction brought to reflux. Benzyl chloride (6.58 g, 52.0 mmol) was added dropwise with stirring to the reaction vessel over 1 h and the reaction was allowed to reflux overnight. The reaction mixture was cooled and poured into ice-water (150 ml) and allowed to stir for 30 min, a white precipitate **(7)** was collected and recrystallized from an EtOAc:hexane 70:30 mixture. Yield: (8.53 g, 89.5%); mp 124-127 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ =, 1.27 (3H, s, CH_3), 2.20 (3H, s, CH_3), 3.07 (1H, d, CH_2, *J* = 13.6), 3.36 (1H, d, CH_2, *J* = 13.6), 7.11-7.23 (5H, m, ArH), 7.77 (2H, d, ArH, *J* = 8.8), 7.43 (2H, d, ArH, *J* = 8.8), 9.68 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 18.7, 26.3,

39.7, 61.3, 122.3, 126.5, 127.6, 128.0, 128.1, 130.1, 136.7, 137.5, 170.2, 206.3; IR (KBr) 3328, 1720, 1654, 1604, 1543, 1494, 1403, 1081, 826 cm^{-1}.

Preparation of hydrazone derivatives (8, 9a and **f, 10, 11a-j, 12)**

These compounds were prepared according to a general procedure a representative example is given.

***Synthesis of 4-nitrophenyl hydrazone derivative of α,α-dimethyl-4-bromoacetoacetanilide* (11a)**

(4.0 g, 14.0 mmol) of 2,2-dimethyl-4-bromoacetoacetanilide (**5a**) was dissolved in MeOH 75 ml, (2.6 g, 16.8 mmol) of 4-nitrophenylhydrazine was dissolved in warm acetic acid (3.25 ml) and added to the stirring reaction vessel. After 1-2 min a yellow precipitate began to fall from solution, the reaction was allowed to stir overnight and the precipitate collected and dried. Yield (4.01 g, 68 %); mp 197-200 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.48 (6H, s, 2 x CH_3), 1.96 (3H, s, CH_3), 7.27 (2H, d, ArH, *J* = 8.8), 7.50 (2H, d, ArH, *J* = 9.2), 7.65 (2H, d, ArH, *J* = 8.8), 8.12 (2H, d, ArH, *J* = 8.8), 9.43 (1H, s, NH), 9.97 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 13.7, 23.5, 51.3, 111.6, 115.1, 122.5, 125.8, 131.2, 138.1, 138.5, 151.8, 151.9, 173.5; IR (KBr) 3322, 1671, 1596, 1492, 1324, 1260, 1113, 1089, 841, 821 cm^{-1}.

Preparation of azoacetate derivatives (13, 14a and **f, 15, 16a-j, 17)**

These compounds were prepared according to a general procedure a representative example is given.

***Synthesis of azoacetate derivative of α,α-dimethyl-4-bromoacetoacetanilide* (16a)**

(3.0 g, 7.15 mmol) of the hydrazone substrate (**11a**) was dissolved in CH_2Cl_2 (100 ml). To this was added (3.8 g, 8.6 mmol) of lead tetraacetate with stirring. 5 ml of glacial acetic acid was added to aid the dissolution of the lead tetraacetate and the solution was allowed to stir overnight. When the reaction was judged complete (TLC) the solvent volume was reduced by 75% and the remaining CH_2Cl_2 solution was washed alternately with 5 x 50 ml portions of water and 5 % aqueous sodium bicarbonate solution. The organic portion was dried over magnesium sulfate and evaporated to dryness and any persistent lead residues were removed by flash chromatography using a 25 : 75 EtOAc : hexane mixture as eluent. Yield (2.80 g, 82%); mp 202-205 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ =, 1.28 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.86 (3H, s, CH_3), 2.18 (3H, s, CH_3), 7.48 (2H, d, ArH, *J* = 8.8), 7.57 (2H, d, ArH, *J* = 9.2), 7.75 (2H, d, ArH, *J* = 8.8), 8.37 (2H, d, ArH, *J* = 8.8). 9.19 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 17.2, 20.5, 20.6, 21.5, 51.0, 103.6, 115.5, 123.0, 123.2, 125.0, 131.2, 138.0, 148.5, 153.9, 168.8, 171.1; IR (KBr) 3324, 1731, 1687, 1605, 1539, 1344, 1240, 1175, 868 cm^{-1}.

Preparation of azetidinones derivatives (18f and **19) procedure 1**

Synthesis of azetidinone derivative* (18f) *using procedure 1

Anhydrous potassium carbonate (0.5 g, 3.6 mmol) was added to azoacetate **16f** (1.2 g, 3 mmol) in 50 ml of refluxing acetone. The solution was allowed to reflux for 22 h after which time (TLC) showed the

reaction to be complete. The insoluble material was filtered from the solution and the filtrate evaporated to dryness. The azetidinone product **(18f)** was isolated after silica gel column chromatography using $CHCl_3$ as eluent. Yield (0.223 g, 22 %); mp 87-89 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.08 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.92 (3H, s, CH_3), 7.15-7.11 (1H, m, ArH), 7.35-7.03 (2H, m, ArH), 7.43 (2H, d, ArH, *J* = 8.8), 7.88 (2H, d, ArH, *J* = 8.8), 8.37 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 17.3, 19.1, 19.2, 60.5, 90.2, 118.3, 123.4, 124.4, 124.9, 129.1, 137.2, 149.2, 154.1, 170.5; IR (KBr) 1759, 1599, 1527, 1495, 1368, 1346, 862, 756, 690 cm^{-1}.

Synthesis of azetidinone derivative* (19) *using procedure 1

(19a) Yield (8.3%); mp 139-141 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 0.85 (3H, s, CH_3), 1.94 (3H, s, CH_3), 3.01 (1H, d, CH, *J* = 14.4), 3.32 (1H, d, CH, *J* = 14.4), 7.27-7.07 (9H, m, ArH,), 7.78 (2H, d, ArH, *J* = 8.8), 8.29 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 16.8, 17.8, 30.6, 64.6, 91.1, 120.0, 125.3, 124.9, 127.2, 128.5, 128.5, 129.1, 129.9 136.1, 136.6, 149.7, 154.2 170.0; IR (KBr) 1745, 1527, 1494, 1383, 1342, 1091, 827, 692 cm^{-1}. Anal. Calcd. for $C_{24}H_{21}ClN_4O_3$: C, 64.21; H, 4.72; N, 12.48. Found: C; 64.01, H; 4.84, N; 12.18.

(19b) Yield (11.6%); mp 137-139 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.43 (3H, s, CH_3), 1.99 (3H, s, CH_3), 2.67 (1H, d, CH, *J* = 14.4), 2.90 (1H, d, CH, *J* = 14.8), 7.27-7.10 (9H, m, ArH,), 7.87 (2H, d, ArH, *J* = 8.8), 8.36 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 15.1, 15.9, 36.3, 62.7, 89.2, 118.0, 122.0, 123.3, 125.2, 126.7, 127.5, 127.9, 128.4, 134.2, 134.7,147.8, 152.3, 168.0; IR (KBr) 1757, 1528, 1494, 1367, 1345,1180, 1092, 820, 745 cm^{-1}.

