APPROACHES TO THE SYNTHESIS OF

β-LACTAM ANTIBIOTICS

by

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To all my family

and

friends

DECLARATION

This thesis is my own composition and, unless otherwise stated, the results described are original, and have not been submitted, in whole or in part, for any other degree at this or any other university.

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COURSES ATTENDED

While working on this thesis, I attended the following courses:-

Spectroscopy	Various Lectures
Application of X-ray crystallography	Various Lectures
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Nuclear Magnetic Resonance spectroscopy	Dr. I. Sadler
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Organic Departmental seminars	Various Lectures
Cell Biology	Dr. J. Phillips
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ABSTRACT

The historical, biological and synthetic aspects of β-lactam antibiotics are reviewed.

This is a study of a novel approach to bisnorpenicillin, involving an intramolecular Michael addition of a thiol onto an acrylate moiety. The development of novel protecting groups, for the thiol functional group, in 4-thioazetidin-2-one, such as trimethylsilyl-, trimethylstannyl - and phenylselenenyl-, were investigated. The application of solid phase methodology to β- lactam derivatives was also examined.

CONTENTS

CONTENTS

CONTENTS

CHAPTER ONE – INTRODUCTION

1.0.0 Introduction

The family of β-lactam antibiotics has now been available on the market for more than four decades. The low toxicity to mammalian cells (with the exception of the small percentage of allergy sufferers) and good activity against a broad spectrum of bacterial pathogens, make them the antibiotics of choice for the treatment of most common infections.

At an early stage in the use of these antibiotics, most treatable bacteria were found to produce enzymes, called β-lactamases, which inactivated the antibiotic. This prompted a great deal of research in the hope of understanding and overcoming this growing problem of bacterial resistance. The financial rewards in obtaining an antibiotic in this class may be very high, if a derivative is produced which possesses non allergenic properties, the required in vivo stability, β-lactamase resistance or β-lactamase inhibition properties, and potent activity towards a broad spectrum of bacteria. Consequently, a great deal of work has been undertaken by the major pharmaceutical companies.

The β-lactam area of research is active on many fronts, as evident from the growing literature. It would be a formidable task to attempt a comprehensive account on each of the researched areas.

This review will be divided into three parts. The first part will deal with a brief history of these antibiotics, and will be confined to the screening aspect, as this has been the most successful method of producing novel structures with bioactive properties. The second part will be concerned with the biological aspects of the cell wall, in respect to the lethal effect of β-lactam antibiotics, and β-lactamase inhibition

properties of some common β-lactam derivatives and also
the biosynthesis of these antibiotics.   The third part
will deal with the synthetic aspects of this area.   The
synthesis of the β-lactam ring will be given briefly by
both total synthesis and by penicillin degradation
methods.   The major part of this final section will
concentrate on approaches to the four-five and the four-
six fused systems.   The final part of the last section
will outline the future trends in this area of research.

1.1.0   Historical Development of β-lactam Antibiotics

It was the fortunate observation, first reported
by Fleming[1] in 1929, that the presence of a contaminant
mould (<u>Penicillium notatum</u>) facilitated the lysis of
surrounding bacterial cells which led workers such as
Florey, Chain and Heately to finally isolate the active
ingredient and to the production of "penicillin"
commercially by 1945.   Penicillin G (1) (Scheme 1) was
the first commercially available β-lactam antibiotic.
Soon after the availability of a pure crystalline sample,
its structure was confirmed by X-ray diffraction by
Crowfoot.[2]   The early history of penicillin affords
an interesting account of the early endeavours in
isolation, purification and chemical modification of
penicillin.[3]
It was soon discovered that by changing the growth
medium of the mould, a modified penicillin could be
formed.   This differed from penicillin G in its
acylamino side-chain in the C-6 position, (Scheme 1).
The alteration gave rise to a change in acid stability
and a slight change in the spectrum of activity.   Only a
limited number of changes could be made in this way,
(Table I), since the modification of the side chain

(1)
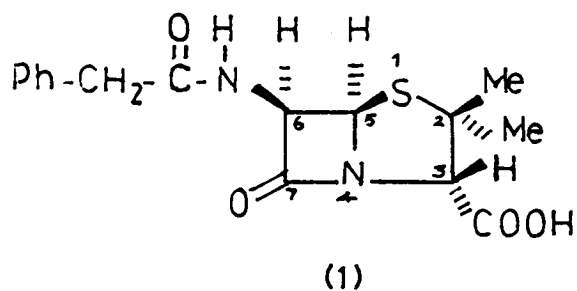
Scheme 1   Structure of benzylpenicillanic acid –
Penicillin G.

Table I   Side chains of natural penicillins
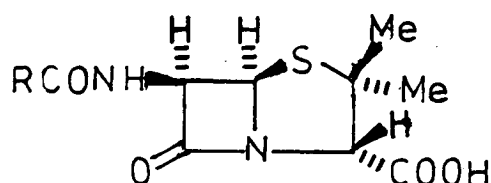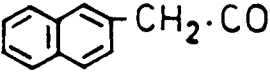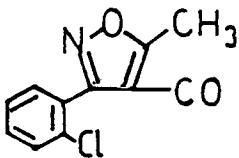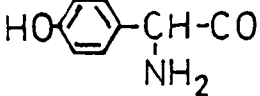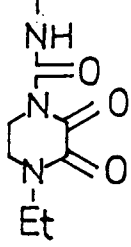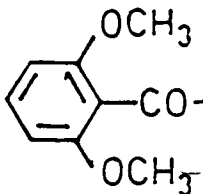
R	Name	Penicillin
$CH_3CH_2CH{=}CHCH_2-$	2-Pentenyl	F
$CH_3(CH_2)_3CH_2-$	n-Amyl	Dihydro F
$CH_3(CH_2)_5CH_2-$	n-Heptyl	K
$CH_2{=}CHCH_2SCH_2-$	Allylthio	O
$CH_3CHCl{=}CHCH_2SCH_2-$	3-Chloro-2-butenyl-thiomethyl	S
$CH_3(CH_2)_3SCH_2-$	nButylthiomethyl	BT
$NH_2CH(CO_2H)(CH_2)_2CH_2-$	α-Aminoadipyl	N
$C_6H_5CH_2-$	Benzyl	G (1)
$C_6H_5.O.CH_2-$	Phenoxymethyl	V (2)
$HO.C_6H_4.CH_2-$	4-Hydroxybenzyl	X

depended upon the ability of the mould to take up the
substrate from the medium and to incorporate it into the
penicillin molecule. This type of modification resulted
in the acid stable penicillin V (2), which was superior
to penicillin G (1) in that it could be administered
orally. Initial attempts to modify the side chain
chemically were restricted due to the shortage of
penicillin G which was a result of the latter's
contemporary high demand as a therapeutic agent. Work
began on the enzymatic removal of the side chain. This
was soon successful,[4] but the important intermediate,
6-amino-penicillanic acid (6.A.P.A.) (3) (Table II) was
still in short supply. Workers at Beecham[5] found that
(3) could be isolated, in high yield, from a
precursor-starved medium. Furthermore, improvements in
fermentation techniques and the use of a high yielding
penicillin strain from Penicillium chrysogenum, obtained
by mutation experiments, made it possible to satisfy the
high demand relatively easily and very cheaply. The
greater supply of this important intermediate (3) gave
rise to thousands of penicillin derivatives. These
contained a wide variety of C-6 acyl-amino side chains,
which had hitherto been unobtainable.[6] Of these, only a
few, namely, ampicillin (4) and carbenicillin (5) had any
improved activity compared to penicillin G (1) and
penicillin V (2).. A few other examples are given in
Table II.

It was soon realised by clinicians that Gram-
positive bacteria, such as Staphylococcus aureus, which
were once very sensitive to penicillins, were becoming
insensitive. These bacteria produced enzymes which
inactivated the penicillins by hydrolysis to penicilloic
acids (11) (Scheme 2). The problem was quite serious in
hospitals where penicillin antibiotics were administered
liberally and where penicillin-resistant strains were
first detected. This discovery gave rise to the imple-
mentation of controls on the use of these antibiotics.
Research was deemed necessary and urgent towards

TABLE II
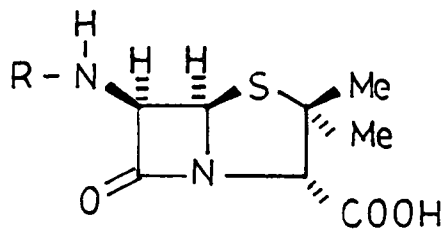
R	Name	Compound No.
H	6-Amino penicillanic acid	(3)
PhCHNH$_2$.CO	Ampicillin	(4)
PhCHCO$_2$H.CO	Carbenicillin	(5)
CH$_2$·CO (naphthyl)	Napthacillin	(6)
(isoxazole with CH$_3$, CO, o-Cl-phenyl)	Cloxacillin	(7)
HO—⟨⟩—CH-CO with NH$_2$	Amoxicillin	(8)
PhCHCO with NH, piperazinedione, Et	Piperacillin	(9)
2,6-(OCH$_3$)$_2$-phenyl-CO	Methicillin	(10)

development of some penicillinase resistant derivatives,
either by total or semi-synthesis.   Efforts in this
direction resulted in a few resistant penicillins (10),
(6) and (7) from semi- synthesis.   However, totally
synthetic attempts were unfortunately rewarded with
derivatives which had considerably lower activity than
the parent nucleus.

The screening of moulds from all sources had begun
soon after the isolation of penicillin.   A sample of
mould isolated by Brotzu from a sewerage outlet in
Sardinia was sent to Abraham at Oxford in 1945.   This
was identified as Cephalosporium acremonium.   Work on
this sample resulted in the isolation of a substance
which had poor activity but was stable to the
penicillanases.   It was only after a great deal of
perseverance that the structure of this substance was
finally identified and reported by Abraham in 1963.[7]
The novel structure was named cephalosporin C (12)
(Scheme 3) and was found to be related to penicillin, but
had a dihydrothiazine ring, rather than a thiazolidine
ring fused to the β-lactam ring.   The experience gained
with penicillins suggested that the activity of this
antibiotic may be improved by modification of the
acylamino side chain in the C-7 position.   It was not
possible to remove the side chain, either enzymatically
or from a precursor-starved medium, in order to obtain
the corresponding intermediate 7-amino cephalosporanic
acid (7.A.C.A.) (13) (Scheme 4).   The acylamino side
chain removal was finally achieved chemically by the use
of nitrosyl chloride (NOCl), for diazotisation of the
α-amino group of the α-aminoadipoyl side chain in
cephalosporin C (12).   This then underwent an intra-
molecular cyclisation to result in an imino lactone which
readily hydrolysed to the amino derivative (13) on
aqueous work-up.   Poor yields were initially obtained
but were improved by workers at Eli Lilly.[8]   Availability
of (13) on a large scale, enabled workers to produce many
acylamino side clain analogues of higher activity than
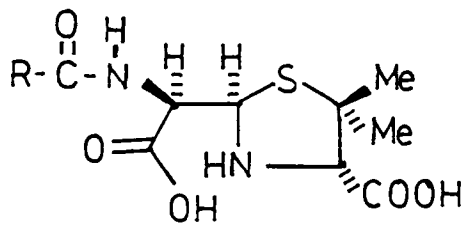
(11)

Scheme 2   Structure of penicilloic acid.
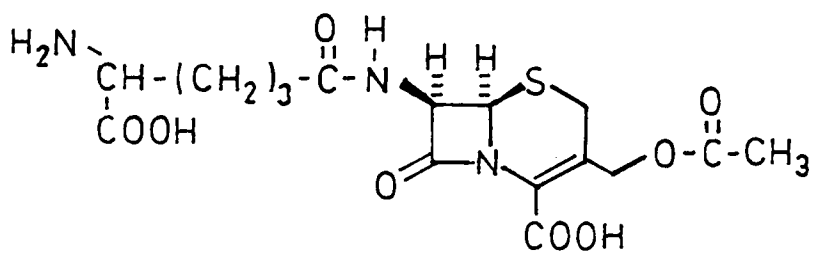
(12)

Scheme 3   Structure of cephalosporin C.
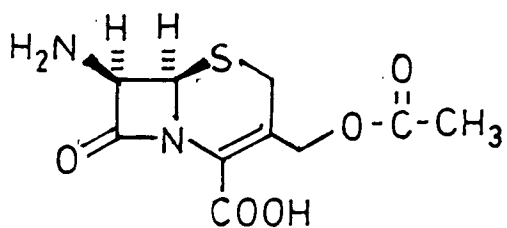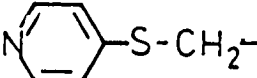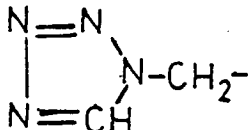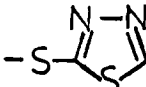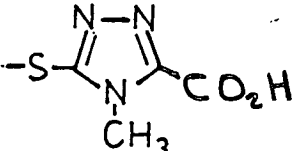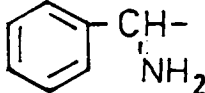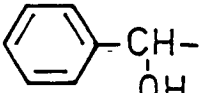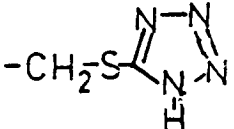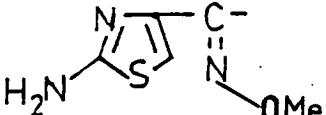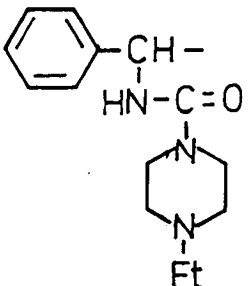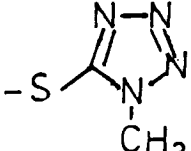
(13)

Scheme 4   Structure of 7-amino cephalosporanic acid.

the parent system (Table III). In addition, the
cephalosporin derivatives were more expensive than
penicillins due to the low yielding strains and also due
to the extra chemical step involved in the manufacture of
the intermediate (13). The first commercially available
cephalosporin was cephalothin (14) (Scheme 5), which
was shortly followed by cephaloridine (15) (Scheme 6).
These derivatives were found to be active against
penicillinase-producing bacteria and also in treating
patients possessing allergy to penicillins. Furthermore,
the cephalosporins were found to be more acid stable, and
had a broader spectrum of activity than penicillin,
however, they were less potent than penicillins against
non-resistant organisms. During the use of
cephalosporins, it became apparent that some bacteria
also produced cephalosporinases. This made these
derivatives useless against the growing number of
resistant strains of bacteria. Work progressed with the
same urgency, as when the penicillinases were first
discovered, although now, with controls on the use of the
these antibiotics, there was less abuse of these drugs
and consequently, the problem of resistance was not so
wide-spread.

The chemical investigations on the stereochemical
requirements, at this stage of development for the
β-lactam antibiotics, concluded that for a β-lactam
derivative to possess activity a number of stereo-
chemical aspects and the nature of peripheral
substituents must be satisfied in the molecule.
Firstly, the β-lactam bond must be intact and strained.
The infra-red spectrum was an essential monitor in this
respect, since the β-lactam carbonyl stretching frequency is
a characteristic feature of these derivatives. The
exact position of the carbonyl stretching signal varied
in accordance with the strain involved in the β-lactam
ring. A higher β-lactam carbonyl stretching frequency,
in the infra-red spectrum (1790 cm^{-1}) indicated a more
reactive nature of the β-lactam bond which suggested a

Table III Side chains of semi-synthetic cephalosporins

R[1]	R[2]	Name
$N\equiv C \cdot CH_2-$	$-OCOCH_3$	cephacetrile
(pyridyl)$-S-CH_2-$	$-OCOCH_3$	cephapirin
(tetrazolyl)$-CH_2-$	$-S-$ (thiadiazole)	cefazolin
$CF_3 \cdot S \cdot CH_2-$	$-S-$ (triazole, CH_3, CO_2H)	cefazaflur
(phenyl)$-CH(NH_2)-$	H	cephalexin
(phenyl)$-CH(OH)-$	$-CH_2S-$ (tetrazole)	cefamandole
(aminothiazole, $=N-OMe$)$-C-$	$-OCOCH_3$	cefotaxime
(phenyl)$-CH(HN-C=O, piperazine, Et)-$	$-S-$ (tetrazole, CH_3)	cefoperazone

(14)

Scheme 5   Structure of cephalothin.
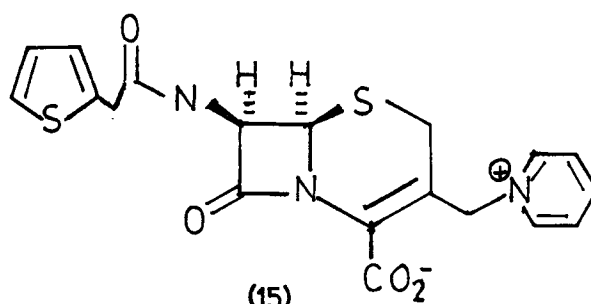
(15)

Scheme 6   Structure of cephaloridine.
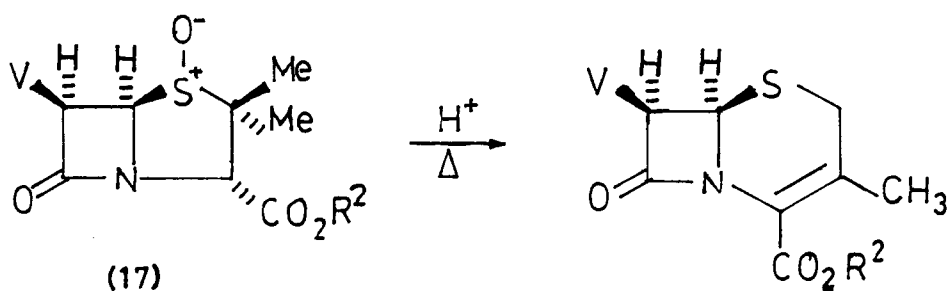
(17)

Scheme 7   Penicillin to cephalosporin conversion.

highly active antibiotic. The relative stereochemistry
of the two β-lactam ring protons was expected to be cis
and with R configuration for both penicillins and
cephalosporins. The second chiral postion (C-3) in
penicillins was also expected to be R. The fused ring
to the β-lactam ring could only be five or six atoms in
size and with a sulphur atom present. Larger rings were
found to be devoid of any activity and replacement of
sulphur, in penicillins, by oxygen, nitrogen or carbon
resulted in very unstable derivatives which were also
devoid of any bioactivity. In addition, an acidic
functional group in the α-position to the β-lactam
nitrogen, was deemed essential in the fused system. The
acylamino side chain was the only type of side chain that
was found to impart biological activity in both
penicillins and cephalosporins. In the case of
cephalosporins,it. was found that only the Δ^3-double bond
imparted biological activity and the substituent in the
C-3$'$ position needed to be a good leaving group.

At this stage, only total synthetic approaches
were attempted towards modified fused systems. Attempts
to selectively open the thiazolidine ring in penicillins,
also resulted in opening of the β-lactam ring. An
important finding was reported by Morin in 1963.[9] This
involved the first chemical conversion of a penicillin to
a cephalosporin derivative by refluxing a penicillin
sulphoxide (16), (Scheme 7), under acidic conditions and
in an inert solvent [This transformation will be
discussed further in the Synthesis section]. This was an
important discovery for a number of reasons. Firstly,
cheap penicillins could be converted to the more
expensive cephalosporin derivatives. Secondly, it was a
means of opening the thiazolidine ring without cleaving
the β-lactam system which then made it possible to
undertake many semi-synthetic approaches towards novel
structures. The use of these cheap chiral starting
materials with the correct stereochemistry around the
β-lactam ring was a distinct advantage over the totally

synthetic approach. Control of the reaction conditions
resulted in chiral products reasonably efficiently, and
these could be used to explore structure-activity
relationships much more rapidly compared to earlier
methods.

Initially, screening   , of micro-organisms
for novel β-lactam antibiotics were confined to a variety
of fungi from different sources. However, in majority
of the cases only penicillins or cephalosporins with a
variety of acylamino side chains were isolated. As a
result, the screening procedures were expanded to various
strains of bacteria.

The Actinomycete species were the only Gram-
positive bacteria which were found to produce β-lactam
containing derivatives. The first of these novel
structures was reported in 1971 by Nagarajan, and
isolated from a strain of Streptomyces lipmani.[10] These
structures, (scheme 8), possessed β-lactamase resistant
properties. This discovery gave rise to the development
of many methods for functionalisation of the C-6 and C-7
position of penicillins and cephalosporins respectively.

In 1976, Japanese workers published a report on
the isolation of a monocyclic β-lactam derivative from a
strain of Nocardis uniformis.[11] This they called
nocardicin A (18), (scheme 9). Later investigations
resulted in a number of related structures (B-G) (scheme
10). Only nocardicin A (18) had any significant·
activity, compared to the rest of the derivatives, but
even this was very weak compared to the penicillins,
cephalosporins and cephamycins. In the same year,
workers at Beecham reported a novel strcture from a
strain of Streptomyces clavuligerus (ATCC 27064).[12]
This was called clavulanic acid (19) (scheme 11) and was
found to have moderate antibacterial activity but
possessed a very high specificity for β-lactamases.
This important observation started a novel approach to
the treatment of infections from β-lactamase producing
bacteria. It was envisaged that by combining a

$$R^1 = O-\overset{O}{\underset{\|}{C}}-CH_3$$

$$= O-\overset{O}{\underset{\|}{C}}-ONH_2$$

(17)

Scheme 8   Structure of cephamycins.

(18)

Scheme 9   Structure of nocardicin A.

Scheme 10   Structure of nocardicin isolates.

β-lactamase inhibitor, such as clavulanic acid (19), and a highly active penicillin or cephalosporin, depending upon the physiological and biochemical compatability, the resistance could be overcome and the infection treated successfully. The intriguing structural aspect of clavulanic acid was that it possessed no acylamino side chain. The ring fused to the β-lactam bond contained an oxygen rather than a sulphur atom and the gem-dimethyl group of penicillins was replaced by an allyl alcohol group.

Two years later, workers at Merck, Sharp and Dohme reported the isolation of a very highly active substance from Streptomyces cattleya.[13] This potent broad spectrum antibiotic possessing natural β-lactamase resistant properties was called thienamycin (20) (scheme 12). It also had the added property of being highly active against normally resistant Gram-negative bacteria such as Pseudomonas aeruginosa and Serratia marcescens. However, the main drawback was its instability in aqueous solutions, even under neutral conditions. Access to (20) is only possible by synthesis, since isolation by fermentation methods leads to low yields and a mixture of products. After investigation of many synthetic analogues of (20), Merck have finally managed to obtain one which retains the potency and the β-lactamase resistance properties of (20) but also a greater stability to aqueous solutions. This was called formimidothienamycin (21) (scheme 13) and is commonly known as "Imipenem".[14] The striking features of these carbapenem systems were the novel R-1-hydroxyethyl side chain at C-6; the trans stereochemistry and 6S and 5R configuration of the β-lactam protons; the Δ^2 double bond in the fused ring and also the methylene group in place of a sulphur atom. All these features appear to contradict the requirements that, only a few years earlier, were thought to be essential for any biological activity of the penicillin/cephalosporin type system. Furthermore, workers at Beecham, searching for
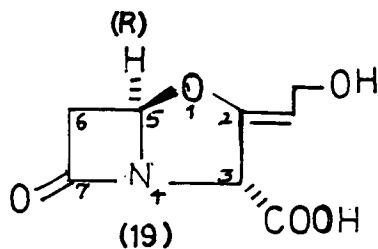
Scheme 11   Structure of clavulanic acid.
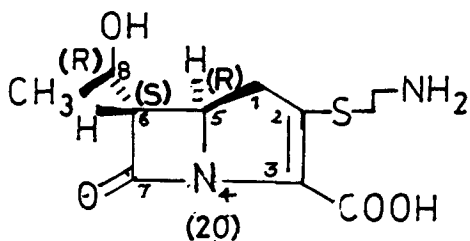
Scheme 12   Structure of thienamycin.
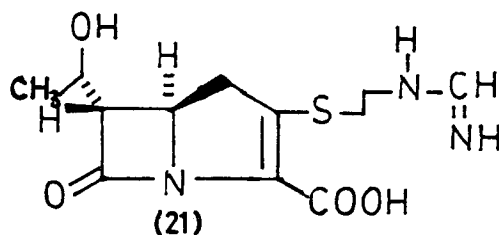
Scheme 13   Structure of formimidothienamycin.
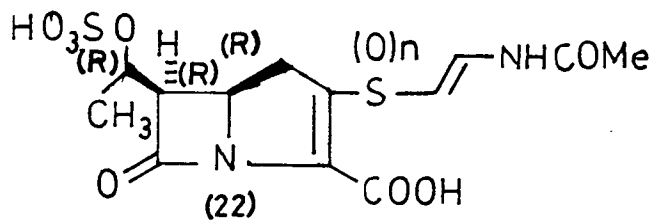
n = 0
  = 1

Scheme 14   Structure of olivanic acid .

β-lactamase inhibitors, also reported an independent discovery of a carbapenem system, from a strain of Streptomyces olivaceus.[15]   This was called olivanic acid (22) (scheme 14), and was found to be a potent β-lactamase inhibitor, similar to clavulanic acid.

The most significant finding in the 1980 s was made simultaneously by two independent research teams at Takeda and Squibb.[16]   They reported the isolation of a monocyclic β-lactam derivative, from various strains of Gram-negative bacteria.   This monobactam was called sulfazecin (23) (scheme 15), and was found to be highly resistant to β-lactamase producing bacteria.   This was attributed to the 3-methoxy substituent.   However, (23) was found to have low biological activity against Gram-negative bacteria.   It was hoped that this could be improved by modification of the acetamido side chain. Work with a variety of 3-acylamino substituents finally resulted in aztreonam (25) (scheme 16), a parenterally administered antibiotic with high activity against Gram-negative bacteria, particularly Pseudomonas species, but minimal activity against Gram-positive bacteria.

Further detailed investigations of the recently identified β-lactam producing bacteria have shown that these bacteria produce many analogues of the parent systems, such as the carbapenem system, which has resulted in a number of analogues, namely epithienamycins (S. flavogriseus),[17] carpetimycins (Streptomyces species),[18] and more recently, asparenomycins (S. cattleya).[19]

With the rapid developments in the area of microbiology, particularly the field of "genetic engineering", work is underway to produce mutant moulds or bacteria which would hopefully synthesise novel β-lactam derivatives which are as potent as those presently used, and which possess novel side-chain substituents.   These side chains might give rise to physiological compatibility for other potent derivatives which are made useless at present due to their

Takeda   Squibb
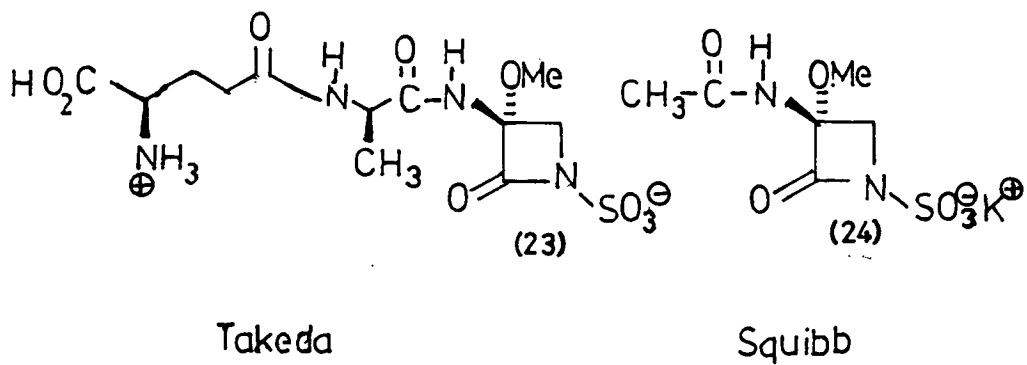
Scheme 15   Structure of monobactams.
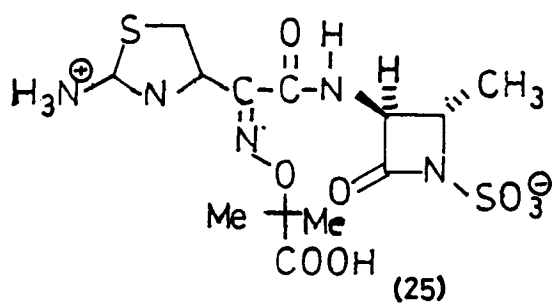
Scheme 16   Structure of aztreonam.

instability under <u>in vivo</u> conditions.

In concluding this section, a word of warning must be said.   Unless chemists can develop a potent unnatural antibiotic, for which bacteria are unable to find a resistance, future bacteria may become super-resistant and our present armoury of antibiotics, including tetracycline, aminoglycosides, macrocycles, and the β- lactam family, may all become useless.   It is vitally important, for future generations, that strict controls on the use of these antibiotics be brought into practice to prevent wide spread resistant strains from developing.   Furthermore, the adaptable nature of bacteria to their environment makes it inevitable for them to produce resistance to the antibiotics in use. Consequently, understanding the methods of resistance employed by bacteria and means of "switching them off" selectively, when the bacteria have infected a host, may allow the control of this serious problem, rather than allowing it to escalate to epidemic proportions for future generations to solve.   Finally, screening methods have been very successful in producing novel natural structures, at the same time giving rise to a higher resistance problem; the solution to this paradox, however, lies in the 'hands of the chemist'.

2.0.0 Biological aspects of β-Lactam Antibiotics

2.1.0 Mode of Action of β-Lactam Antibiotics

The bacterial cell differs from that of the
mammalian cell in that the former possesses a thick cell
wall. It has been found that the cell wall of
Gram-positive bacteria is largely composed of a
peptidoglycan layer with which are associated
polysaccharides and teichoic acids. The rigid structure
of the cell-wall is attributed to cross linkings within
the peptidoglycan layer. The cell wall of the
Gram-negative bacteria possess two distinct layers.
The outer layer of the wall consists of
proteins, lipopolysaccharides
and lipoproteins. The inner layer of the wall consists
of a thin rigid layer of peptidoglycan. Early
experiments on bacteria treated with sub-lethal doses of
penicillin showed that large amounts of cell wall
constituents were released into the media. This type of
observation led Strominger and Tipper,[20] in 1965, to
propose the structural analogue theory for penicilins.

The peptidoglycan layer of the cell wall is
composed of a complex network of linear heteropolymers.
These are cross-linked by short pentapeptide strands
(consisting of glycine in S. aureus), in order to produce
a rigid overall structure. This is essential for a
growing bacterium. The terminal end of the pentapeptide
strand is found to be connected to a D-alanyl-D-alanine
unit, the peptide chain being elongated via the
C-terminus, the terminal D-alanine is removed by a
carboxypeptidase and the glycan strand joined with an
amino group from another polymer strand, via a
transpeptidase. This process occurs throughout the
matrix of the peptidoglycan.

Strominger and co-workers proposed that D-alanyl-
D-alanine was the portion of the cell wall to which
penicillin was an analogue. By acylating either the
carboxypeptidase or the transpeptidase, the cell wall
structure would weaken and consequently, this would

rupture the growing bacterium due to the enormous rise in
the internal osmotic pressure.

This simple model is a good explanation for the
observations.   However, the activity of such diverse
structures as nocardicins and monobactams, clearly shows
that the above model is limited.   Furthermore, improved
purification and separation techniques in the field of
microbiology have shown that the exact process of the
action of β-lactam antibiotics is much more complex.[21]

2.2.0 β-Lactamase Inhibitors and their mode of action

The increasing number of bacteria becoming
resistant to the β-lactam antibiotics has become a growing
problem for the clinician.   The methods of resistance
employed by bacteria are as follows: (i) production of
enzymes called β-lactamases, which selectively cleave the
β-lactam bond;   (ii) modification of the cell wall in
order to inhibit the penetration of the antibiotic and
(iii) a reduction of the growth rate in the presence of
the antibiotic.

This section will only be concerned with the first
method, since the study of inhibition of β-lactamases is
probably the easiest for a chemist.

The β-lactamases produced by various resistant
bacteria have one factor in common;   they selectively
cleave the β-lactam bond in this class of antibiotics.
The exact nature of these enzymes varies a great deal
between species and also subspecies.   This is possibly
the reason for the absence of an ideal inhibitor to these
enzymes.   Excellent reviews are available regarding the
types of β-lactamases produced by organisms and their
modes of inactivation.[22,23]

This account will only give the summary of the
chemical aspects of β-lactamase inhibition by the various
derivatives that are available.

Inhibition of β-lactamases occurs either reversibly or irreversibly (scheme 17). Normally, β-lactamase sensitive derivatives (such as penicillins) are inactivated rapidly, followed by regeneration of the β-lactamase by hydrolysis of the intermediate complex (path a). The β-lactamase inhibitor also forms an intermediate complex of the type (28). This complex may involve a covalent bond (30) or simply a strong interaction between the enzyme and the inhibitor (28). Hydrolysis of complex (28) may be reversible or irreversible. The reversible hydrolysis may be sufficiently slow to allow the concentration of the active β-lactam antibiotic to rise above the tolerance level required for the survival of the bacterium thus leading to the death of the organism.

The covalent complex (30) can carry out a number of possible pathways (a - c) (scheme 17). The most common is Path a for most β-lactamase sensitive antibiotics where the enzyme is regenerated and the β-lactam derivative is decomposed; the rate of hydrolysis for the inhibitor is usually much lower than for a non-inhibitor. Good β-lactamase inhibitors normally undergo Paths b or c in which the enzyme gets inactivated and the β-lactam derivative undergoes a number of rearrangements and remains bonded to the enzyme.

Clavulanic acid (19) was the first β-lactamase inhibitor discovered by workers at Beecham.[12] This was found to undergo the rearrangement as in scheme 18. The enzyme would become irreversibly inactivated by this inhibitor. However, an excess of the inhibitor was usually necessary to inactivate the enzyme irreversibly.

Synthetic studies of penicillin derivatives which were thought to have β-lactamase inhibiting properties, fortuitously, led to the 6-β-bromo-penicillanic acid (34) (scheme 19) and later to penicillanic acid sulphone (36) (scheme 20). Both these derivatives are found to have good β-lactamase inhibiting properties. (34) was believed to further rearrange to a thiazolidinone-enzyme
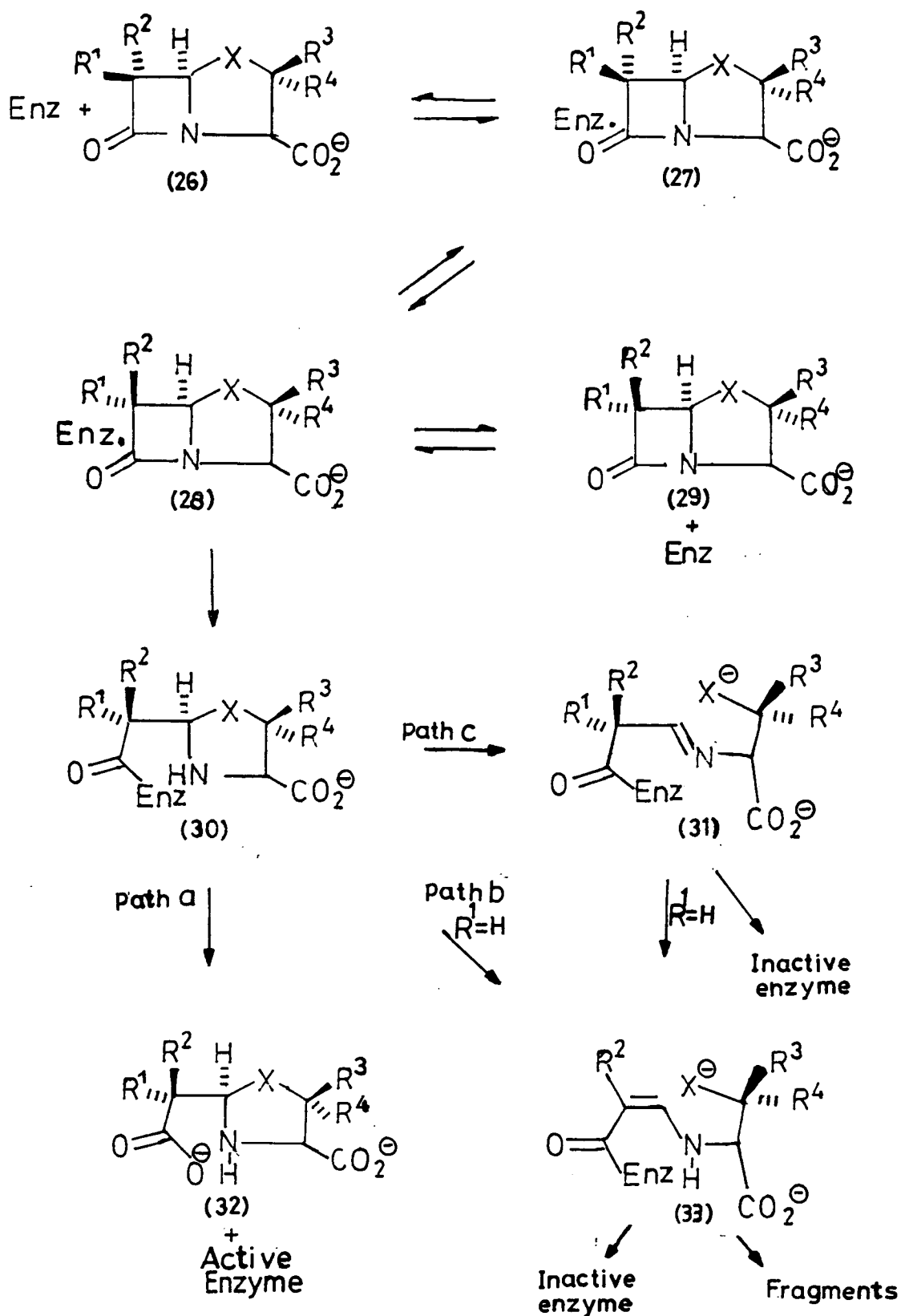
Scheme 17 General scheme for β-lactamase inactivation of bicyclic β-lactams.

derivative (35) (scheme 19).