Preparation of azetidinones derivatives (18a-j) procedure 2

Synthesis of azetidinone derivative* (18a) *using procedure 2

(1g, 2.1 mmol) of the azoacetate derivative **(16a)** was dissolved in hot propanol (40 ml) to which (0.15 g, 2.3 mmol) of potassium cyanide was added. The solution was allowed to reflux for 30 min, after which TLC showed development of product spots and total consumption of the starting material, this was evaporated to dryness. Water (100 ml) was added to the reaction vessel and this was extracted with EtOAc (2 x 25 ml) followed by CH_2Cl_2 (2 x 25 ml). The combined extracts were dried over magnesium sulfate and upon evaporation to dryness an orange oil was collected. The product was isolated after column chromatography. Yield (0.14 g, 16 %); mp 155-158 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.19 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.62 (3H, s, CH_3), 7.18 (2H, d, ArH, *J* =8.8), 7.55 (2H, d, ArH, *J* =8.8), 7.76 (2H, d, ArH, *J* = 9.2), 8.28 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 13.3, 17.8, 24.4, 49.3, 83.0, 122.5, 123.4, 124.9, 127.7, 130.8, 132.4, 153.8, 174.0, 180.1; IR (KBr) 1755, 1520, 1489, 1362, 1347, 827, 753 cm^{-1}. Anal. Calcd. for $C_{18}H_{17}BrN_4O_3$: C, 51.81; H, 4.11; N, 13.43, Found: C; 52.10, H; 4.28, N; 13.21.

Synthesis of azetidinone derivative* (18b) *using procedure 2

Yield (0.15 g, 20 %); mp 95-98 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 0.98 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.77 (3H, s, CH_3), 3.71 (3H, s, OCH_3), 6.77 (2H, d, ArH, *J* =*9.2*), 7.28 (2H, d, ArH, *J* =*9.2*), 7.79 (2H, d, ArH, *J* = 9.2), 8.28 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 17.2, 19.1, 19.2, 55.5, 60.4, 90.3, 114.3, 120.3 123.3, 124.8, 130.2, 149.2, 154.1, 156.5, 170.2; IR (KBr) 1759, 1513, 1392, 1346, 1247, 1184, 1164, 1031, 860, 828, 751, 688 cm^{-1}.

Synthesis of azetidinone derivative* (18c) *using procedure 2

Yield (0.21 g, 25 %); mp 112-116 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.08 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.62 (3H, s, CH_3), 2.24 (3H, s, CH_3), 2.25 (3H, s, CH_3), 7.06-6.96 (3H, m, ArH), 7.76 (2H, d, ArH, *J* = 9.2), 8.29 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 15.2, 17.4, 19.3, 19.9, 20.7, 59.6, 91.8, 123.2, 124.3, 124.9, 125.8, 129.8, 132.9, 135.1, 138.5, 149.1, 154.2, 171.6; IR (KBr) 1757, 1526, 1471, 1366, 1345, 1134, 1093, 869,792, 752, 688 cm^{-1}. Anal. Calcd. for $C_{20}H_{22}N_4O_3$: C, 65.56; H, 6.05; N, 15.29, Found: C; 65.61, H; 6.02, N; 15.20.

Synthesis of azetidinone derivative* (18d) *using procedure 2

Yield (0.13 g, 15%); mp 103-107 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.07 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.89 (3H, s, CH_3), 2.33 (3H, s, CH_3), 7.13 (2H, d, ArH, *J* =8.0), 7.32 (2H, d, ArH, *J* =8.4), 7.88 (2H, d, ArH, *J* = 9.2), 8.37 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 17.3, 19.1, 19.2, 21.0, 60.4, 90.1, 118.3, 123.4, 124.9, 129.6, 134.1, 134.6, 149.2, 154.1, 170.3; IR (KBr) 1751, 1523, 1513, 1347, 1164, 1131, 861, 817, 752, 688 cm^{-1}. Anal. Calcd. for $C_{19}H_{20}N_4O_3$: C, 64.76; H, 5.72; N, 15.90, Found: C; 64.81, H; 5.73, N; 15.85.

Synthesis of azetidinone derivative* (18e) *using procedure 2

In the case of azetidinone derivative **(18e)** *it was the propanol transesterification product that was isolated*

Yield (0.10 g, 11 %); mp 97-100 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 0.94 (2H, t *J* =7.6), 1.00 (3H, s, CH_3), 1.29 (2H,m), 1.31 (3H, s, CH_3), 1.72-1.67 (1H, m), 1.88 (3H, s, CH_3), 4.18 (1H, t, *J* =6.4), 4.27 (1H, q, *J* =5.2), 7.38 (2H, d, ArH, *J* =8.8), 7.78 (2H, d, ArH, *J* =8.8), 7.91 (2H, d, ArH, *J* = 9.2), 8.27 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 9.5, 13.3, 16.5, 17.9, 18.1, 59.8, 65.5, 89.2, 116.3, 122.4, 123.9, 124.9, 129.7, 140.0, 148.3, 152.9, 165.0, 169.6; IR (KBr) 1749, 1709, 1509, 1352, 1270, 849, 769 cm^{-1}.

Synthesis of azetidinone derivative* (18f) *using procedure 2

Azetidinone derivative **(18f)** was prepared using procedure 2 as outlined above; two additional compounds were isolated during the chromatographic separation **(20f** and **21f)**.

(18f) Yield (0.10 g, 12 %). Spectra identical to those reported using *procedure 1*

(20f) Yield (0.14 g, 16 %); mp 195-197 ºC ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.49 (3H, s, CH_3), 1.53 (3H, s, CH_3), 1.92 (3H, s, CH_3), 6.96 (1H, s br, ArH),7.07 (1H, t, ArH, *J* = 7.2), 7.33-7.26 (3H, m, ArH), 7.61 (2H, d, ArH, *J* = 7.6), 8.15 (2H, s br, ArH), 9.06 (1H, s br, NH), 9.48 (1H, s br, NH); ^{13}C NMR (100MHz, DMSO-d_6) 21.7, 22.9, 24.3, 65.6, 110.5 (d, *J* = 222), 120.8, 123.5, 126.2 (d, *J* = 69), 128.3, 138.7, 138.9, 154.2, 171.8, 172.8. IR (KBr) 3338, 3288, 1647, 1592, 1522, 1329, 1272, 1108, 750, 695 cm^{-1}.