The carbapenem derivatives have the unique property of being highly active antibiotics with potent β-lactamase inhibition or β-lactamase resistant properties. The olivanic acids (22), discovered by workers at Beecham,[15] were found to be potent β-lactamase inhibitors; the inhibition was observed to be irreversible. The detailed mechanism has not been reported as yet. However, a possible mechanism is given in Scheme 21. The instability of the pyrroline system may give rise to a reaction within the active site of the β-lactamase, thus, leading to irreversible inactivation of the enzyme.
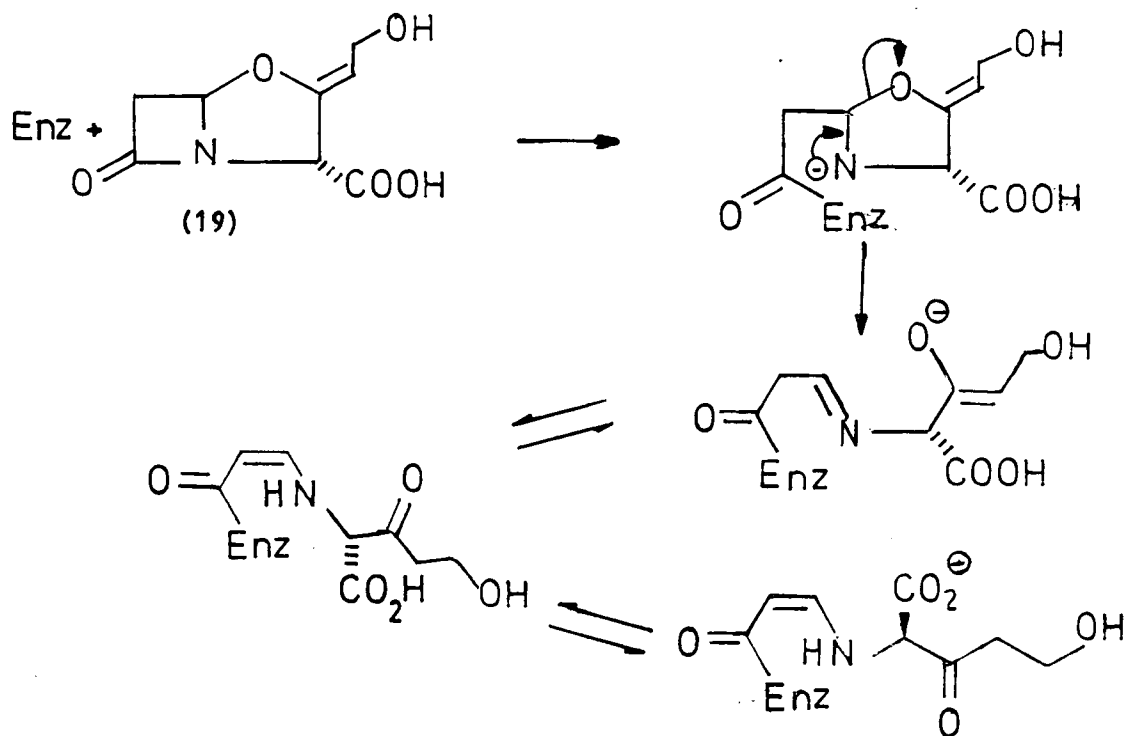
Scheme 18
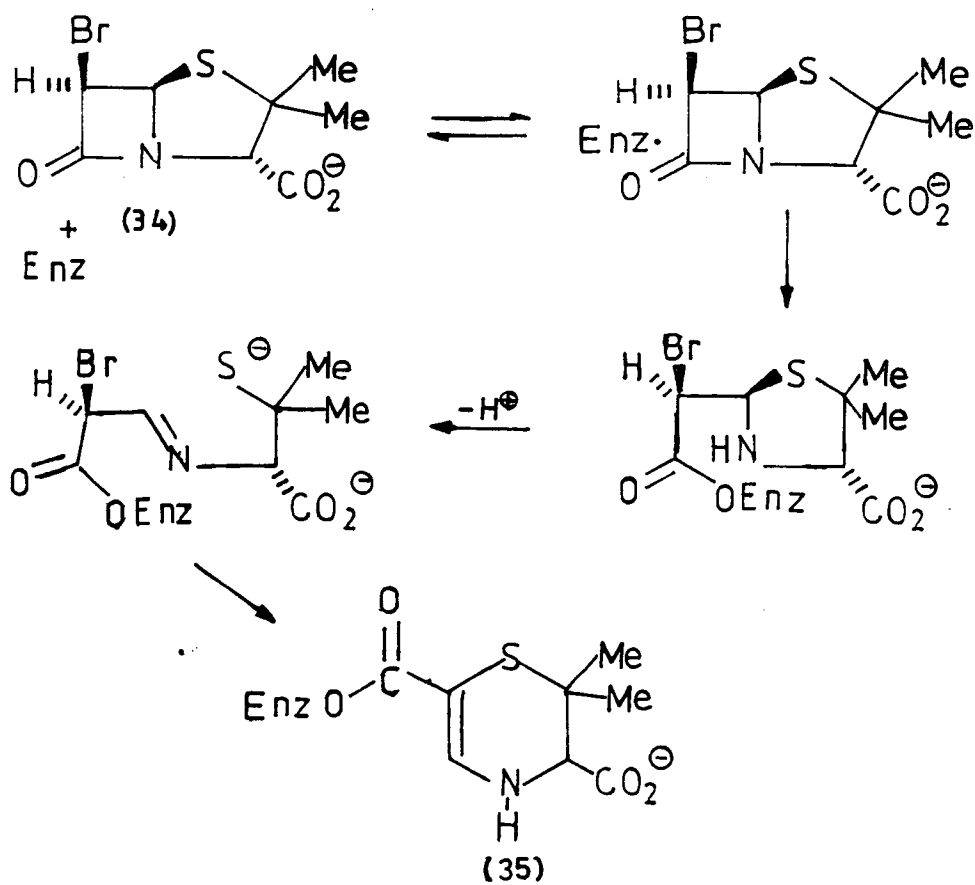
Scheme 19
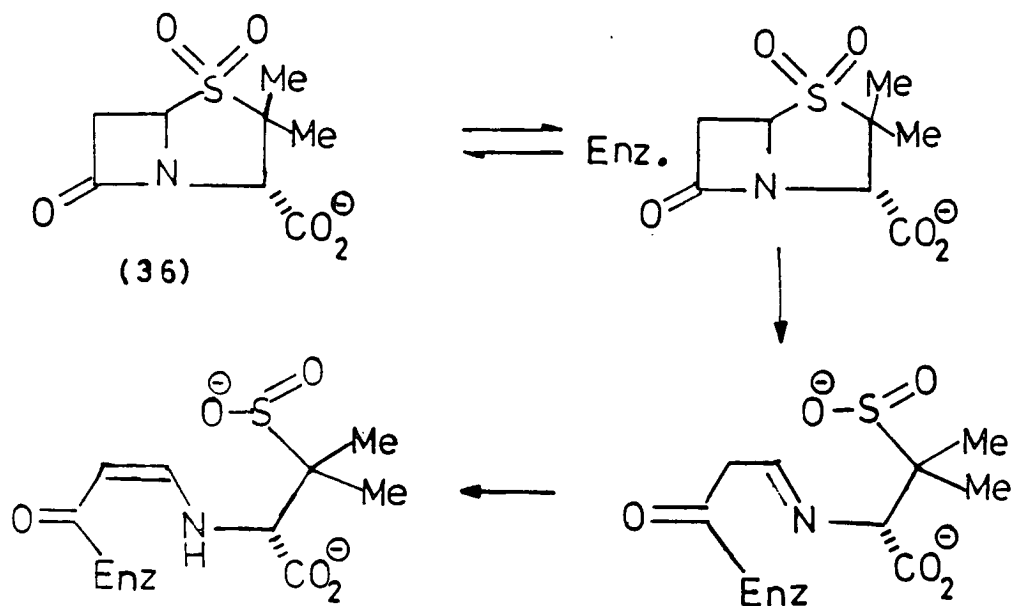
Scheme 20
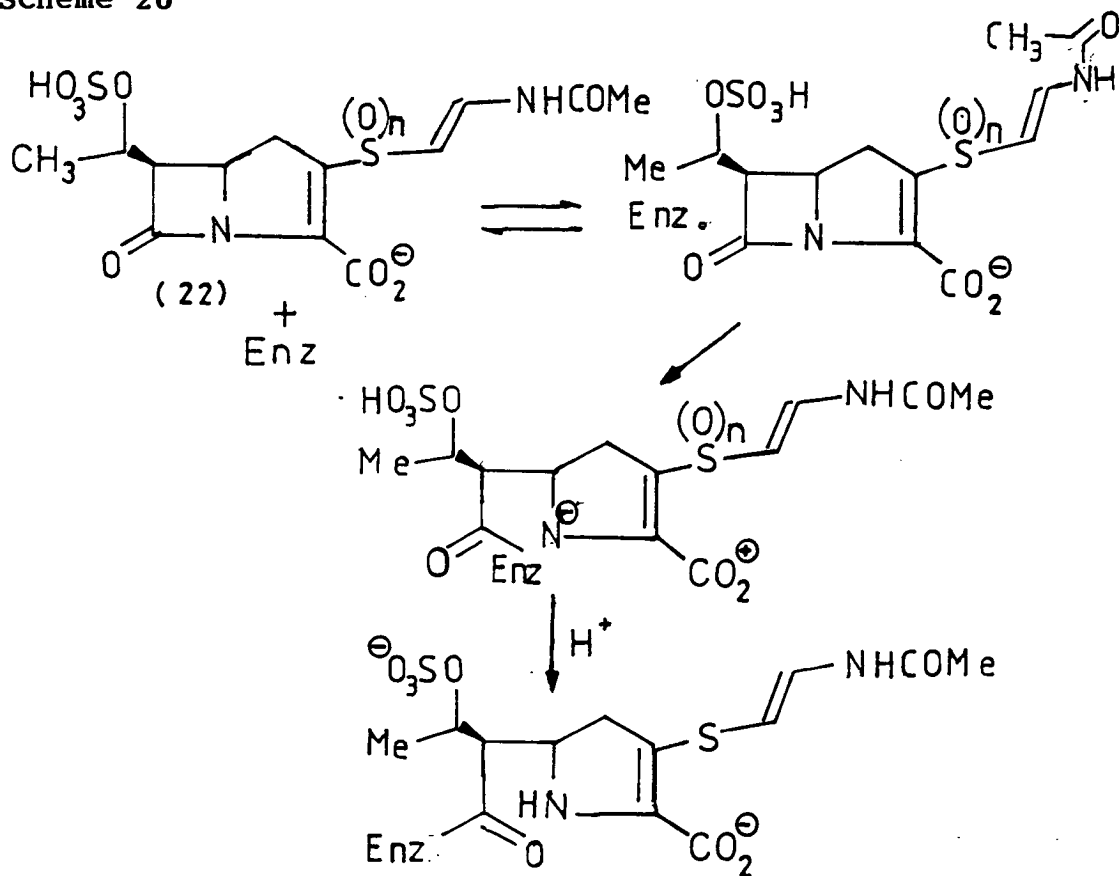
Scheme 21

2.3.0 BIOSYNTHESIS OF β-LACTAM ANTIBIOTICS

2.3.1 Introduction

Penicillin biosynthesis has been studied since its first isolation from a Penicillium mould. The purpose of this research was to determine the mechanism of its biosynthesis and to find out the use of these metabolites by the organism and finally to investigate conditions which would maximise the production of penicillin.

The early work, during the 1940's involved intact cells of a Penicillium chrysogenum culture. Consequently, progress was slow due to the problems involved in the interpretation of the inhibitive or activating effects of the substrates to penicillin synthesis, and to the poor uptake of isotopically labelled amino acids or short peptides (thought to be potential precursors) by the intact cells.

The work by Arnstein and co-workers[24] in the 1960 s, suggesting that a tripeptide, α-aminoadipoyl-cysteinyl-valine (37) (scheme 22), was a precursor to penicillin has since been substantiated.[25] The significant breakthrough in this area came in the seventies when cell free extracts and enzymes responsible for the transformations on the biosynthetic pathway were isolated in a purified form from Penicillium chrysogenum and Cephalosporium acremonium.[26,27,28]

The biosynthesis of penicillin (1), cephalosporin C (12) and cephamycin (17) is now believed to be involved in a single stepwise pathway (scheme 23). A great deal of work has been done with these purified enzymes as may be seen from the number of publications in the literature.[29] Furthermore, the isolation of novel β-lactam structures, including nocardicin A (18), clavulanic acid (19) and thienamycin (20) from a variety of Gram-positive bacterial species, has stimulated further work on the isolation of the enzymes involved in the biosynthesis of the respective structures. Work with isotopically-labelled substrates

(37)
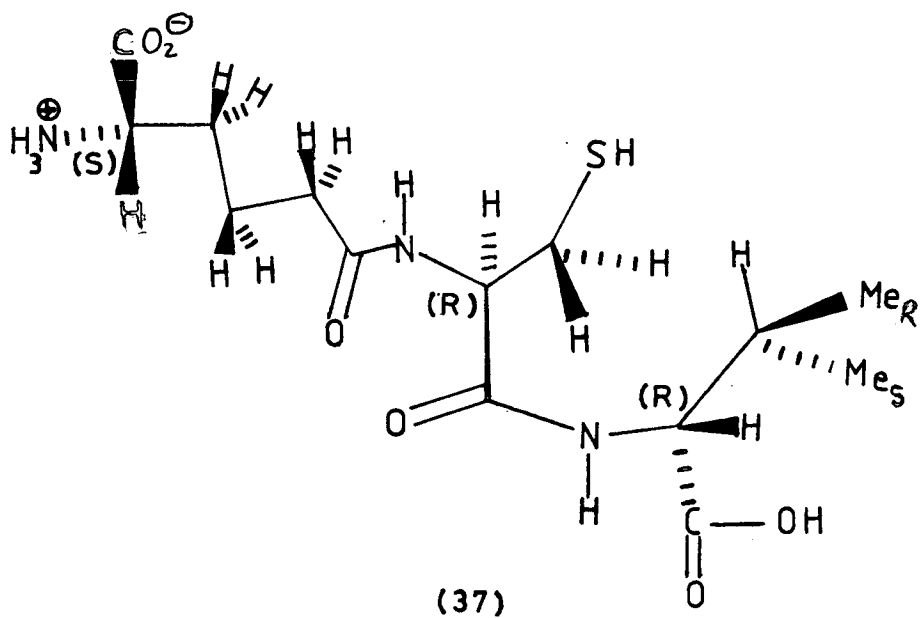
Scheme 22   Structure of Arnstein's ACV tripeptide.

was undertaken in order to determine the biosynthetic pathways to these useful natural products.[30]

The presently accepted penicillin and cephalosporin biosynthetic pathway (scheme 23) will be dealt with briefly; only the conclusions and some relevant experimental data in support of them will be given. The biosynthetic studies on the novel structures, nocardicins, clavulanic acid and thienamycin, are still in their very early stages.

2.3.2 Biosynthesis of penicillin, cephalosporin and cephamycin

The availability of isopenicillin N synthetase, in a purified form from both <u>Penicillium chrysogenum</u> and <u>Cephalosporium acremonium</u>, has made it possible to uncover many of the misunderstandings of the proposed biosyntheses of penicillins and cephalosporins. It is established that L-α-amino adipic acid (38), L-cysteine (39) and L-valine (40) are the starting precursors to the (LLD) (A.C.V.) tripeptide (37), as first suggested by Arnstein in 1957.[24] The interesting aspect is the inversion of L-valine to D-valine in the tripeptide. Labelling experiments with L-[2-^{3}H]-valine resulted in complete loss of label in the tripeptide.[31] Furthermore, double [^{18}O] labelled L-valine loses one of the oxygen atoms on being converted to isopenicillin N. This suggested that during the incorporation into the tripeptide, the carboxyl group of valine was protected as a thioester derivative. In addition, treatment of (37) with isopenicillin N synthetase in [$H_2^{18}O$] enriched medium did not incorporate any oxygen label in isopenicillin N (43), thus, confirming that the loss of ^{18}O had occurred during the formation of the tripeptide (37) rather than during the ring closure steps to isopenicillin N (43).
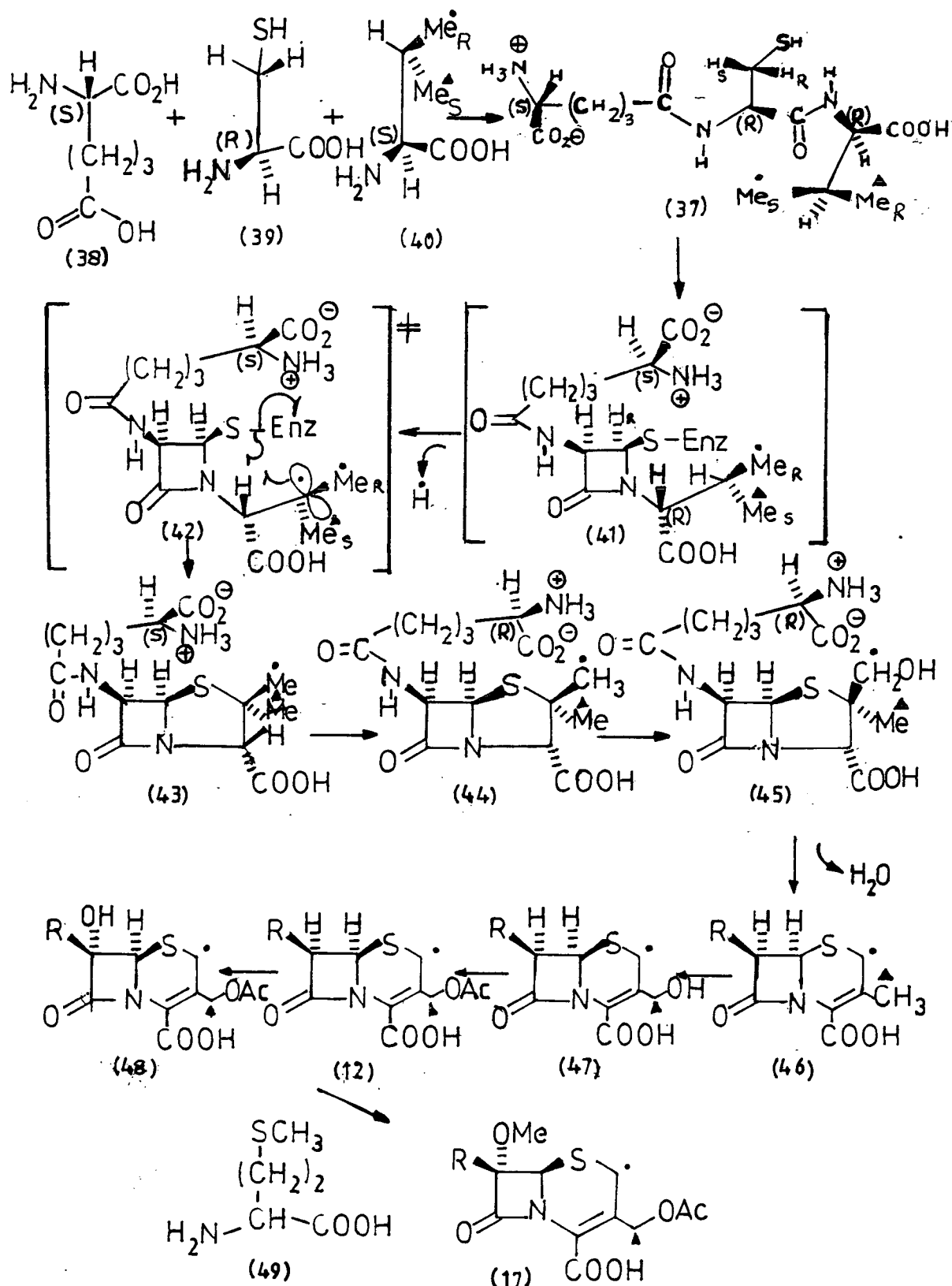
Scheme 23   Biosynthetic Pathway of Penicillins,
Cephalosporin C and Cephamycins

The β-lactam ring is formed first. The exact method is not known but it has been deduced from experiments in which the $3H_S$ proton of cysteine is lost stereospecifically. Consequently, the process occurs with retention.[32] Propositions have been made which suggest hydroxylation at C-3 of the cysteinyl residue followed by activation by phosphorylation which would result in nucleophilic displacement of the phosphate derivative by an amidino nitrogen and lead to ring closure. Intermediates of the type (50) (scheme 24) have been synthesised but cyclisations with the enzyme isopenicillin N synthetase have been unsuccessful. However, recent reports have indicated the successful cyclisation of (50) by use of biomimetic conditions using ascorbic acid, ferrous sulphate, oxygen and ethylenedia-minetetraacetic acid (EDTA); these make up the Udenfriends reagent. These experiments suggest that a free intermediate of the type (50) does not exist and is very likely to be present in an enzyme bound form, bound via the sulphur atom as in (41).[33] Consequently, intermediates of the type (50) would not be able to participate in the catalytic cycle. Although, it has been reported that an intermediate of type (50) has been isolated,[34] later reports have shown this finding to be unlikely due to the great instability of (50) even under neutral conditions.[35]

A great deal of evidence is available which suggests that the cyclisation of (41) to form the thiazolidine ring in isopenicillin N (43) occurs through a radical process.[36] Labelling experiments suggest that this cyclisation occurs with retention, and the methyl groups remain in the relative positions they occupied in the valine residue. This suggests that the rate of cyclisation must be much faster than that of rotation about the C-2 - C-3 bond of valine (42). On the other hand, the enzyme surface may be such that clefts are available for the methyl groups to fit into, and the steric factors present, would prevent any possible rotation about the C-2 - C-3 bond. The sulphur-carbon

(50)
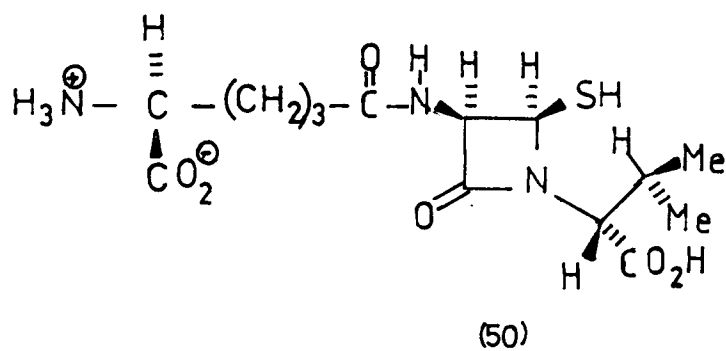
Scheme 24   Structure of a potential intermediate in the
biosynthetic pathway.

(53)
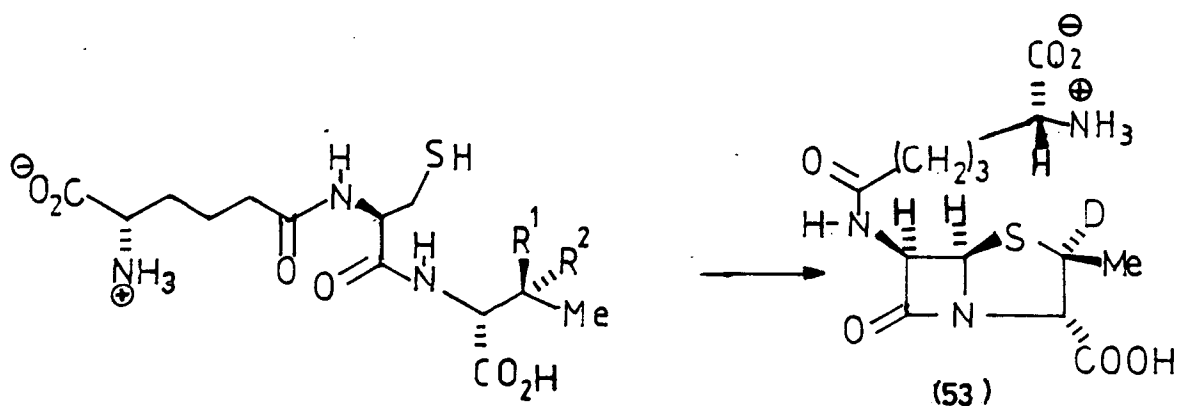
(51) $R^1 = D, R^2 = H$

(52) $R^1 = H, R^2 = D$

Scheme 25   Kinetic Isotope effect involved in the
thiazolidine ring cyclisation.

bond would thus be formed with retention, <u>via</u> a radical species. Work relating the nature of the intermediate involved in the cyclisation of the thiazolidine step (41 - 43), and the selectivity of isopenicillin N synthetase to available substrates, has shown that modified tripeptides can be converted to bicyclic fused ring systems with this enzyme. The distribution of the products confirms the intermediacy of a radical species (scheme 26). In addition, a large kinetic isotope effect is observed for intermediates isotopically labelled at the C-3 position corresponding to C-3 of valine (with deuterium), (Scheme 25), both substrates (51) and (52) result in (53). It must be noted that a 1:1 mixture of a tripeptide substrate labelled at C-3 on the valine residue, with deuterium, and an unlabelled tripeptide, was converted to isopenicillin N (43) without any isotopic discrimination between the two available substrates. This experiment also indicates that the β-lactam ring formation occurs prior to the thiazolidine ring formations.

The conclusions from these experiments suggested that during the ring closure with the enzyme, isopenicillin N synthetase, the carbon-hydrogen bond that would be preferentially broken is determined by the strength of the bond being broken, stability of the radical generated, and the steric requirement of the group adopting the α-face in the penicillin. It has been found that the more sterically demanding group adopts the more crowded β-face in the penicillin molecule. This would suggest that on the enzyme surface, the opposing side to the cleft must be sterically limiting; the strain generated by this situation may be the driving force for closing the thiazolidine ring in order to produce the highly rigid penicillin molecule. The fusion of the second ring to the β-lactam ring is, thus, determined by the subtle interplay between the strength of the bond being broken and the steric requirements of the appropriate groups.
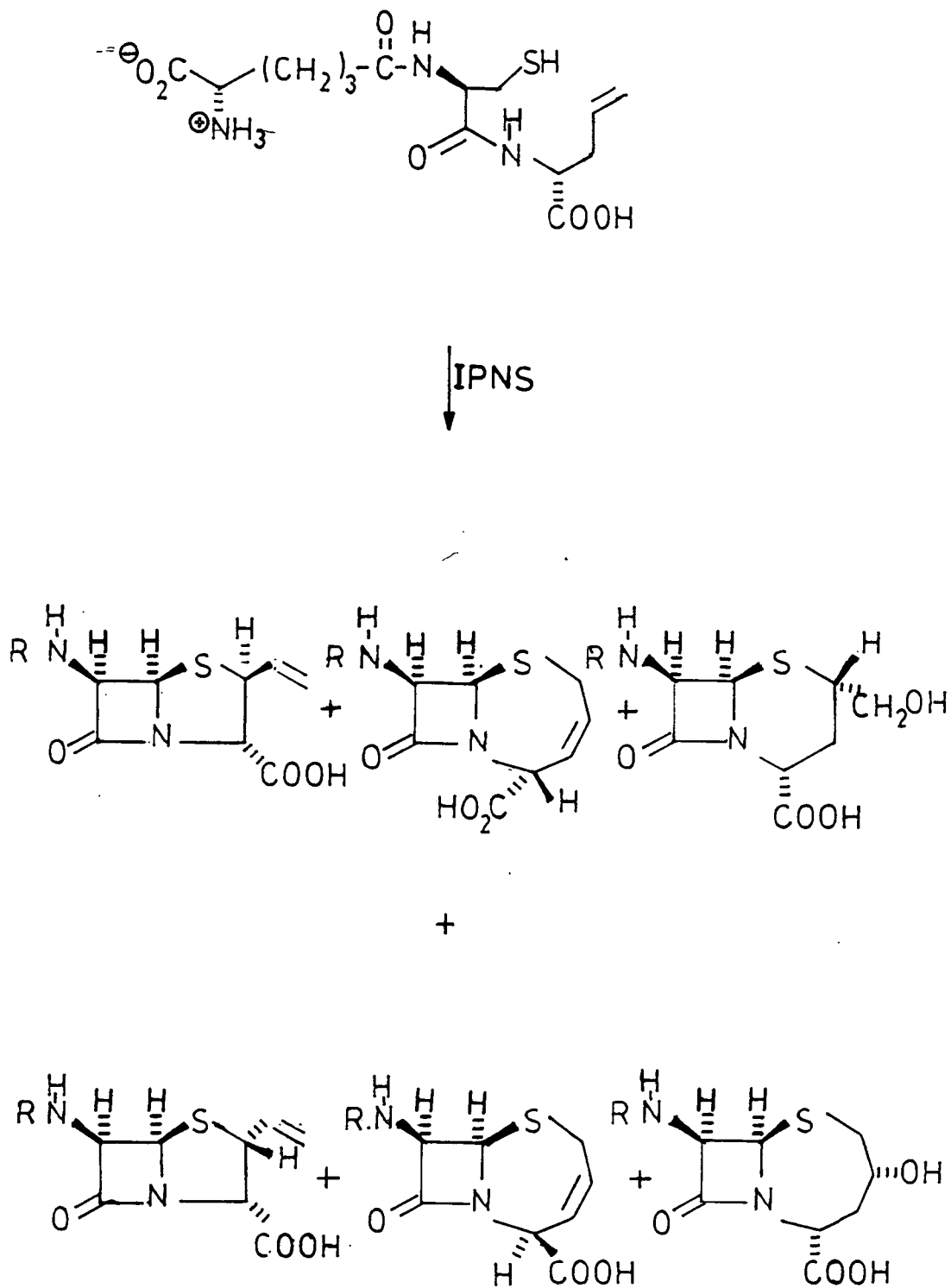
Scheme 26   Modified tripeptide cyclisation with
             isopenicillin N synthetase (IPNS)

The biosynthetic conversion of penicillin to cephalosporin is found to involve prior inversion of configuration of the α-amino adipyl side chain from (S) in isopenicillin N (43) to (R) as in penicillin N (44). An unstable epimerase has been detected in protoplast lysates of C.acremonium that converts isopenicillin N (43) to penicillin N (44), prior to its conversion to cephalosporin C (12). Consequently, in Penicillium moulds, only penicillin is produced from isopenicillin N. In Cephalosporium moulds, the epimerase would result in penicillin N (44) which would then be converted to cephalosporin C (12).

The exact process of the ring-expansion step is still a little doubtful, but the evidence favours hydroxylation. It has been found that the β-methyl group is involved in the ring-expansion step and becomes part of the cephalosporin nucleus, whilst the α-methyl group becomes the acetoxymethyl group and hence, the C-3$'$ position of the cephalosporin nucleus. Two enzymes are found to be responsible for the ring expansion and the hydroxylation steps. These have been identified as being dioxygenases[37] which require molecular oxygen, iron, ascorbate and 2-oxoglutarate for activity in both C.acremonium and Steptomyces clavuligerus. An attempt to separate the two enzymes present in C.acremonium failed. However, recently, the two enzymes from S.clavuligerus have been separated.[38]

The acetylation of (47) has been reported to involve acetyl Co-enzyme A.[39]

The biosynthesis of cephamycin (17) is reported to involve a further hydroxylase,[40] with similar co-factors for the ring expansion and successive steps to those of cephalosporin C (12). The methylation of the C-7 hydroxy derivative (48) involved methionine (49).[41] Aspects of the biosynthesis of cephalosporins and penicillins have been reviewed in detail.[42] Further excellent reviews have been published recently.[43,44]

2.3.3 Biosynthesis of the novel β-lactam derivatives

Nocardicin A (18) has been reported[45,46] to have derived its skeleton from two molecules of tyrosine, with a loss of the carboxyl group from each amino acid residue. Tyrosine is utilised via L-(p-hydroxyphenyl) glycine (54). The precursor of nocardicin A (18) is suggested as (55) (Scheme 27).

Clavulanic acid (19) has been reported[47,48] to be biosynthesised from a C_3 and a C_5 unit (scheme 28). The C_3 unit is formed from a C_3 intermediate of glycolysis, whilst the C_5 unit has its origins in a C_5 amino acid that is directly related to 2- oxoglutarate (56). Further results show that amino acids [ornithine (60), and arginine] of the urea cycle, particularly ornithine (60), are much better (15-20 times) precursors for Clavulanic acid (19) in S.clavuligerus than are glutamic acid (57), 5-hydroxynorvaline (58) and proline (59).[47] In an interconnected series of experiments, ^3H and ^{14}C labelled glycerol, pyruvate, serine, glycerate, and glycine, have been examined as precursors for the C_3 unit in clavulanic acid. The results have indicated that glycerate (61) is the most direct precursor to the C_3 unit of clauvlanic acid.[48]

The biosynthetic origins of the carbapenem antibiotics are not fully known and will thus not be dealt with. The recent review[44], however, does indicate some findings in this area which are still under investigation.

Scheme 27   Biosynthesis of Nocardicin A.

Scheme 28   Biosynthesis of Clavulanic Acid.

3.0.0 SYNTHESIS OF β-LACTAM ANTIBIOTICS

Bacterial resistance to β-lactam antibiotics has made it necessary for chemists to obtain derivatives which overcome this problem. Screening of micro-organisms has resulted in some novel structures which possess superior properties to the penicillins and cephalosporins.

The first modification that is usually undertaken on a novel structure involves altering the peripheral substituents on the parent ring system. This has been the most successful method of producing commercially acceptable antibiotics. The second option is to change the atoms within the fused ring system. The sensitivity of the β-lactam ring to acids and bases and to nucleophilic reagents makes the second type of modification particularly challenging. Furthermore, the atoms that can successfully replace sulphur in the penam and cephem systems are limited to carbon, oxygen and nitrogen, but, even these derivatives usually give rise to unstable bicyclic fused systems, particularly under physiological conditions.

A large number of excellent reviews covering the many developments in the synthesis of the β-lactam antibiotics are available.[49]   It is,therefore,intended that all peripheral modifications of the parent systems be excluded in this account.

The two major approaches that have been used for the synthesis of β-lactam antibiotics are total-, and semi-synthesis. The total-synthesis approach possessed many problems in the early years, particularly with the synthesis of the β-lactam ring, and of its conservation during the subsequent synthetic scheme to the target molecule, (either a penicillin or a cephalosporin). The overall yields were usually low, due to the large number of steps involved or due to the inefficiency of some approaches. A further problem was the lack of stereochemical control in most routes. Recently, this

problem has diminished since many asymmetric syntheses of β-lactam derivatives are being developed. On the other hand, the semi-synthetic approach involved the use of penicillin as the starting material and had the advantage that the substrate possessed the correct sterochemistry around the β-lactam ring. However, the major disadvantage was the necessity for selective cleavage of the thiazolidine ring in the penicillin molecule, whilst keeping the β-lactam ring intact.

It is intended that the scope of this account be limited to a brief resume of some important traditional routes to monocyclic β-lactam intermediates <u>via</u> both total synthesis and penicillin degradation methods, and then, primarily, this section will be concerned with the synthetic strategies which give rise to five or six membered rings fused onto the β-lactam ring. Since many novel derivatives have been synthesised, this account is restricted to the fused ring systems which commonly have biological activity.

3.1.0 MONOCYCLIC β-LACTAMS

Interest in monocyclic β-lactams has traditionally been aroused because of their potential for the synthesis of bicyclic fused systems, such as penicillins and cephalosporins. However, recently interest in their synthesis has been stimulated, in their own right, due to the isolation of monocyclic β-lactam derivatives which possess some biological activity such as nocardicin A (18) and the monobactams (23).

The synthesis of monocyclic β-lactam derivatives, with a variety of functional groups substituted around the ring, has been successfully achieved by many routes (schemes 29-32).[50-52]

The most successful and commonly used procedures involve [2+2] cycloaddition reactions, such as the ketene - imine reaction and the alkene - isocyanate method, and an intramolecular amide bond formation utilising the activating agent - dicyclohexylcarbodiimide (DCCI), as used in peptide synthesis. Furthermore, monocylic β - lactam derivatives are obtainable by the selective degradation of the penicillins, and then transformed into the desired derivatives. The semi- synthetic route is limited by the ability of the chemist to convert the available functional groups on the monocyclic β-lactam derivative (obtained from penicillin) into the required functional groups, under mild conditions which do not cleave the β-lactam bond.

The following section is confined to these two approaches for the synthesis of monocyclic β-lactam intermediates.

β- amino acids

β- halo acylamido

α- halo acyl-N,N-methyl-
carbonylalkylamido
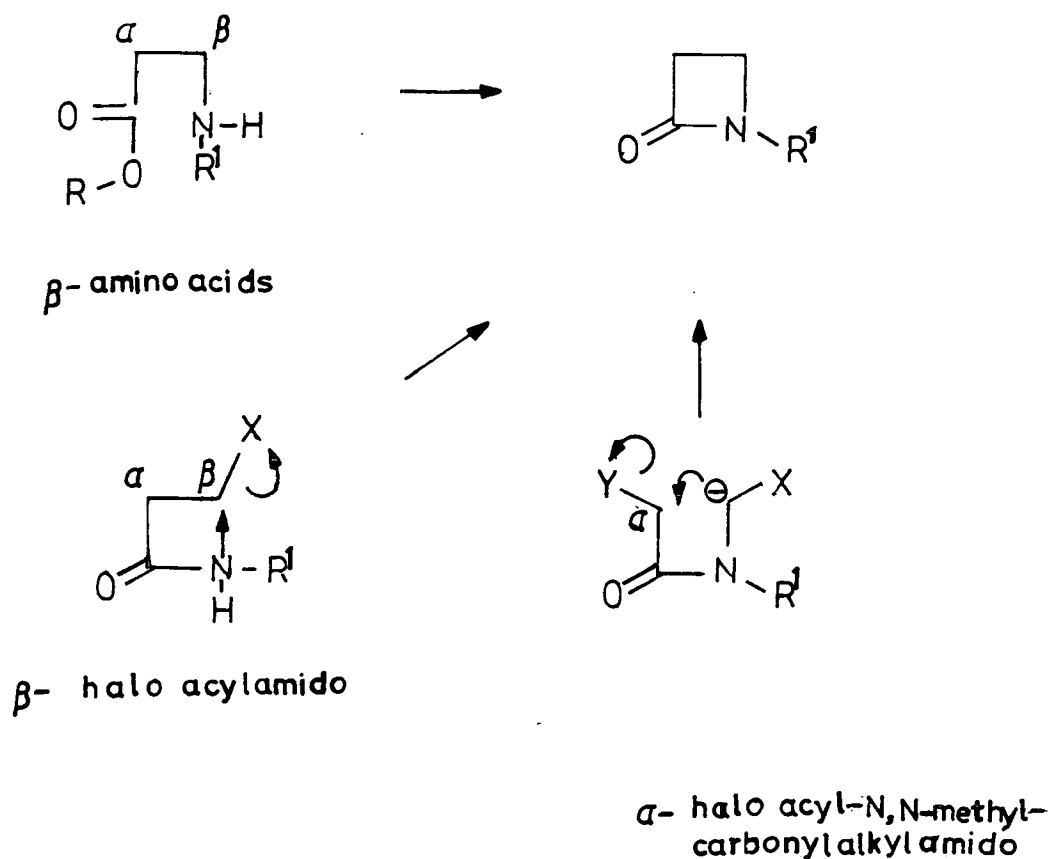
Scheme 29   Cyclisation of the requisite acyclic
            precursors.

Scheme 30   Ring Expansion.
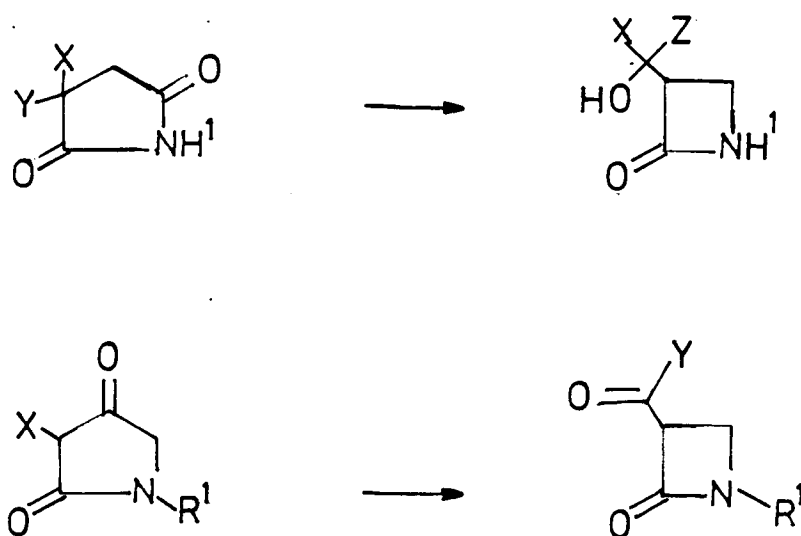
Scheme 31   Ring contractions.

Scheme 32   Oxidation of Azetidines.

(62)   (63)   (64)
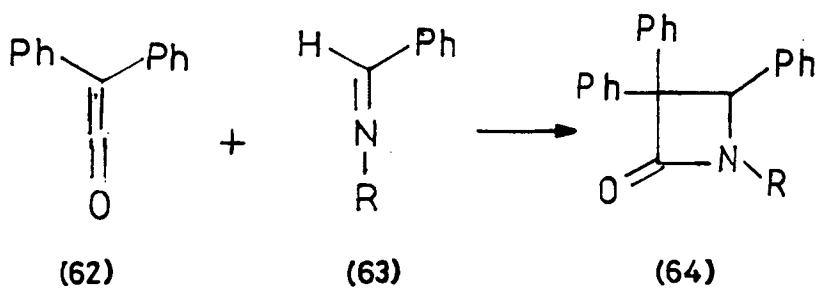
Scheme 33   Ketene-Imine Cycloaddition for β-lactam
             synthesis.

3.1.1 TOTAL SYNTHESIS OF MONOCYCLIC β-LACTAMS

a) The ketene-imine method for β-lactam ring formation.

The earliest synthesis of a β-lactam derivative (3) was reported by Staudinger in 1907.[53] This involved a ketene (62) addition to a Schiff base (63) (scheme 33). It was only after the β-lactam – thiazolidine structure had been established for penicillin that a resurgence of interest in this synthetic route occurred. This was the most direct route to the formation of the β-lactam ring, but it possessed some problems. The β-lactam ring is very sensitive to acidic and basic conditions in the fused ring system. It was,thus, necessary to have functional groups present in the starting materials (ketene and Schiff base) which could readily be converted to the desired ones, in the bicyclic fused system, and under mild conditions. Furthermore, handling of the ketene was made difficult due to the possibility of a [2+2] cycloaddition reaction involving two ketene molecules. Consequently, high dilution conditions were necessary. Unstable ketenes were much more difficult to handle. The imine derivative was required to be electron rich but with substituents present which would stabilise a possible carbocation or a radical intermediate. The [2+2] cycloaddition may occur via a diradical or zwitterion mechanism and suitable conditions were necessary for their successful stabilisation in order to obtain acceptable yields.

One of the earliest uses of this route in penicillin synthesis was reported by Sheehan and Corey in 1950 (scheme 34).[54] This method incorporated the amino group protected as a succinimide (65). The acyl chloride (65) readily generated the ketene (67) under basic conditions. Addition of the ketene (67) to the thiazoline (66) generated the bicyclic fused system (68), and, by subsequent hydrolytic cleavage of the amino
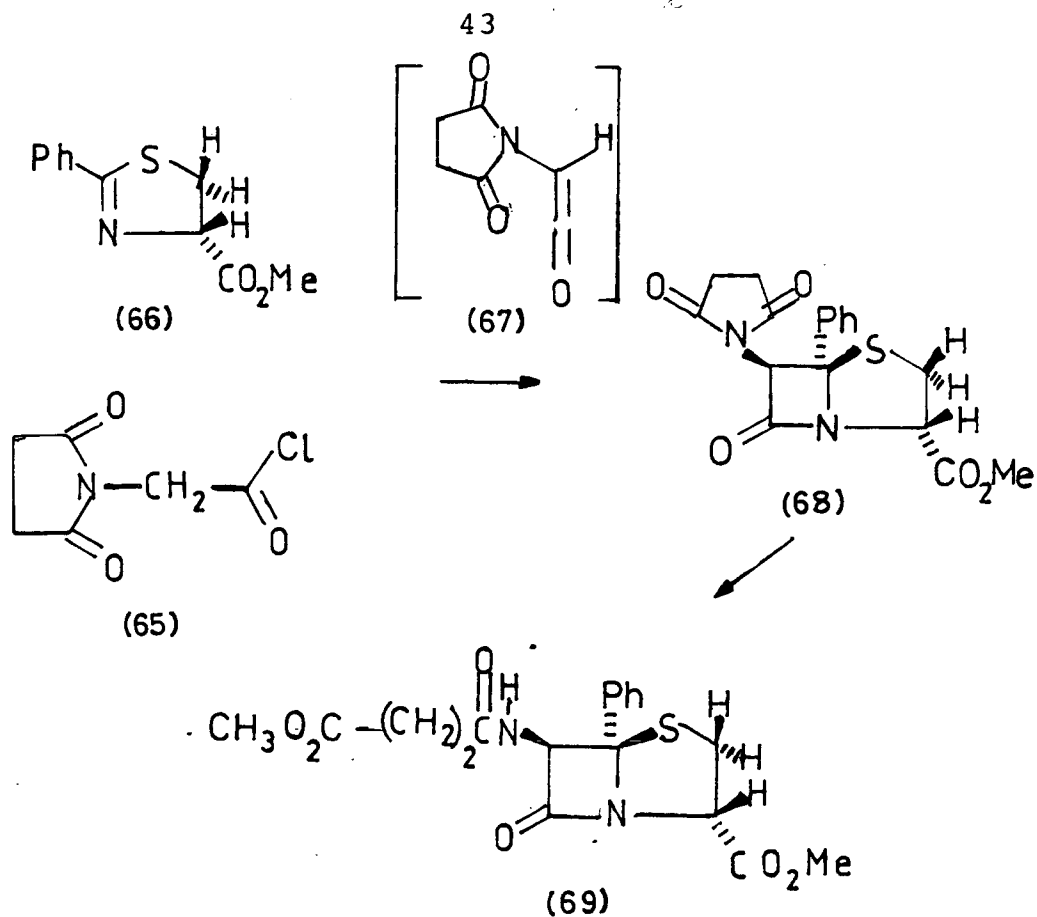
Scheme 34   Use of Ketine-Imine method for penicillin
             synthesis.
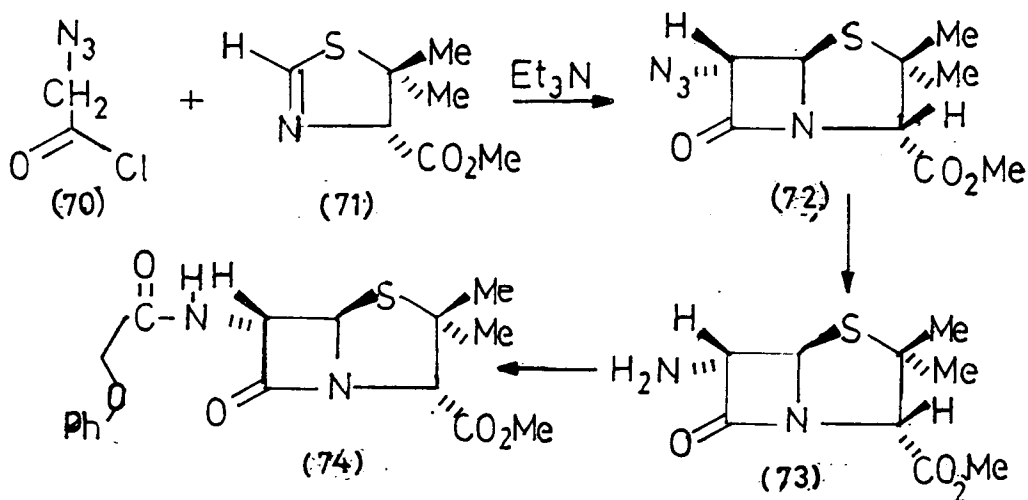
Scheme 35   Use of the azido group as a 'latent amino
             function' in the Ketine-Imine synthesis of
             penicillin.

(75)   (76)   (77)   (78)
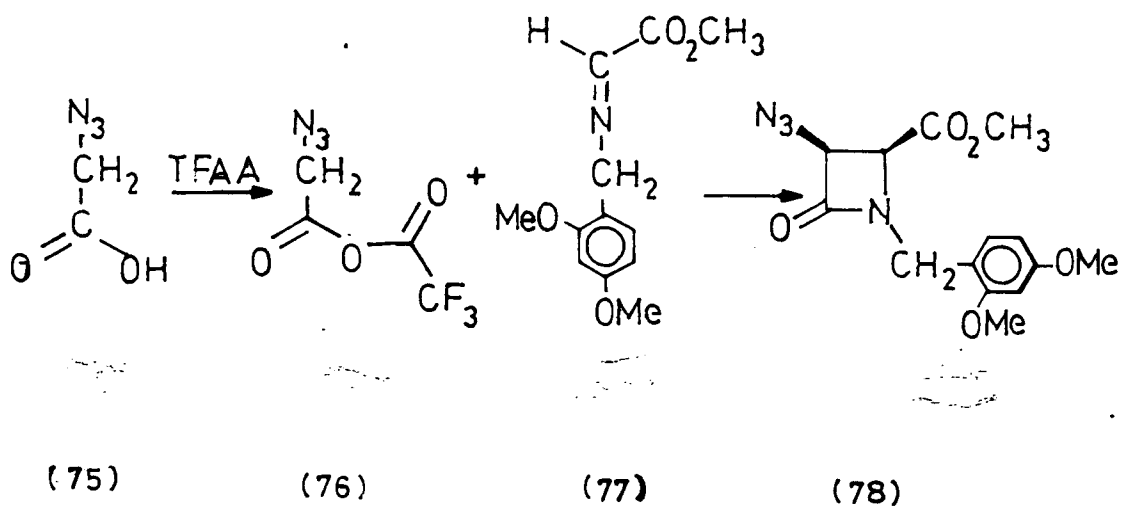
Scheme 36   Modification of the Ketine-Imine method.

(79)

+

O=C=N=SO$_2$Cl

(80)

(81)

(82)   R^1=R^2=Me

(83)   R=H,R^2=C=CH$_2$

(84)   R=H,R^2=OCOCH$_3$
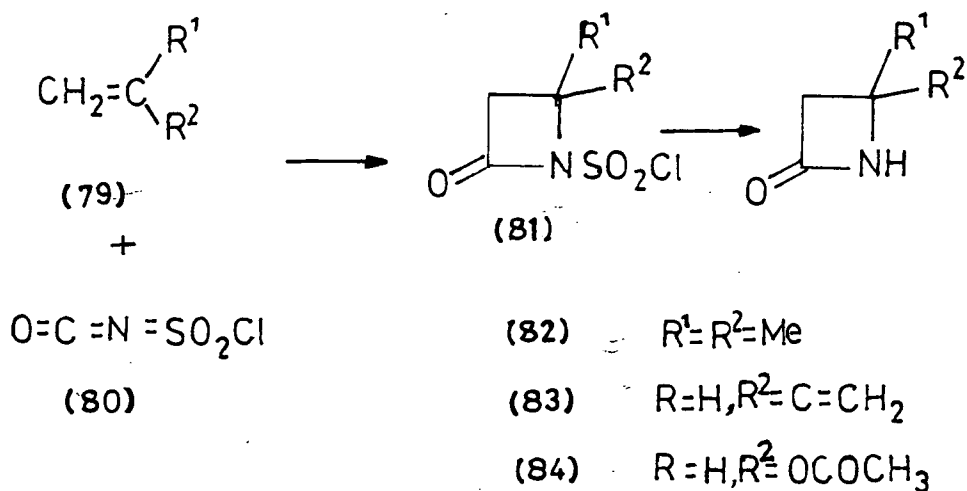
Scheme 37   N-chlorosulphonyl isocyanate addition to an alkene for β-lactam synthesis.

protecting group, followed by diazomethane, *treatment,* 5-phenyl
penicillin (69) was prepared.   The yields were low due
to the requirement for hydrolytic conditions for the
cleavage of the succinimide protecting group and due to
dimerisation problems of the ketene.   The yields of the
monocylic β-lactam derivative could be improved by the
use of the azido group as the "latent amino function", as
reported by A.K. Bose in 1968, (scheme 35).[55]   The azido
group could readily be reduced to the amino group, by
hydrogenolysis using Adam's catalyst.   Further
improvements were made by using the required side chain
directly, such as **p**-nitrobenzyloxycarbonylglycyl
chloride[56], thus removing two stages in the synthesis.
However, this modification made the ketene addition more
difficult due to steric interactions.

          A great deal of variation of the substituents in
each reagent is possible.   The acyl chloride, for
instance, could be replaced by a mixed anhydride (76)
(scheme 36)[57] and the imine could be acyclic (77) or
cyclic (66) or (71), and with a variety of substituents.
The stereochemistry of the addition is determined by the
steric interactions involved.   Normally, large groups
adopt a trans geometry about the β-lactam ring.   In
fused systems, the sterically-demanding group adopts the
α-stereochemistry rather than occupying the crowded
β-face, as required in the natural penicillin.   This
unfortunately makes it necessary to isomerise the C-3
position, of the azetidinone, to obtain the biologically
active   and thermodynamically less stable **(cis)**
derivative.   However, this isomerisation is not
necessary for intermediates which are used to synthesise
thienamycin (20), which possesses trans ring
stereochemistry in contrast to penicillins and
cephalosporins.

b) **The alkene-isocyanate method of β-lactam ring formation.**

In the late 1950's, Graf found that N-chlorosulphonyl isocyanate (CSI) (80) was a powerful reagent for the synthesis of β-lactams (scheme 37).[58] Starting with isobutylene, 4,4-dimethylazetidin-2-one (82) is formed via the N-chlorosulphonyl addition product (81). Butadiene gives the corresponding 4-vinylazetidin-2-one (83) and vinyl acetate affords 4-acetoxyazetidinone (84). 4-Acetoxyazetidinone (84) is a very useful intermediate, as will be seen later. The acetoxy group in (84) can readily be displaced by nucleophiles such as amines, thiols, alcohols and more recently carbanions and is, thus, a means of introducing nitrogen, sulphur, oxygen and carbon atoms respectively in the 4 or 5-position of a bicyclic fused system composed of a β-lactam ring and either a five or six membered ring respectively.

c) **Functionalisation of the C-3 position of the azetidinone.**

The need to functionalise the C-3 position of the azetidin-2-one (85) (scheme 38) to obtain the relevant intermediate which would impart biological activity in the final compound is made possible by use of strong base methodology,[59] such as lithium diisopropylamide in an aprotic solvent and at low temperature (-78°C), and trapping the carbanion at C-3 with an electrophilic reagent, for example acetaldehyde or a halogen (which can then be further substituted by a nucleophile).

(d) **The use of the N,N-dicyclohexylcarbodiimide coupling reagent for β-lactam ring formation.**

The first totally synthetic route to penicillin V

was reported by Sheehan in 1959.[60]   The important
β- lactam ring formation step was carried out at the end
of the synthetic scheme and involved a
dicyclohexylcarbodiimide coupling reaction (scheme 39).
This synthetic strategy will be covered later in the
penam section.
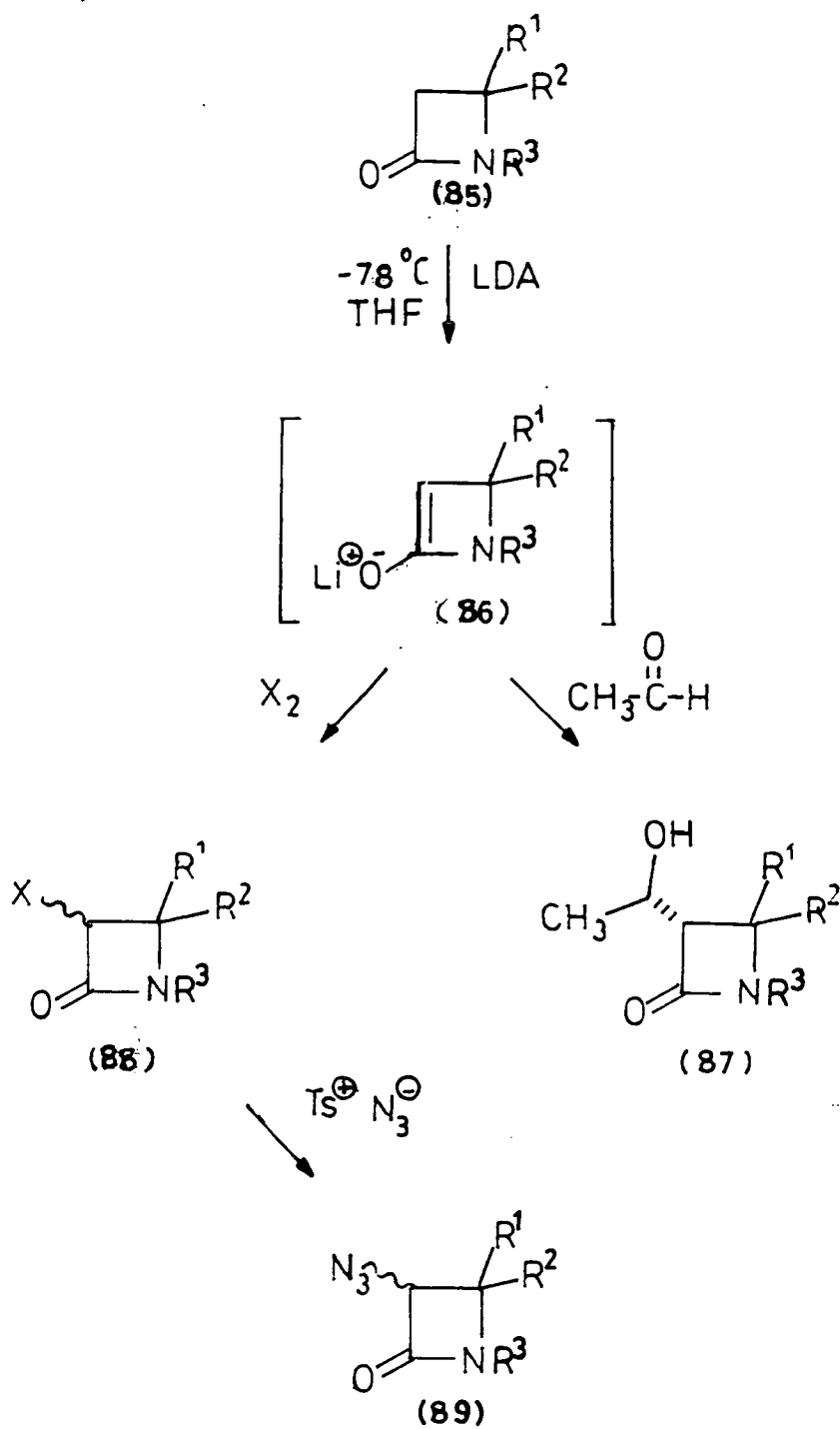
Scheme 38   Functionalisation of the C-3 position an
             azetidinone, using strong base methodology.
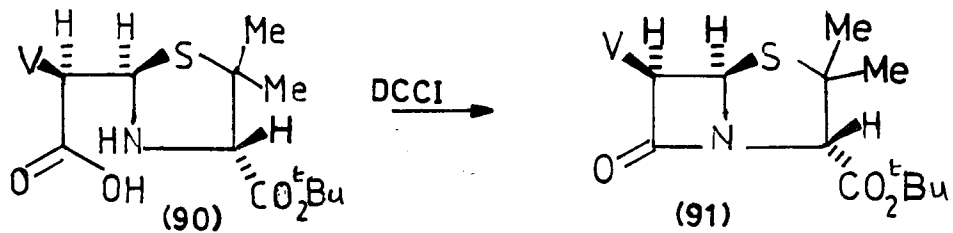
Scheme 39   Sheehan's classical synthesis of a β-lactam
             derivative.
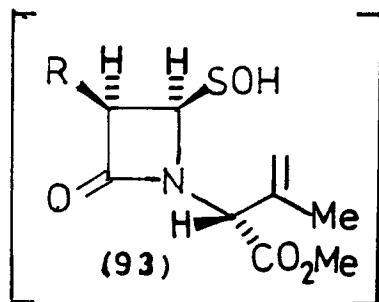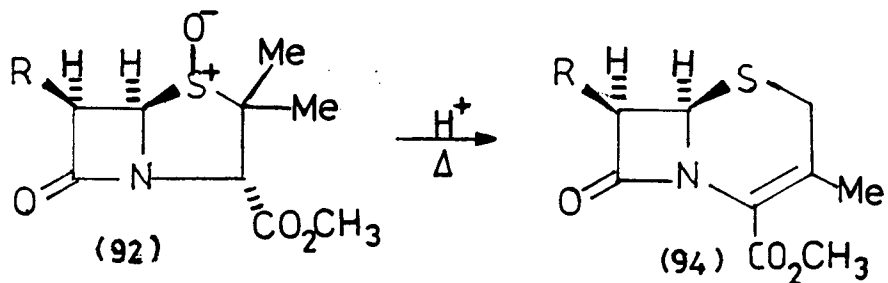
Scheme 40   Selective cleavage of the thiazolidine ring
             in penicillins.

3.1.2 Degradation methods of penicillin.

Penicillins are the cheapest and the most readily available β-lactam derivatives obtainable by fermentation methods. Consequently, all semi-synthetic strategies to the variety of novel β-lactam antibiotics, have been attempted <u>via</u> the penicillins.

a)   Selective cleavage of the thiazolidine <u>via</u> a penicillin sulphoxide.

The selective cleavage of the thiazolidine ring in penicillins was finally accomplished by R.B. Morin and co-workers in 1963 (scheme 40).[61]   Refluxing methyl phenoxyacetamidopenicillin sulphoxide (92) in xylene in the presence of acetic acid resulted in the isolation of a cephalosporin derivative (94).   This novel rearrangement was investigated in great detail and was concluded to involve a sulphenic acid intermediate (93).[62]   This intermediate (93) has since been found to be very versatile.   It has been trapped by both electrophilic[63-65] and nucleophilic[66-69] reagents.   The sulphenic acid (93) has been reduced with trimethylphosphite to thiazoline (102)[70] and in one instance, it has been possible to isolate and characterise a crystalline sample (103) (scheme 41).[71]   Each of these intermediates is a potential source for further modification and functionalisation in order to obtain a bicylic fused system.

b) **Rearrangement involving selective opening of the thiazolidine ring.**

In 1963, Wolfe and co-workers reported[72] a novel rearrangement, involving an opening of the thiazolidine ring (scheme 42). Working with the acid chloride derivative (104), it was found that on base treatment, (104) would undergo a rearrangement to (105). This derivative was found to have an identical mass to a dehydrated penicillin molecule. These derivatives were, thus, called anhydropenicillins. They possessed no biological activity and were relatively inert to most reagents. However, the lactone ring could be opened with mercuric acetate and acetic acid. The product distribution was sensitive to temperature. Ozonolysis of the double bond resulted in only decomposition products. As an intermediate (105) was of limited use.

c) **Thiazolidine ring cleavage by the Curtius rearrangement.**

Selective opening of the thiazolidine ring in penicillins was also reported by Sheehan in 1965 (scheme 43).[73] This involved the Curtius rearrangement of the azido derivative (106) to the isocyanate (107) which readily afforded the carbinol derivative (108) under mildly acidic conditions.(108) existed primarily in the ring open form (109).

d) **Beecham method of selective cleavage of the thiazolidine ring _via_ a β-elimination method.**

Workers at Beecham[74] also developed a very useful means of opening the thiazolidine ring, with the advantage of having removed the oxidation step of the mercaptan to the sulphoxide. This procedure involved a
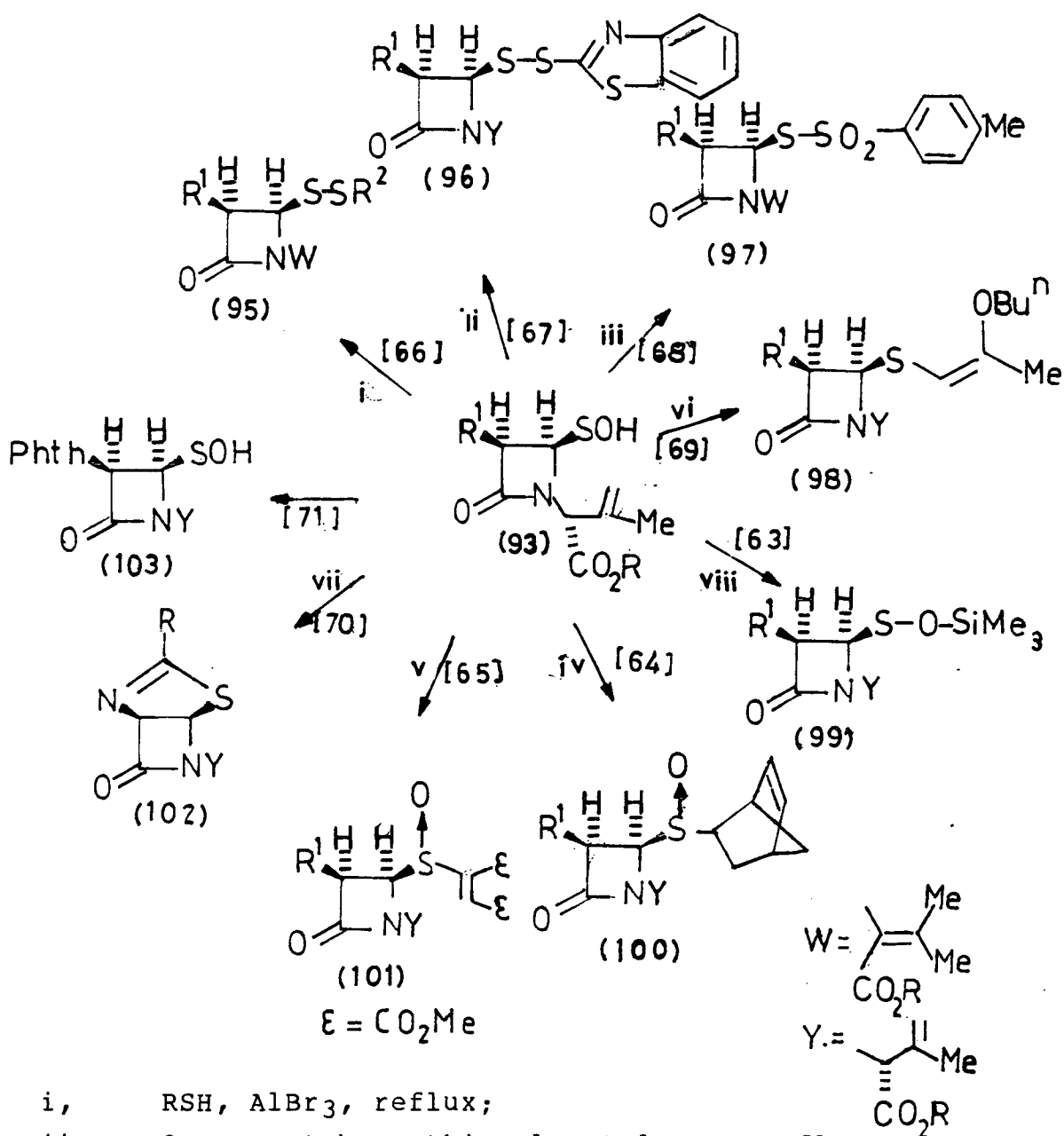
i,   RSH, AlBr$_3$, reflux;

ii,   2-mercaptobenzothiazole, toluene, reflux;

iii,   P-TsOH, benzene, diethylaniline, reflux;

iv,   norbornadiene, reflux;

v,   MeOOC.C $\equiv$ C.COOMe, Benzene;

vi,   CH$_2$ = C(OBu)Me, dioxane, THF, AlCl$_3$;

vii,   P(OMe)$_3$. benzene, reflux;

viii,   Me$_3$SiCl, hexamethyldisilazane benzene.

Scheme 41   Trapping experiments for Sulphenic acid (93).

V = PhOCH₂CONH
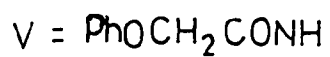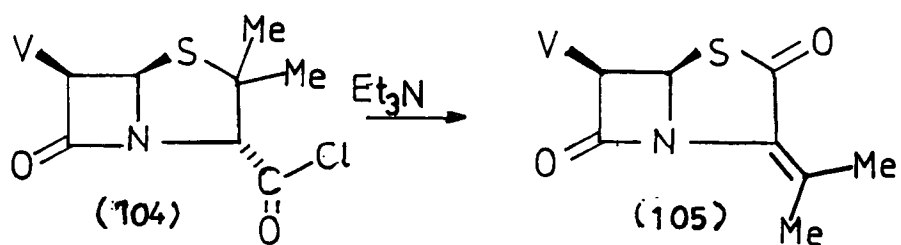
Scheme 42   Penicillin to anhydropenicillin
            rearrangement.
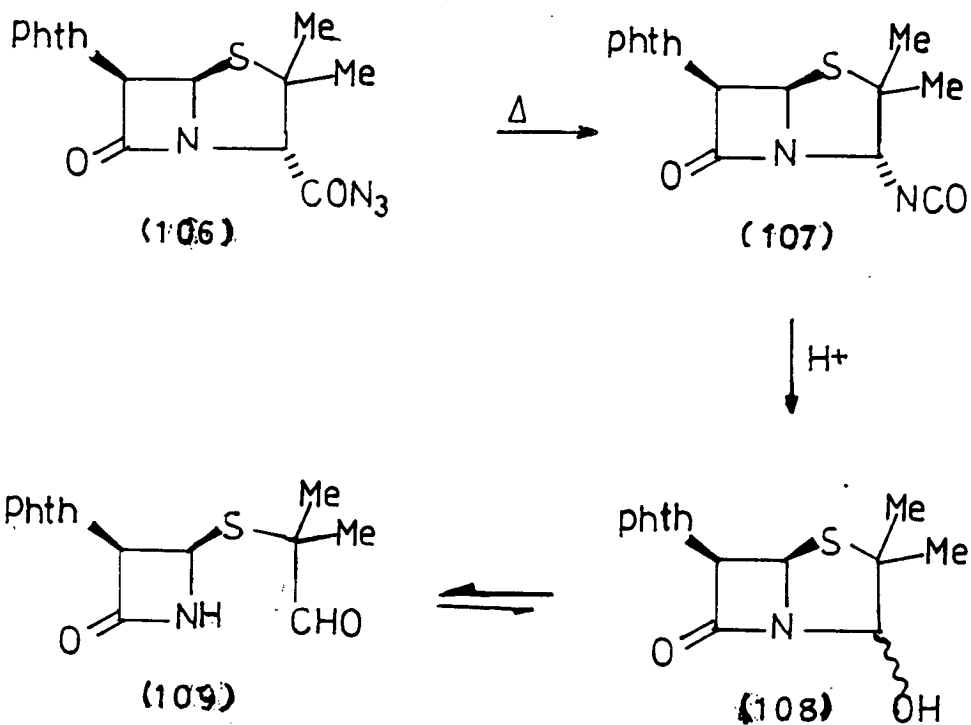
Scheme 43   Selective cleavage of the thiazolidine ring in
             penicillins - Sheehan's method.
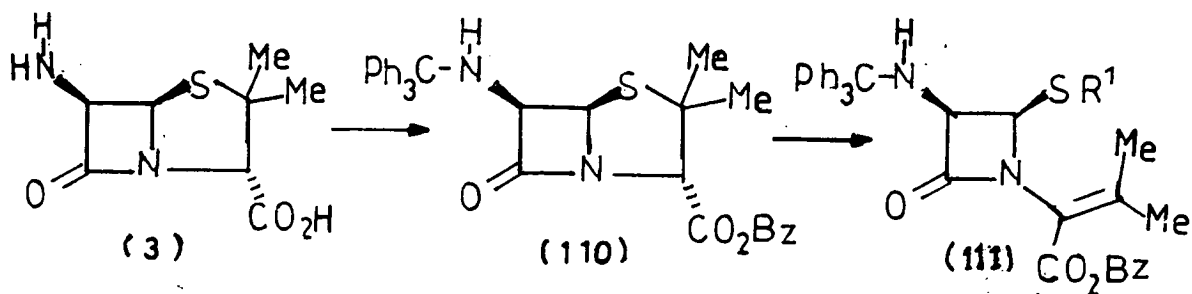
Scheme 44   Selective cleavage of the thiazolidine ring
             in penicillins - Beecham's method.

β-elimination mechanism, by the use of primary alkyl halide and a strong base on a protected pencillin derivative (110), obtained from 6-APA (3), to afford the 1,2-secopenicillanate (111) in excellent yields (scheme 44).

e)   **Methods developed for the removal of the β-lactam N-substituent.**

Many methods have been devised to remove the N-substituent on the β-lactam nitrogen to obtain the monocyclic β-lactam intermediate (115) (scheme 45).. The most common method was developed by the Lilly group[75] and involved an isomerisation of the double bond, in intermediate (112) with triethylamine, to the conjugated system (113), followed by ozonolysis to obtain the   . moderately stable oxonamide derivative (114) which is hydrolysed with aqueous methanol to result in the N-unsubstituted drivative (115).   A one-step removal of the butenoate group has also been developed by research workers at Beecham.[76]   This involves a permanganate oxidation, under basic conditions, of the double bond to a 1,2 diol which spontaneously fragments to the monocyclic azetidinone (115), under the reaction conditions.   A further method of removing the butenoate substituent on the ring nitrogen was reported by Barton and co-workers in 1973.[77]   This involved the 1,3-dipolar addition of diazomethane to the conjugated double bond to result in the pyrazoline (116) followed by either hydrolysis, or reduction with zinc and acetic acid, to result in the azetidinone (115) (scheme 45).

f)   **Functionalisation of the azetidinone derivative.**

The procedure used to replace a substituent on the

Scheme 45   Methods for the removal of the substituent on the β-lactam nitrogen.

ring nitrogen is now a classical one, and was first developed by R.B. Woodward and co-workers and reported in 1972 (scheme 46).[78]   This method involved the condensation of the azetidinone (115) with a glyoxylic ester (117) in refluxing benzene to afford the hemiaminal (118).   This is readily purified by chromatography and converted to the phosphorane (120) via a two step procedure involving chloro derivative (119).   The phosphorane (120) is a very important intermediate for the synthesis of fused ring systems, as will be seen later.

Functionalisation of the C-3 position in the azetidinone (121) (scheme 47), is not normally necessary for azetidinones which are obtained from degradation of penicillins, and which are used for the synthesis of novel penicillins or cephalosporins.   However, where the amino group is not required, the free amino group can be replaced by a variety of nucleophiles, via the diazonium ion, such as halide,[79] and hydroxide.[80]   The 3-halo azetidinone can be reduced to a C-3 unsubstituted derivative with zinc and ammonium chloride[81].   The 3-unsubstituted azetidinione can then, using the strong base methodology, be substituted electrophilically.[59]

The thiomethyl group in (122) can readily be removed by chlorinolysis[82] (scheme 48), and the C-4 position of the 4-chloroazetidinone (123) is then functionalised.   However, the intermediacy of the azetidinonium ion (124) usually results in a mixture of 4R/4S substituted products (125).   Replacement of sulphur by oxygen or nitrogen atoms is readily possible using the appropriate nucleophiles.   The formation of a carbon-carbon bond in the C-4 position of the azetidinone (123) is of particular interest, as applied to the synthesis of thienamycin (20).   Use of carbanions, such as Grignard or alkyl lithium derivatives, would result in β-lactam cleavage.   However, Japanese workers[83] have reported an interesting method of carbon-carbon bond formation   at the C-4 position of the azetidinone
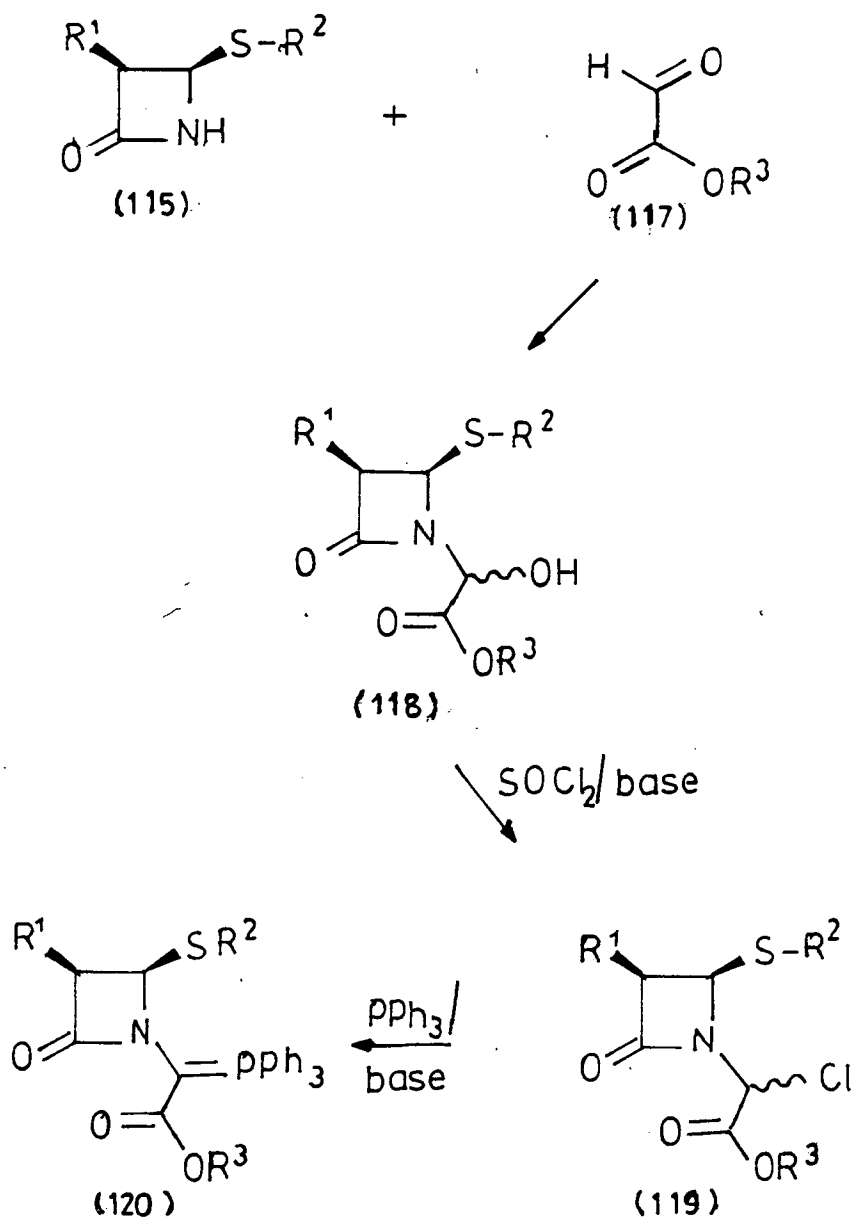
Scheme 46   Woodward's method of replacing the
             substituent on the β-lactam nitrogen.

$R^1 =$ acyl, H, Protecting group

$R^2 = -H-$, alkyl,-H-,-H-

$R^3 =$ Butenoate, H

(121)
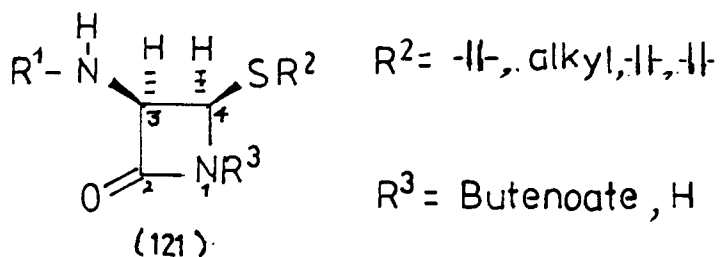
Scheme 47 **Azetidinone obtained from degradation of penicillins.**

(122)

Cl_2

(123)

(124)

$\overset{\ominus}{Nu}\overset{\oplus}{M}$

(125)
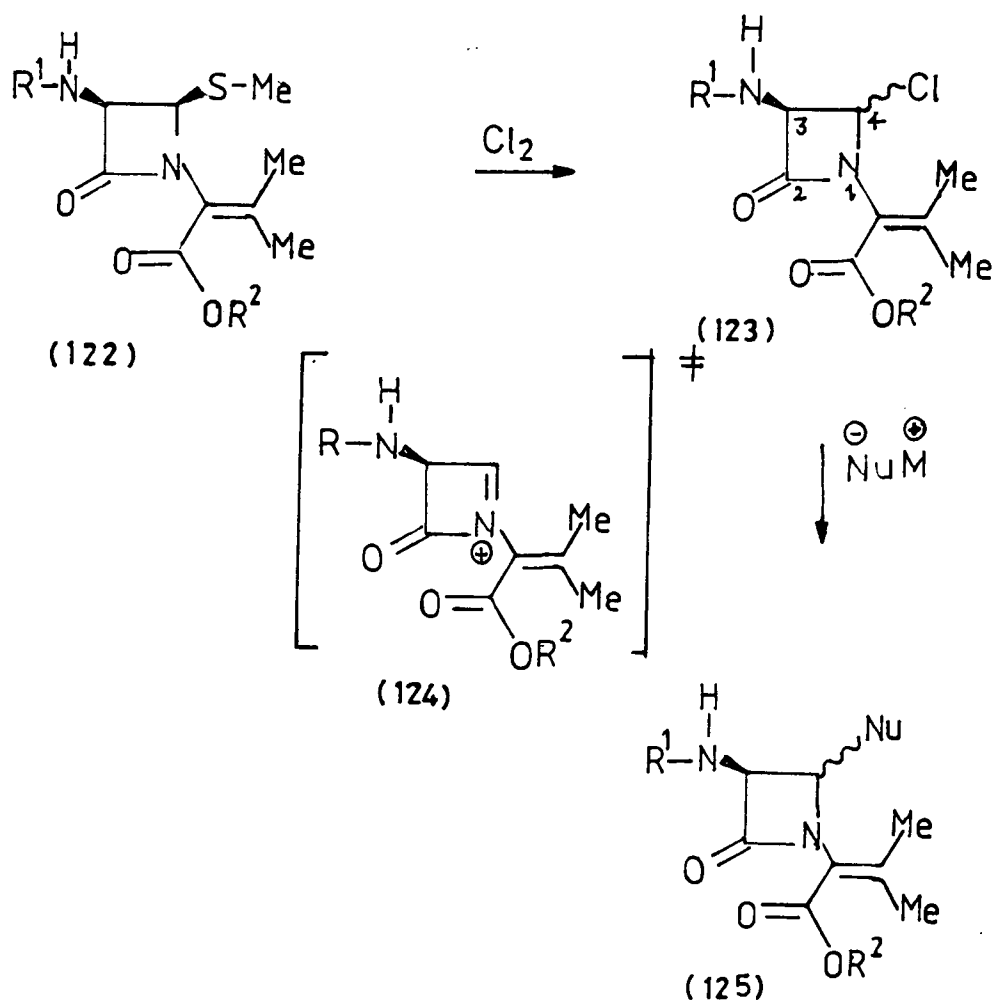
Scheme 48 **Modification of the C-4 position of the azetidinone.**

(scheme 49). This route involves chlorinolysis of a
seco-penicillanate derivative (126) to $\overset{give}{\wedge}$ the
4-chloroazetidinone (127). Treatment of (127) with an
alkyl cuprate derivative (128) results in a
diastereomeric mixture of the 4-alkylazetidinoneS (129)
which is readily transformed through subsequent steps to
the carbapenem nucleus (20).

More recently workers at Sankyo in Japan[84] and at
Merck, Sharp and Dohme[85] have developed a similar
functionalisation of the C-4 position of the azetidinone ,
(127) which they have transformed to thienamycin by
standard methodology.

However, due to the poor stereochemical control of
the C-4 substitution by the above methods, intermediates
for carbapenem synthesis are normally obtained by totally
synthetic methods, rather than via semi-synthetic routes.

The degradation of pencillins to monocyclic
β-lactam intermediates is a well explored area and
comprises a great deal of the fascinating chemistry of
penicillins. Many rearrangements of penicillin result
in non-β-lactam containing products, but these are
outside the scope of this account.

6 – APA

(126) → (127)

CuCH₂CH=CH₂
(128)

Thienamycin ← (129)
(20)
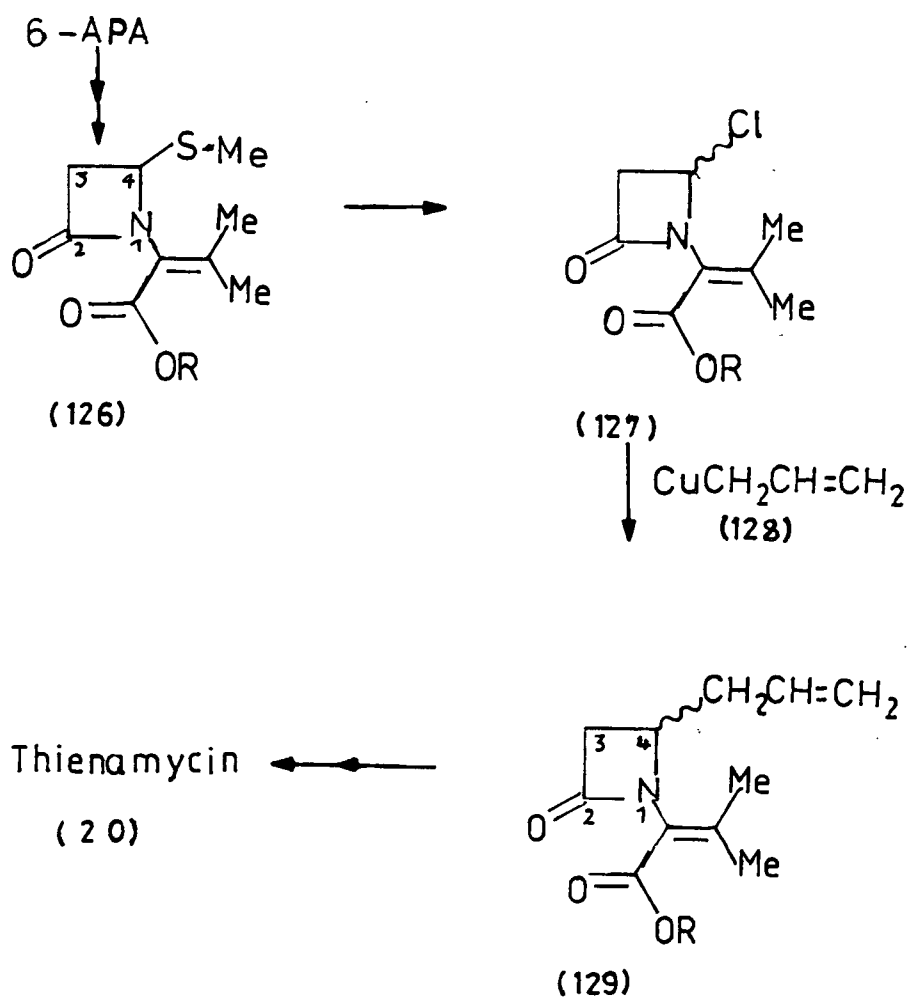
Scheme 49

3.2.0 SYNTHESIS OF BICYCLIC FUSED SYSTEMS

Prior to the discovery of antibacterially active monocyclic β-lactam antibiotics, such as nocardicin. A and monobactams, only fused ring systems such as penicillins, cephalosporins and cephamycins were known to possess any activity against pathogenic bacteria. Consequently, synthesis involving monocyclic β-lactam intermediates were normally directed only towards novel bicyclic fused systems, which were not obtainable from natural sources (scheme 50). This situation changed during the mid-seventies, with the discovery of the novel β-lactam structures from a variety of Gram positive bacteria. Recently, work has started on suitably functionalised derivatives which possess no β-lactam ring, these being γ-lactams and 1,2-diazetidin-2-one analogues of β-lactam antibiotics. These will be discussed in the section on future trends.

Totally synthetic procedures usually involved a large number of steps and resulted in low yields of the final product. Consequently, these routes would not be commercially viable. However, if the particular derivative possessed sufficient activity, attention would then be diverted towards determining a viable route. Imipenem, a derivative of thienamycin is a case in point.

Most synthetic routes that will be considered start with a monocyclic β-lactam intermediate. It is assumed that the monocyclic β-lactam intermediate is available by either total synthesis, or penicillin degradation, procedures described in an earlier section of this account. The important consideration of this section is concerned with the methods employed to annelate the second ring system onto the β-lactam ring. This section will be divided according to the size of the ring to be formed, and further sub-divided according to whether the fused system belongs to the penam, oxapenam, carbapenam, azapenam,
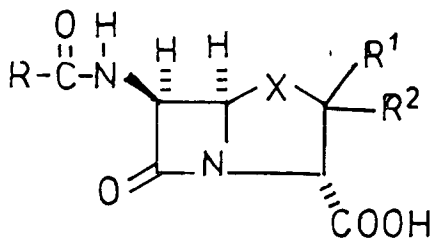
$$X = S, O, NR, CR_2$$
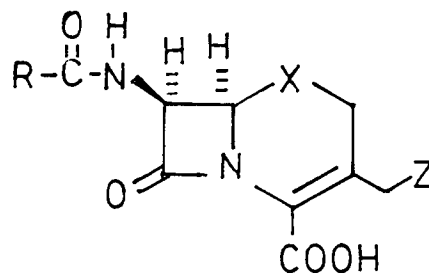
$$Z = OAc, P_y, SR_3$$
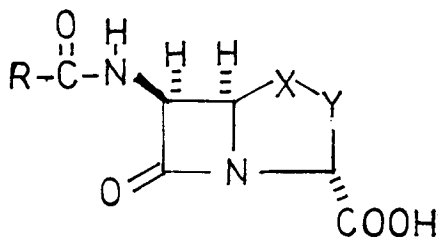
$$X = S, O, NR, CR_2$$
$$Y = S, O, NR, CR_2$$
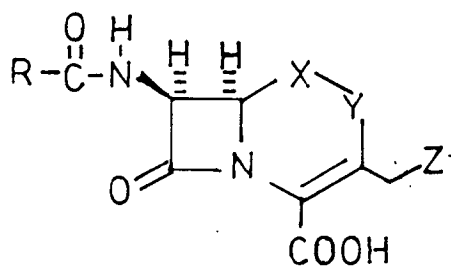
$$Z = OAc \quad P_y \quad SR_3$$

Scheme 50   Structures of modified fused systems in the β-lactam area.

penem, clavem/oxapenem, carbapenem, azapenem, or cephem, oxacephem, carbacephem or azacephem categories. The modes of cyclisation will also be given according to Baldwin's rules.