(21f) Yield (0.17 g, 18 %); mp 142-145 ºC ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.19 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.61 (3H, s, CH_3), 7.34-7.28 (2H, m, ArH), 7.41-7.39 (1H, m, ArH), 7.52-7.47 (2H, m, ArH,), 7.76 (2H, d, ArH, *J* = 8.8), 8.26 (2H, d, ArH, *J* = 9.2); 9.00 (1H, s NH); ^{13}C NMR (100MHz, DMSO-d_6) 13.3, 17.9, 24.4, 49.3, 83.1, 123.4, 124.8, 126.3, 128.7, 129.2, 131.9, 149.3, 153.9, 174.3, 180.5; IR (KBr) 1661, 1528, 1377, 1346, 1161, 1107, 862 cm^{-1}

Synthesis of azetidinone derivative* (18g) *using procedure 2

Yield (0.21 g, 25%); orange oil; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.06 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.63 (3H, s, CH_3), 2.23 (3H, s, CH_3), 2.32 (3H, s, CH_3), 6.88 (1H, d, ArH, *J* =8.0), 7.02 (2H, d, ArH, *J* =8.0), 7.75 (2H, d, ArH, *J* = 9.2), 8.28 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 17.5, 18.9, 19.3, 19.8, 21.1, 59.5, 91.7, 123.1, 124.9, 126.3, 127.1, 130.5, 131.9, 136.2, 138.1, 149.1, 154.2, 171.5; IR (liquid film) 1762, 1527, 1504 1370, 1347, 1139, 862, 753, 739 cm^{-1}.

Synthesis of azetidinone derivative* (18h) *using procedure 2

Yield (0.18 g, 22%); mp 108-110 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.06 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.65 (3H, s, CH_3), 2.37 (3H, s, CH_3), 7.22-7.06 (4H, m, ArH,), 7.76 (2H, d, ArH, *J* = 8.8), 8.29 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 17.6, 19.1, 19.3, 19.9, 59.6, 91.8, 128.2, 124.9, 126.2, 126.4, 128.2, 131.3, 133.3, 136.4, 149.1, 154.1, 171.3; IR (KBr) 1761, 1530, 1493, 1346, 1133, 874, 769 cm^{-1}. Anal. Calcd for $C_{19}H_{20}N_4O_3$: C, 64.76; H, 5.72; N, 15.90. Found: C; 64.66, H; 5.69, N; 15.83.

Synthesis of azetidinone derivative* (18i) *using procedure 2

Yield (0.14 g, 16 %); mp 98-100 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.04 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.67 (3H, s, OCH_3), 6.89-6.85 (2H, m, ArH,), 7.19-7.15 (1H, m, ArH,), 7.60-

7.58 (1H, m, ArH,), 7.79 (2H, d, ArH, *J* = 9.2), 8.29 (2H, d, ArH, *J* = 8.8); ^{13}C NMR (100MHz, $CDCl_3$) 16.9, 18.9, 19.9, 55.6, 59.8, 91.5, 112.0, 120.8, 123.1, 123.9, 124.9, 127.6, 128.4, 149.0, 153.9, 154.4, 172.4; IR (KBr) 1761, 1524, 1497, 1390, 1345, 1242, 1106, 1025, 858, 770, 752.

Synthesis of azetidinone derivative* (18j) *using procedure 2

(18j) Yield (0.17 g, 20%); mp 132-134 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 0.98 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.82 (3H, s, CH_3), 7.18 (2H, d, ArH, *J* =8.8), 7.29 (2H, d, ArH, *J* =9.2), 7.78 (2H, d, ArH, *J* = 8.8), 8.28 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 17.3, 19.0, 19.1, 60.7, 90.3, 119.5, 123.4, 124.9, 129.1, 129.5, 135.8, 149.3, 154.0, 170.3; IR (KBr) 1753, 1534, 1346, 1144, 869, 851, 705 cm^{-1}. Anal. Calcd for $C_{18}H_{17}ClN_4O_3$: C, 57.99; H, 4.60; N, 15.03. Found: C; 57.99, H; 4.73, N; 14.81.

(21j) Yield (0.21 g, 23%); mp 201-203 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.07 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.60 (3H, s, CH_3), 7.32 (2H, d, ArH, *J* =8.4), 7.38 (2H, d, ArH, *J* =8.4), 7.89 (2H, d, ArH, *J* = 9.2), 8.40 (2H, d, ArH, *J* = 9.2), 9.08 (1H, s, NH); ^{13}C NMR (100MHz, DMSO-d_6) 13.8, 17.9, 22.9, 48.5, 81.6, 123.2, 125.1, 128.8, 129.3, 132.3, 132.8, 148.7, 154.0, 165.3, 179.1. IR (KBr) 3300, 1742, 1661, 1532, 1492, 1344, 1102, 1085, 713.7, 687.2 cm^{-1}.

Synthesis of N-acyl hydrazide derivative as rearrangement product (24)

(1g, 2.8 mmol) of the azoacetate derivative **(15)** was dissolved in hot EtOH (40 ml) to which (0.24 g, 3.7 mmol) of potassium cyanide was added. The solution was allowed to reflux overnight, before being evaporated to dryness. Water (100 ml) was added to the reaction vessel and this was extracted with EtOAc : EtO_2 (15:85). The combined extracts were dried over magnesium sulfate and upon evaporation to a reduced volume a pale brown solid precipitated. The precipitate was filtered off and washed with petroleum ether and dried. Yield (0.31 g, 35 %); mp 172-174 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.23 (3H, t, CH_3, *J* = 6.8), 1.27 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.88 (3H, s, CH_3), 4.16-4.06 (2H, m, CH_2), 6.90 (1H, d, ArH , *J* = 7.6), 7.15 (1H, d, ArH, *J* = 7.6), 8.11 (1H, d, ArH, *J* = 8.0), 8.17 (1H, d, ArH, *J* = 7.6), 9.58 (1H, s, NH); anal. Calcd. for $C_{13}H_{19}N_3O_5$ requires C, 54.36; H, 6.14; N, 13.58; found C, 54.53; H, 6.29; N, 13.51.

Synthesis of N-acyl hydrazide derivative (25) by decarboxylation

(0.5 g, 1.6 mmol) of N-acyl hydrazide derivative **(24)** was added to a 2.5 molar solution of sodium hydroxide (3.99 g dissolved in 40 ml of water) the solution was allowed to reflux for 4 h. The pH was adjusted to 7-8 with conc. HCl to give a yellow precipitate which was extracted into EtOAc (3 x 20 ml) dried over magnesium sulfate and evaporated to dryness Yield (0.41 g, 90 %); mp 215-216 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.21 (3H, s, CH_3), 1.32 (3H, s, CH_3), 1.94 (3H, s, CH_3), 6.90-6.85 (1H, m,

ArH), 7.15-7.10 (1H, m, ArH), 8.27-8.23 (2H, m, ArH) 9.50 (1H, s, NH); IR (KBr) 3347, 1710, 1619, 1599 cm^{-1}; Anal. Calcd. for $C_{12}H_{15}N_3O_5$ requires C, 51.24; H, 5.38; N, 14.94. Found C, 51.12; H, 5.50; N, 14.71.