3.2.1 FOUR-FIVE FUSED SYSTEMS

3.2.2 PENAM

a) Synthesis of natural penicillins

The pencillin nucleus is   known as the penam system. Few attempts have been made to synthesise the natural penicillins since they can now be readily obtained by fermentation methods. However, the classic synthesis of pencillin V (2) by Sheehan in 1959 (Scheme 51),[60] was a tremendous step for chemists in the art of handling these sensitive compounds. Pencillamine (130) was condensed with a protected malonaldehyde derivative (131) to generate the thiazolidine intermediate (132). The phthalimide protection was removed prior to the formation of the β-lactam ring, by treatment with hydrazine and hydrochloric acid to generate (133). Acylation of the amino group with phenoxyacetyl chloride and triethylamine in dimethyformamide resulted in (134). Acid mediated removal of the t-butyl group with formic acid resulted in the diacid. Generation of a monosodium salt and then treatment with N,N-dicyclohexylcarbodiimide (DCCI) in dioxane-water finally afforded racemic penicillin V (2). This was found to have 50% the activity of the natural product. Furthermore, the confidence generated by this route stimulated more chemists to take on the challenges provided in this area. A route involving only four steps to methyl penicillin V (2) (scheme 52)
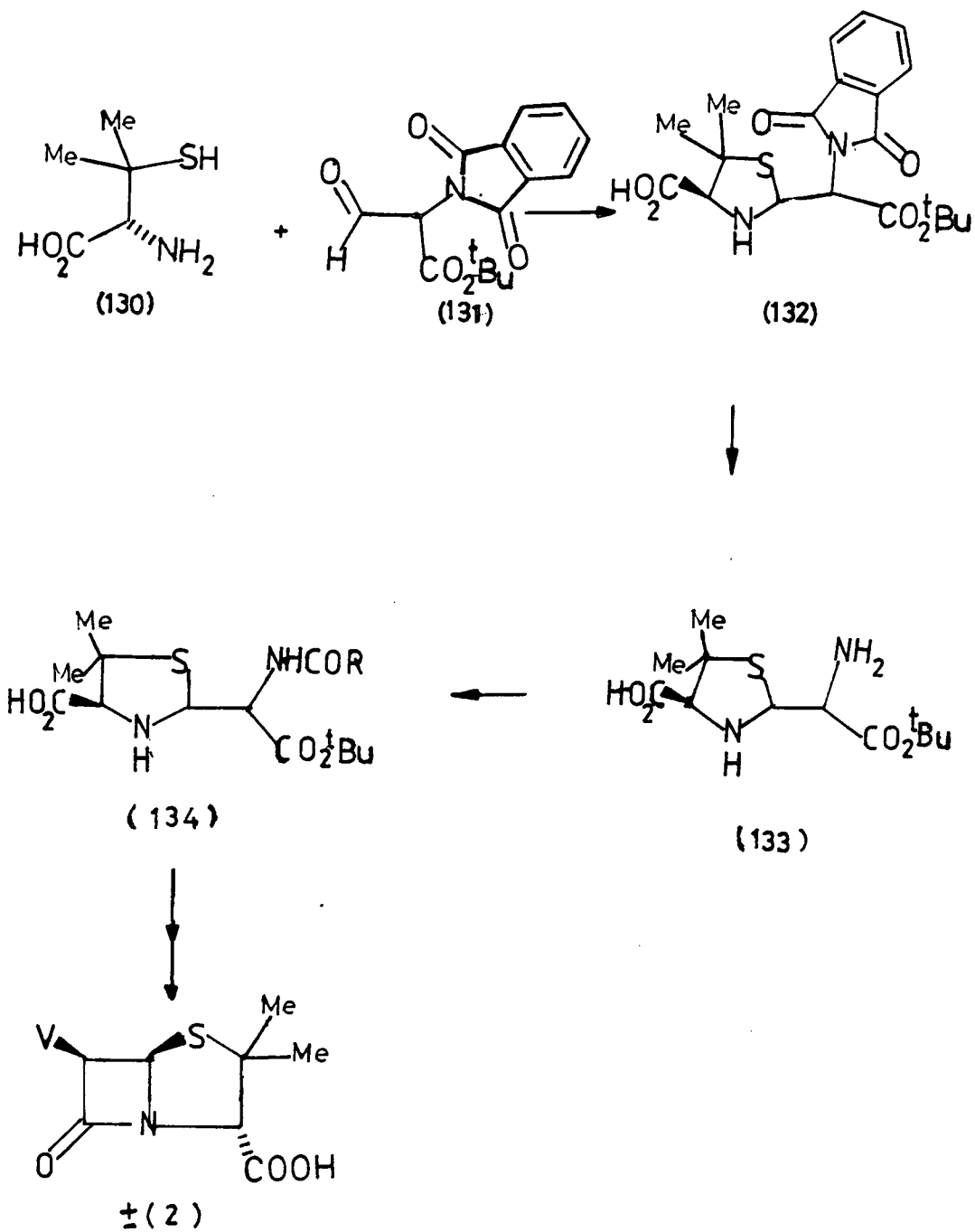
Scheme 51   Synthesis of penicillin V by Sheehan's
method.

was soon developed by A.K. Bose and co-workers.[55]   The important step involved the [2+2] cycloaddition reaction, utilising the ketene-imine method, for generating the β-lactam ring, and isomerisation of the C-6 position of the penam nucleus, using the strong base methodology,[59] to obtain the β-isomer incorporating the natural stereochemistry.

b)   Synthesis of bisnorpenicillins

Synthesis of penam systems lacking the geminal methyl groups, present in the penicillin series, has been successfully achieved by a number of workers.   These are called bisnorpenicillins (130) (scheme 53).   The earliest route[86] to (130) involved the condensation of cysteine (132) with a masked formylglycine derivative (131).   This method is similar to that of Sheehan's route to pencillin V (scheme 54).[60]   The bisnorpenicillin V (137) was found to have much reduced activity relative to penicillin V (2).   However, recently workers at Beechams described a much shorter route to (137) (scheme 55).[87]

The azetidinone (138) is readily obtained from degradation of penicillin V (2).   This route, however, suffers from the unfortunate problem of the absence of any stereochemical control, during the build-up of the second ring.   This gives rise to a mixture of products (141) and (142) which have to be separated from the desired product (140).   Further work[88] at Beecham has shown that changing the C-6 acetamido side chain from the phenoxyacetamido to the protected phenylglycyl type (143), (scheme 56), resulted in increased stability to the β-lactamases produced by many bacteria, compared to the penicillin counterpart, piperacillin (9).   Recently, a further synthesis of a penam system lacking the geminal methyl groups has been reported (scheme 57).[89] The intermediate (144) was obtained by degradation of a
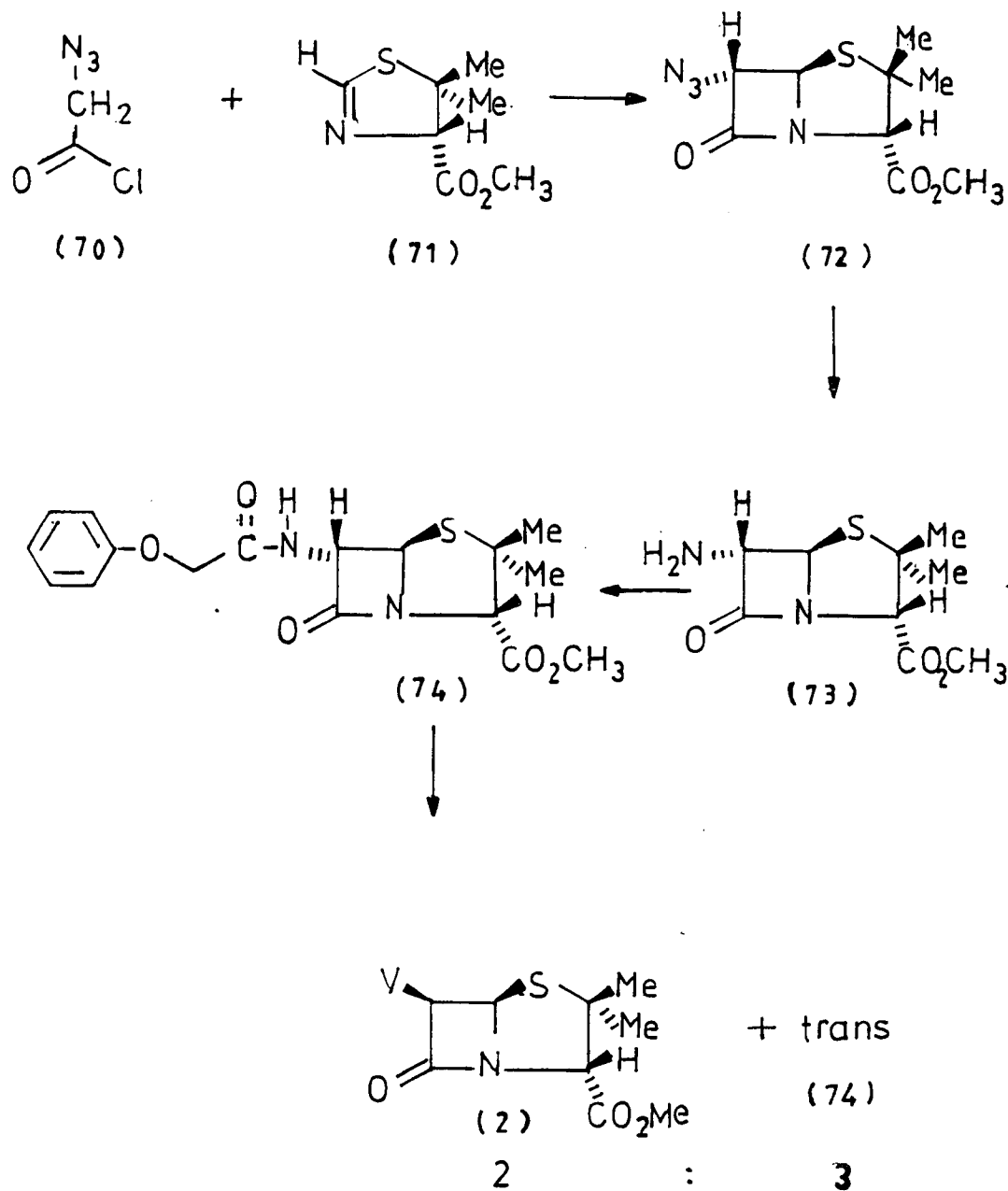
Scheme 52   Ketene-Imine procedures to pencillins, as used by A.K. Bose.
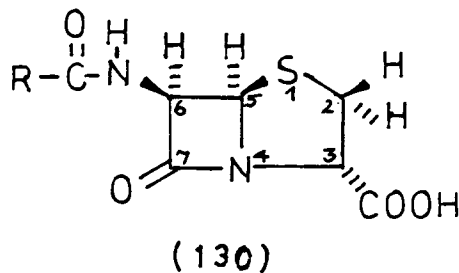
Scheme 53   Structure of the bisnorpenicillin nucleus.

Ft = Phtalimide

Ft = Phthalimide
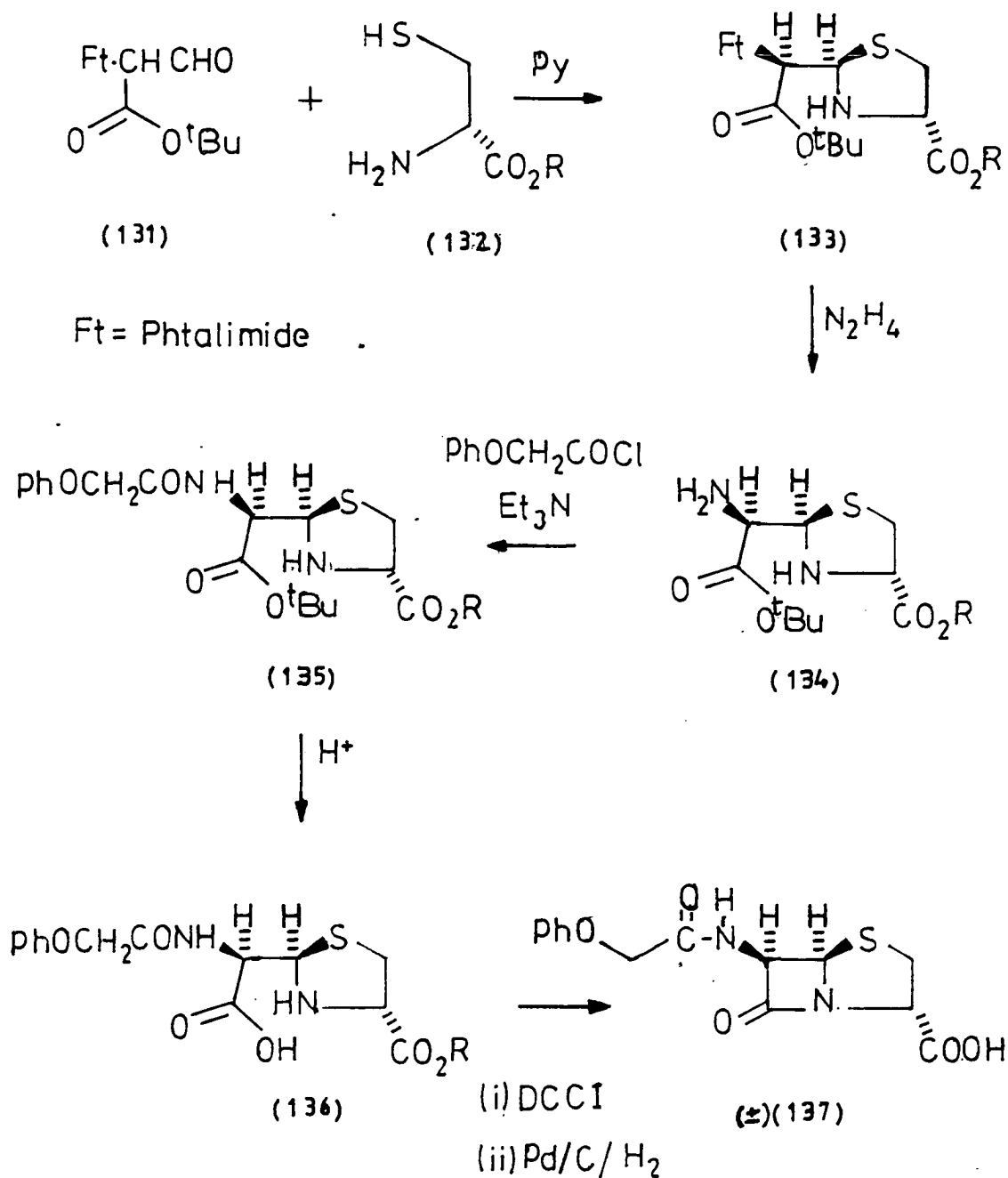
Scheme 54 **Synthesis of bisnorpenicillin V _via_ Sheehan's approach.**

(141)

+

(138) (139) (140)

V = PhOCH$_2$CONH

Bz = CH$_2$Ph

+

(142)

Scheme 55   Beecham's synthesis of bisnorpenicillin V.

(143)

Scheme 56   Structure of piperacillin's side chain.
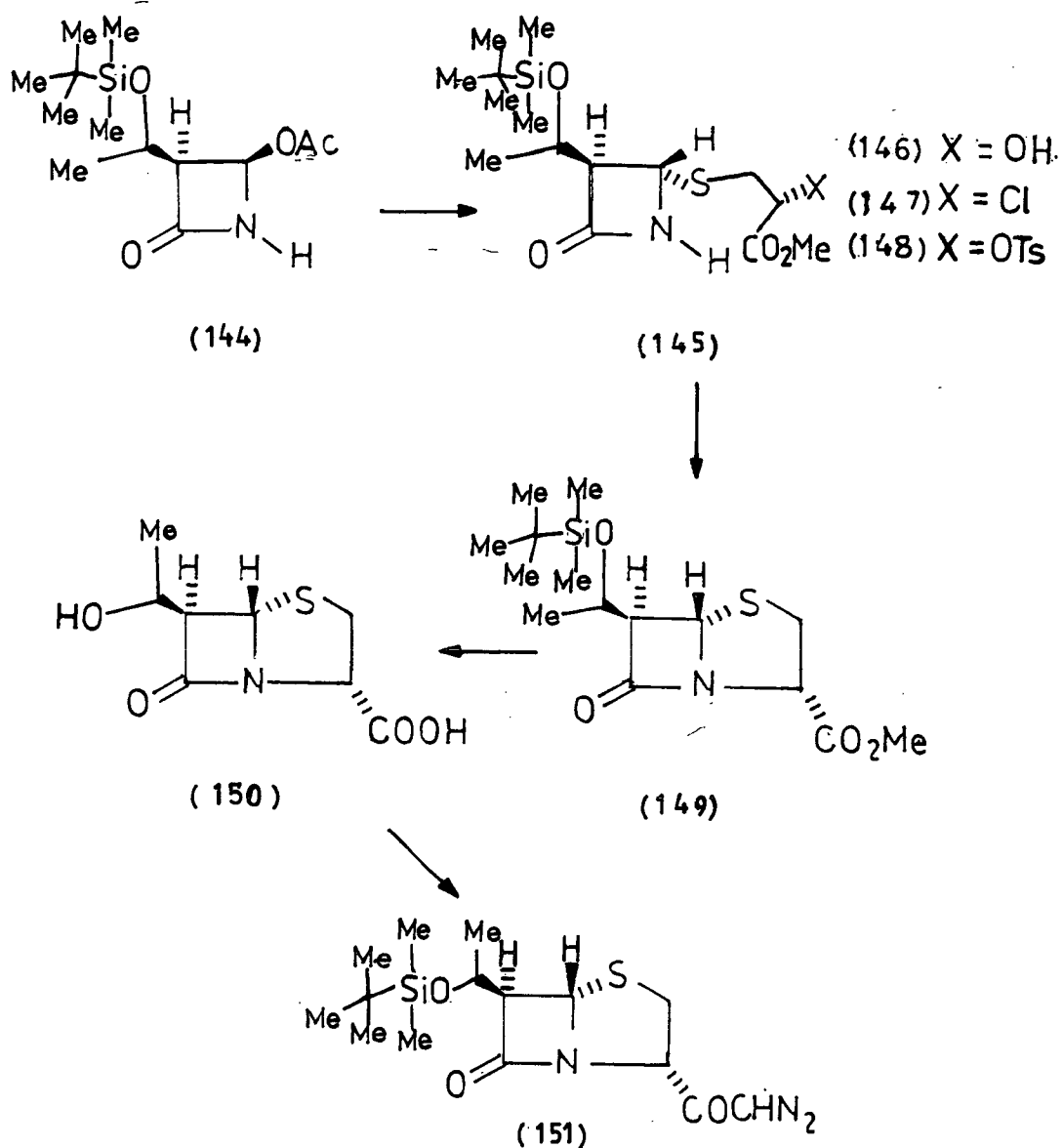
Scheme 57   Synthesis of a bisnorpenicillin derivative by
             a 5-exo-tet cyclisation involving the
             β-lactam nitrogen.
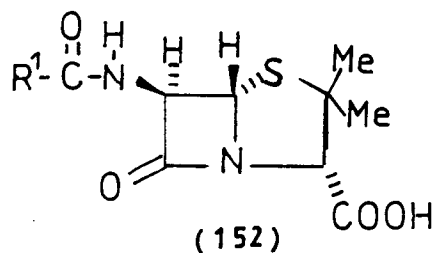
Scheme 58   Structure of C-5 epipenicillins.

side chain modified penicillin, by sequential treatment
with mercuric acetate,[90] ozone,[75] and methanol/triethyla-
mine, although it is possible to obtain (144) by a
totally synthetic strategy. Nucleophilic displacement
of the acetoxy group in (144) with racemic methyl
2-hydroxy-3- mercaptopropanoate[91], affords (145) as a
mixture of diastereomers with trans configuration of
substituents on the β-lactam ring. The mixture was not
separated but taken through the synthesis. The
subsequent cyclisation was difficult and occurred only in
low yield, as had been obtained by many other workers
previously.[92] Treatment of (146) with tosyl chloride in
the presence of dimethylaminopyridine afforded an
unstable mixture of diastereomeric chlorides (147) and
tosylates (148). Immediate exposure of a mixture of
(147) and (148) to 2 Molar Sodium hydroxide and
tetra-n-butylammonium bromide in dichloromethane provided
a mixture of the bisnorpenicillin esterS(149) in only
8-10% yield. The derivative (150) was not tested for
biological activity but was further converted to a
diazoketone derivate (151) for the investigation of
diazoketone rearrangement. This is outside the scope of
this account. It could be anticipated that the 5S
isomer, being unnatural, is devoid of any activity, as
has been concluded in previous investigations involving
epipenicillin derivatives (152) (scheme 58).[93] It must
be noted that the 5-exo-tet mode of intramolecular
cyclisation involving an intermediate of the type (153)
has not proved possible with cis geometry of the
β-lactam ring and with a sulphur atom in the C-4 position
of the azetidinone (153) (scheme 59).[94] A possible
rationale for this conclusion is that the cyclisation,
with the cis-isomer, would require a highly crowded and
rigid transition state which may be too unstable to form.

c)   **Synthesis of an isopenam derivative by a 5-exo-tet cyclisation.**

Moving the sulphur atom to the 2 position of the penam nuclus has also been reported.[95]   The isopenam (155) (scheme 60), has been synthesised by a versatile route involving a very important intermediate (78) (scheme 61); which is readily obtained by the ketene-imine method (scheme 36)[57] and which incorporates a very useful β-lactam nitrogen protecting group, (2,4-dimethoxybenzyl), which can be removed oxidatively under relatively mild conditions, ($K_2S_2O_8$ pH 5-6.6).   Further modification of (78) by standard procedures allows the formation of (156). Glyoxylate condensation with the azetidinone (156) resulted in a diastereomeric mixture of hemiaminals (157), which, on treatment with thionyl chloride and a base, generated a mixture of the chloro diastereomers (158).   Reaction of (158) with potassium thioacetate in a mixture of dimethylformamide and tetrahydrofuran afforded a mixture of thioester diasteromers (159).   Deprotection of the thioester with cyclohexylamine in dichloromethane caused the spontaneous intramolecular cyclisation via a 5-exo-tet mode, and simultaneous deprotection of the benzyl group generated the cyclohexylammonium salt (160).   It has been possible to obtain this derivative by a different strategy, which involved the reaction of derivative (158) with hydrogen sulphide and a base to afford the ring closed product.[96]   The isopenam derivative is reported to exhibit good activity against Gram-negative organisms as well as S.aureus.[97]
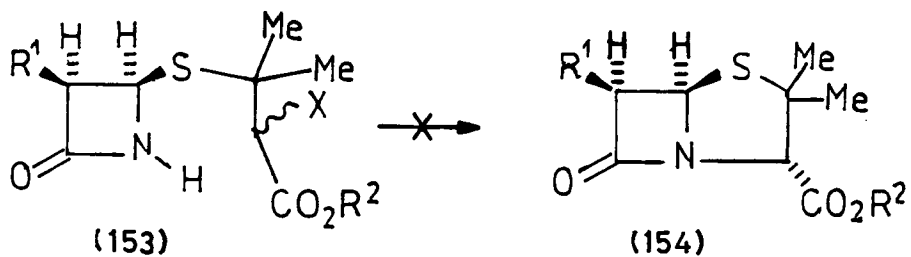
Scheme 59 5-exo-tet cyclisation in the above azetidinone has not been possible.
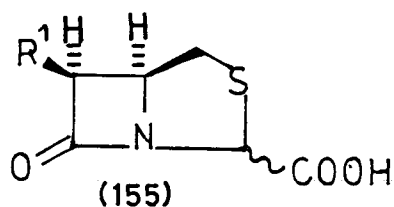
Scheme 60 Structure of an isopenam nucleus.
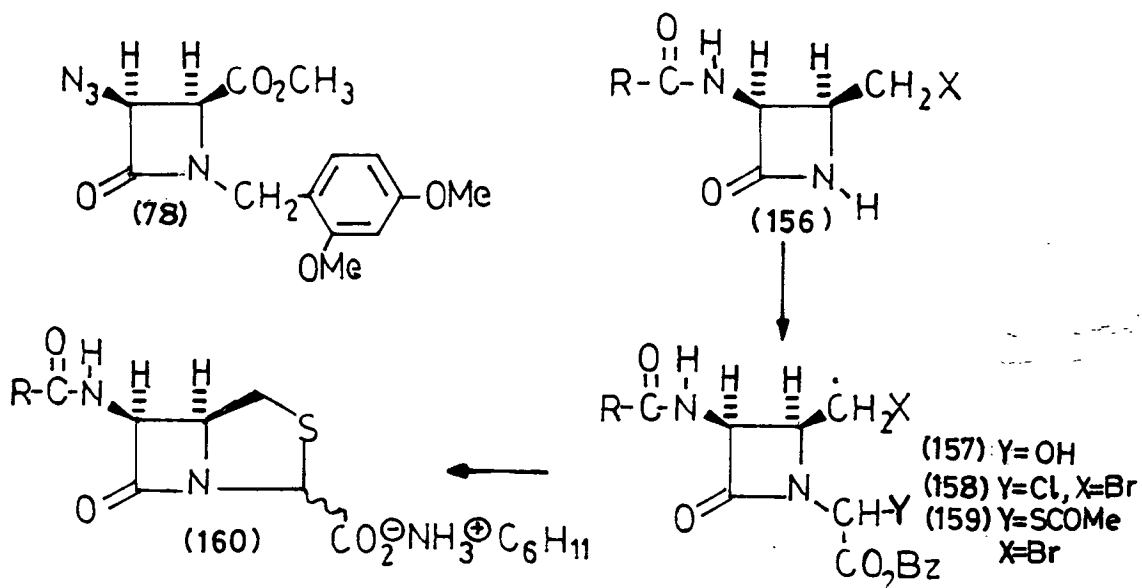
Scheme 61 Synthesis of the isopenam system.

(161)
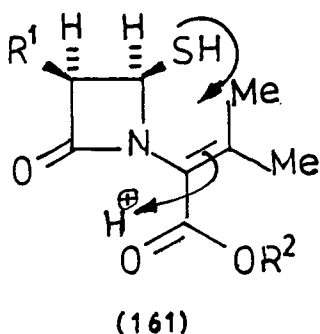
Scheme 62   Structure of intermediate capable to
             undergoing a Michael addition reaction _via_ a
             5-endo-trig cyclisation.
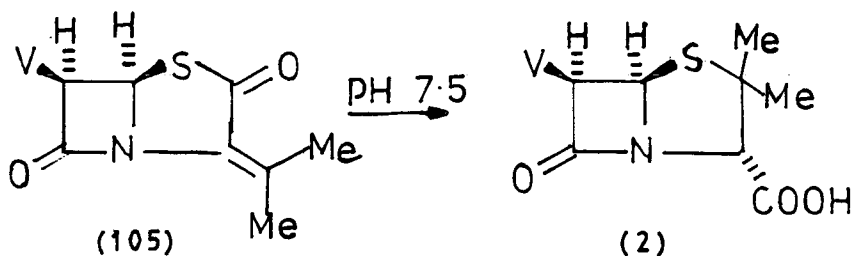
Scheme 63   Conversion of the anhydropenicillin V to
             penicillin V.
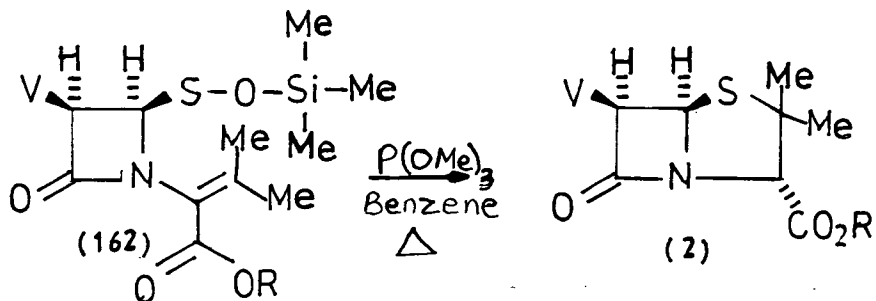
Scheme 64   Conversion of the sulphenic acid intermediate
             to penicillin V (via a thiolate intermediate).

d)   **Synthesis of the pencillin system _via_ a**
        5-endo-trig cyclisation.

A further method of cyclisation which has been
reported to afford the penam system involves the Michael
addition of a thiol onto the butenoate moiety,
intramolecularly, in derivative (161) (scheme 62).   This
5-endo-trig mode of cyclisation is an exception to the
empirical "Baldwin rules".[98]   The first approach,
involving a conversion of an anhydropenicillin V (105) to
penicillin-V (2) under buffered conditions, was described
by S. Wolfe in 1963 (scheme 63).[99]   It was reported that
large amounts of anhydropenicillin V were recovered and
the characterisation of the products (2) was by its
activity towards penicillin sensitive bacteria.   This
work has come under a great deal of criticism due to
insufficient characterisation data.   Furthermore, this
work has not been found to be reproducible and is
generally considered to be invalid.   A further method
was reported more recently (scheme 64)[100].   This
involved the trapped sulphenic acid intermediate (162)
which on heating afforded penicillin V (2).   It was
suggested that the intermediate involved was of the type
(161).   Again, this work has not been reproduced.
Generally, the above reports are considered to have been
due to the derivatives (105) or (162) being contaminated
by the starting material (2).   Purification of β-lactam
intermediates is very often a time consuming process, and
usually requires a great deal of skill to remove trace
impurities, particularly when dealing with small scale
preparations.   A thorough investigation was undertaken
by Baldwin[101], and this type of 5-endo-trig cyclisation
was found to be unsuccessful for the intermediates of
type (161).   This has been rationalised to be due to
unfavourable stereoelectronics in (161) by Baldwin.[102]

e) **Synthesis of a modified penicillin molecule <u>via</u> a 5-exo-trig cyclisation.**

The corresponding allowed route involving a 5-exo-trig cyclisation has been realised (scheme 65).[103]   The mercapto acid (163) was cyclised to the 2-oxopenam (164) using N,N-diisopropylcarbodiimide.

f)   **Synthesis of a penam nucleus by a 5-exo-tet cyclisation involving a thiol at C-4.**

A further route to the penam system that could be envisaged, would involve a 5-exo-tet mode of cyclisation of : the thiol group and the β-lactam nitrogen substituent by an internal S$_N$2 mechanism (165) (scheme 66).   This strategy consisting of a nucleophilic substitution <u>via</u> an S$_N$2 type mechanism to form a penam system, through the formation of an S-C2 bond, has not been reported.   However, in intermediate (166), cyclisation has been successfully achieved by a radical method, as described by Baldwin and co-workers (scheme 67).[104]   This approach supported the biosynthetic synthesis of pencillins described by the same authors earlier.

g)   **Synthesis of a penam nucleus by a 5-exo-tet cyclisation involving a carbanion intermediate.**

Thus far, the cyclisation approaches to afford the penam system have involved either a sulphur or a nitrogen atom as the nucleophile.   Workers at Beecham[105] have undertaken considerable research on the chemistry of the penicillins and of modified structures particularly with electron withdrawing substituents at the C-2 position.
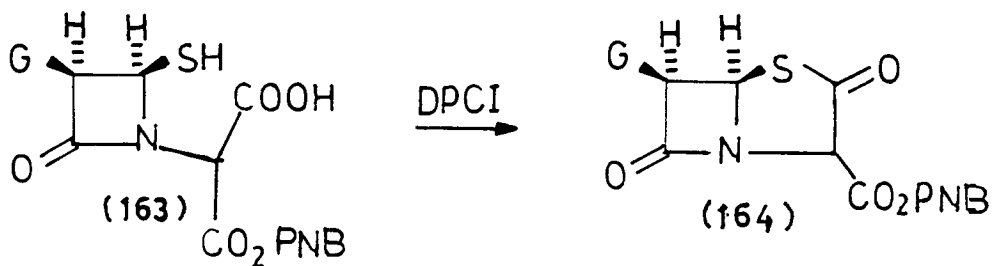
DPCI=diisopropylcarbodiimide

Scheme 65   Use of a thiol   intermediate for a
successful 5-exo-trig cyclisation.
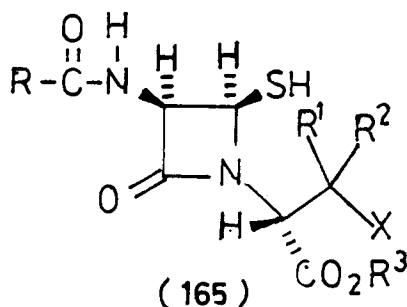
Scheme 66   Structure of intermediate capable of
undergoing a 5-exo-tet cyclisation <u>via</u> the
free thiol.
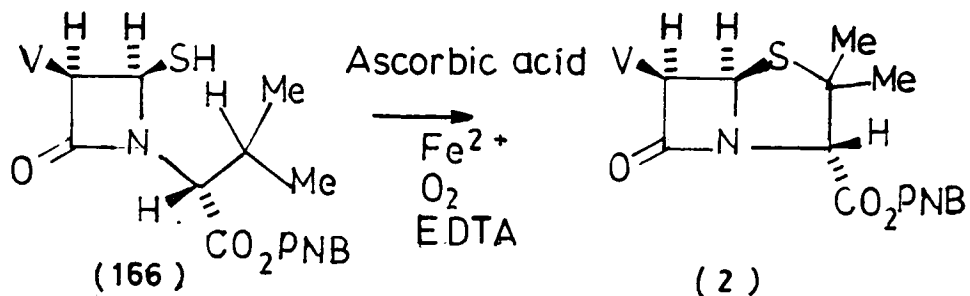
Scheme 67   Radical mode of cyclisation of intermediate
(166).

The ring closure involved a carbanion as the nucleophile (scheme 68). Intermediate (167) is readily obtained <u>via</u> a penicillin degradation procedure. Activation of the methylene substitutent of the alkyl-α- thioacyl. derivative was accomplished <u>via</u> the use of the sulphoxide derivative (168), which made it easier to generate the α-carbanion under mild conditions. The chloro — substituted substituent on the nitrogen was incorporated by the well known method devised by Woodward and co-workers.[78] The intramolecular cyclisation involving a 5-exo-tet mode was effected by the use of potassium tert-butoxide at -20°C. Derivatives (172) and (173) were found to have activity, but to a lesser extent than their penicillin V (2) and ampicillin (4) counterparts. Attempts to generate the diacid derivative (174) by acid treatment of (170) resulted in decomposition. However, Baldwin and co-workers[106] have recently managed to obtain the diacid derivative (175) (scheme 69). This suggests that the earlier workers may have used too vigorous conditions and perhaps a change of the ester functions might have given them success. The method employed by Baldwin and co-workers involved oxidation of the β-methyl group in an intact penicillin V molecule to afford the β-carboxylate derivative (175). (175) was an intermediate on route to the synthesis of a novel structure, the 2-exomethylene penam system (177) (scheme 70) by a decarboxylative Pummerer reaction involving the sulphoxide (176).

A derivative possessing two ester functions in the C-2 position of the penam system (184) was also developed (scheme 71)[107]. This approach involved the thiazoline (178),[108] since it was found that the Beecham method of ring opening of penicillin by alkylating the sulphur atom in the presence of a base, was largely limited to primary halides.[74] Work with thiazolines (178) by Barton and co-workers[109] had shown that when an analogue of (179), derived from penicillin V (2), was treated with certain alkyl halides in aqueous dimethylformamide, containing

(167)a) n = o
(168)b) n = 1
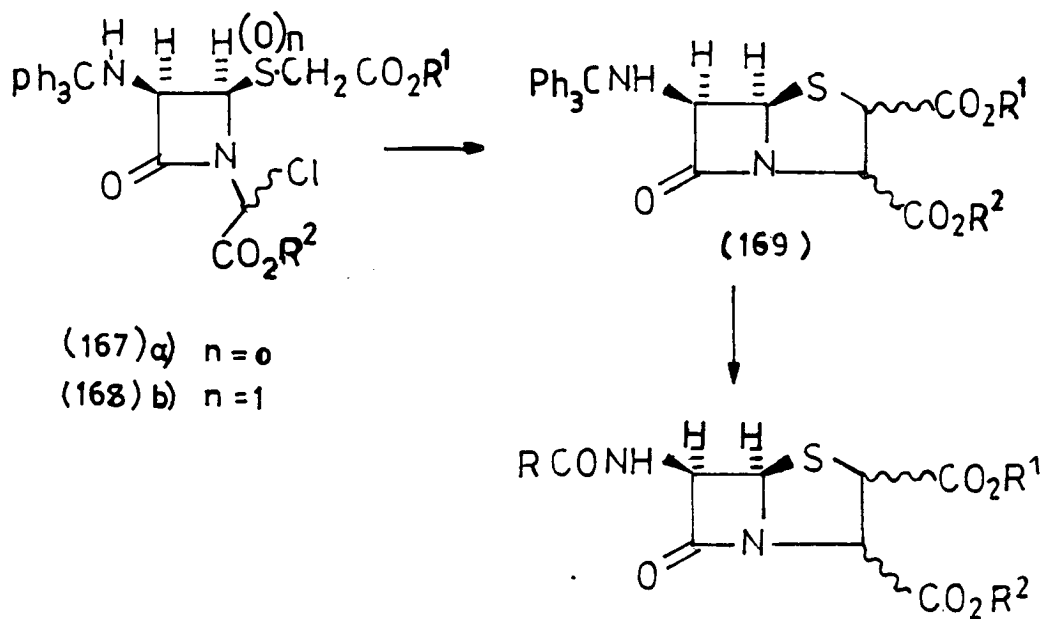
(170)   R = PhOCH$_2$, R^1,R^2 = ^{t}Bu

(171)   R = PhOCH$_2$, R^1 = Me, R^2 = PNB

(172)   R = PhOCH$_2$, R^1 = Me, R^2 = H

(173)   R = PhCHNH$_2$, R^1 = Me, R^2 = H

(174)   R = PhOCH$_2$, R^1, R^2 = H

Scheme 68   Use of a carbanion intermediate for a successful cyclisation.
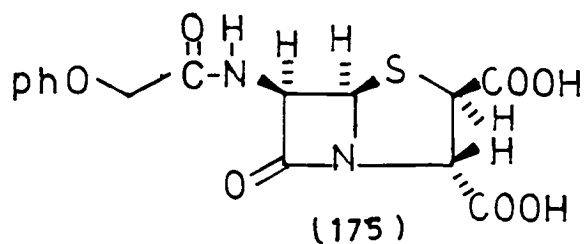
Scheme 69   Structure of penicillin diacid.

(176)   (177)
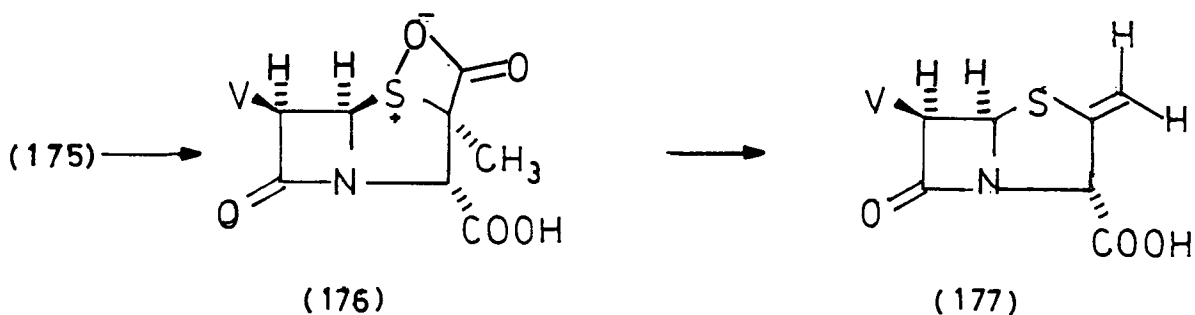
Scheme 70   Synthesis of the 2-exomethylene penam system.

(178) R =

(179) R = H

(180) R = H
(181) R = CHOHCO₂CMe₃
(182) R = CHClCO₂CMe₃

(183) R = CMe₃
(184) R = H
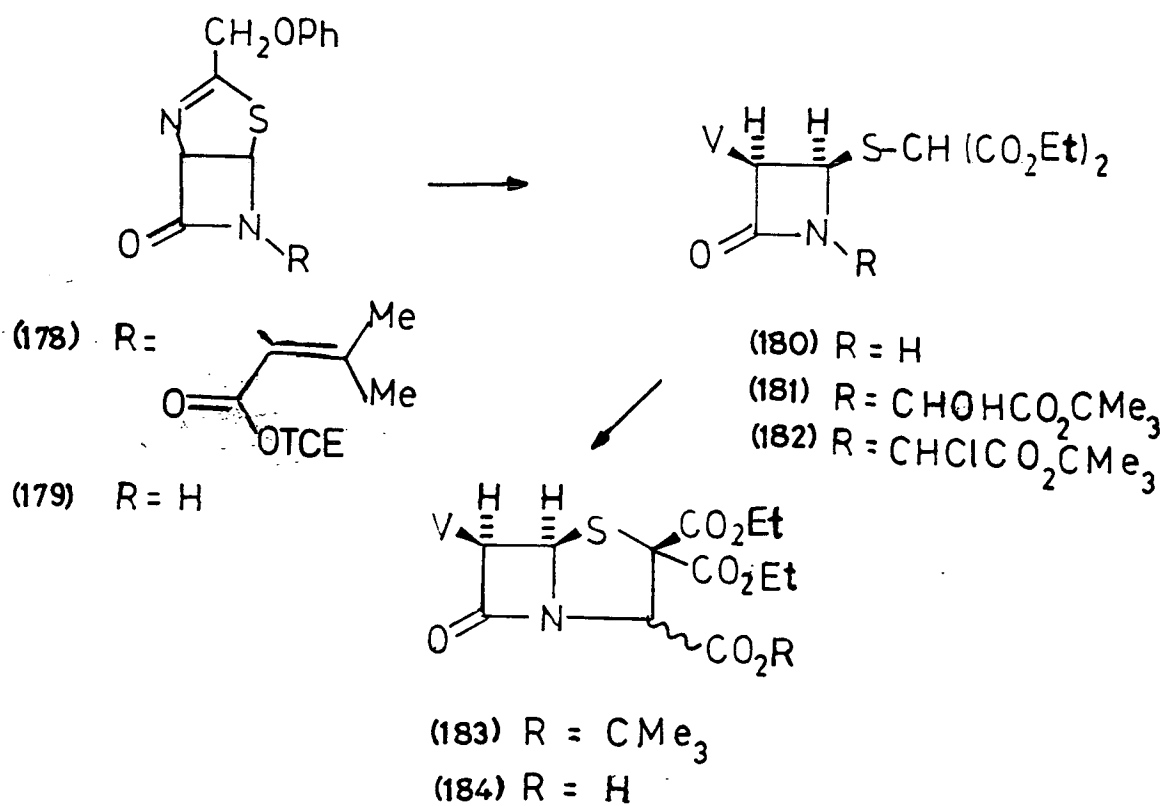
Scheme 71   Synthesis of a β-lactam derivative possessing
             a diester functionality at C-2.

urea and an antioxidant, at 50°C, S-alkylation occurred
with hydrolytic opening of the thiazoline ring.   This
procedure was applied using diethyl bromomalonate and the
thiazoline (179), resulting in (180) which was taken
through subsequent transformations to afford (182).   The
cyclisation step from (182) to (183) avoided the need to
oxidise the sulphide to the sulphoxide, since the methine
proton was sufficiently acidic, (due to the electron with-
drawing effect of the two ester groups), to generate the
carbanion and spontaneously cyclise under the mild
reaction conditions.   Attempts to obtain the tri-acid
derivative failed.

h)   Synthesis of a penam nucleus via a 5-exo-tet
        cyclisation involving a substitution by a sulphur
        nucleophile at the C-4 position.

        A synthesis of the penam system has been
reported,[110] which involves a 5-exo-tet cyclisation onto
the 4-position of the azetidinone (186) (scheme 72).
The method affords a mixture of diastereomers and results
in a number of isomeric products (187).   Intermediate
(185) is obtained by total synthesis, via the
ketene-imine method.   Chlorinolysis of the
sulphur-carbon bonds afforded a diastereomeric mixture of
(186).   This was cyclised[111] by treatment with one
equivalent of anhydrous tin (II) chloride in dioxane at
room temperature to afford (187) as a diastereomeric
mixture.
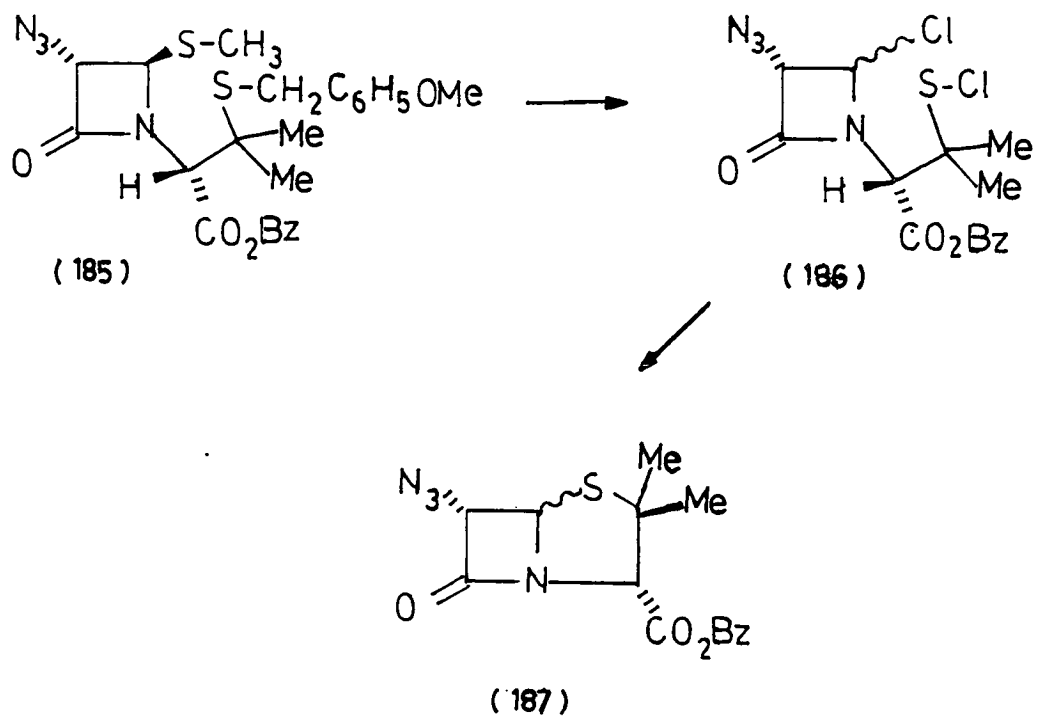
Scheme 72   A 5-exo-tet cyclisation involving a
nucleophilic substitution at C-4 of the
azetidinone (186).

3.2.3   OXAPENAM

The oxapenam derivatives are the oxygen analogues of pencillins. They are usually less stable, and possess lower activities than the parent systems. The different chemical properties of oxygen in relation to sulphur has necessitated different methods of cyclisation to afford bicyclic fused systems.

a)   Synthesis of an oxapenam molecule _via_ a 5-endo-trig cyclisation.

An early approach[112] (scheme 73) involved the transformation of the oxazoline (188), obtained by degradation of penicillin G (1) with mercuric acetate at room temperature,[113] to oxapenicillin G (189) _via_ treatment with a lithium alkyl thiolate in hexamethyl phosphoramide, followed by acidification to pH 2.3. The product (189) was not isolated but possessed similar polarity to penicillin G, and had similar biological activity to pencillin G which was lost on exposure to a pencillinase. A further method which was reported by Wolfe[114] involved the 5-endo-trig cyclisation of (192) to (189) (scheme 74). This, however, has proved impossible in the sulphur analogue (161). In the opinion of the author, both of the above reports are questionable on the grounds that if the stereoelectronics of the sulphur analogue (161) are incorrect, as described by Baldwin,[102] then the oxygen analogue (192), which would require a much tighter angle of "nucleophilic trajectory", would not be possible, particularly when considering the nucleophilicity and size of oxygen compared to sulphur. Secondly, the transition state for the oxygen analogue would be expected to be less stable, hence more difficult to form, due to the electronegativity of oxygen which would give rise to a more strained bicyclic fused system,
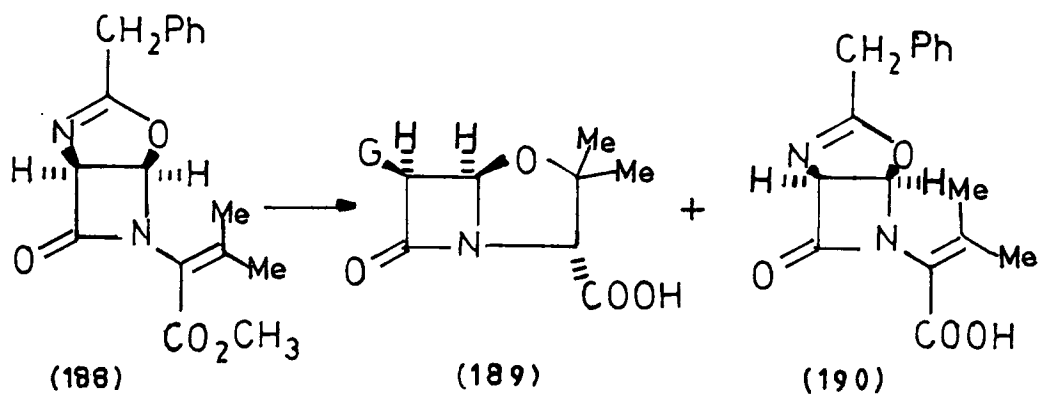
Scheme 73
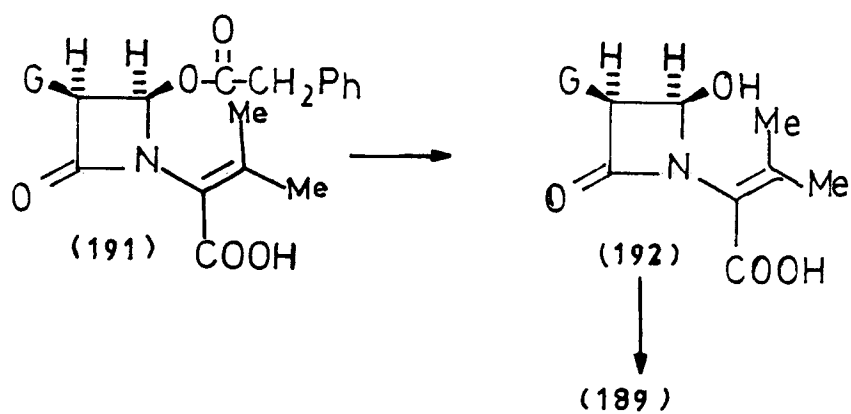
Scheme 74
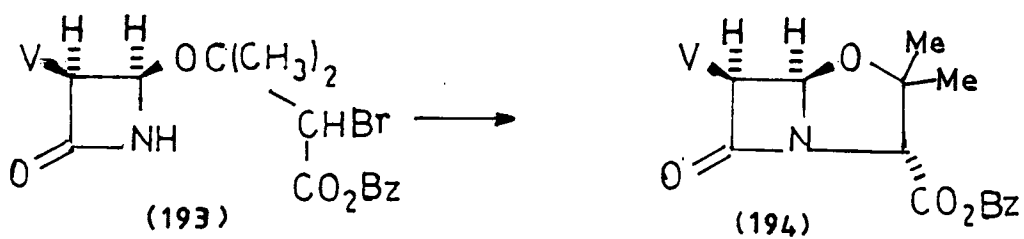
Scheme 75

compared to the sulphur system. Finally, the
characterisation data available for the products are not
sufficient for an unequivocal representation of the
structure.

**b)   Synthesis of the oxapenam derivative via a 5-exo-
        tet cyclisation involving the β-lactam nitrogen.**

The 5-exo-tet mode of cyclisation (scheme 75),
involving the β-lactam nitrogen in a displacement of a
halide, has not been possible in the sulphur analogue
(153), where the <u>cis</u> geometry of the β-lactam
substituents exists.   However, in the oxaderivative
(193), this method of cyclisation has been realised by
base treatment to generate (194).[115]

**c)   Synthesis of the oxapenam derivative involving a
        carbene insertion reaction.**

A novel method of preparing bisnordethiaoxa-
penicillin G (196) has been reported by Cama and
Christensen,[116] involving a carbene insertion into the
β-lactam nitrogen-hydrogen bond (scheme 76).   This
approach has not been possible for the sulphur analogues,
owing to complications involving the polarisable lone
pairs on the sulphur atom and the electrophilic carbene
intermediate.

**c)   A 5-exo-trig cyclisation to an oxapenam
        derivative.**

A recent report[117] indicated a 5-exo-trig mode of
cyclisation of (199) to (200), involving the β-lactam

(195)   (196)
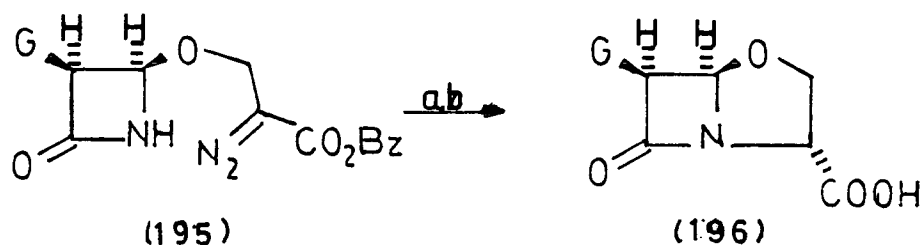
G=PhCH₂CONH

a)   Rh₂(OAc)₂

b)   H₂/Pd/C

Scheme 76

(84)   (197)   (198)

(200)   (199)
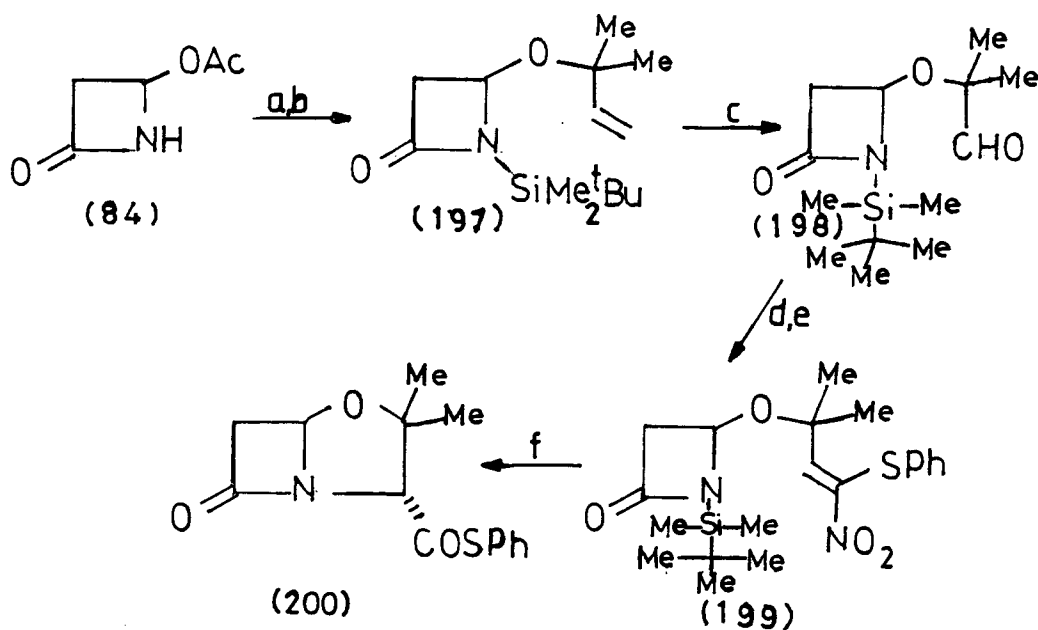
a)   Me₂C(OH)CH=CH₂, Zn(OAc)₂.2H₂O,PhMe,80°C

b)   Me₂ᵗBuSiCl, EtN(ⁱPr)₂, CH₂Cl₂, 25°C

c)   O₃,CH₂Cl₂, -78°C;   Me₂S

d)   PhSCH₂NO₂, tBuOH, THF tBuOK (0.1 equiv.) 0°C

e)   MsCl, Et₃N, CH₂Cl₂, -78°C to 0°C

f)   Bu₄N⁺F⁻, THF, -55°C;   O₃, THF-CH₂Cl₂, -78°C

Scheme 77

ring nitrogen to generate an oxapenam derivative (200) in high yield (scheme 77).

3.2.4   CARBAPENAM

The carbapenam derivatives possess a methylene group in place of a sulphur atom. These are very unstable in comparison to the penam system. They do, however, possess biological activity.

a)   **Synthesis of a carbapenam nucleus by a ring contraction method.**

An important route to these derivatives involves the ring contraction of a 3-diazopyrrolidin-2,4-dione derivative (201) (scheme 78), to result in the carbapenam (202).[118]

b)   **Synthesis of a carbapenam nucleus by a 5-exo-trig cyclisation.**

A more recent method[117] involves the azetidinone (203),which undergoes a 5-exo-trig mode of cyclisation involving the β-lactam nitrogen to generate (204) in high yield (scheme 79).

c)   **Synthesis of a carbapenam nucleus by a 5-exo-tet cyclisation.**

A further example of a 5-exo-tet mode by cyclisation to generate the carbapenam system has been reported (scheme 80)[119]. The derivative (205) was obtained from (207) (scheme 81)[120] by standard procedures, for example alkylation of the β-lactam nitrogen with diethyl bromomalonate, ozonolysis (with a

Scheme 78

(203)

R=SiMe₂t-Bu

X=C(SPh)NO₂

Scheme 79

ε=CO₂Et , J=OTs
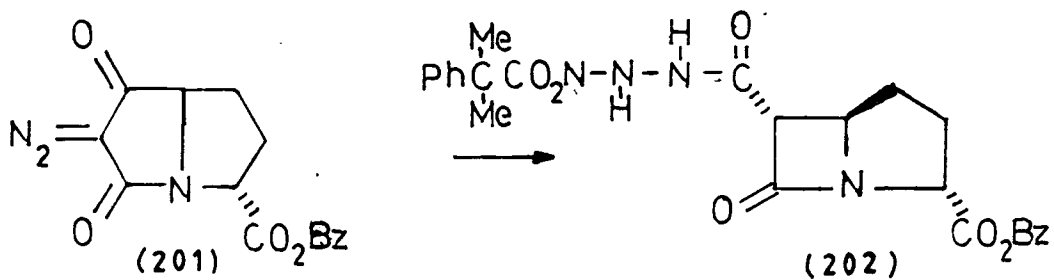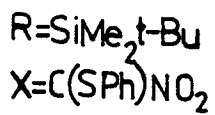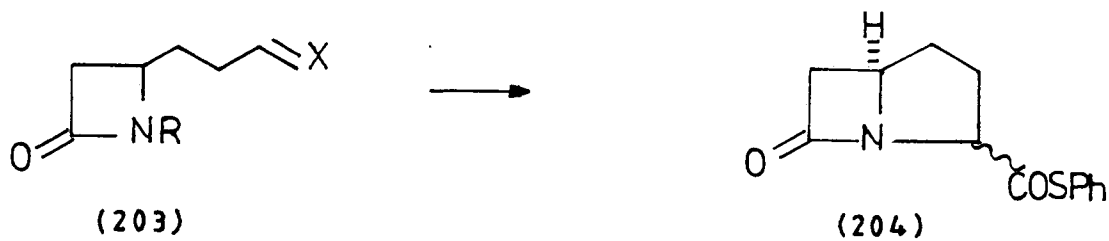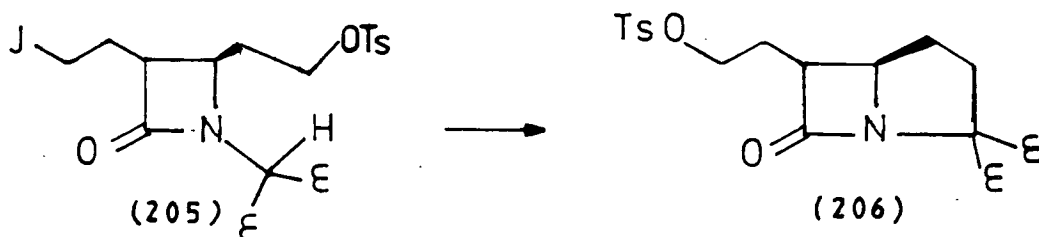
Scheme 80

reductive work-up), followed by tosylation. The cyclisation of (205) was effected by treatment with base at 0°C.

3.2.5   AZAPENAM

The azapenam derivatives are the nitrogen analogues of penams. Their unstable nature has attracted only a few synthetic attempts. The biological activity of these analogues is not very promising. However, some weak growth inhibiting properties of bacteria are evident.

A total synthesis of a 2-azapenam system has been reported by Huffman and co-workers (scheme 82).[121] The versatile intermediate (78), as utilised for the synthesis of the isopenam (155), was again used by the same authors. The interesting step involves the 5-endo-trig cyclisation by the β-lactam nitrogen onto the imine to result in the bicyclic system (209). (210) was reported to be unstable, but showed some bacterial growth inhibiting properties.

3.2.6   PENEM

The penem system (211) (scheme 83) was the first unnatural unsaturated fused β-lactam system to be obtained synthetically. This structure encompasses both virtues of the natural products: the ring strain, present in the penicillins, and the electron withdrawing aspects of the enamide group present in cephalosporins. This novel structure was pioneered by R.B. Woodward and was delivered in his lecture presented at the first symposium dedicated to advances in the β-lactam
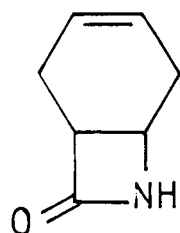
(207)

Scheme 81   Structure of intermediate for carbapenam and carbapenem synthesis.
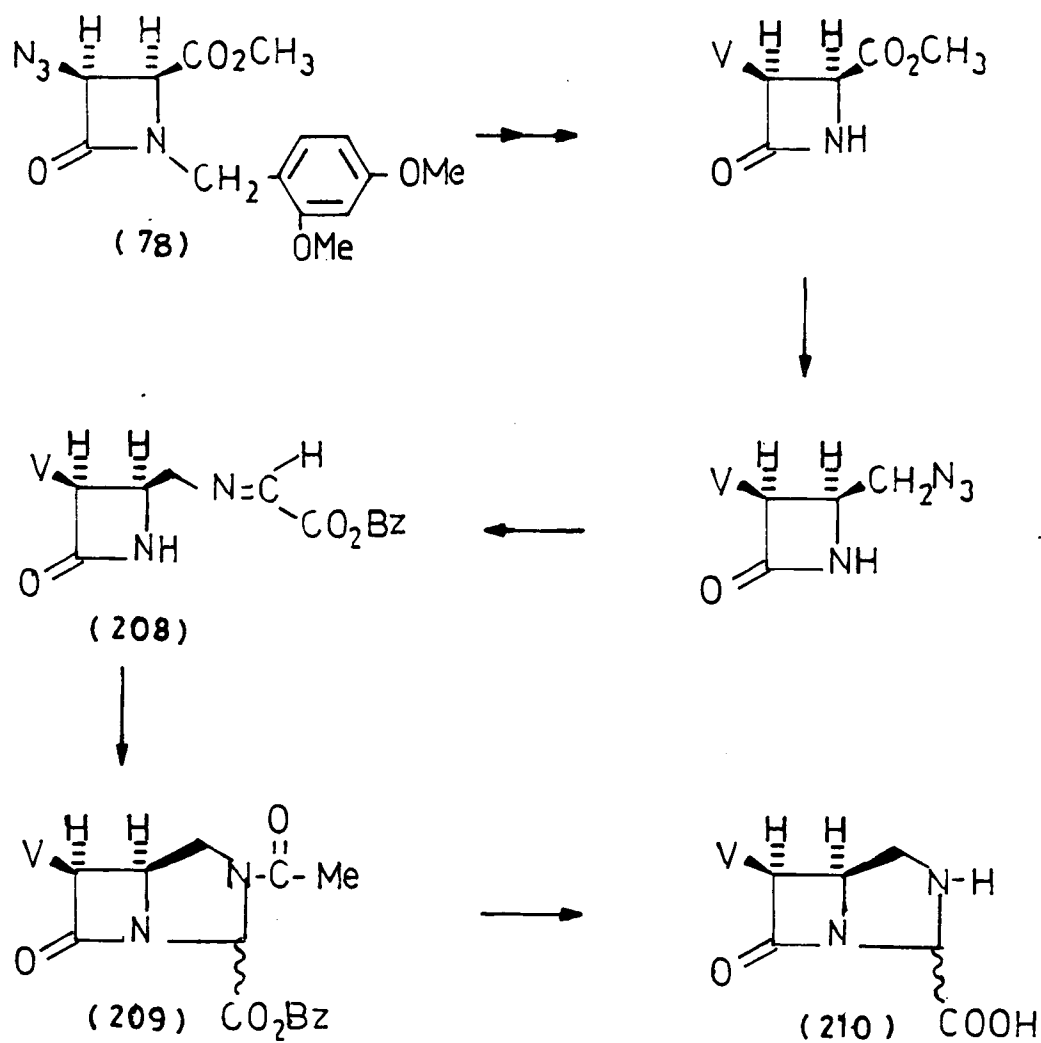
Scheme 82

antibiotics.   The penem derivatives were found to have inherent biological activity.   However, their instability to physiological conditions questions their use as potential antibiotics for commercial use.

a)   Wittig reaction for penem synthesis.

The synthesis of the penem system has been attempted by many workers, but only a few strategies have been employed to obtain the bicyclic nucleus.   The earliest attempt was made by Woodward (scheme 84)[123], which involved an intramolecular Wittig reaction of the phosphorane derivative (212) to afford the penem (213). The phosphorane (212) was readily obtained by degradation of naturally occurring penicillins by well known procedures followed by functionalisation of the β-lactam nitrogen, as described earlier.[78]   The success of the cyclisation was very dependent on the electron withdrawing capacity of the carboxylate protecting group (R^3), strongly electron withdrawing groups requiring longer reaction times and higher temperatures.   The 6β-acetamidopenem derivatives obtained showed some biological activity against Gram-positive strains of bacteria, but unfortunately, penem (214) was found to be labile under acidic conditions, even on silica gel, resulting in decomposition to a ketene (215) and a thiazole (216) (scheme 85).

The Wittig method has been a very popular one for constructing highly modified bicyclic fused systems and has been used by many workers.

(211)
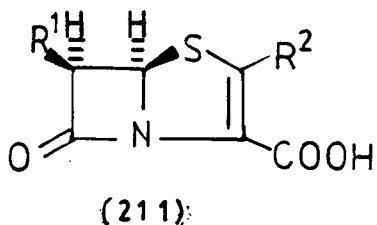
Scheme 83   Structure of a penem nucleus.

(212)   (213)

Scheme 84

(214)   (215)   (216)
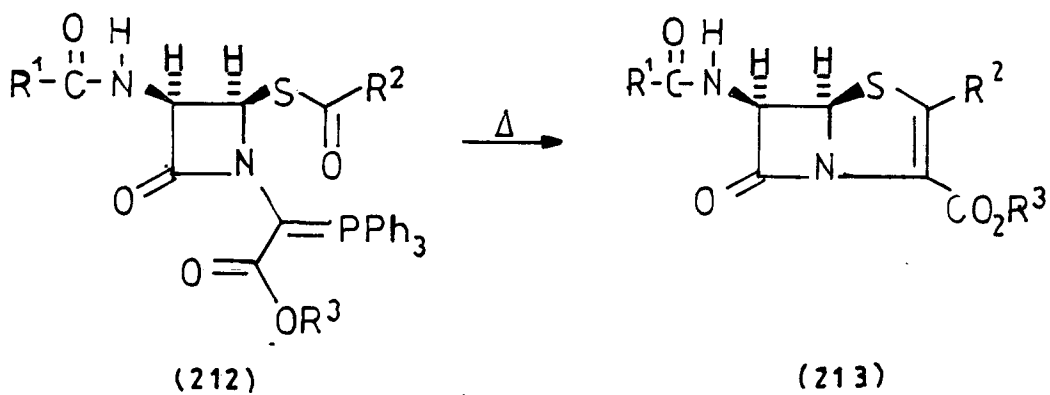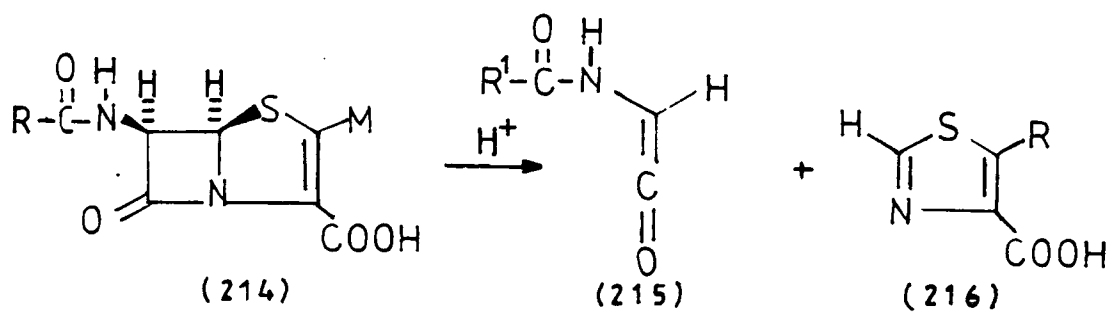
Scheme 85

b)   Nucleophilic substitution at C-4 for a penem synthesis via 5-exo-tet cyclisation.

Another approach to the penem system was devised by the Glaxo research group[124] and involved an internal nucleophilic displacement at the C-4 position of the azetidinone (218) to afford the penem (219) (scheme 86).   One major drawback of this method was the diastereomeric mixture that resulted due to epimerisation at C-5 of (219).   Intermediate (218) was obtained from the clavulanate (217), by a route involving a novel betain intermediate (221) (scheme 87).   In-vitro experiments on (220) suggested that it had very good activity.   However, in in-vivo test results were disappointing.

c)   Penem synthesis involving a sulphur extrusion process.

A novel method to the penem system (223) has been developed by the Farmitalia group,[125] involving a sulphur extrusion process from intermediate (222) (scheme 88) which can be obtained quite readily from a threonine-derived 4-benzoyloxyazetidinone derivative (224) (scheme 89).   This method has recently been used to obtain a 1-dethia-1- selenapenem derivative (225) (scheme 90).[126]   The mechanism of this reaction, as proposed[126] in scheme 91, explains the lack of stereochemical control at C-5.   It has been reported that by appropriate choice of solvent, one isomer can be produced in excess, particularly if the hydroxyethyl side chain is unprotected (R=H).
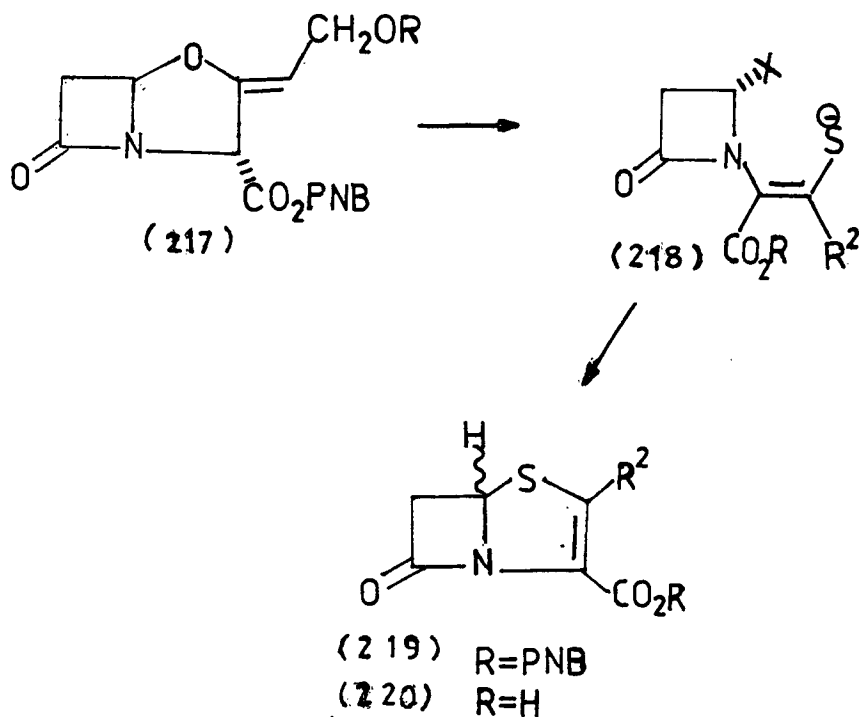
Scheme 86
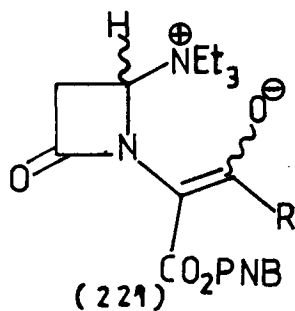
Scheme 87   Structure of betaine derivative obtained from clavulanic acid.

(222) → (223)

Scheme 88

(224)
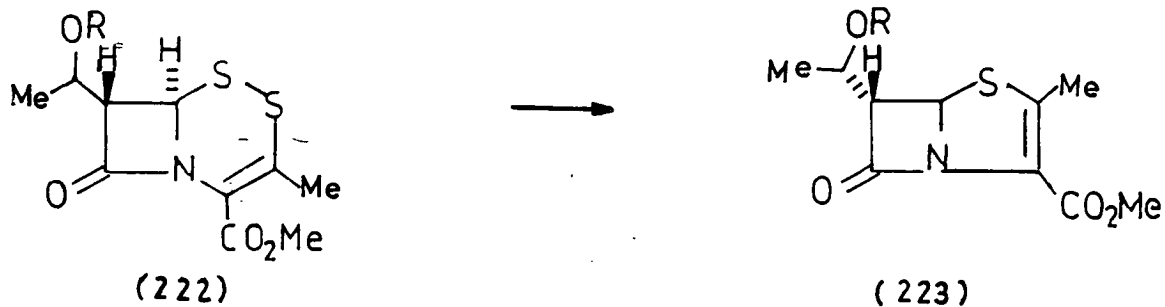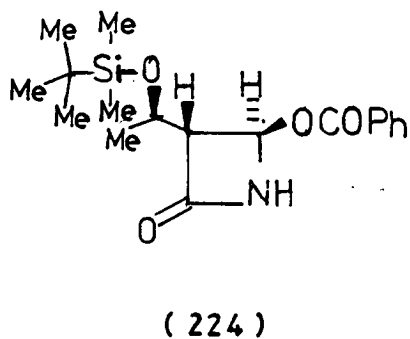
Scheme 89   Structure of azetidinone used for penem synthesis.

(225)
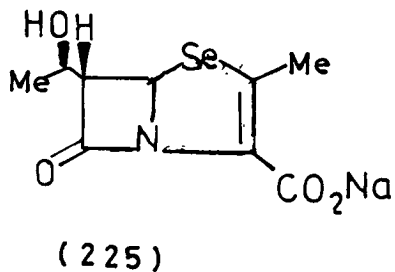
Scheme 90   Structure of a selenopenem derivative.

d)   **Synthesis of a penem nucleus _via_ a 5-exo-trig cyclisation involving the β-lactam nitrogen.**

Wasserman and Hans[127] have recently reported a very interesting approach to a penem derivative (236) by a route which is sterochemically controlled at C-5 and C-6 (scheme 92).   Derivative (228) is readily obtained by a nucleophilic displacement of the acetoxy group in derivative (237) by dimethyl thioacetonedicarboxylate and base (scheme 93).   Protection of the N-H group by tert-butyldimethylsilyl chloride, followed by ozonolysis at low temperature resulted in (230).   Treatment of (230) with N,N-dimethylformamide dimethyl acetal and base gave the enamino derivative (231).   The enamine (231) was subjected to photooxidation and afforded the tricarbonyl system (232).   Desilylation led directly to the bicyclic system (234).   The hydroxyl group in (234) was removed by conversion to the chloride followed by treatment with zinc and aqueous acetic acid to form (235).   The thiolactone (235) was etherified by treatment with diazomethane to afford the penem system (236).

e)   **Synthesis of a penem nucleus _via_ a carbene insertion reaction.**

It is interesting to note an unsuccessful approach to the penem system (scheme 94)[128] which involved intermediate (238), where it was hoped that a carbene insertion would give the bicyclic fused system (239). However, all attempts at cyclisation failed.   This is surprising since, as will be seen later, in the carbapenem synthesis this ring closure takes place successfully.   However, an efficient chiral synthesis to a 2-alkylthiopenem derivative (243) has been reported which involves a carbene insertion into a
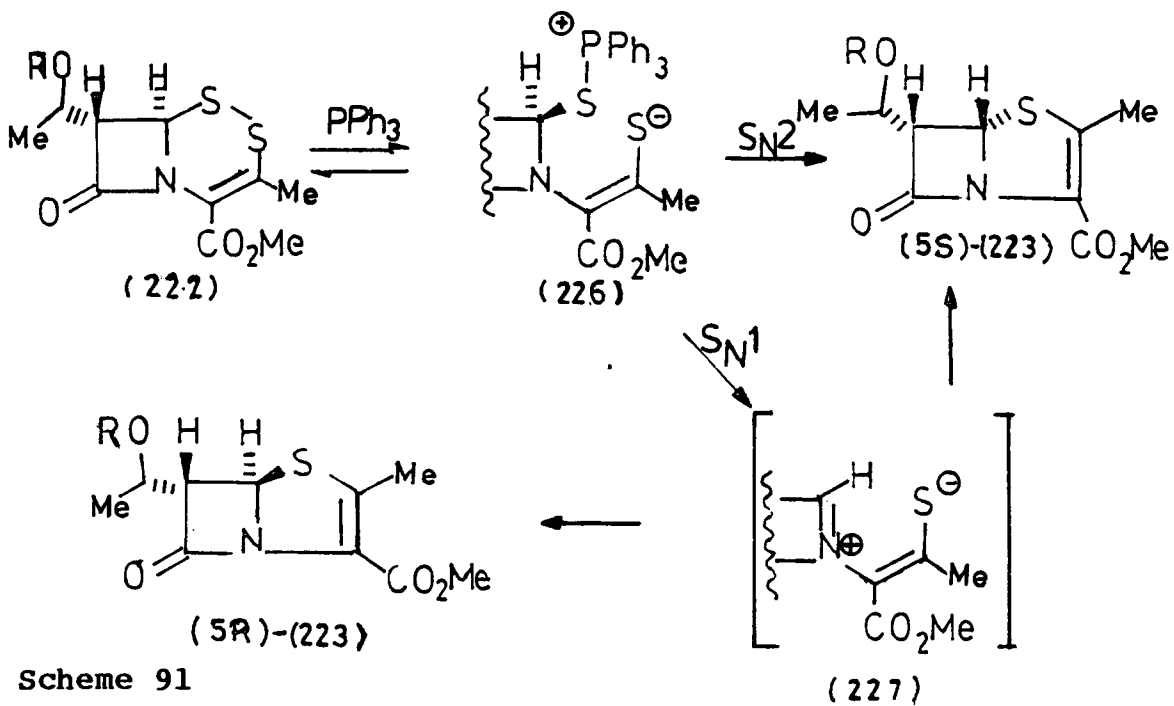
Scheme 91
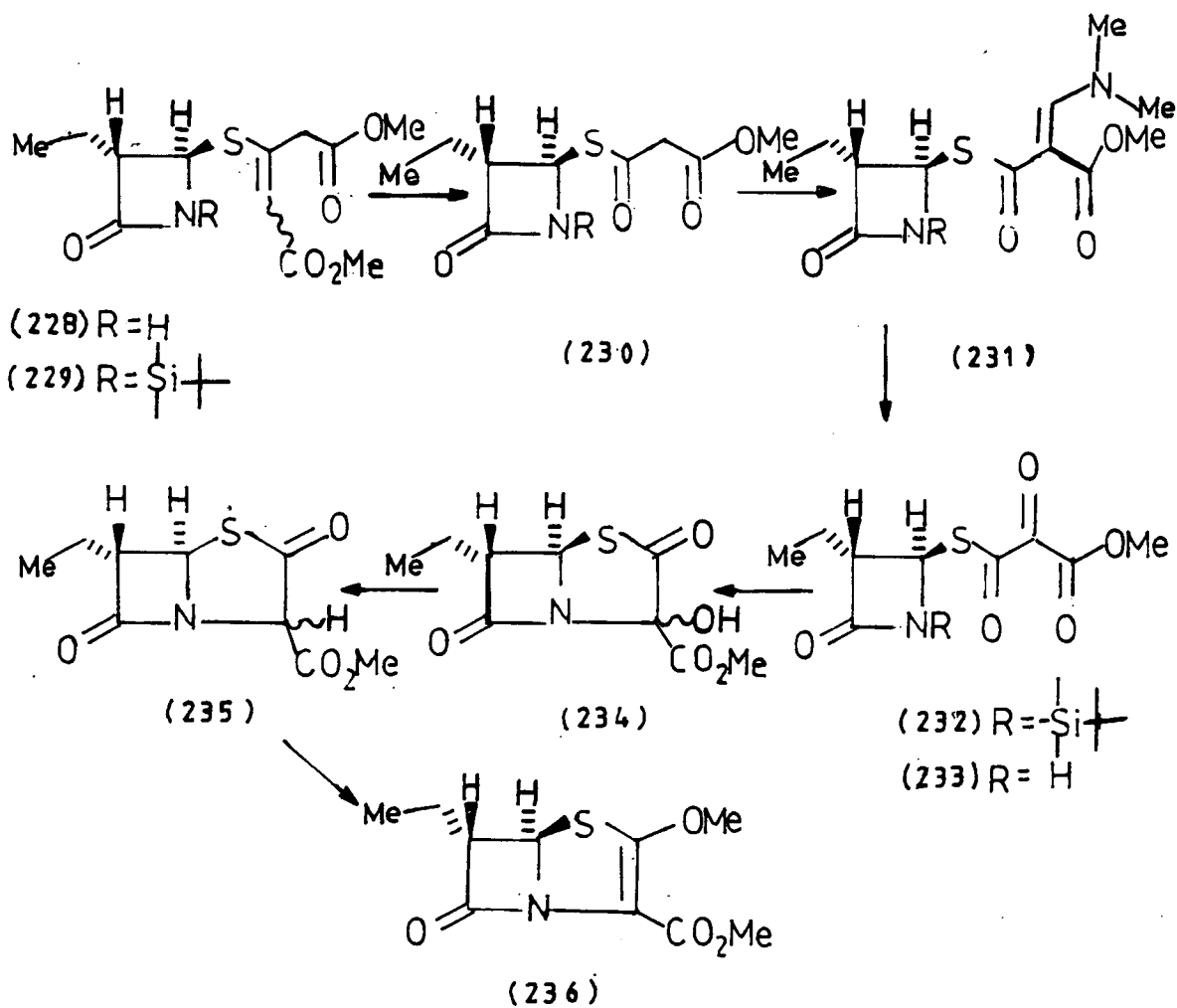
Scheme 92
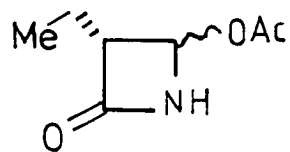
(237)

Scheme 93
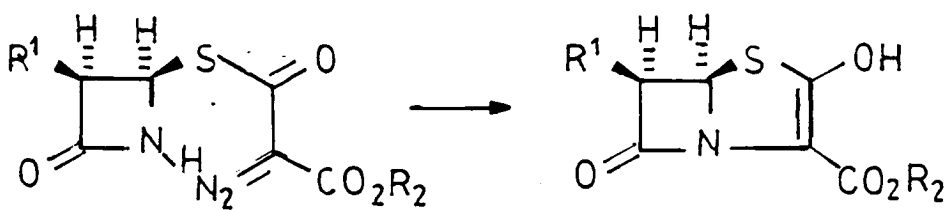
(238)   (239)

Scheme 94
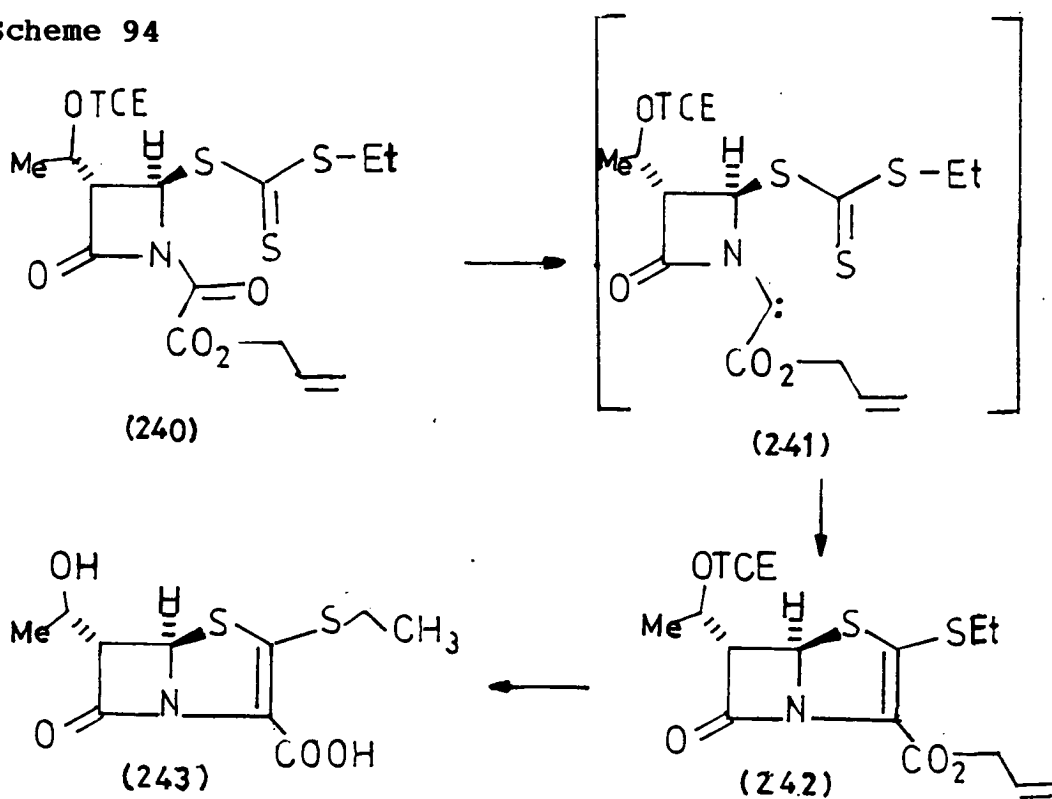
(240)   (241)

(243)   (242)

Scheme 95

trithiocarbonate protecting group in moderate yields (scheme 95).[129] This route is adaptable for a large scale synthesis of (243) which is a potent antibiotic with broad spectrum activity and β-lactamase resistant properties. This compound is now commercially produced by Schering.[130] Derivative (240) is readily obtained as outlined in scheme 96.

f)   Structural aspects of the penem system.

The penem system was found to possess inherent biological activity.[123] However, early attempts at utilising the acylamino side chain as in penicillins and cephalosporins did not impart the stability in this system that was necessary for _in-vivo_ activity. The 6-unsubstituted penem derivative was also synthesised,[131] but although this exhibited high activity in _in-vitro_ testing, _in-vivo_ results were disappointing. Furthermore, it was established[132] that the 5R isomer of the penem system was essential for biological activity. Consequently, routes affording a racemate would not be acceptable for commercial synthesis.   It was the discovery of thienamycin[13] that prompted the introduction of the hydroxyethyl group at the C-6 position of the penem system[133] and which finally led to the highly active derivative (243).