Synthesis of diazetidinone derivative (26)

A 0.05 M solution of N-acyl hydrazide derivative **(25)** was made up in MeCN (0.85 g/60 ml). (3.7g, 18 mmol) of dicyclohexylcarbodiimide was added and the reaction refluxed overnight under a nitrogen atmosphere. After removal of the bulk of the excess dicyclohexylcarbodiimide and dicyclohexylurea by filtration, the remaining organic elements were evaporated to dryness and subjected to flash column chromatography to separate any persistent dicyclohexylurea. The desired diazetidinone derivative **(26)** was isolated and further purified by recrystallization from pet. ether : EtOAc 20 : 80. Yield (0.35g, 45%); mp 112-114 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.70 (6H, s, 2 x CH_3), 2.20 (3H, s, CH_3), 7.33-7.28 (2H, m, ArH), 8.30-8.22 (2H, m, ArH); IR (KBr) 1796, 1696, 1627 cm^{-1}; anal. Calcd. for $C_{12}H_{13}N_3O_5$ requires C, 54.75; H, 4.98; N, 15.96; found C, 54.38; H, 4.93; N, 15.84.

Synthesis of succinimide derivative (27f)

(200 mg, 0.54 mmol) of the 2-iminopyrrolidine-5-one derivative **21f** was dissolved in 10 ml of aqueous acetic acid. The solution was heated to 50 ^{o}C for 24 h, 100 ^{o}C for a further 24 h and finally refluxed for 4 h after which TLC showed total consumption of the starting material. The solution was allowed to cool and added to ice-water (100 ml) which induced precipitation of a pale yellow solid that was filtered from solution. Yield (153 mg, 78 %); product decomposed in air over 24 h; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.19 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.61 (3H, s, CH_3), 7.30-7.25 (2H, m, ArH), 7.35-7.32 (1H, m, ArH), 7.43-7.37 (2H, m, ArH), 7.76 (2H, d, ArH, *J* = 8.8), 8.26 (2H, d, ArH, *J* = 9.2); ^{13}C NMR (100MHz, $CDCl_3$) 13.3, 17.9, 24.4, 49.3, 83.1, 123.4, 124.8, 126.3, 128.7, 129.2, 131.9, 149.3, 153.9, 174.3, 180.5; IR (KBr) 1719, 1529, 1396, 1374, 1346, 1146, 1126 cm^{-1}.

Synthesis of azetidinone derivative (28h)

(100 mg, 0.28 mmol) of the azetidinone derivative **(18h)** was dissolved in glacial acetic acid (25 ml) and heated to 80 ^{o}C, to this was added 0.5 ml of hydrogen peroxide solution (35% by weight) at 15 min intervals over 3 h. After 5 h the reaction mixture was treated with 2 ml of the hydrogen peroxide solution and allowed to stir at 80 ^{o}C overnight. A further 2 ml of hydrogen peroxide solution was added to the reaction mixture and the reaction allowed to stir for a further 3 h after which no starting material could be detected by TLC The reaction was cooled to room temperature and poured into 150 ml of ice-water which induced precipitation of a pale yellow solid that was filtered from solution. Yield (66 mg, 63.9 %); mp 132-134 ºC; ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.29 (3H, s, CH_3), 1.51(3H, s, CH_3), 1.69 (3H, s, CH_3), 2.37

(3H, s, CH_3), 7.24-7.17 (3H, m, ArH,), 7.38 (1H, d, ArH, *J* = 8.8), 8.26 (2H, d, ArH, *J* = 9.6), 8.29 (2H, d, ArH, *J* = 10.0); ^{13}C NMR (100MHz, $CDCl_3$) 14.5, 17.7, 18.7, 19.0, 60.3, 86.3, 123.4, 124.6, 126.6, 127.2, 128.6, 131.3, 132.5, 136.7, 149.9, 150.4, 171.0; IR (KBr) 1752, 1535, 1485, 1465, 1385, 1346, 1310, 868, 850, 760, 703 cm^{-1}.

Synthesis of pyrazole derivatives (29 and **30f)**

Synthesis of pyrazole derivative **(29)**

(1 g, 3.1 mmol) of the azoacetate derivative **(13)** was dissolved in hot n-PrOH (40 ml) to which (0.3 g, 4.6 mmol) of potassium cyanide was added. The solution was allowed to reflux for 4 h, before being evaporated to dryness. Water (100 ml) was added to the reaction vessel and this was extracted with CH_2Cl_2 (15:85). The combined extracts were dried over magnesium sulfate and upon evaporation to a reduced volume a pale brown solid precipitated. The precipitate was filtered off and washed with petroleum ether and the product isolated after flash chromatography. Yield (0.13 g, 15 %) mp 210-212 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 1.27 (3H, t, CH_3, *J* = 7.2), 2.26 (3H, s, CH_3), 4.20 (2H, q, CH_2, *J* = 7.2), 6.64 (2H, s br, CH_2), 7.81 (2H, d, ArH , *J* = 7.2), 8.32 (2H, d, ArH, *J* = 7.2); ^{13}C NMR (100MHz, DMSO-d_6) 14.3, 14.4, 59.1, 93.9, 123.0, 124.9, 143.1, 144.9, 150.6, 151.2, 164.0; IR (KBr) 3349, 1676, 1548, 1344, 1286, 1131, 1112, 857, 789 cm^{-1}.

Synthesis of pyrazole derivative **(30f)**

Yield (0.12 g, 15 %); mp 181-183 ºC; ^{1}H NMR (400MHz, DMSO-d_6): δ = 2.94 (3H, s, CH_3), 6.60 (2H, s br, CH_2), 7.06 (1H, t, ArH , *J* = 7.6), 7.32 (2H, t, ArH, *J* = 7.6), 7.63 (2H, d, ArH , *J* = 7.6), 7.88 (2H, d, ArH , *J* = 9.2), 8.35 (2H, d, ArH, *J* = 9.2), 8.85 (1H, s, NH,); ^{13}C NMR (100MHz, DMSO-d_6) 14.2, 98.4, 120.4, 122.6, 123.3, 124.9, 128.5, 138.8, 143.3, 144.7, 148.2, 150.7, 163.3; IR (KBr) 3455, 3429, 3313, 1650, 1599, 1543, 1495, 1343, 864, 745, 684 cm^{-1}.

ACKNOWLEDGEMENTS

This work was supported by the National Institute for Cellular Biotechnology (NICB) and the Irish Research Council for Science Engineering and Technology (IRCSET). The authors would also like to thank Mr. Damien McGuirk for assistance with NMR spectroscopy and Dr. Helge Müller-Bunz for X-Ray crystallography.