Scheme 96

(19)

Scheme 97

(244)

(245)
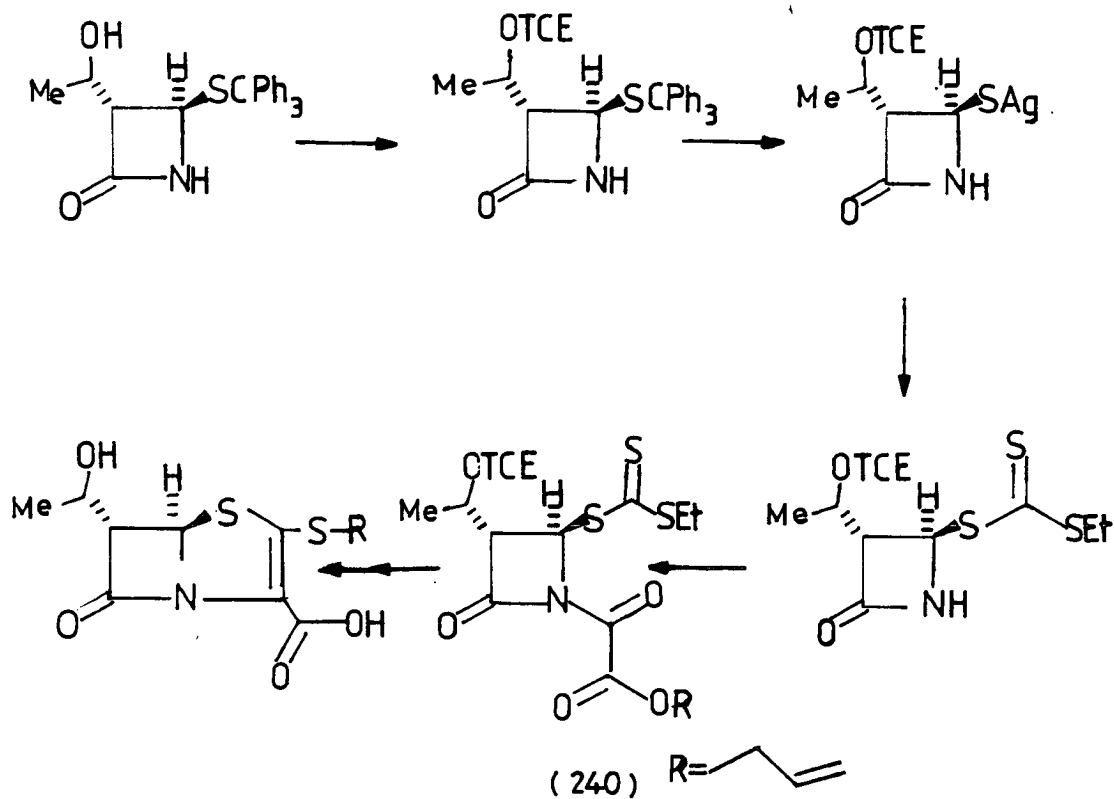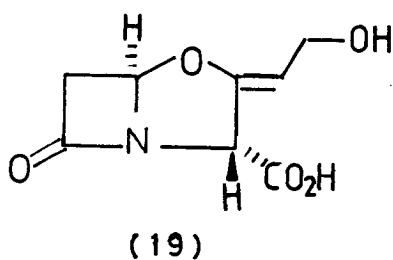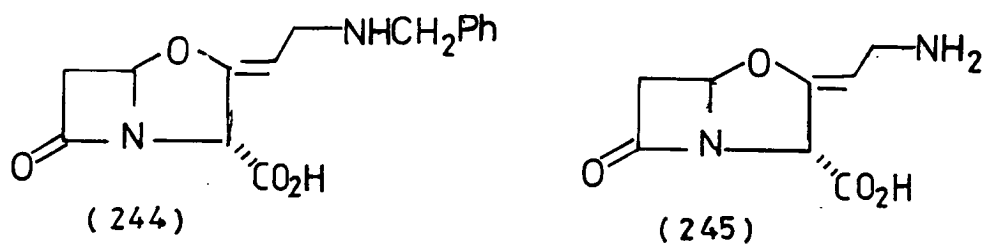
Scheme 98 Structures of modified clavulanic acid.

3.2.7   CLAVEM-OXAPENEM

Clavulanic acid (19) (scheme 97) is the parent compound of this class of antibiotics. It possesses moderate antibacterial activity but shows very high specificity for β-lactamases. It is for this reason that it is used as an inhibitor in conjunction with a pencillin, such as amoxycillin (8) or ampicillin (4), to treat patients infected with β-lactamase producing bacteria.

Beecham workers have undertaken a thorough investigation into its modification and its chemistry.[134]   It would appear that the isolated structure (19) possesses near optimal features for the maximisation of its important properties. A derivative which has five times the activity of (19), is the N-benzyl protected amino analogue (244) (scheme 98).[135] This is believed to be due to increased blood levels and half-life, as is found with the free amino derivative (245) (scheme 98), relative to clavlanic acid (19).

a)   **Synthesis of clavulanic acid by a 5-exo-tet cyclisation.**

The first racemic synthesis of clavulanic acid (19) was reported by workers at Beecham, this involved a 5-exo-tet cyclisation of an enol onto the C-4 position of the azetidinone (246) to result in (247) (scheme 99).[136]   No chiral synthesis of clavulanic acid is available at present.[137]
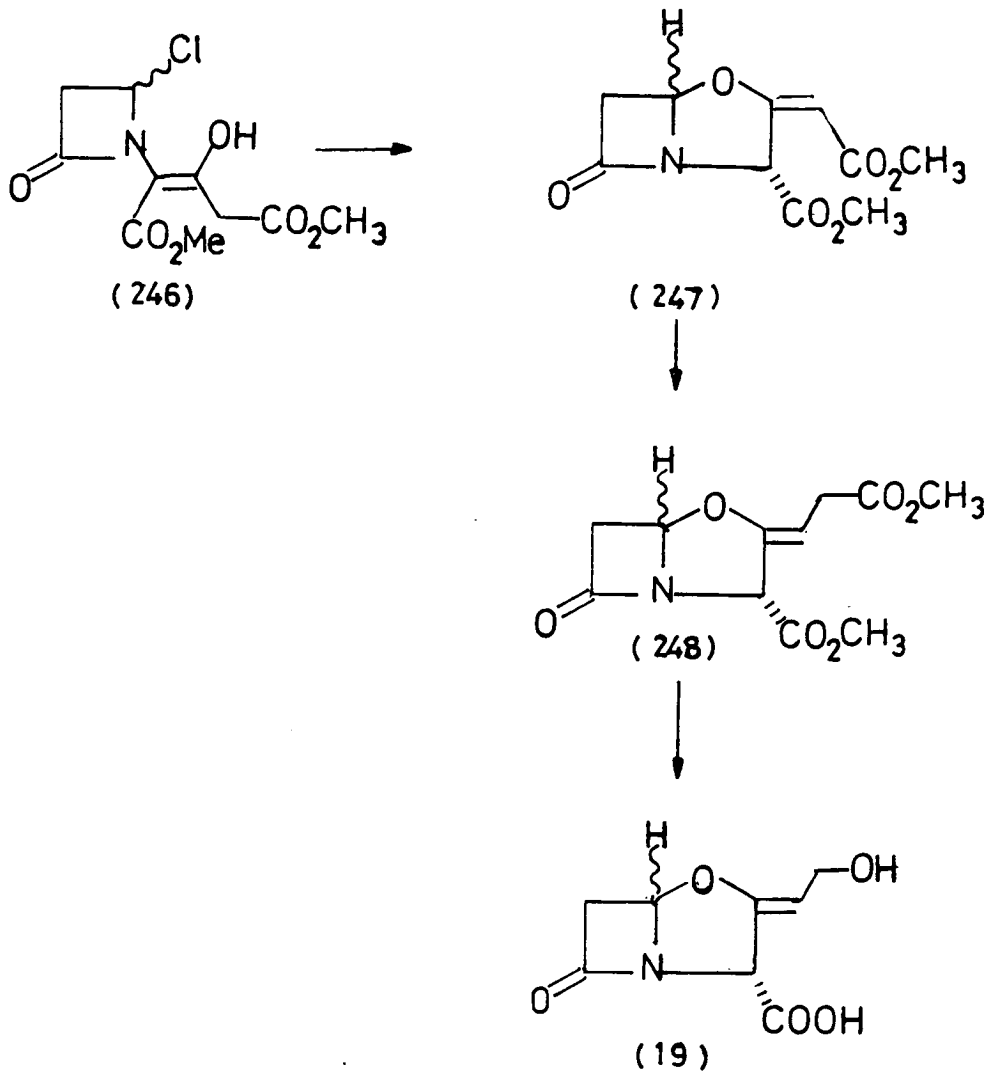
Scheme 99
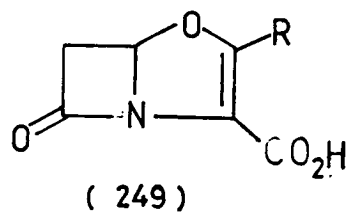
Scheme 100 Structure of an oxapenem derivative.

b)   **Synthesis of an oxapenem by isomerisation of clavulanate.**

The oxapenem derivative (249) (scheme 100) could be expected to be obtained by base treatment of clavulanic acid.   Attempts to carry out this isomerisation on a model compound (250) resulted in a very interesting oxazoline ring opening reaction (scheme 101).[138]   The Glaxo group found that treatment of (250) with triethylamine resulted in the betaine (251) which,on heating in ethyl acetate,furnished the endocylic bicyclic dervative (252).   This derivative was reported to be a powerful inhibitor of cell free β-lactamases.   The betaine (251) was found to be quite useful in that the C-4 position of the azetidinone could be substituted by a variety of nucleophiles.   Furthermore, the ring could be easily reformed by heating.   This procedure was used to synthesise penems,as described earlier.[124]

c)   **Stability aspects of oxapenem derivatives.**

Oxapenems were found to be highly unstable to normal physiological conditions.   Their synthesis, for antibiotic purposes, is **beset** with problems, particularly their instability to acids and bases, and only workers at Beecham and Glaxo have continued to concentrate their efforts on these derivatives.[139-142]

3.2.8   CARBAPENEM

Thienamycin (20) and olivanic acid (22) were the first compounds isolated in this class of antibiotics. Since their discovery in the late   **1970's   ,**
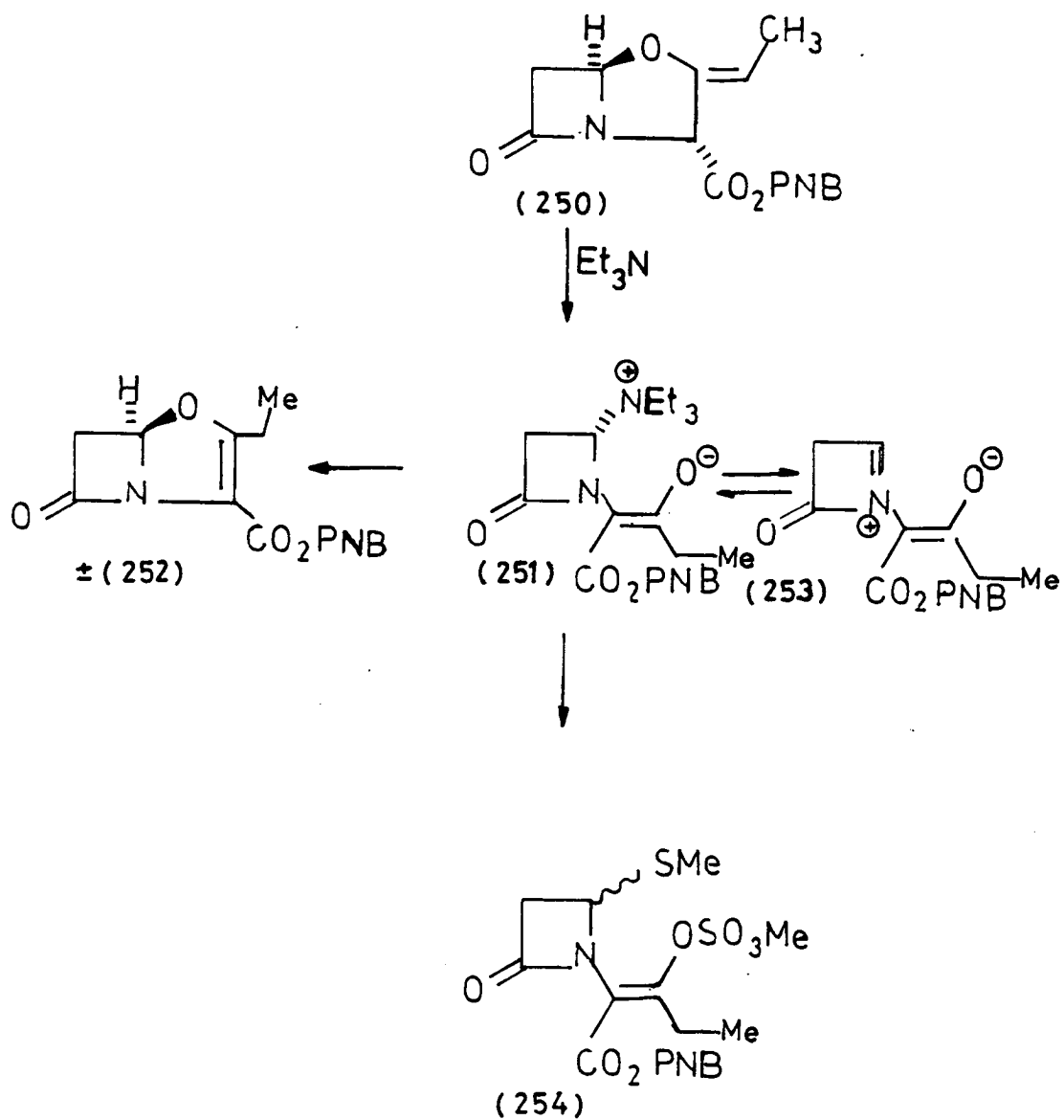
Scheme 101

several similar structures which vary according to the C-2 and C-6 substituents, and to the substituent stereochemistry of the β-lactam ring, have been isolated.   Examples include carpetamycin (255),[143] asparenomycin (256),[144] and PS-5 (257)[145] (scheme 102). The characteristic properties of high potency, broad spectrum activity and β-lactamase resistance have made the carbapenem derivatives potentially extremely valuable antibiotics.   Unfortunately, these antibiotics suffer from physiological instability, particularly to enzymes (the dehydropeptidases) present in the mammalian kidneys.   Thienamycin has been found to possess the optimal stereochemical requirements for maximum activity (namely anR-hydroxyethyl group, trans geometry around the β-lactam ring and the Δ^2 double bond), in the carbapenem series.[146]   Consequently, this section will only be concerned with synthetic approaches to thienamycin (205).

a)   Synthesis of a carbapenem molecule by a 5-exo-tet cyclisation involving a carbanion.

The first synthesis of thienamycin (20) was reported by the Merck group in 1978 (scheme 103)[147]. Derivative (258) was obtained from a long synthesis starting from chlorosulphonyl isocyanate and 1-acetoxybutadiene.   Cyclisation was accomplished via the bromonium ion (259), by treatment with base, to furnish (260), which was then dehydrobrominated to (261).   The 8R and 8S isomers could be separated at this stage.   Decarboxylation of the 8R-isomer gave (262) which, after isomerisation to a 4:1 mixture of (263) and (262), was separated by chromatography.   Finally, hydrogenolytic removal of protecting groups from (263), yielded synthetic racemic thienamycin (20).
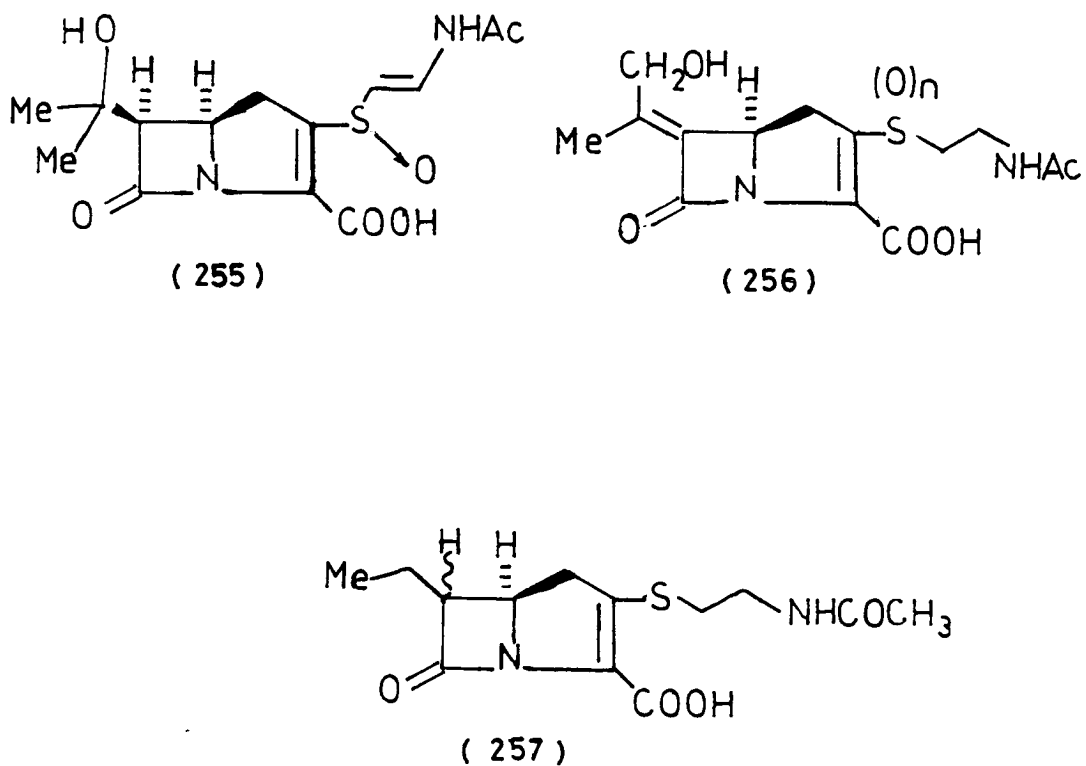
Scheme 102   Structure of recently isolated carbapenem
derivatives.
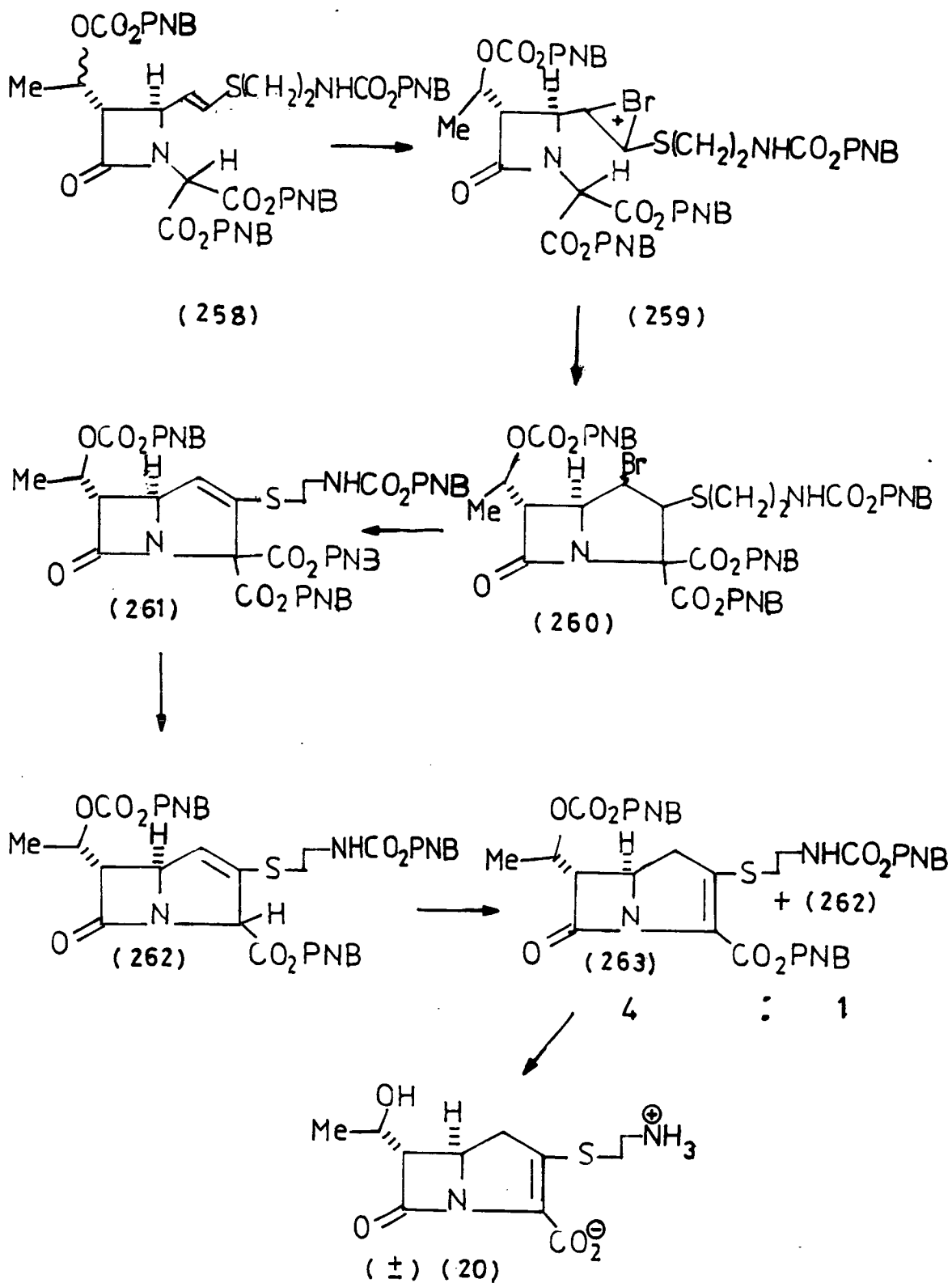
Scheme 103

b) Carbapenem synthesis involving the Wittig reaction.

The intramolecular Wittig reaction, as devised by
Woodword for penem synthesis,[123] has been utilised to
obtain the thienamycin nucleus (265) (scheme 104).[148]
An important intermediate (266) (scheme 105) that has
been developed for carbapenem synthesis has been
reported.[149]   This requires the use of the Wittig
methodology for a cyclisation to occur.   Ozonolysis of
(266) generates a dialdehyde derivative which
spontaneously cyclises to the carbapenem nucleus and the
aldehyde group at the C-6 position is available for
further modifications.   Recently, this derivative (266)
has been employed for the synthesis of a number of
carbapenem analogues.[150]

**c) Carbapenem synthesis involving a carbene insertion
reaction.**

Another important route that was developed for the
synthesis of carbapen-2-em ring system was reported by
the Merck group (scheme 106).[151]   The important aspect
of this synthesis is the rhodium acetate catalysed ring
closure of (269) to (270).   The cyclisation involves a
carbene insertion into the azetidinone N-H bond.
Reaction of (270) with phosphonyl chloride/base and also
a protected cysteamine followed by deprotection of all
the protecting groups readily generates thienamycin
(20).   This approach has been used for the synthesis of
the asparenomycins.[152]   This route is generally the
method of choice for carbapenem derivatives.[153-156]
Consequently, much work is at present directed towards
economic chiral syntheses of intermediates such as
(267)[157-159] and (271)[160-161] (scheme 107).   The high
sensitivity of the carbapenem nucleus to acids and bases

(264)   (265)

Scheme 104

(266)
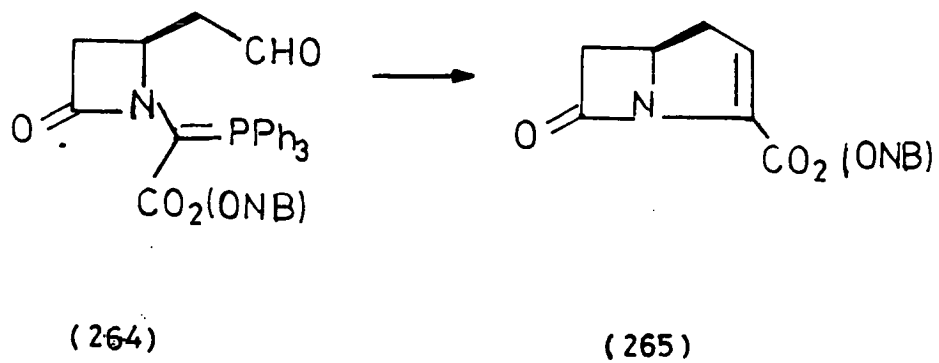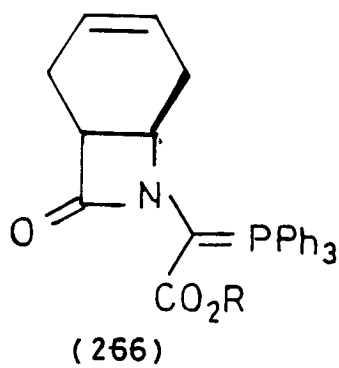
Scheme 105   Structure of intermediate for carbapenem
             synthesis.
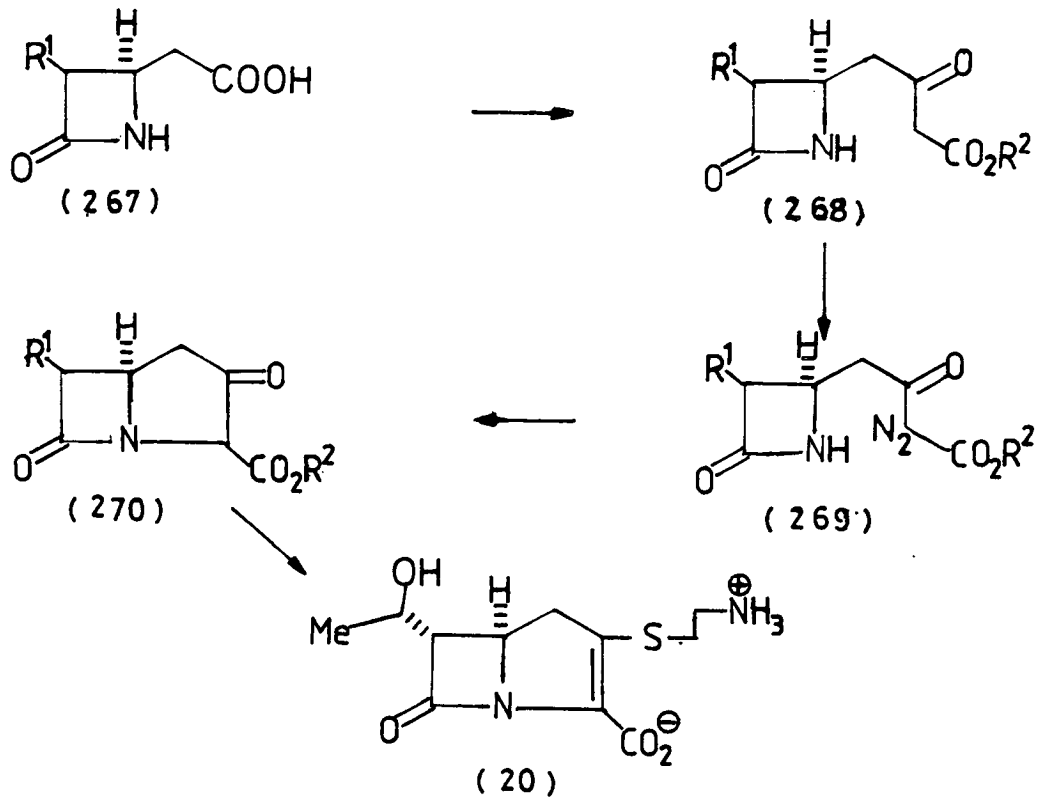
Scheme 106
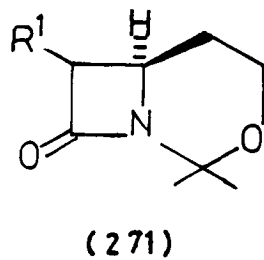
Scheme 107 Structure of intermediate for carbapenem synthesis.
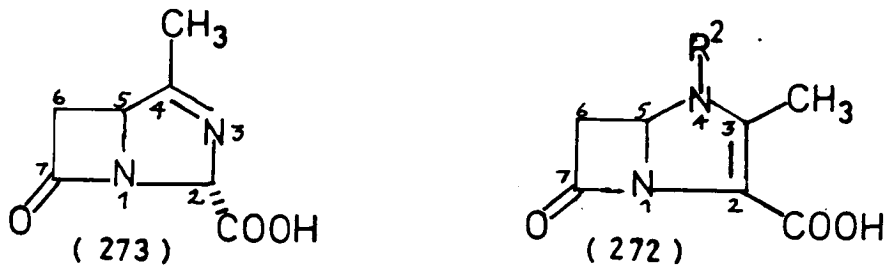
Scheme 108 Structure of azapenem derivatives.

makes it necessary to incorporate the correct
stereochemistry and substituents at an early stage of the
synthesis.

3.2.9   AZAPENEM

The l-azapenem derivative (272) (scheme 108) with
a nitrogen atom replacing the sulphur atom of the penam
system has been reported recently.[162]

The l-azapenem derivative (272) was obtained by
desulphurisation, with triphenylphosphine in acetonitrile
at room temperature, of the azathiacephem derivative
(274) (scheme 109). The ring closure proceeds <u>via</u> the
azetidinonium intermediate (275) followed by nucleophilic
subsitution at C-4, by the enamide nitrogen to afford a
racemic product. Attempts to remove the p-nitrobenzyl
ester to generate the free acid, under a variety of
conditions gave, after work-up, only decomposition
products, thus confirming the low stability of this
system.

In addition the nitrogen analogue (273) (scheme
108) of the carbapen-3-em derivative has been
reported.[163] This compound was found to be biologically
inactive. The 6-acylamino derivative (279) has been
synthesised by Pearson.[164] This route involved an
interesting intramolecular cyclisation involving a
reactive nitrene intermediate (277) (scheme 110).
Intermediate (276) was readily obtained by the
ketene-imine method. (279) was also devoid of any
biological activity.
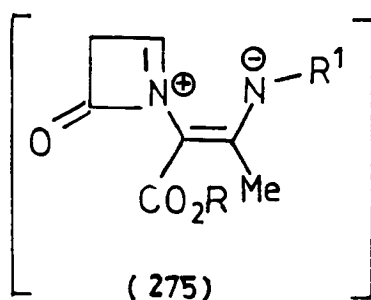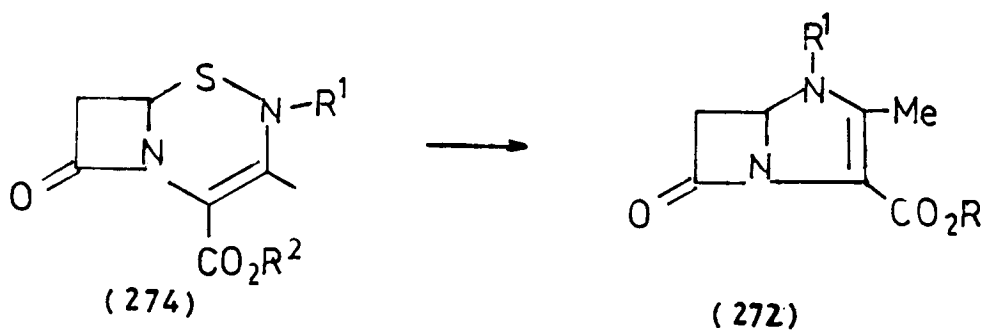
Scheme 109

Scheme 110

$$(280)$$

(12) $R^1 = H_3\overset{\oplus}{N}CH\overset{\ominus}{CO}_2(CH_2)_3, R^2 = H, R^3 = OAc$

(14) $R^1 = C_4H_3SCH_2, \qquad R^2 = H, R^3 = OAc$

(15) $R^1 = C_4H_3SCH_2, \qquad R^2 = H, R^3 = C_5H_5N$
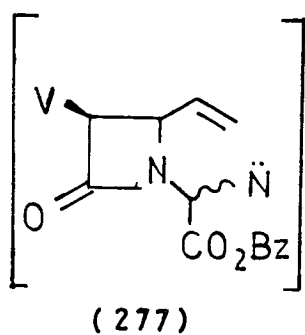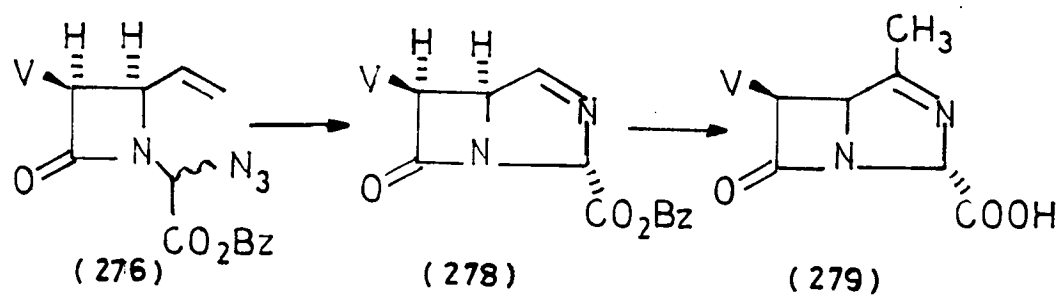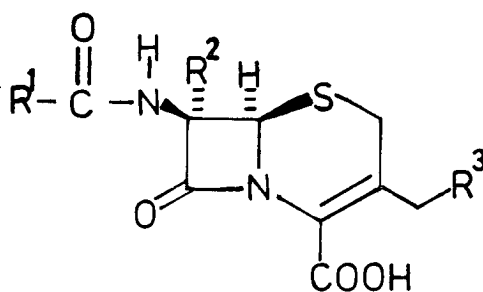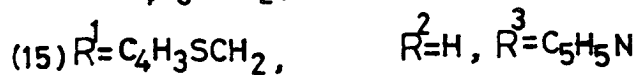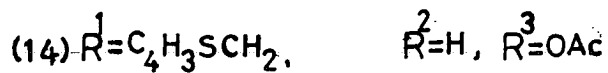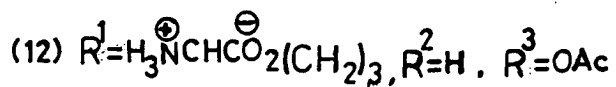
Scheme 111   Structure of Cephalosporin derivatives.
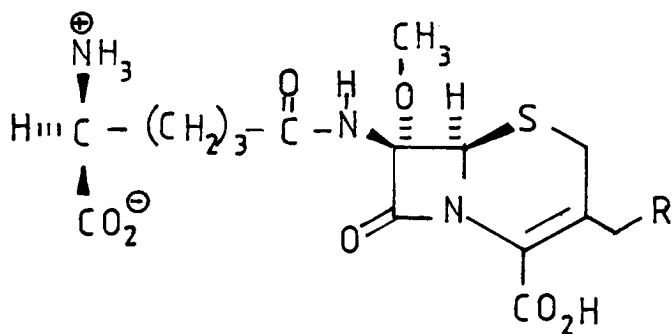
$$(17)$$

Scheme 112   Structure of Cephamycins.

3.3.0 FOUR-SIX FUSED SYSTEMS

3.3.1 SYNTHESIS OF CEPHALOSPORINS

The cephalosporin nucleus (280) (scheme 111) is comprised of a β-lactam ring, fused onto a dihydrothiazine system.   Its discovery was prompted by its stability to penicillinase producing bacteria which had become resistant to penicillins.   Cephalosporin C (12) was the first member isolated in this class of compounds.   Its biological activity was quite low. However, changing the acylamino side chain (R^1) to a 2-thienylacetyl group resulted in a highly active derivative with a broader spectrum of activity than penicillins G and V.   This was called cephalothin (14) (scheme 111).   Many modified side-chain derivatives were subsequently produced (Table III).   These had improved biological properties, such as acid stability, high blood levels, increased potency, a broader spectrum of activity and stability to Penicillinases..[165]   Treatment with pyridine produced another derivative which was discovered to have very high potency.   This derivative was found to have the pyridine displacing the acetoxy group to result in a betaine derivative called cephaloridine (15) (Scheme 111).   Consequently, the acetoxy group was substituted by many nucleophiles such as alkyl or aryl thiols, amino compounds and alcohols to afford modified derivatives which improved the biological properties of the cephem system.[166]

The discovery of cephalosporin derivatives containing a 7 α -methoxy group, the cephamycins (17) (scheme 112), imparted a much required property of β-lactamase resistance into the system.   This discovery made it necessary to obtain systems with 7 α-substituents.[167]   It is intended that the peripheral modifications of this system remain outside the scope of this account, since there are already a number of reviews

available.[168]   However, since the acylamino, 7 α-C and C-3 positions impart important biological properties to these derivatives, synthetic approaches which allow convenient modification of these positions in the cephalosporin nucleus will be discussed.

The cephalosporins are obtained by fermentation methods, using a high yielding strain of <u>Cephalosporium acremonium</u>.   The isolated derivative has an α-aminoadipyl side chain at C-7 and an acetoxy group at C-3.   Since this derivative has minimal activity, it is necessary to carry out chemical modifications, such as removal of the 7-acyl group to obtain 7-aminocephalo-sporanic acid (7-ACA) (13), or removal of both the 7-acyl group and the 3'-acetoxy group to obtain 7-amino-deacetoxycephalosporanic acid (7-ADCA) (281) (scheme 113).   Consequently, cephalosporin derivatives are much more expensive than penicillins, for which the intermediate 6-amino penicillanic acid (6-APA) (3) is obtained directly from precursor-starved fermentation media.   It was for these economic reasons that methods of converting penicillins to cephalosporins were sought. In addition, direct modification of cephalosporins is limited due to the problems of isomerisation of the Δ^3-double bond to the Δ^2-position (scheme 114), and to the problems of lactone formation in Δ^3-isomers under acidic conditions (scheme 115).   The Δ^2-isomer (283) and the lactone (284) are devoid of any activity.   In order to undertake a thorough investigation of the structure-activity relationship of the cephem system, totally synthetic approaches were developed.

a)   Conversion of natural penicillins to cephalosporins.

The earliest penicillin to cephalosporin conversion was reported by R.B. Morin and co-workers in

116
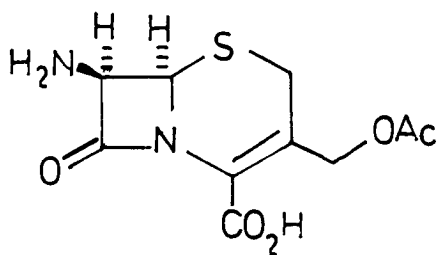
(13)
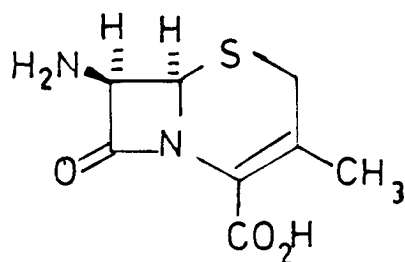
(281)

Scheme 113   Structures of 7-aminocephalosporanic acid
(7-ACA) and 7-aminodeacetoxycephalosporanic
acid (7-ADCA).
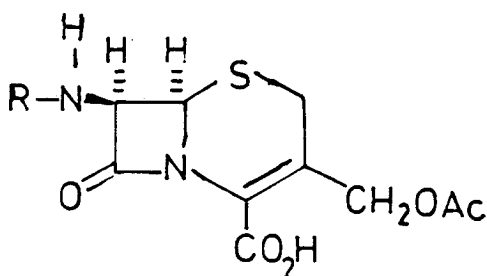
(282)
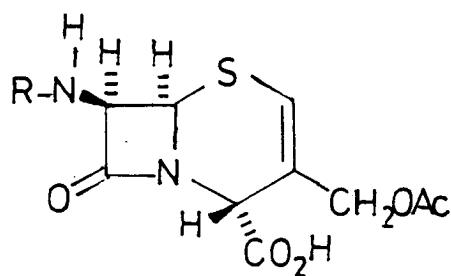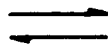
(283)

Scheme 114   Isomerisation of the double bond
in the cephem system.
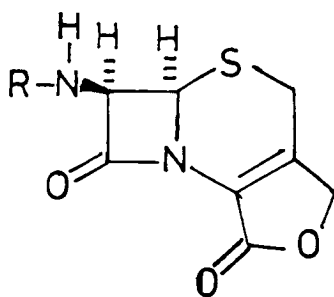
(284)

Scheme 115   Lactone formation in the cephem system.

(285) R^1 = PhOCH$_2$CONH
R^2 = TCE

(286) R^1 = PhCH$_2$CONH
R^2 = SiMe$_3$

(287) R^1 = PhOCH$_2$CONH
R^2 = TCE

(288) R^1 = PhCH$_2$CONH
R^2 = H

Scheme 116

(285)

(290)

(291)

(293)

(289)

(292)

(287)
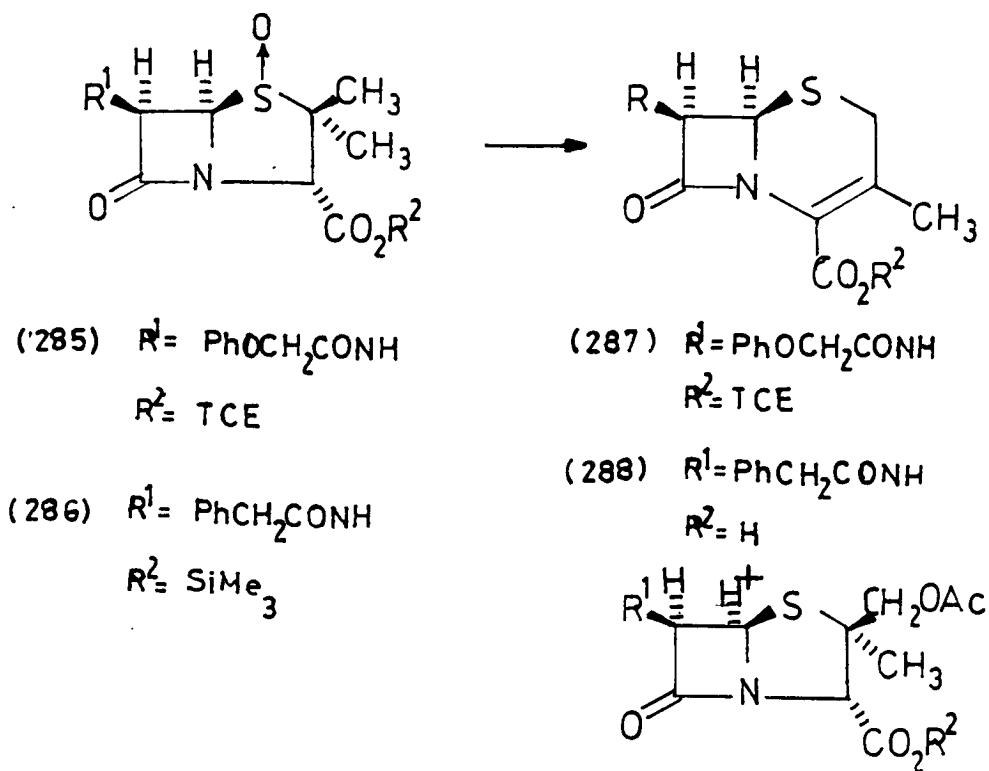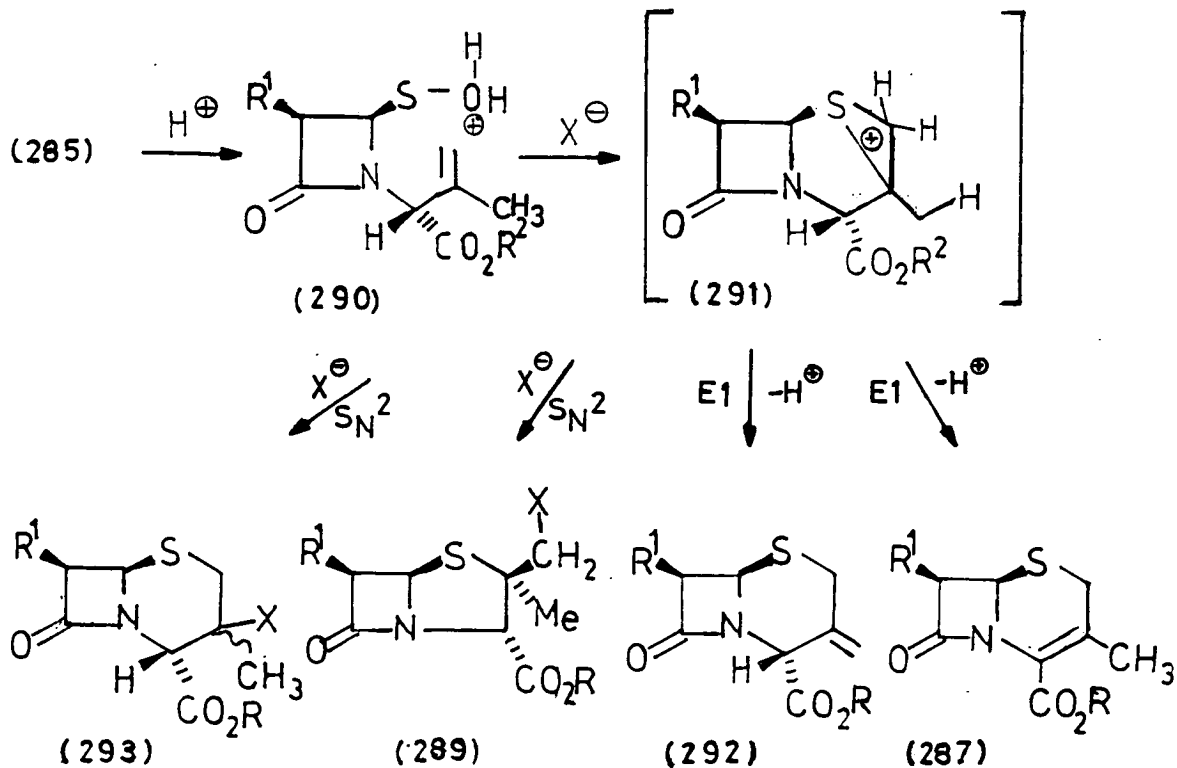
Scheme 117

1963 (scheme 116)[9], as described in an earlier section.
This conversion involved refluxing a penicillin V
sulphoxide ester in xylene in the presence of an acid,
such as p-toluenesulphonic acid, to result in a mixture of
products, from which the cephalosporin derivative (287)
was identified.   The mechanism of this reaction was
thoroughly studied by Morin and co-workers[169] and also
Cooper[170], and was reviewed by Sammes[171] (scheme 117).
The important intermediate that was generated was the
sulphenic acid (290).   This has been trapped by many
methods as described earlier.   The intermediate (290)
undergoes an electrophilic addition to the double bond to
result in the episulphonium ion (291).   Decomposition of
(291) is dependent on the size of the side chain (R^1) and
the size of the conjugate base (X^-) of the acid used.
The different pathways available allow the formation of a
variety of products.   Recent developments have allowed
this conversion from penicillin G sulphoxide derivative
(286) to deacetoxycephalospranic acid (288) to be
possible in yields in excess of 80%, with transient
protection of the carboxylic acid as its trimethylsilyl
ester.[172]   The deacetoxycephalosporanic acid derivatives
are also found to possess good activity and are readily
absorbed through the intestinal wall and can thus be
orally administered.   They are also valuable
intermediates allowing further modifications to afford
novel cephalosporins.