REFERENCES

1. D. M. Smith, A. Kazi, L. Smith, T. E. Long, B. Heldreth, E. Turos, and Q. P. Dou, *Mol Pharmacol*, 2002, **61**, 1348; L. Sun, N. I. Vasilevich, J. A. Fuselier, S. J. Hocart, and D. H. Coy, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 204; B. K. Banik, I. Banik, and F. F. Becker, *Bioorg. Med. Chem.*, 2005, **13**, 3611; I. Banik, F. F. Becker, and B. K. Banik, *J. Med. Chem.*, 2003, **46**, 12.
2. T. Sperka, J. Pitlik, P. Bagossia and J. Tözséra, *Bioorg. Med. Chem. Lett*., 2005, **15**, 3086.
3. A. K. Halve, D. Bhadauria, and R. Dubey, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 341.
4. C. A. Downey, J. P. James, J. Lawler, P. O'Malley, and S. Wolfe, *J. Chem. Soc., Chem. Commun.,* 1992, **5**, 454.
5. T. Duff, J. P. James, and H. M. Bunz, *Heterocycles*, 2006, **68**, 465.
6. W. C. Groutas, M. J. Brubaker, L. S. Chong, R. Venkataraman, H. Huang, J. B. Epp, R. Kuang, and J. R. Hoidal, *Bioorg. Med. Chem.,* 1995, **3**, 375.
7. F. Misani, and M. Taylor-Bogert, *J. Org. Chem.,* 1947, **10**, 347.
8. J. Buckingham, *Q. Rev. Chem. Soc.*, 1969, 23, 37.
9. R. N. Butler, *Chem. and Ind.*, 1968, **6**, 437; A. Kotali, *Curr. Org. Chem.*, 2002, **6**, 965.
10. K. A. Tehrani, and N. De Kimpe, *Tetrahedron Lett*., 2000, **41**, 1975.
11. M. Okimoto, Y. Takahashi, and T. Kakuchi, *Synthesis*, 2003, **13**; 2057; J. G. Schantl, H. Gstach, *Monatsh. Chem*., 1985, **116**, 1329.
12. C. Nisole, P. Uriac, L. Toupet, and J. Heut, *Tetrahedron*, 1993, **49**, 889.
13. E. Vilsmaier, R. Adam, C. Tetzlaff, and R. Cronauer, *Tetrahedron*, 1989, **45**, 3683.
14. D. H. R. Barton, J. C. Jaszberenyi, W. Liu, and T. Shinada, *Tetrahedron,* 1996*,* **52** 14673.
15. E. Vilsmaier, R. Adam, P. Altmeier, R. Cronauer, *Tetrahedron*, 1989, **45**, 131; A. R. Battersby, and S.W. Westwood, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1679.
16. A. -F. A. Harb, H. H. Abbas, and F. H. Mostafa, *Chem. Pap*., 2005, **59**, 187.
17. A. S. Shawali, H. M. Hassaneen, M. Sami and H. M. Fahham, *J. Heterocycl. Chem.*, 1976, **13**, 1137.
18. M. B. Smith and J. March, 'March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure,' 6th Edition, Wiley-Interscience, a John Wiley & Sons, Inc., Publication, Hoboken, New Jersey, 2007, pp. 1284-1285.

References

1. J. Lister, *Lancet*, 1867, **90**, 353; J. Lister, *Br. Med. J.*, 1871, **1**, 30.

2. L. Pasteur, and J. Joubert, *Bull. Acad. Med.*, 1877, **6**, 781.

3. S. A. Waksman, *Mycologia*, 1947, **39**, 565; W.R. Strohl, '*Biotechnology of antibiotics'*, 2nd ed., p. 1.

4. R. Emmerich, *Science*, 1885, **5**, 75.

5. S. Duckett, *Lancet*, 1999, **354**, 2068.

6. A. Fleming, *Br. J. Exp. Path.,* 1929, **10**, 226.

7. D. C. Hodgkin, *Adv. Sci*., 1949, **6**, 85.

8. F. P. Doyle, J. H. C. Nayler, H. Smith, and E. R. Stove, *Nature,* 1961, **191**, 1091.

9. M. J. Basker, K. R. Comber, R. Sutherland, and G. H Valler, *Chemotherapy*, 1977, **23**, 424.

10. G. Brotzu, *Lav. Ist. Ig. Cagliari*, 1948, 1.

11. G. G. F. Newton, and E. P. Abraham, *Biochem. J*, 1956, **62**, 651.

12. N. D. Shah, L. C. Vermeulen, J. P. Santell, R. J. Hunkler, and K. Hontz , *Am. J. Health-Syst. Pharm.,* 2002, **59**, 131; K. Outterson, *U. Pitt. L. Rev.*, 2006, **67**, 67.

13. R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. R. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, *Antimicrob. Agents Chemother.*, American Society for Microbiology, Detroit, Mich., 1962, p. 687.

14. J. S. Kahan, F. M. Kahan, R. Goegelman, S. A. Currie, M. Jackson, E. O. Stapley, T. W. Miller, A. K. Miller, D. Hendlin, S. Mochalest, S. Hernandezt, H. B. Woodruff, and J. Birnbaum, *J. Antibiot.*, 1979, **32**, 1.

15. R. B. Woodward, *Recent Advances in Chemistry of β-lactam Antibiotics*, Special pub. 28, Elks, J., Ed. *The Chemical Society*, London, 1977, 167.

16. J. Regos, CIBA-Geigy, unpublished results, 1977; N. M. Samarendra, P. Ruppa, B. Kamalesh, and S. Rudong, *Top. Heterocycl. Chem.,* 2006, **2**, 207.

17. J. Basu, R. Chattopadhyay, M. Kundu, and P. Chakrabarti, *J. Bact.*, 1992, **174**, 4829.

18. J. Clayden, N. Greeves, S.Warren, P. Wother, Organic Chemistry, 2001, Oxford UP, Oxford, p. 366.

19. C. Darwin, 'On the origin of species by means of natural selection.' London: J Murray Company, 1859.

20. A. G. Brown, D. Butterworth, M. Cole, G. Hanscomb, J. D. Hood, C. Reading, G. N. Rolinson, *J. Antibiot.,* 1976, **29**, 668.

21. Z. Zhang, J. Ren, D. K. Stammers, J. E. Baldwin, K Harlos, and C. J. Schofield, *Nat. Struct. Biol.*, 2000, **7**, 127.

22. Y-l. Lin, N-y. Chang, and C. Lim, *J. Am. Chem. Soc.,* 2002, **124**, 12042.

23. H. Aoki, H. Sakai, M. Kohsaka, T. Konomi, J. Hosoda. Y. Kubochi. E. Iguchi, and H. Imanaka*, J. Antibiot.,* 1976, **29**, 492; M. Hashimoto, T. Komori. and T. Kamiya, *J. Am. Chem. Soc*., 1976, **98**, 3023; M.Hashimoto, T. Komori, and T. Kamiya, *J. Antibiot.,* 1976, **29**, 890.

24. C. A. Koppel, L. McShane, F. Jose, and R. D. C. Cooper, *J. Am. Chem. Soc.,* 1978, **100**, 3933.

25. H. H. Wasserman, and D. J. Hlasta, *J. Am. Chem. Soc.*, 1978, **100**, 6780.

26. (a) R. B. Sykes, C. M. Cimarusti, D. P. Bonner, K. Bush, D. M. Floyd, N. H. Georgopapadakou, W. H. Kostar, W. C. Liu, W. L Parker, P. A. Principe, M. L. Rathnum, W. A. Slusarchyk, W. H. Trejo, and J. S. Wells, *Nature*, 1981, **291**, 489; (b) W. L. Parker, W. H. Koster, C. M. Cimarusti, D. M. Floyd, W. C. Liu, and M. L. Rathnum, *J. Antibiot.,* 1982, **35**, 189; (c) J. S. Wells, W. H. Trejo, P. A. Principe, K. Bush, N.

Georgopapadakou, D. P. Bonner, and R. B. Sykes, *J. Antibiot.,* 1982, **35**, 184; (d) W. L. Parker, and M. L. Rathnum, *J. Antibiot.,* 1982, **35**, 300.

27. J. S. Wells, W. H. Trejo, P. A. Principe, K. Bush, N. Georgopapadakou, D. P. Bonner, and R. B. Sykes *, J. Antibiot.,* 1982, **35**, 295;
28. D. M. Floyd, A. W. Fritz, J. Pluscec, E. R. Weaver, and C. M. Cimarusti, *J. Org. Chem.*, 1982, **47**, 5160.
29. J. Pitlik and C. A. Townsend, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 3129.
30. T. Sperka, J. Pitlik, P. Bagossia, and J. Tözséra, *Bioorg. Med. Chem. Lett*., 2005, **15**, 3086.
31. J. W. Clader, D. A. Burnett, M. A. Caplen, M. S. Domalski, S. Dugar, W. Vaccaro, R. Sher, M. E. Browne, H. Zhao, R. E. Burrier, B. Salisbury, and H. R. Davis, Jr., *J. Med. Chem.,* 1996, **39**, 3684.
32. G. Veinberg, I. Shestakova, M. Vorona, I. Kanepe, and E. Lukevics, *Bioorg. Med. Chem. Lett.,* 2004, **14**, 147.
33. D. M. Smith, A. Kazi, L. Smith, T. E. Long, B. Heldreth, E. Turos, and Q. P. Dou, *Mol Pharmacol,* 2002, **61**, 1348.
34. B. K. Banik, I. Banik, and F. F. Becker, *Bioorg. Med. Chem*., 2005, **13**, 3611; I. Banik, F. F. Becker, and B. K. Banik, *J. Med. Chem.,* 2003, **46**, 12.
35. H. Staudinger, J. Meyer, *Helv. Chim. Acta.*, 1919, **2**, 635.
36. Josef Klarer, and Fritz Mietzsch, United States Patent 2,085,037, June 29, 1937.
37. C. Scheurlen, H. Allgayer, W. Kruis, E. Erdmann, and T. Sauerbruch, *Clin Investig.* 1993, **71**, 286.
38. P. Krogsgaard-Larsen, T. Liljefors, U. Madsen 'A textbook of drug design and discovery'; 3rd ed.; Taylor and Francis: New York, 2002; Chapter 14, pp. 410-458.
39. J. Hefner, and P. Langer, *Tetrahedron Lett*., 2008, **49**, 2262.

40. P. W. Baures, S. A. Peterson, and J. W. Kelly, *Bioorg. Med. Chem.,* 1998, **6**, 1389.

41. J. H. Jorgensen, S. A. Crawford, and G. A. Alexander., *Antimicrob. Agents Chemother.*, 1982, **22**, 162.

42. R. N. Jones, H. W. Wilson, and W. J. Novick Jr., *J. Clin. Microbiol.*, 1982, **15**, 677.

43. D. Hamilton, J. H. Wu, and G. Batist, *Mol. Pharmacol.*, 2007, **71**, 1140.

44. D. Mitra, M. Kar, R. Pal, and A. Basak, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4514; M. Kar, and A. Basak, *J. Chem. Soc., Chem. Commun.*, 2006, 3818.

45. P. J. Garratt, and S. B. Neoh, *J. Org. Chem.* 1979, **44**, 2667; Y. S. P. Cheng, P. J. Garratt, S. B. Neoh, V. H. Rumjanek, *Isr. J. Chem.*, 1985, **26**, 101; S. Braverman, Y. Duar, and D. Segev, *Tetrahedron Lett.*, 1976, **36**, 3181.

46. A. M. Maxam, and W. Gilbert, *Methods Enzymol.*, 1980, **65**, 499; A. M. Maxam, W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.*, 1977, **74**, 560.

47 M. S. Butler, and A. D. Buss, *Biochem. Pharmacol.*, 2006, **71**, 919.

48. I. L. Knunyants, and N. P. Gambaryan, *Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk,* 1958, **52**, 3816.

49. M. S Manhas, and S. J. Jeng*, J. Org. Chem.*, 1967, **32**, 1246.

50. H. H Wasserman, D. J. Hlasta, A. W Tremper, and J. S. Wu, *Tetrahedron Lett.,* 1979, **6**, 549.

51. P. G Mattinghly, J. F. Kerwin, Jr., and M. J. Miller, *J. Am. Chem. Soc.* 1979, **101**, 3943.

52. M. J. Miller, P. G Mattinghly, M. A. Morrison, and J. F. Kerwin, Jr., *J. Am. Chem. Soc.* 1980, **102**, 7026.

53. R. O. Garay, and M. C. Cabaleiro, *J. Chem. Soc., Perkin Trans. 2,* 1988, 1643.

54. C. A. Downey 'Metallic acetate oxidations of substituted hydrazones of acetoacetanilides and their conversion to β-lactams.' PhD. Thesis, 1993, Dublin City University.

55. C. A. Downey, J. P. James, J. Lawler, P. O' Malley, and S. Wolfe, J. Chem. Soc., Chem. Commun., 1992, 454.

56. D. H. R. Barton, J. C. Jaszberenyi, W. Liu, and T. Shinada, *Tetrahedron,* 1996*,* **52** 14673.

57. A. R. Battersby, and S. W. Westwood*, J. Chem. Soc. Perkin Trans. I,* 1987, 1679.

58. C. Nisole, P. Uriac, L. Toupet, and J. Huet, *Tetrahedron*, 1993, **49**, 889.

59. D. A. Coulter, J. R. Huguenard, and D. A. Prince., *Neurosci. Lett.,* 1989, **98**, 74.

60. D. A. Coulter, J. R. Huguenard, and D. A. Prince., *Br. J. Pharmacol.*, 1990, **100**, 800; P. G. Kostyuk, E. A. Molokanova, N. F. Pronchuk, A. N. Savchenko, and A. N. Verkhratsky, *Neuroscience,* 1992, **51**, 755.

61. J. Herrington, and C. J. Lingle, *J. Neurophysiol.*, 1992, **68**, 213; R. J. Sayer, A. M. Brown, P. C. Schwindt, and W. E. Crill, *J. Neurophysiol.*, 1993, **69**, 1596.

62. M. Park, J. Lee, and J. Choi, *Bioorg. & Med. Chem. Lett.*, 1996, **6**, 1297.

63. A. Zejc, J. Obniska, M. Wilimowski, M. Rutkowska, J. Witkowski, L. Barczyńska, L. Kędzierska-Goździk, W. Wojewódzki, K. Orzechowska-Juzwenko, T. Plawiak, E. Duś, J. Gryska, and M. Gliniak, *Pol. J. Pharmacol. Pharm*., 1990, **42**; 69; J. Obniska, K. Kulig, and A. Zejc, *Acta Pol. Pharm.-Drug Res.*, 1998, **55**, 223; J. Obniska, A. Zejc, and J. Karolak-Wojciechowska, *Farmaco*, 1999, **54**, 423; J. Obniska, A. Zejc, and A. Zagórska, *Acta Pol. Pharm.-Drug Res.*, 2002, **59**, 209; J. Obniska, and A. Zagórska, *Farmaco*, 2003, **58**, 1227.