     The penicillin sulphoxide to cephalosporin route
can be modified by involving an intermediate such as a
disulphide (294), which is obtained by trapping the
sulphenic acid (290) with 2-mercaptobenzothiazole.[67]
The disulphide (294) can be converted to modified
penicillins or be transformed to cephalosporins.
Transformations to the cephem system involving this type
of intermediate (294) usually involve an episulphonium
ion (291), which is also capable of affording other by-
products, such as the penam system (298).   Consequently,
this route has limited usefulness, particularly regarding

(296)

(297)

(294) R = CH₂CCl₃ = TCE

(295) R = H

MBT = S—benzothiazole

G = PhCH₂CONH

(298)

(300)

(299)

Scheme 118

(301)

(302)

(303)
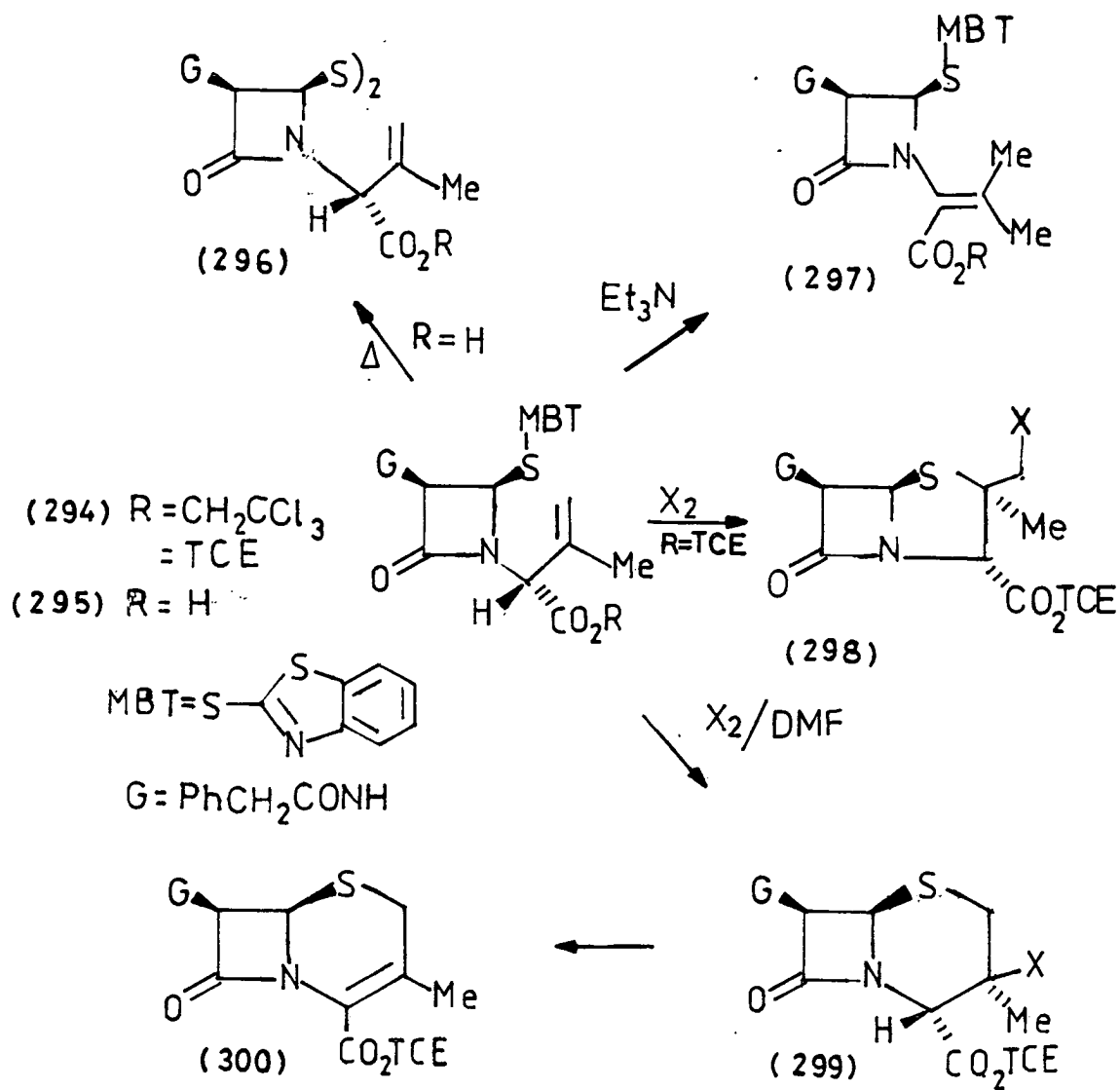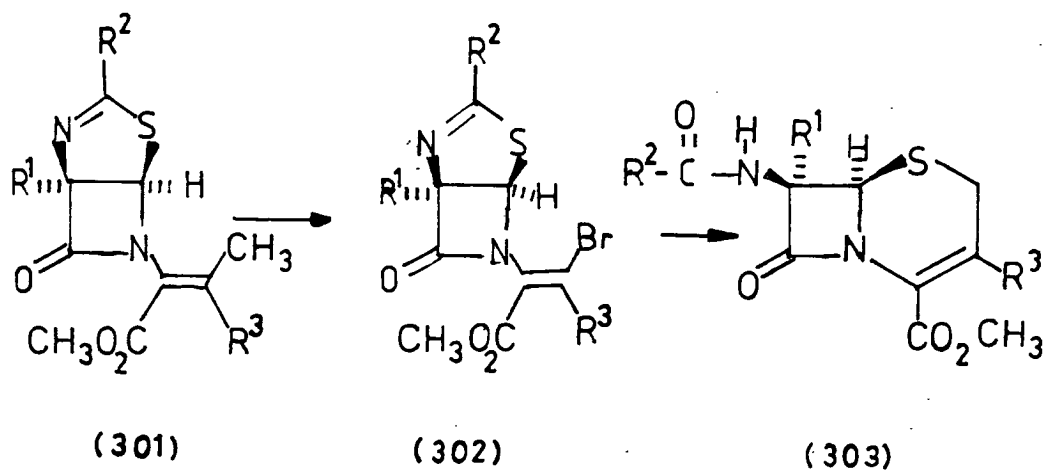
Scheme 119

the functionality available at the C-7 and the C-3 positions.

b) Synthesis of a cephem nucleus via a 6-exo-tet cyclisation.

A much better route to the cephem system (303) involves the use of the thiazoline (301) (scheme 119).[173]   Allylic bromination of the methyl group in (301) affords (302), and generation of the free thiol results in a 6-exo-tet cyclisation to afford (303). This route allows the functionalisation of the cephem nucleus at the C-7 (α and β) and C-3' positions. However, the success of this cyclisation is dependent on the presence of an enolisable group (R_3) on the double bond, particularly if R^1 is a hydrogen atom.

c) Synthesis of a cephem nucleus via a Wittig reaction.

The Beecham group have also devised an important route to the cephem system (306), involving an intramolecular Wittig reaction,[174] as used by R.B. Woodward for the penem system.[123]   This approach allowed a variety of functional groups to be incorporated in the C-3' position (scheme 120).   Intermediate (305) was obtained by degradation of pencillin G to the azetidinone (304) (scheme 120).   The β-lactam N-substituent was attached by the procedure previously described by Woodward and Heusler.[78]   The ketone group was generated by a catalytic hydration of the triple bond using mercuric chloride and piperidine followed by hydrolysis.   The cyclisation was successfully completed by heating in dioxane or toluene.
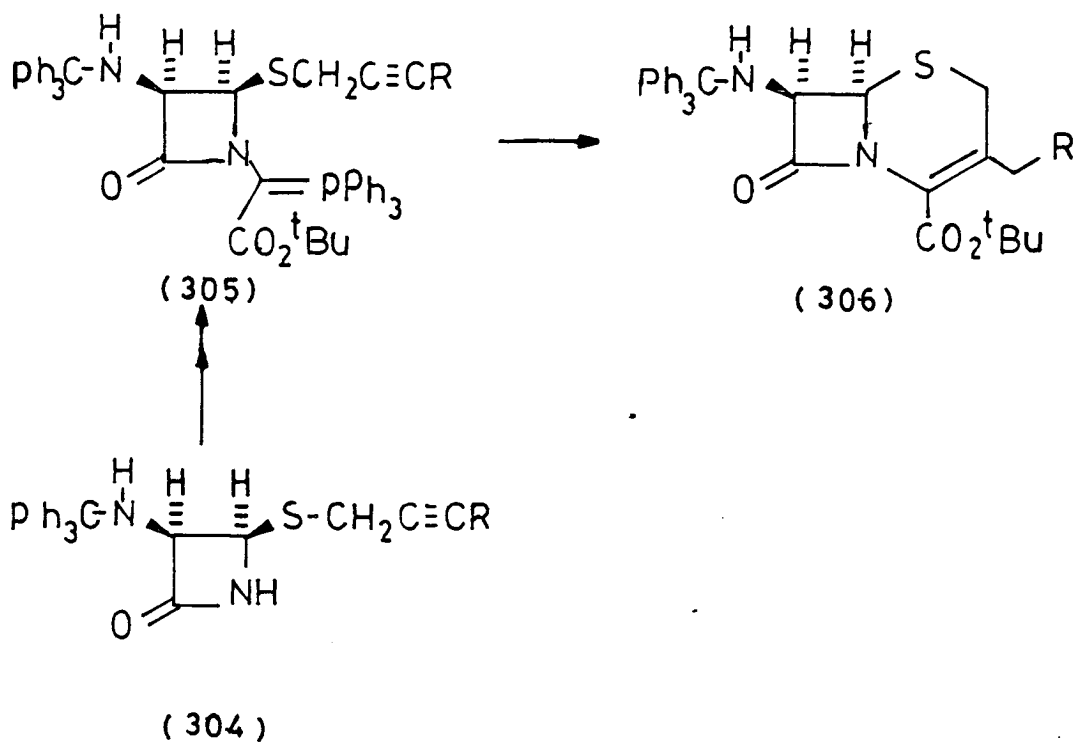
Scheme 120
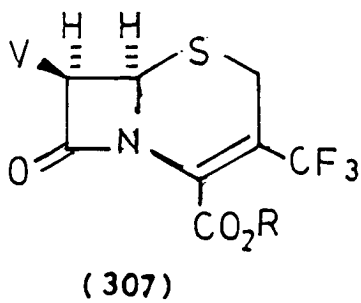
Scheme 121   Structure of 3-trifluoromethyl cephalosporin.

The intramolecular Wittig reaction approach has been used by workers at Shionogi,[175] to successfully synthesise the 3-trifluoromethyl cephalosporin (307) (scheme 121). This type of derivative could not be synthesised from penicillins prior to this approach.

d)   Total synthesis of cephalosporin derivatives *via* a 6-exo-trig cyclisation.

Totally synthetic approaches to the cephem system were attempted soon after the identification of cephalosporin C. The first synthsis of cephalosporin C was described by R.B. Woodward in 1966 (scheme 58).[176] This elegant synthesis clearly shows the difficulty involved in obtaining the intermediates with the correct stereochemistry at the chiral positions in the synthesis of a natural product.

L-Cysteine was protected at its three reactive sites (308) and functionalised at the unactivated methylene position, adjacent to the sulphur atom, with complete stereochemical control using dimethyl azodicarboxylate to result in a trans substituted intermediate (309), oxidation of which with lead tetraacetate, followed by hydrolysis of the trans acetoxy derivative (310) to the trans hydroxy derivatives (311) and activation of the hydroxyl group with methane sulphonyl chloride, and subsequent displacement of the mesylate with sodium azide resulted in the cis azido derivative (312). Reduction of the azido group with aluminium amalgam/1M sodium hydroxide resulted in the amino derivative (313) which was cyclised to the versatile β-lactam intermediate (314) with triethylaluminium. The β-lactam nitrogen was reacted *via* a Michael addition with a reactive unsaturated dialdehyde derivative (315); (obtained from malonaldehyde (320) condensed with a trichloroethyl

(308) X=H
(309) X=NCO₂Me HNCO₂Me
(310) X=OAc
(311) X=OH

(312) Y= N₃
(313) Y= NH₂

(314)

(315)

(316)

(317)

(318)

(319)

(12)
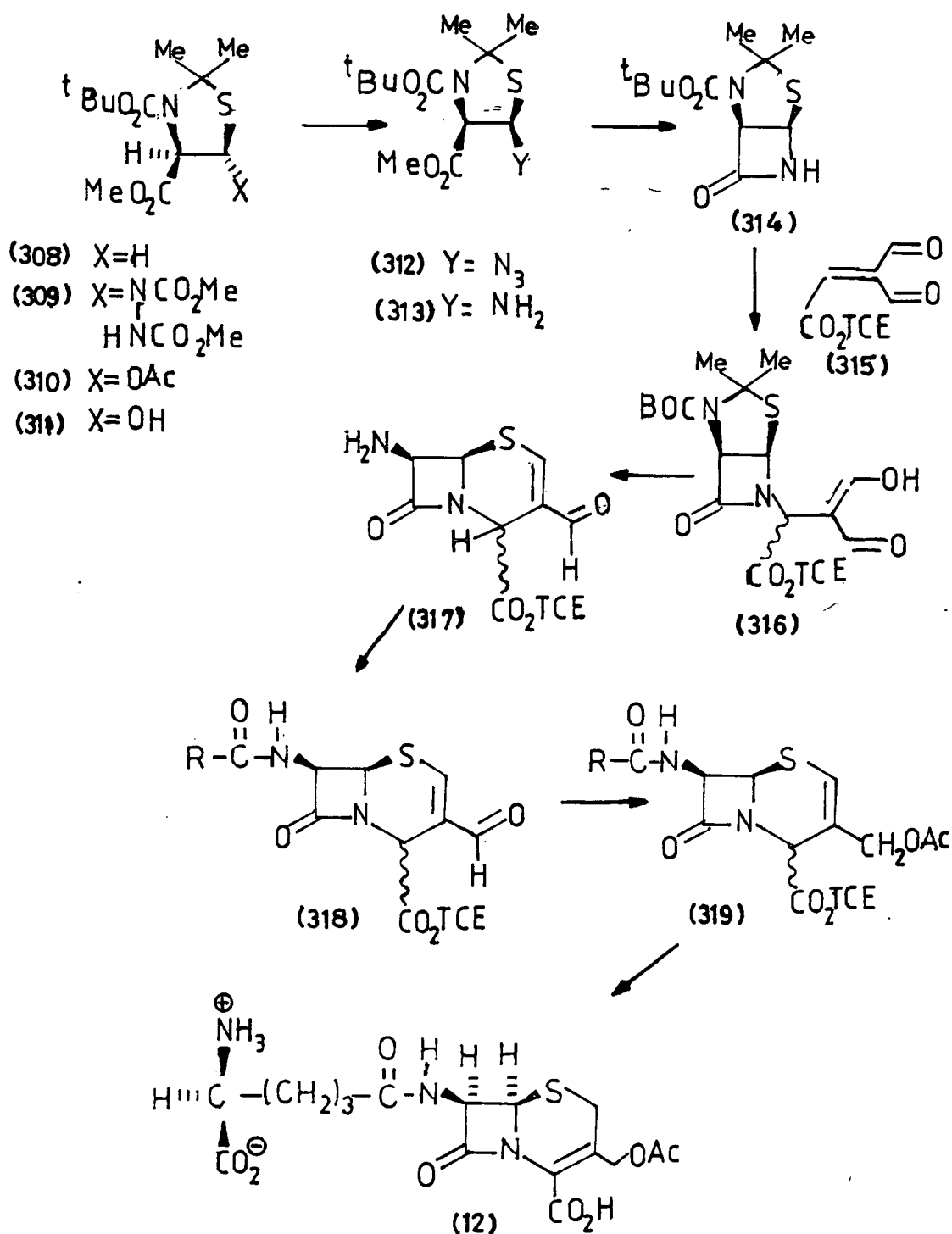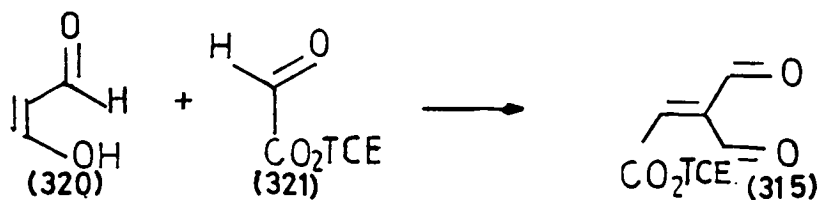
Scheme 122

Scheme 123   Synthesis of a dialdehyde derivative.

glyoxylate (321), (scheme 123)), to afford the N-substituted diastereomeric mixture (316). Deprotection of the acetonide and the t-butyloxycarbonyl groups with trifluroacetic acid resulted in the Δ^2-cephem system (317) <u>via</u> a 6-exo-trig cyclisation. The amino group at the C-7 position was reacted with a trichloroethyl-α- amido-adipylacyl chloride derivative to generate (318). Diborane reduction of the α,β -unsaturated aldehyde followed by acylation with acetic anhydride resulted in the Δ^2-acetoxy derivative (319). Equilibration of the double bond with pyridine and treatment with zinc and acetic acid removed the trichloroethyl protecting groups to afford synthetic chiral cephalosporin C (12).

The synthesis of cephalosporin C by Woodward was too long to be a means of studying structure-activity relationships of cephalosporins. However, a very important intermediate was obtained; the thiazolidine (314). The stereochemistry and the functionality around the β-lactam bond in (314) is identical to both penicillins and cephalosporins and it is thus a very important intermediate for both of these systems. Work commenced on obtaining an intermediate of this type from readily available penicillins. This transformation was accomplished, initially, by Woodward and Heusler (scheme 124)[177] and later by the Eli Lilly group (scheme 125)[178]. The thiazoline (329) could be reduced with moist aluminium amalgam in an ethereal solvent, or with sodium borohydride, to give the corresponding thiazolidine (330). The thiazoline (102) could readily be obtained by the action of heat and trimethyl phosphite on penicillin V sulphoxide followed by ozonolysis and methanolysis, as developed by the Ciba group.[70] Using the intramolecular Wittig reaction approach (314) or (330) could be functionalised appropriately to obtain many novel cephem derivatives in relatively few steps.
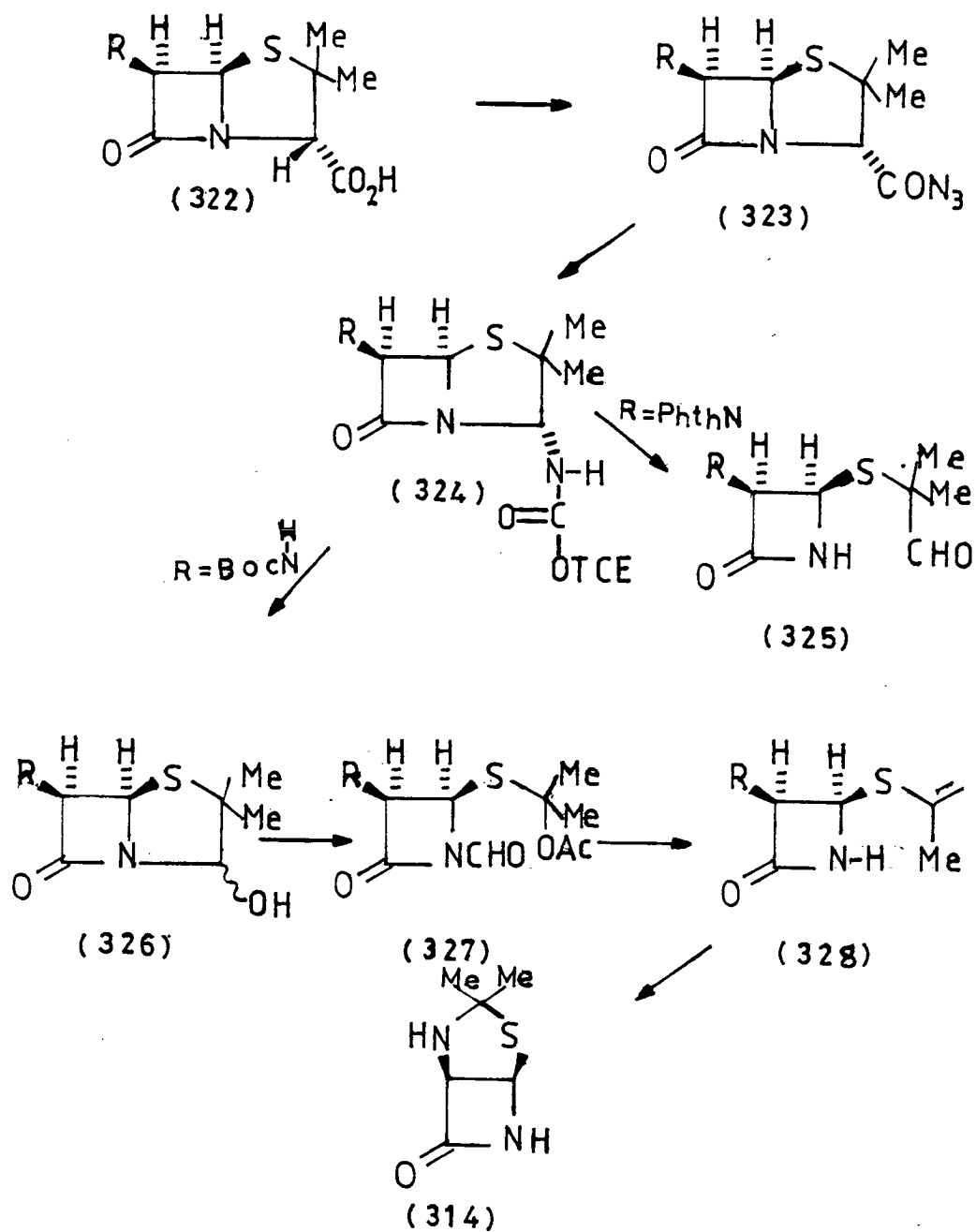
Scheme 124
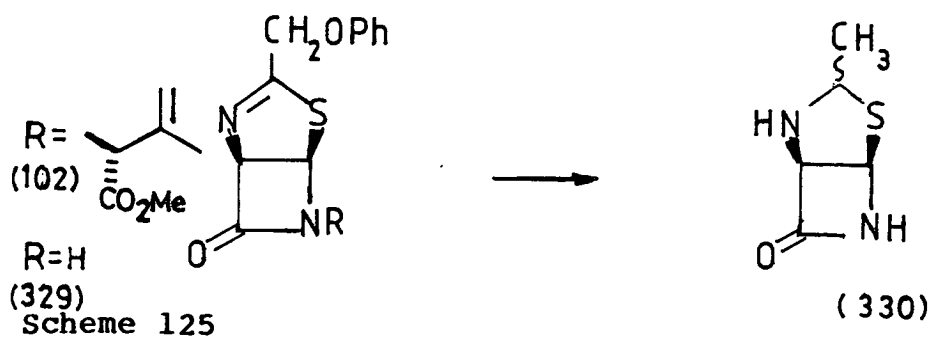
Scheme 125

e)   Synthesis of the cephem nucleus <u>via</u> a N,N-
        dicyclohexylcarbodiimide coupling reaction.

        The general approach devised by Sheehan for the
synthesis of penicillins was also successfully adapted
for the synthesis of cephalosporins by Roussel-Squibb
(scheme 127).[179-180]   Intermediate (336) was obtained by
total synthesis starting from pyruvic acid, formaldehyde
and dimethylamine to form the Mannich base (331), which
was then converted to the thiolacetate (332) by
displacement of dimethylamine with thioacetic acid.
Removal of the thiol protecting group under acidic
conditions and condensation with the amine derivative
(334) gave both the erythro- and threo-isomers of the
condensation product (335).   Treatment with hydrazine to
remove the phthalimide group, and then acid to remove the
t-butyl group, gave only the desired threo amino acid
(336).   Tritylation of the free primary amino group
greatly facilitated β-lactam formation.   Cyclisation was
accomplished <u>via</u> treatment with N,N- dicyclohexyl-
carbodiimide.   After acid removal of the trityl group
from (337) the product was resolved with (+)-tartaric
acid and then the free amino group acylated with
2-thienylacetyl chloride.   The lactone (338) was
converted to the hydroxy-acid (339) by base hydrolysis.
The yields for the final step were low, generally
approximately 30%.

f)   Synthesis of the cephem nucleus <u>via</u> the
        ketene-imine method.

        The Merck group[181] also devised a versatile route
to the cephalosporins using the method popularised by
A.K. Bose,[55] involving a ketene-imine cyclisation and a
latent amino group (scheme 127).   The important aspect
of this synthesis is the manner in which the thiazine

Scheme 126

(70)   (340)
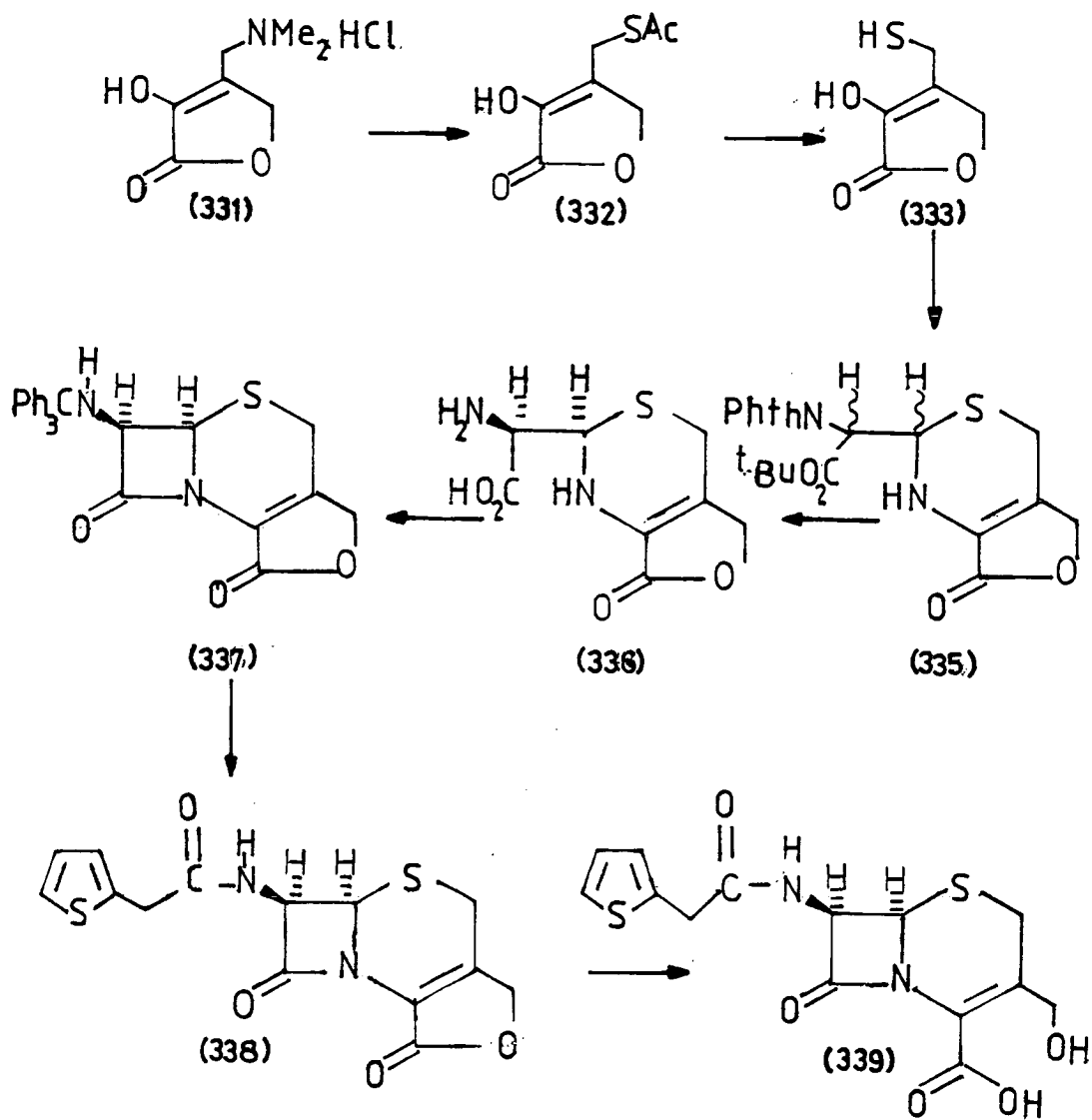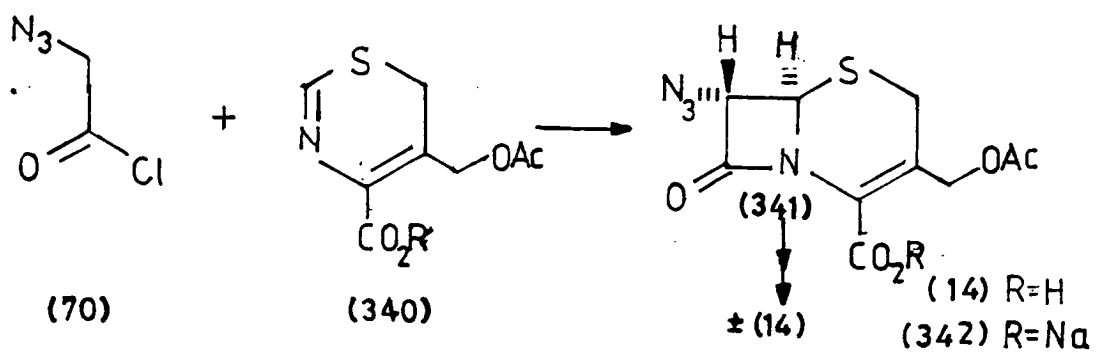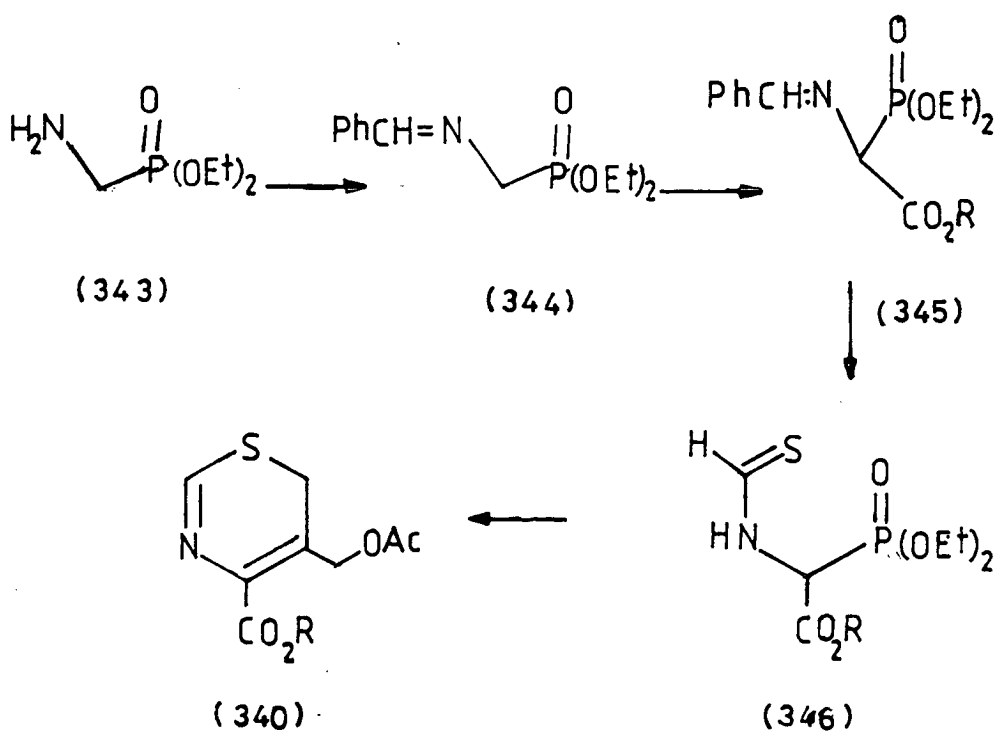
Scheme 127

Scheme 128
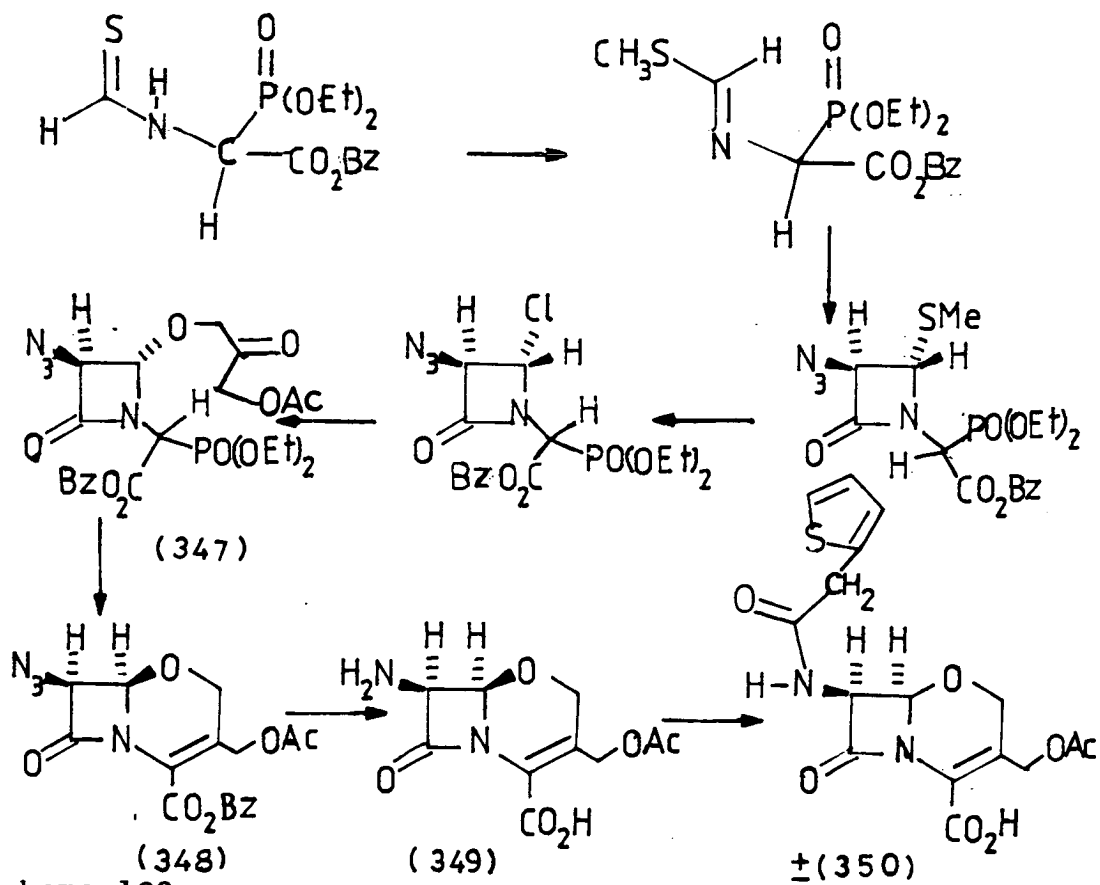
Scheme 129

derivative (340) was obtained in high yield (scheme 128). However, the drawback to this synthesis (scheme 127) is the racemic product (14) that is obtained.

3.3.2   OXACEPHEMS

a)   Synthesis of an oxacephem derivative <u>via</u> a ketene-imine approach.

The oxacephems are the oxygen analogues of cephalosporins.   The first synthesis of an oxacephem derivative was described by Wolfe in 1974,[182] and was shortly followed by that of the Merck group (scheme 129).[183]   The racemic synthesis of 1-oxacephalothin (350) involved the ketene-imine method to form the β-lactam ring, followed by an Emmons condensation using a phosphonate and a protected α,α-dihydroxy acetone derivative in the C-4 position of the azetidinone (347), to form the dihydrooxazine ring system (348).   (348) was then converted through standard procedures to 1-oxa cepthalothin (350).   This was found, after correction for the racemic mixture, to have comparable biological activity to sodium cephalothin (342).

b)   Synthesis of an oxacephem derivative <u>via</u> a Wittig reaction.

The Beecham group also utilised their versatile pencillin degradation methods to obtain a 1-oxacephem derivative (354) (scheme 130).[184]   This route used penicillin G as the starting material and involved an intramolecular Wittig reaction to form the second ring.

(351) → (352)

(354) ← (353)
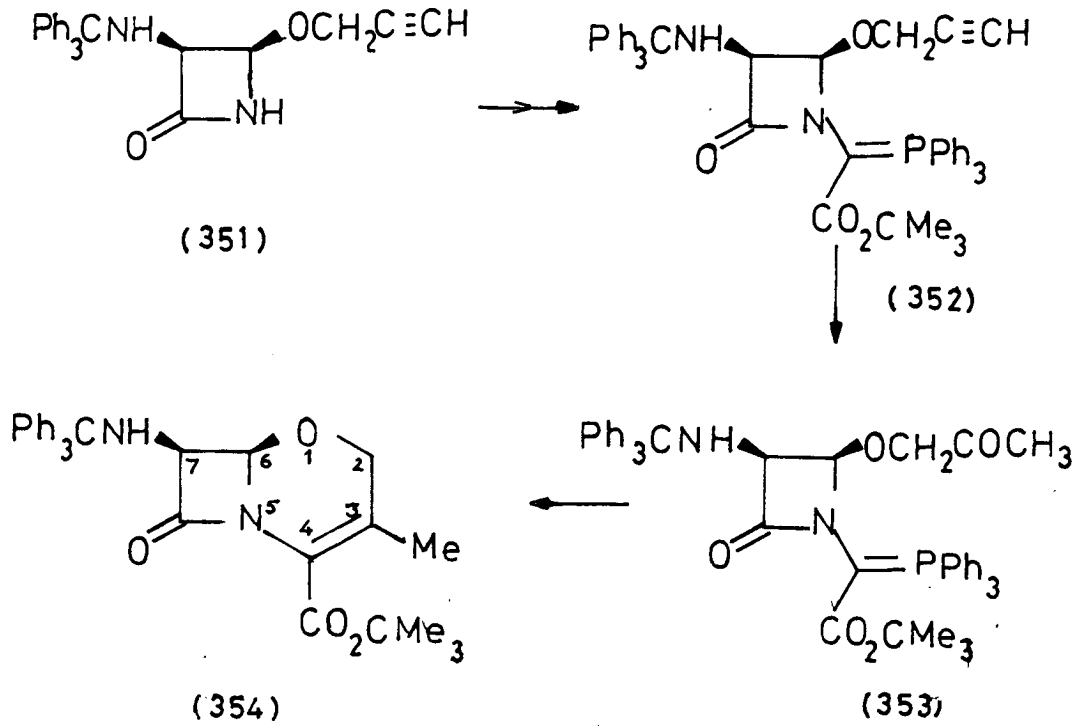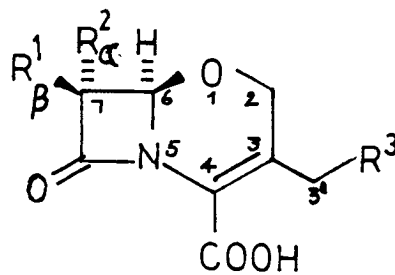
Scheme 130

Scheme 131 Modification sites in the oxacephem nucleus.

(355)
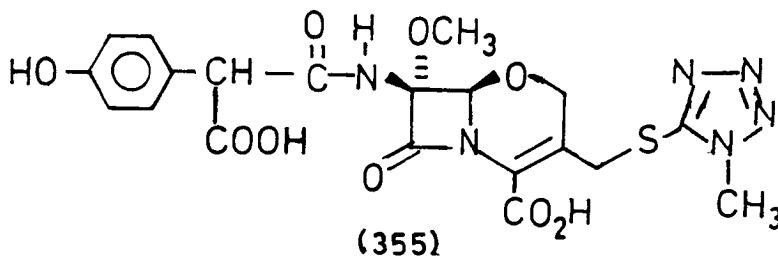
Scheme 132 Structure of moxalactam.

The ketone (353) was generated by hydration of the alkyne substituent using mercuric chloride and piperidine followed by aqueous work-up. It was found that this method of hydration was only successful for terminal alkyne substituents, in the oxygen analogues of cephems. Consequently, in order to obtain the appropriately functionalised ketone, a different approach was necessary. This modified route involved protecting the ketone as an olefin. The ketone was readily regenerated by ozonolysis at low temperature. The final oxacephem derivatives were found to be optically active and with the cis stereochemistry about the β-lactam ring. The oxacephem derivatives, thus, prepared were found to have only moderate activity compared to their cephalosporin analogues.