64. M. Montembault, G. Vo-Thanh, A. Deyine, V. Fargeas, M. Villiéras, A. Adjou, D. Dubreuil, D. Esquieu, C. Grégoire, S. Opi, J-M. Péloponèse, G. Campbell, J. Watkins, J. de Mareuil, A-M. Aubertin, C. Bailly, E. Loretc, and J. Lebretona, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1543.

65. M. C. Hsu, U. Dhingra, J. V. Earley, M. Holly, D. Keith, C. M. Nalin, A. R. Richou, A. D. Schutt, S. Y. Tam, M. J. Potash, D. J. Volsky, and D. D. Richman, *Proc. Natl. Acad.*

Sci. U.S.A., 1993, **90**, 6395; A. Lapidot, E. Ben-Asher, and M. Eisenstein, *FEBS Lett.,* 1995, **367**, 33.

66. C. A. Miller, H. I. Scholl, and L. M. Long, *J. Am. Chem. Soc.*, 1951, **73**, 5608; C. A. Miller, and L. M. Long, *J. Am. Chem. Soc.,* 1953, **75**, 373.
67. A.S. Shawali, H.M. Hassaneen, M. Sami and H.M. Fahham, *J. Heterocyclic Chem.,* 1976, **13**, 1137.
68. F. Gosselin, P. D. O'Shea, R. A. Webster, R. A. Reamer, R. D. Tillyer, and E. J. J. Grabowski, *Synlett*, **2006**, 3267; S. T. Heller, and S. R. Natarajan, *Org. Lett*., 2006, 8, 2675.
69. J-F. Li, Y-Q. Zhu, X. Wang, H-Z. Yang, *J. Heterocyclic Chem.,* 2007, **44**, 749.
70. N. K. Terrett, A. S. Bell, D. Brown, and P. Ellis, *Bioorg. Med. Chem. Lett.,* 1996, **6**, 1819.
71. D. B. Grant, A. E. Chalmers, M. A. Wolff, H. B. Hoffman, D. F. Bushey, *Rev. Toxicol.*, 1998, **2**, 147.
72. S. K. Meegalla, D. Doller, D.-Y. Sha, R. Soll, N. Wisnewski, G. M. Silver, and D. Dhanoa, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 4949.
73. K. A. Rossi, J. A. Markwalder, S. P Seitz, C-H. Chang, S. Cox, M. D. Boisclair, L. Brizuela, S. L. Brenner, and P. F. W. Stouten, *J. Comput. Aided Mol. Des.,* 2005, **19**, 111.
74. J. A. Markwalder, M. R. Arnone, P. A. Benfield, M. Boisclair, C. R. Burton, C-H. Chang, S. S. Cox, P. M. Czerniak, C. L. Dean, D. Doleniak, R. Grafstrom, B. A. Harrison, R. F. Kaltenbach III, D. A. Nugiel, K. A. Rossi, S. R. Sherk, L. M. Sisk, P. Stouten, G. L. Trainor, P. Worland, and S. P. Seitz, *J. Med. Chem.*, 2004, **47**, 5894.
75. M. J. Barnes, R. Conroy, D. J. Miller, J. S. Mills, J. G. Montana, K. P. Parminder, G. A. Showell, L. M. Walsh, and J. B. H. Warneck, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 354.
76. M. J. Graneto, R. G. Kurumbail, M. L. Vazquez, H-S. Shieh, J. L. Pawlitz, J. M. Williams, W. C. Stallings, L. Geng, A. S. Naraian, F. J. Koszyk, M. A. Stealey, X. D. Xu,

R. M. Weier, G. J. Hanson, R. J. Mourey, R. P. Compton, S. J. Mnich, G. D. Anderson, J. B. Monahan, and R. Devraj, *J. Med. Chem.*, 2007, **5**, 5712.

77. M. B. Smith and J. March, 'March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure,' 6th Edition, Wiley-Interscience, John Wiley and Sons, Inc., Publication, Hoboken, New Jersey, 2007, pp. 1284-1285.
78. P. F. O' Malley, 'Oxidation, cyclisation and rearrangements of hydrazone derivatives of β-ketoesters,' PhD. Thesis, 1997, Dublin City University.
79. C. F. H. Allen, *J. Am. Chem. Soc.*, 1930, **52**, 2955; O. L. Brady, *J. Chem. Soc*., 1931, 756.
80. R. N. Butler, *Chem. and Ind*., 1968, **6**, 437; A. Kotali, *Curr. Org. Chem.*, 2002, **6**, 965.
81. W. A. F. Gladstone, and R. O. C. Norman, *J. Chem. Soc.,* 1965, 5177.
82. M. Behforouz, J. L. Bolan, and M. S. Flynt, *J. Org. Chem.*, 1985, **50**, 1187.
83. F. Misani, and M. Taylor-Bogert, *J. Org. Chem.,* 1947, **10**, 347.
84. N. Zinin, *J. Prakt. Chem.,* 1845, **36***,* 98.
85. B. W. Langley, B. Lythgoe, and N. V. Riggs, *Chem. Ind.,* 1951, 75; B. W. Langley, B. Lythgoe, and N. V. Riggs, *J. Chem. Soc.*, 1951, 2309; F. Yagi, and K. Tadera, *Agricul. Biol. Chem.*, 1987, **51**, 1719; P. Lindblad, K. Tadera, and F. Yagi, *Environ. and Exper. Botany*, 1990, **30**, 429.
86. M. Gamberini, M. R. Cidade, L. A. Valotta, M. C. S. Armelin, and L. C. C. Leite, *Carcinogenesis* 1998, **19**, 147; G. R. Hoffmann, and R. W. Morgan, *Environmental Mutagenesis* 1984, **6**, 103; E. S. Fiala, R. Czerniak, A. Castonguay, C. C. Conaway, and A. Rivenson, *Carcinogenesis* 1987, **8**, 1947; W. Lijinsky, J. E. Saavedra, and M. D. Reuber, *Cancer Research* 1985, **45**, 76.
87. M. Yamato, H. Iinuma, H. Naganawa, Y. Yamagishi, M. Hamada, T. Masuda, H. Umezawa, Y. Abe, and M. Hori, *J. Antibiot.*, 1986, **39**,184.

88. B. T. Gillis, and K. F. Schimmel *J. Org. Chem.,* 1962, **27**, 413; B. T. Gillis, and K. F. Schimmel *J. Org. Chem.*, 1967, **32**, 2865.

89. P. E. Gagnon, and B. T. Newbold, *Can. J. Chem.*, 1959, 37, 366.

90. A. M. Churakov, O. Y. Smirnov, S. L. Ioffe, Y. A. Strelenko, and V. A. Tartakovsky, *Eur. J. Org. Chem.*, 2002, 2342.

91. J. N. Brough, B. Lythgoe, and P. Waterhouse, *J. Chem. Soc.*, 1954, 4069.

92. J. P. Freeman, *J. Org. Chem.,* 1963, **28**, 2508.

93. H. C. Brown, *Pure Appl.Chem.,* 1982, 54, 1783.

94. M. W. Moon, *J. Org. Chem.*, 1972, **37**, 2005; M. W. Moon; *J. Org. Chem.,* 1972, **37**, 386; M. W. Moon; *J. Org. Chem.,* 1972, **37**, 383.

95. M. S. Manhas, and S. J. Jen, *J. Org. Chem.,* 1967, **32**, 1246.

96. R. Joyeau, H. Molines, R. Labia, and M. Wakselman, *J. Med. Chem.*, 1988, **31**, 370.

97. L. Gaonkar, and K. M. L. Rai, *Tetrahedron Lett.*, 2005, **46**, 5969.

98. P. G. Mattingly, J. F. Kerwin Jr., and M. J. Miller, *J. Am. Chem. Soc.*, 1979, **101**, 3983.

99. M. J. Miller, P. G. Mattingly, M. A. Morrison, and J. F. Kerwin Jr., *J. Am. Chem. Soc.*, 1980, **102**, 1021

100. H. Kessler, D. Liebfritz, and C. Burk, *Tetrahedron*, 1970, **8**, 1805; P. Kittnel, and J. Warkentin, *Can. J. Chem.*, 1975, **53**, 2275.

101. D. W. K. Yeung, and J. Warkentin, *Can. J. Chem*., 1976, **54**, 1349; A, S, Nazran, and J. Warkentin, *J. Am. Chem. Soc.,* 1982, **104**, 6405.

102. Y-M Chang, R. Profetto, and J. Warkentin, *J. Am. Chem. Soc*., 1981, **103**, 7189; A, S, Nazran, and J. Warkentin, *J. Am. Chem. Soc*., 1981, **103**, 236.

103. M. Bodanszky, *Principles of Peptide Synthesis*, Springer-Verlag, New York, 1984, pp. 28-36.

104. D. H. R. Barton, J. D. Elliott, S. D. Géro, *J. Chem. Soc., Chem. Comm.* 1981, 1136; D. H. R. Barton, J. D. Elliott, S. D. Géro, *J. Chem. Soc., Perkin Trans. I*, 1982, 2085.

105 . M. J. Harrison, R. 0. C. Norman, and W. A. F. Gladstone, *J. Chem. Soc. (C),* 1967, 735.

106 . L. Mathew, and J. Warkentin, J. Am. Chem. Soc., 1986, 108, 7981.

107. M. Bodanszky, and A. Bodanszky, *The Practice of Peptide Synthesis*, 2nd ed., 1994, Springer-Verlag, New York.

108. A. V. Stachulski, Tetrahedron Lett., 1982, 23, 3789.

109. P. Benovsky, G. A. Stephenson, and J. R. Stille, J. Am. Chem. Soc., 1998, 120, 2493; Y. Oikawa, T. Nishi, and O. Yonemitsu, J. Chem. Soc. Perkin Trans*., 1*, 1985, 1; H-H. Tso, T. Chou, and S. Chun Hung, *J. Chem. Soc. Chem. Commun.*, 1987, 1552; E. B. Vilhauer, and R. C. Anderson, *J. Org. Chem.*, 1987, **52**, 1186; R. J. Giguere, G. von Ilsemann, and H. M. R. Hoffmann, *J. Org. Chem.*, 1982, **47**, 4948; D. A. Lightner, J. K. Gawronski, and T. B. Bouman., *J. Am. Chem. Soc.*, 1980, **102**, 1983; T. Sugimura, and L. A. Paquette, *J. Am. Chem. Soc.*, 1987, **109**, 3017; A. G. Shultz, W. G. McMahon, and R. K. Kullnig, *J. Org. Chem.*, 1987, **52**, 3905; E. J. Corey, and W. J. Suggs, *Tetrahedron Lett.*, 1975, **31**, 2647; R. Ratcliffe, and R. Rodehorst, *J. Org. Chem.*, 1970, **35**, 4000.

110. C. Di Bello, F. Filira, V. Giormani, and F. D'Angello, *Gazz. Chim. Ital.*, 1972, **102**, 180; K. E. Pfitzner, J. G. Moffatt, *J. Am. Chem. Soc.*, 1965, **87**, 5661.

111. D. B. Dess, and J. C. Martin, J. Org. Chem., 1983, 48, 4156; D. B. Dess, and J. C. Martin, J. Am. Chem. Soc., 1991, 113, 7277.

112. F. Yokokawa, K. Izumi, J. Omata, and T. Shioiri, Tetrahedron, 2000, 56, 3027.

113. C. Hartman, and V. Mayer, Chem. Ber., 1893, 26, 1727.

114. S. De Munari, M. Frigerio, and M. Santagostino, J. Org. Chem., 1996, 61, 9273.

115. M. Shibuya, S. Ito, M. Takahashi, and Y. Iwabuchi, *Org. Lett.*, 2004, **6**, 4303.

116. R. Saladino, M. Mezzetti, E. Mincione, I. Torrini, M. Paglialunga Paradisi, and G. Mastropietro, J. Org. Chem., 1999, 64, 8468; W. Adam, R. Curci, J. O. Edwards, Acc. Chem. Res., 1989, 22, 205; R. W. Murray, Chem. Rev., 1989, 89, 1187.

117. M. Dal Colle, G. Distefano, D. Jones, A. Guerrino, G. Seconi, and A. Modelli, *J. Chem. Soc. Perkin Trans. 2*, 1994, **4**, 789.

118. R. W. Layer, *Chem. Rev.,* 1963; **63**(5); 489.

119. E. Vilsmaier, R. Adam, P. Altmeier and R. Cronauer, *Tetrahedron,* 1989, 1, 131.

120 P. J. Hore, "Nuclear Magnetic Resonance", Oxford chemistry primers 32, Oxford University Press, 1995, pp 44-55.

121. John Coates, "Interpretation of Infrared Spectra, A Practical Approach" *Encyclopedia of Analytical Chemistry R.A. Meyers (Ed.)*; 2000; © John Wiley & Sons Ltd, Chichester, pp 10815–10837.

122. "Advanced Organic Chemistry: Reactions, Mechanisms and Structure", (6th Ed), Jerry March, © John Wiley & Sons Ltd, Chichester, pp 24-26; "A guidebook to mechanisms in organic chemistry" (6th Ed), Peter Sykes, pp 11-12.

123. F. Gosselin, P. D. O'Shea, R. A. Webster, R. A. Reamer, R. D. Tillyer, and E. J. J. Grabowski, *Synlett*, **2006**, 3267; S. T. Heller, and S. R. Natarajan, *Org. Lett*., 2006, 8, 2675.

124. R. Pearson, and G, Songstad, *J. Am. Chem. Soc.*, 1967, **89**, 1827.

125. "Advanced Organic Chemistry: Reactions, Mechanisms and Structure", (4th Ed), Jerry March, © John Wiley & Sons Ltd, Chichester, pp 261-263.