The moderate activity of oxacephem derivatives stimulated workers at Shionogi,[185] to undertake a detailed structure-activity investigation of these derivatives, in the hope of finding a highly active antibiotic. The investigation involved modification of substituents at C-7 (α and β) and at C-3 positions of the nucleus (scheme 131). The systematic investigation into the structure-activity relationship of the oxacephem system[186] resulted in the following findings: the 7α-arylmalonylamino group (as in carbenicillin (5)), the 7α-methoxy substituent (as in cephamycins) and the C-3-N-methyltetrazolyl thiomethyl group, (as in the highly active cephalosporins such as cefoperazone, SCE-1365, Cefazaflur, cefamandole, cefmetazole and others) caused an enhancement of the potency and the pharmo-kinetics of the oxacephem system towards a broad spectrum of Gram-negative bacteria. This work led to the first commercially available oxacephem derivative, moxa-lactam (355) (scheme 132). The synthetic strategy employed involved the Beecham method, as discussed earlier, but deviated in that the propargyl group was converted to an epoxide, which could be opened by acid or base to a diol derivative. This could subsequently be

converted to a carbonyl moiety, which would have the
appropriate substituent in the α-position and which could
be used in an intramolecular Wittig cyclisation as
discussed earlier.

**c)   Chiral synthesis of an oxacephem nucleus <u>via</u> a
        6-exo-tet cyclisation involving an alkoxy
        nucleophile at the C-4 position.**

An excellent chiral synthesis of the 1-oxacephem
system from 6-aminopenicillanic acid (6-APA) has been
reported (scheme 133).[187]   The important step in this
strategy is the enantioselective cyclisation of the
oxazoline intermediate (358) to the 6R fused system
(359), catalysed by boron trifluoride etherate.   The
inversion of the stereochemistry at the C-7 position in
(360) to (361) was mediated by the tert-butylhypochlo-
rite/lithium methoxide method.[188]   This strategy is
sufficiently flexible to functionalise the important C-7
(α and β) and the C-3 positions of this fused system.
The oxacephem derivatives are quite effective
antibiotics.   However, relatively few have come onto the
market.   The major reason for this is that the oxacephem
nucleus, being unnatural, requires a great deal of "fine
tuning" to find the correct combination of peripheral
functional groups with physiological compatability.   The
synthetic approaches described clearly indicate the large
number of steps that are usually necessary for their
synthesis.   These two factors are very time consuming
aspects of research and are probably the reasons for the
dearth of oxacephem antibiotics on the market.   Further
work towards the oxacephem system is still necessary in
order to find a much shorter and stereoselective approach
to them, particularly for large scale synthesis.   The
use of penicillins, as raw materials, is probably the
best method at present.

6-APA

(356) → (357) → (358)

(361) ← (360) ← (359)

Scheme 133

(362)
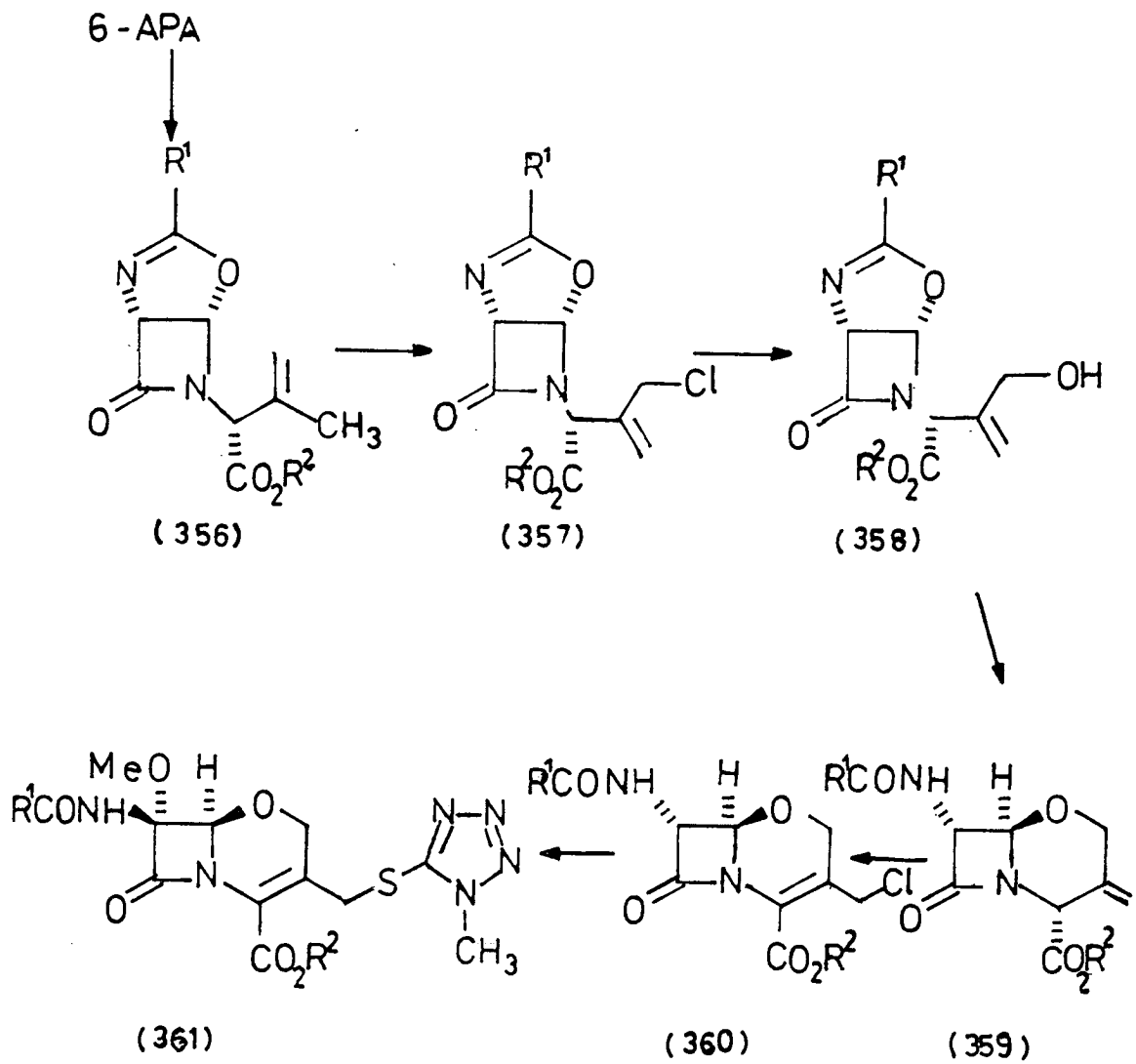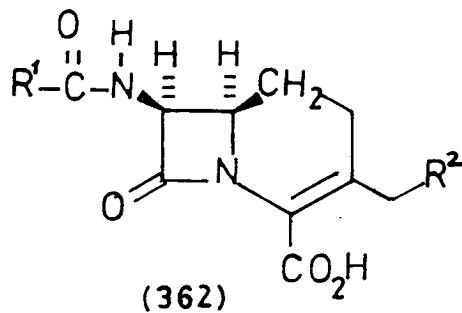
Scheme 134   Structure of the carbacephem nucleus.

3.3.3 CARBACEPHEMS

a) Synthesis of a carbacephem nucleus <u>via</u> the ketene-imine method.

The carbacephem system (362) (scheme 134) has not had the same success as its oxacephem analogue. The first synthesis of a carbacephem derivative was reported by workers at Merck, as part of a study of structural analogues of cephalosporins.[189] This route involved the ketene-imine method for the β-lactam ring formation and an Emmons intramolecular cyclisation (scheme 135). The racemic 1-carbacephalothin product (363) was found to have comparable activity to the 1-thia congener (14) (scheme 5). The results obtained had been corrected taking into consideration the comparison of a chiral sample of cephalothin (14) to the racemic product (363).

b) Total synthesis of a carbacephem nucleus involving the ketene-imine method and the Emmons method.

A total synthesis of 1-carbacephalothin confirmed its high biological activity.[190] Introduction of a methoxy group in the 7 α position of the carbacephem nucleus (scheme 136) considerably improved its biological properties over the 1-thia-congeners. The strategy employed for their synthesis involved the use of the ketene-imine method for the β-lactam ring formation and closure of the second ring by the Emmons method (scheme 137).[191]

Scheme 135

Scheme 136 Structure of 7α-methoxy carbacephem
derivative.

c) **Synthesis of a C-1 substituted carbacephem nucleus.**

The carbacephem system has an additional position for modification, compared with the cephalosporins. The C-1 position has been substituted by a hydroxy group to obtain the 1 α-hydroxy-1-carbacephem derivative (364) (scheme 138) by Colvin.[192] The synthetic strategy adopted was that of the Merck route.[190] This derivative (364) was found to have little activity compared to the 1R-sulphoxides of cephalosporins. It did not possess any β-lactamase inhibitory activity.

d) **Synthesis of the carbacephem nucleus via a carbene insertion reaction.**

An enantioselective synthesis of the carbacephem system (366) has been reported.[193] The important steps involve an asymmetric ketene-imine cycloaddition for the β-lactam ring formation[194] and a carbene insertion into the azetidinone N-H bond, for fusion of the dihydropiperidine ring system to the carbacephem derivative (scheme 139). Intermediate (365) is very useful for undertaking a thorough structure-activity investigation on the biological effects of varying the 7(α,β)and the C-3 substituents.

e) **Structural aspects of a carbacephem nucleus.**

At present, much work is being carried out by Japanese workers in order to find the best combination of substituents around the carbacephem nucleus.[195,196]
Carbacephem derivatives containing the aminothiazolyl group on the acyl substituent are being

Scheme 137   Total   synthesis of   carbacephem
derivatives.

(364)

Scheme 138   Structure of 1α-hydroxy carbacephem
derivative.

(365)

(366)

Scheme 139

investigated.[195]   The biological activity results seem
to be very good against Gram-negative bacteria but rather
poor against Gram-positive bacteria.[196]   Further
successful modifications may soon result in a potent
carbacephem antibiotic being available on the market.

3.3.4   AZACEPHEMS

The nitrogen analogues of cephalosporins (scheme
140) are the azacephems.   The 1-aza cephem derivatives
have usually resulted in biologically inactive
derivatives due to their instability.   However, the
2-,[197] and 3-aza cephems[198] do show some biological
activity, though very little in comparison to the
cephalosporins.

a)   Synthesis of a 2-aza cephem derivative <u>via</u> a
        6-exo-trig cyclisation.

The route employed by Doyle (scheme 141)[197]
involved a ketene-imine strategy for the preparation of
the azetidinone (370).   The aldehyde function at C-4 of
the azetidinone (370) was condensed with an amine to
obtain the schiff base (371).   This was then reduced
with sodium borohydride to give the amine (372).
Attempts to remove the acetal and to induce cyclisation
resulted only in tars and unidentifiable products.   The
approach was modified by protection of the amine (372) as
an amide (373), using zinc chloride in trifluoroacetic
anhydride.   The acetal (373) was converted to the
β-ethoxy acrylate (375) under the conditions of the
reaction.   Previous work by the authors had shown that
the β-hydroxy acrylate (375) could readily be obtained
<u>via</u> the pyrrolidine derivative (374).   Hydrolysis of

$X = NH_1 , Y=CH_2 \; Z=CR_2$

$X=CH_2 , Y=NR , Z=CH$

$X=CH_2 , Y=CH , Z=NR$
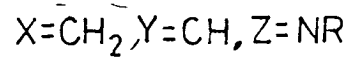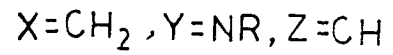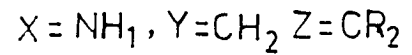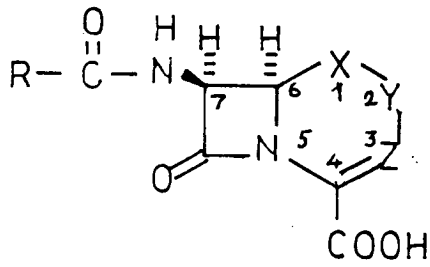
Scheme 140   Structure of azacephem systems.

$X=O$

$R = H$
$\quad = SO_2CH_3$
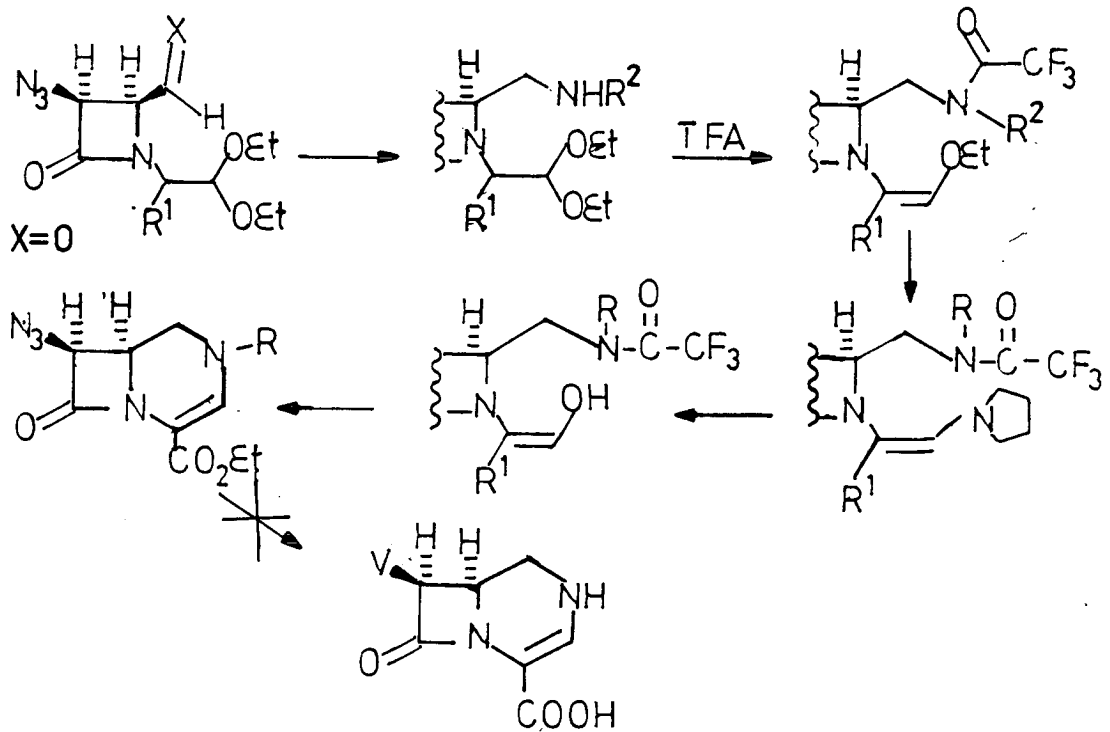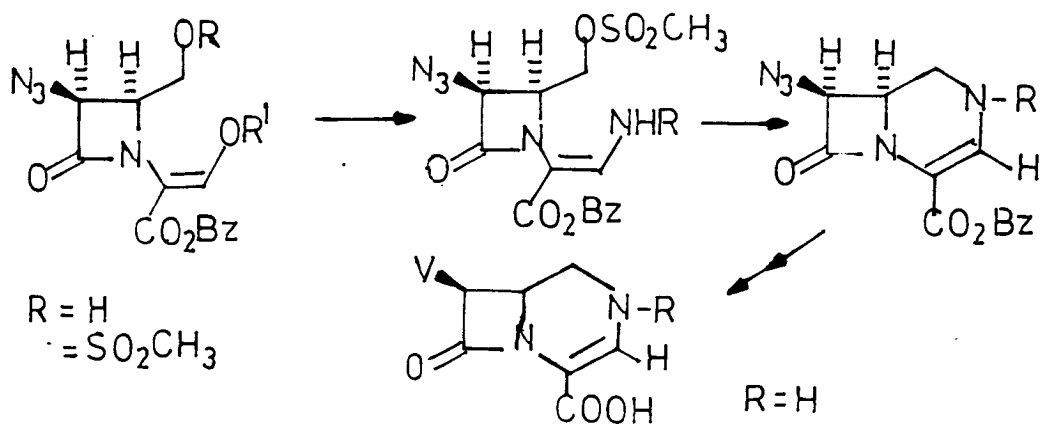
Scheme 141

Scheme 142

(374) with acetone/10% hydrochloric acid gave the enol (375). Removal of the N-trifluoroacetyl group was successfully achieved by treatment with sodium borohydride in ethanol to result in the bicyclic fused system (376). However, this approach made it impossible to obtain the N-unsubstituted derivative (377). A further modification was carried out (scheme 142). This approach allowed the formation of (377). (377) was found to be quite unstable and had weak activity, although this may have been due to low purity.

b)   Synthesis of a 3-azaceph-1-em nucleus _via_ a 6-exo-trig cyclisation.

An approach to the 3-azacephem system has been described by Gleason (scheme 143)[198]. The azetidinone (381), readily obtained by a ketene-imine method, followed by Woodward's route for the incorporation of the β-lactam N-substituent, was smoothly cyclised to the bicyclic system (382). Selective reduction of the azido group followed by acylation resulted in the 3-aza-1-dethiaceph-1-em (383). Catalytic reduction of (382) followed by acylation with 2-thienylacetyl chloride afforded the 3-aza-1-dethiacepham (384). The 3-aza-cephem ester (383) possessed very weak activity against _B. Subtilis_, while the saturated ester (384) was inactive. The use of the methyl esters may have invalidated the biological results. However, earlier reports[197] have shown that the free-acids of the aza-cephem derivatives readily decarboxylate.

(378) $R^1 = OH$

(379) $R^1 = Cl$

(380) $R^1 = \beta- NH C_6H_4CH_3\text{-}p$

(381) $R^1 = \alpha- \quad -||-$

Scheme 143

(385) CO_2R^3

n = 0,1

(387) n = 0,1

(388) $R^3 = H$
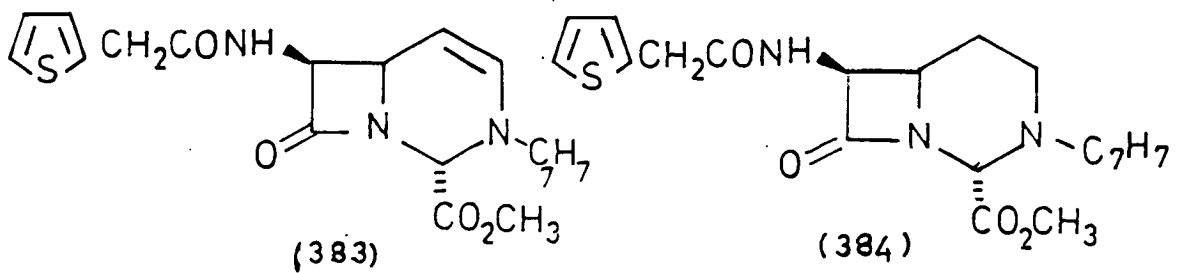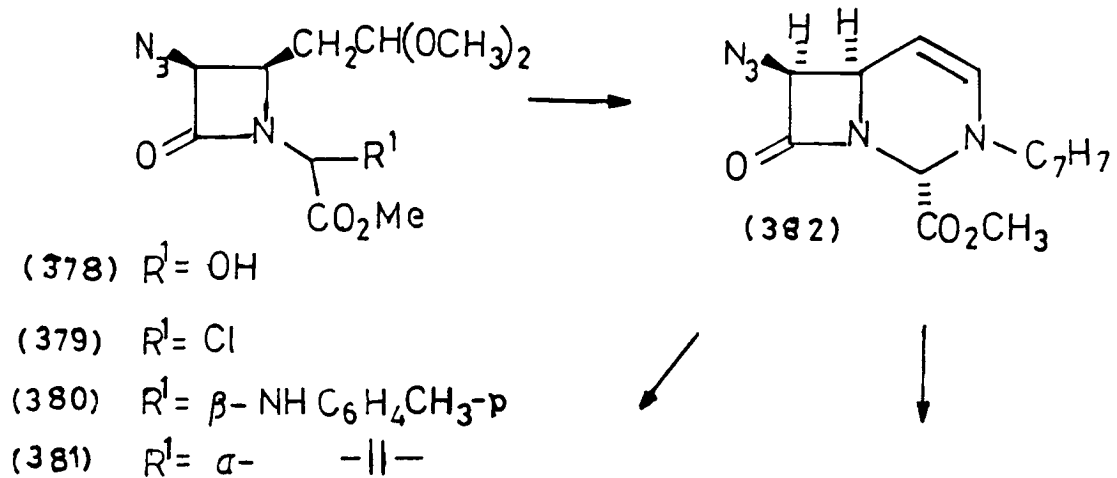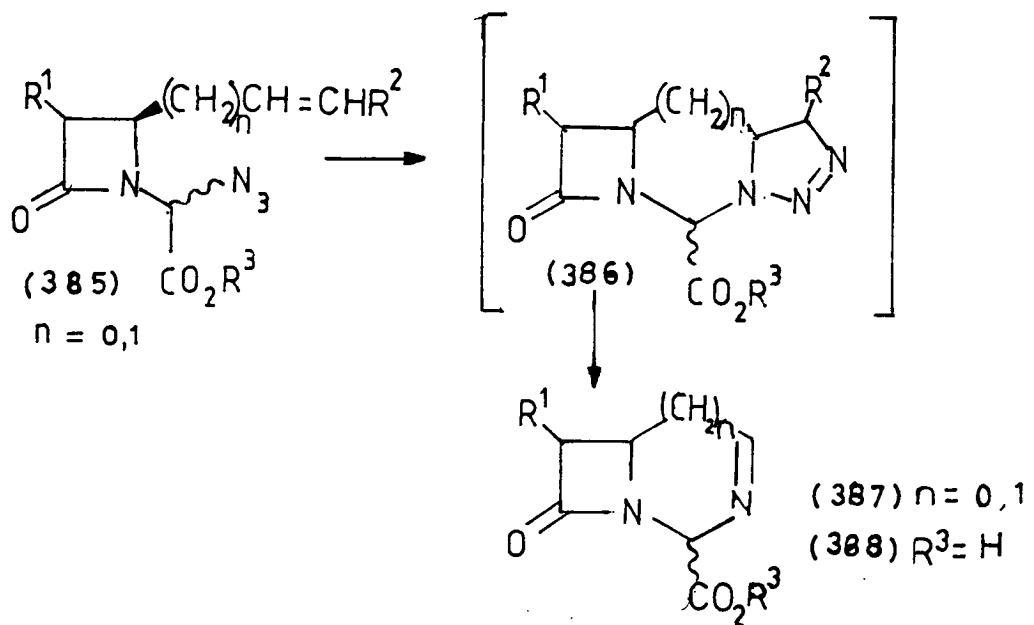
Scheme 144

c)   **Synthesis of the 3-azaceph-2-em <u>via</u> a 1,3 dipolar addition of an azide to an olefin.**

Recently, workers at Beecham have reported a novel route to highly modified azacephem and azapenem derivatives.[199]   This involved a 1,3 dipolar addition of an azide onto an olefin (scheme 144).   The final acid derivatives (388) were found to be devoid of any antibacterial activity.

d)   **Structural aspects of the azacephem nucleus.**

The instability of the azacephem systems has limited their usefulness as antibiotics.   Furthermore, they do not appear to show any inherent strong activity.   It may be necessary to carry out a wider structure-activity relationship study, in order to find the optimum substituents, to impart stability to this system and, perhaps, to improve its activity.   In terms of the economics involved, it would seem that the azacephem systems described may not lead to any commerical antibiotics.

3.4.0   FUTURE TRENDS

The discovery of the novel β-lactam structures, isolated from the screening procedures, has prompted chemists to attempt the synthesis of many highly modified derivatives in this class of compounds.   Better understanding of the mode of action of both the antibacterial activity and the β-lactamase inhibitory properties of β-lactam antibiotics, has resulted in more rational approaches to drug design.

Through the experience with structure-activity investigations, it has been found that a fine balance exists between the activity of the antibiotic and its stability towards physiological conditions. It has also been found that sometimes, as with thienamycin, that in-vitro experiments lead to very promising results, but in-vivo testing results are disappointing.

At present chemists have to synthesise derivatives with particular substituents on the periphery of the particular fused system using either one, or a combination, of the approaches that have been described. The final pure product is then tested for activity against a variety of Gram-negative and Gram-positive bacteria. The spectrum of bacteria used varies from one company to another and also the actual method of testing/detecting may vary. This type of research is very expensive. The need for rational drug design has been brought about, particuarly by the high costs involved in this area of research.

The large variety of structures which are now available and which possess biological activity are very different from the simple structural analogue theory of D-alanyl - D-alanine that Strominger and co-workers had proposed for penicillins, as early as 1965.[24] At present, only two important aspects are being considered in the β-lactam synthetic strategy. The first of these is the acylating ability of β-lactam derivatives. Making the β-lactam bond sufficiently strained, increases its reactivity, thus, giving rise to rapid acylation of the specific enzymes responsible for cell growth which would lead to cell death. Attempts to isolate the enzymes responsible for cell growth are underway, particularly, to obtain structural high resolution X-ray diffraction patterns. This would allow identification of the three-dimensional structure of these enzymes. The second factor is to get the antibiotic to its target. This area has received a great deal of attention and requires compatability of the antibiotic

with the physiological conditions. This, unfortunately, has to be achieved at the expense of the reactivity of the β-lactam ring.

Work is being carried out in many institutes on the γ-lactam derivatives (390) (scheme 145). These derivatives have been found to exhibit weak biological activity.[200-202] Furthermore, they have also been shown[202] to inhibit the cell wall synthesis, as do the β-lactam antibiotics. It can be envisaged that by appropriate functionalisation of these systems, it may be possible to improve their potency.

Over the past few years, Beecham workers have been investigating tricyclic fused systems (390) and (391) (scheme 146)[203-205]. Unfortunately, these have been found to have very weak activity.

Recent work on 1,2-diazetidin-3-ones (392) and (393) (scheme 147) by Taylor,[206] shows the problems that arise when novel systems are sought. It was reported that simple extensions of standard β-lactam chemistry to its aza analogues were fraught with unexpected difficulties.

The β-lactam area of research can be seen to be as active now as it was when penicillin was first isolated. The fascinating chemistry and the important selective antibacterial activity and the bacterial resistance towards the β-lactam antibiotics has ensured active research in this area. The greater understanding of the mode of action, isolation techniques and better synthetic methods may, however, soon make rational drug design a reality.
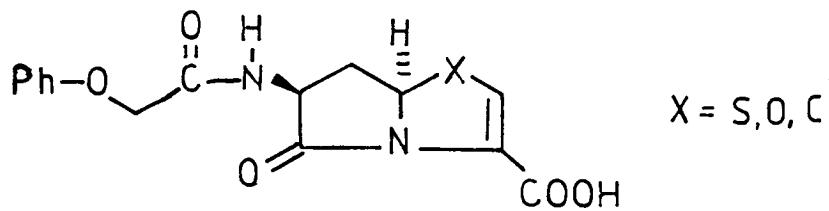
(389)

Scheme 145   Structure of a γ-analogue of a penem system.
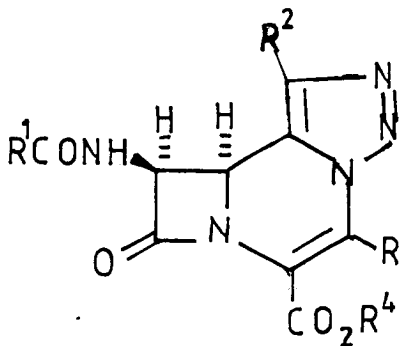
(390)
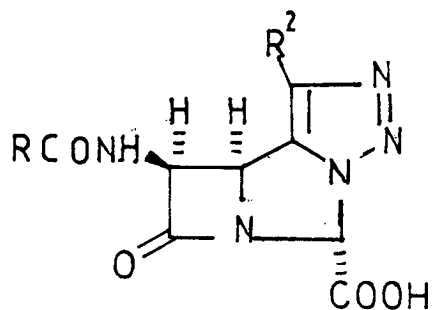
(391)

Scheme 146   Structure of novel tricyclic fused systems.
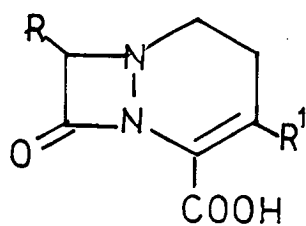
(392)
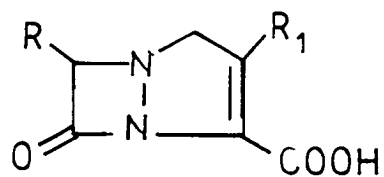
(393)

Scheme 147   Structure of 1,2-diazetidin-3-ones.

CHAPTER TWO - DISCUSSION

1.0.0 INTRODUCTION

Work in these laboratories involving dehydroamino acids suggested an interesting novel route for the synthesis of a bicyclic β-lactam derivative (130) (scheme 148).

Richardson[207] has found that thiol addition to the dehydroalanine derivative (395) occurred quite readily (scheme 149), whereas, addition to the dehydrovaline derivative (396) did not occur. This finding was interesting in relation to the field of β-lactam antibiotics for the following reasons. Firstly, an early proposal[208] for the biosynthesis of penicillins implicated the cyclisation of the monocyclic β-lactam intermediate of the type (394) (scheme 148). However, this transformation has since been shown, by labelling experiments, not to occur in the biosynthesis of penicillins.[209,210] Secondly, careful experimentation by Baldwin and co-workers[101] on the monocyclic β-lactam intermediate (399) (scheme 150), under acidic, basic and radical conditions, has shown that the 5-endo-trig cyclisation does not take place. The rationale of the conclusion was based upon the poor stereoeletronics of this system (scheme 151).[102] These results appeared consistent with the work done in our laboratories. In the acyclic case the steric factor prevented the addition of the thiol to (396). However, Baldwin and co-workers did not point out the influence of the steric hindrance present in their system. We thus questioned their conclusion, since our work had suggested that addition of a thiol to a dehydroalanine derivative (395) occurred readily due to the lack of any steric hindrance. This prompted us to investigate an intermediate of the type (394) (scheme 148), which lacked the steric factor of the methyl substituents and depended only on the
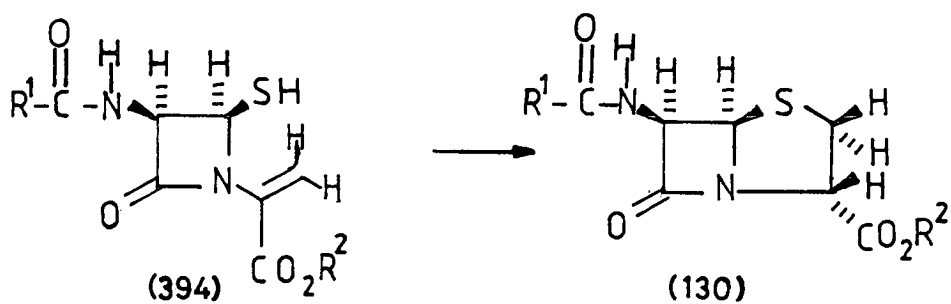
Scheme 148   Novel approach to bisnorpenicillin
              involving a 5-endo-trig cyclisation.

(395) $R^1 = R^{1'} = H$ (397) $R^1 = R^{1'} = H$

(396) $R^1 = R^{1'} = Me$ (398) $R^1 = R^{1'} = Me$
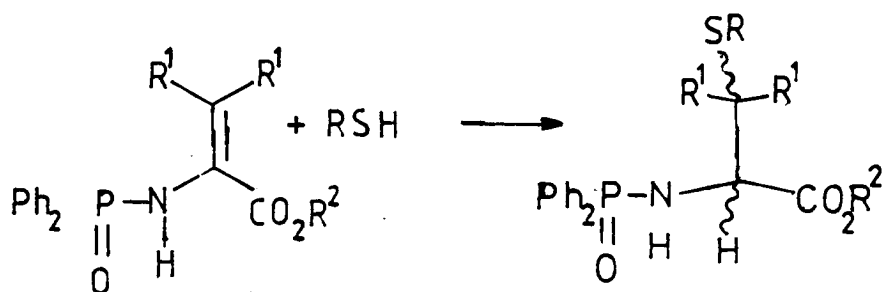
Scheme 149   Thiol addition to dehydroamino acids.
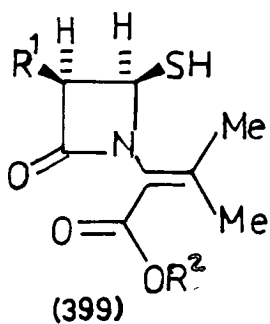
$R^1 = Phthalimido$

$R^2 = Me$

$\quad = CH_2Ph$

Scheme 150   Structure of intermediate used by Baldwin
              and co-workers.

stereoelectronics of the process. Our experience suggested that in the absence of any steric hindrance this process might lead to a successful cyclisation. Our approach was thus designed to study the stereoelectronics in isolation. In addition, workers at Beecham[87] have devised a route to bisnorpenicillin V (140) (scheme 55) which lacks regiospecificity and stereospecificity. It was envisaged that our approach (scheme 148) would possess better stereochemical control at C-3, by variation of the side chain (R^1) and, possibly, the solvent. Furthermore, workers at Beecham[88] have shown that by appropriate modification of the side-chain (R^1) , the bisnorpenicillin derivative has enhanced biological activity over its pencillin counterpart. The chemistry of the bisnorpenicillin system has not been investigated to any great extent. Consequently, the facility of this route would make it possible to study the chemistry of (400) and the bisnorpenicillin sulphoxides (401) (scheme 152), their stereochemistry and biological activity, the effect of base treatment on (400) and (401), typical rearrangements of (400) compared with the pencillins, and possible transformations to the penem system.

1.1.0 **Background to Early Experimental Work**

Initial studies were carried out by Thackery.[211] This work, directed towards the synthesis of (414), and derivatives thereof (scheme 153), utilised the strategy pioneered by R.B. Woodward[212] in the synthesis of β-lactam antibiotics.

The oxidation of penicillin V potassium salt (402) with sodium metaperiodate in an aqueous solution, was followed via the starch-iodide test until a negative result was obtained, typically 1.5 hrs. The reaction mixture was then diluted with ice and acidified to pH 2.5 with 2 Molar hydrochloric acid to afford the precipitated
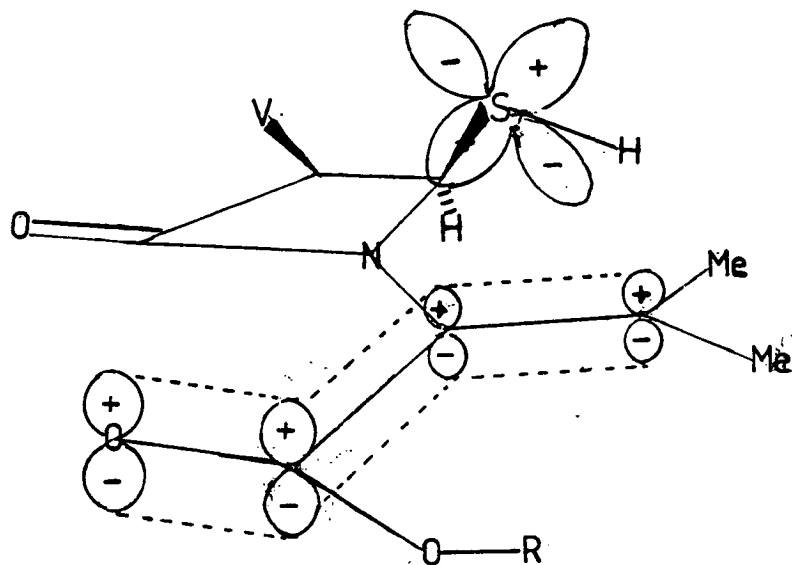
(399)

Scheme 151   Stereoelectronics of the intermediate used
by Baldwin and co-workers.

(400)   n = 0

(401)   n = 1
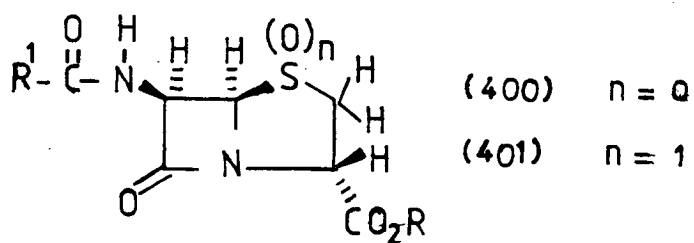
Scheme 152   Structure of bisnorpenicillin and its
sulphoxide.
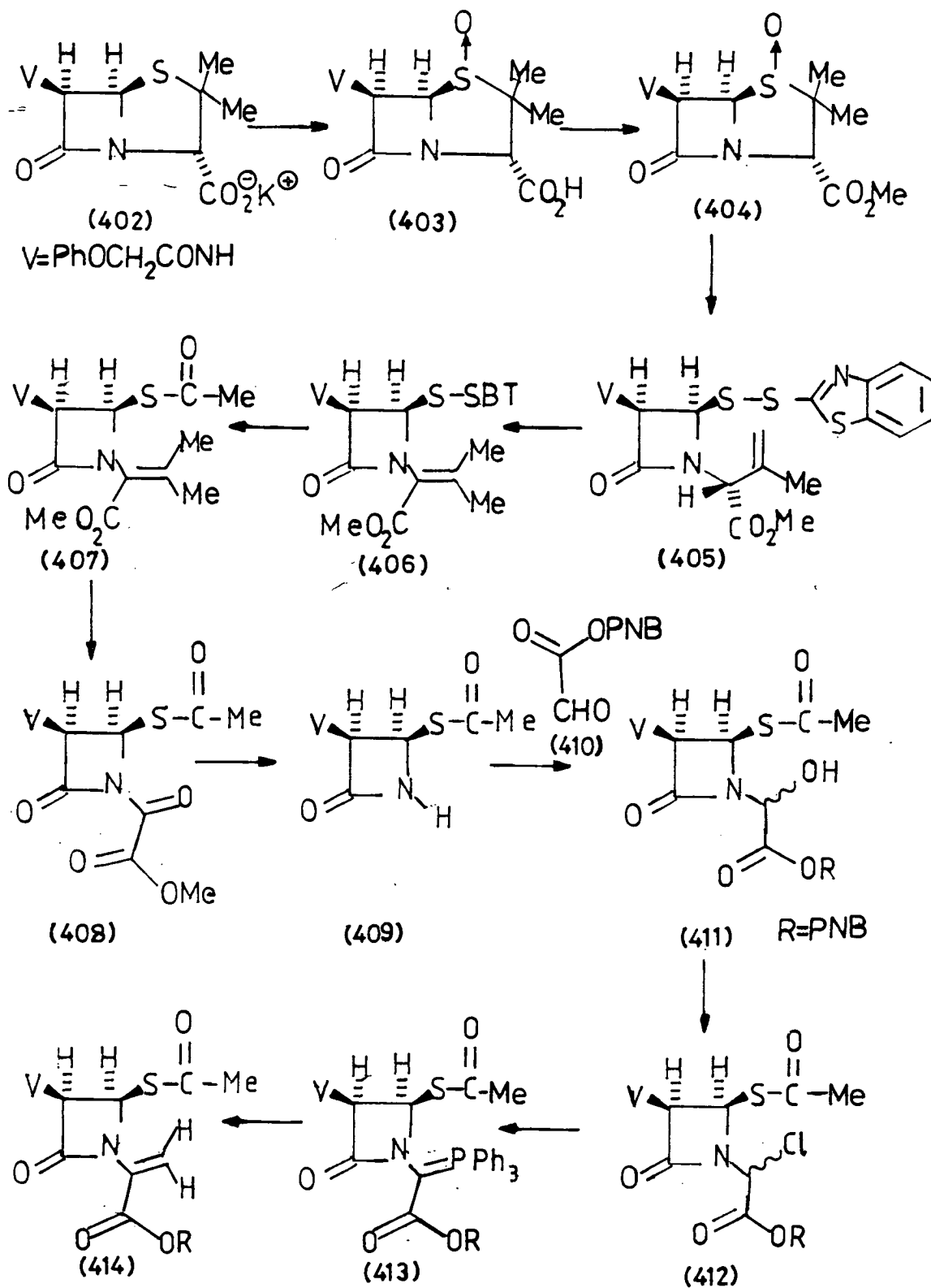
Scheme 153   Synthetic strategy to the target
                molecule (414).

S-sulphoxide (403)[213] which was then filtered and washed
with ice water. The crude product (403) was
crystallised from an acetone/water mixture (1:2) and left
at 5°C overnight. The dried product was then dissolved
in dimethylformamide and treated with 0.5 equivalents of
anhydrous potassium carbonate. To the resulting
solution excess methyl iodide (2 equivalents) in
dimethylformamide was added and the reaction mixture
stirred for 18hrs. The work-up procedure was
modified. Normally, the reaction mixture was diluted
with water and the product (404) was isolated by ethyl
acetate extraction. This method resulted in low yields and
required a large amount of extracting solvent, because of
the solubility of methyl penicillin V sulphoxide (404) in
the water/dimethylformamide system. The modified
procedure involved removal of the dimethylformamide
in vacuo, maintaining the temperature as low as possible,
dissolving the residue in ethyl acetate, filtering off
all insoluble salts followed by washing the filtrate
twice with water and brine, and finally, drying over sodium
sulphate. The resulting product (404) was obtained by
crystallisation from methanol in good yield (70-80%) (mpt
= 116°C) and the reaction could be carried out readily on
a large scale (100g batches). The product (404) was
sufficiently pure to be used directly for the following
step. The sulphoxide (404) to the disulphide (405)
transformation[67] was found to proceed in variable yield
(0-66%), however, the best conditions were found to be on
a 0.1 molar scale using 300 ml of toluene, 1 equivalent
of 2-mercaptobenzothiazole and heating at 95-100°C for
4hrs under nitrogen. The disulphide product (405)
normally crystallised on cooling, recrystallisation from
ethyl acetate and petroleum ether (40-60) resulted in
pure (405) (mpt = 147°C). The maximum yield was never
greater than 66% which may have been due to
disproportionation of the disulphide (405) into the
symmetrical disulphides (415) and (416) (scheme 154).
The isomerisation of the double-bond to the $\alpha - \beta$

unsaturated isomer would appear to be a very straight forward reaction, however, adding a catalytic amount of triethylamine (TEA) to a dichloromethane solution of the disulphide (405) did result in the isomerised product (406), as indicated by the infra-red analysis, in addition to a large amount of precipitate, which was found to be the bis-2-mercaptobenzothiazole disulphide (416) (mpt = 180°C). Work-up involved an ice cold wash with citric acid (5% (w/v)), drying over magnesium sulphate and purification. On a small sale, the yield of product was 60%, but upon scaling up the reaction, from 9mmol to 56 mmol of disulphide (417), the yield dropped to 30%. Much starting material (405) was still present and other by-products were obtained including the symmetrical disulphides (416) and (417). The resulting purification involved a column chromatography using an isocratic eluent of (1:1) chloroform and petroleum ether (40-60) followed by crystallisation from diethylether and cyclohexane, to remove the symmetrical disulphide (417). The required product (406) was then obtained as a white foam. This step was not optimised and it was found that the best method was to carry out several small-scale reactions, (9mmol of (405)), which were then worked-up, combined and purified. The pure product (406) was a foam and was not capable of crystallisation. The symmetrical disulphide (417) was, however, readily crystallisable from diethylether and was characterised. The ^{1}H n.m.r. spectrum of (417) was similar to that of (406) except the allyl methyl signals had a larger difference in their chemical shifts, in addition to fewer aromatic protons being present in the region 6.9-7.8 ppm.

The step involving a conversion of disulphide to the thioester derivative (407) afforded an excellent yield (70%) on a small scale (9mmol). However, on a large scale (45mmol) the yield diminished to 30-45%. The optimised conditions involved dissolving 25mmol of the disulphide (406) in a minimum amount of acetic anhydride,and then adding 3 equivalents of acetic acid
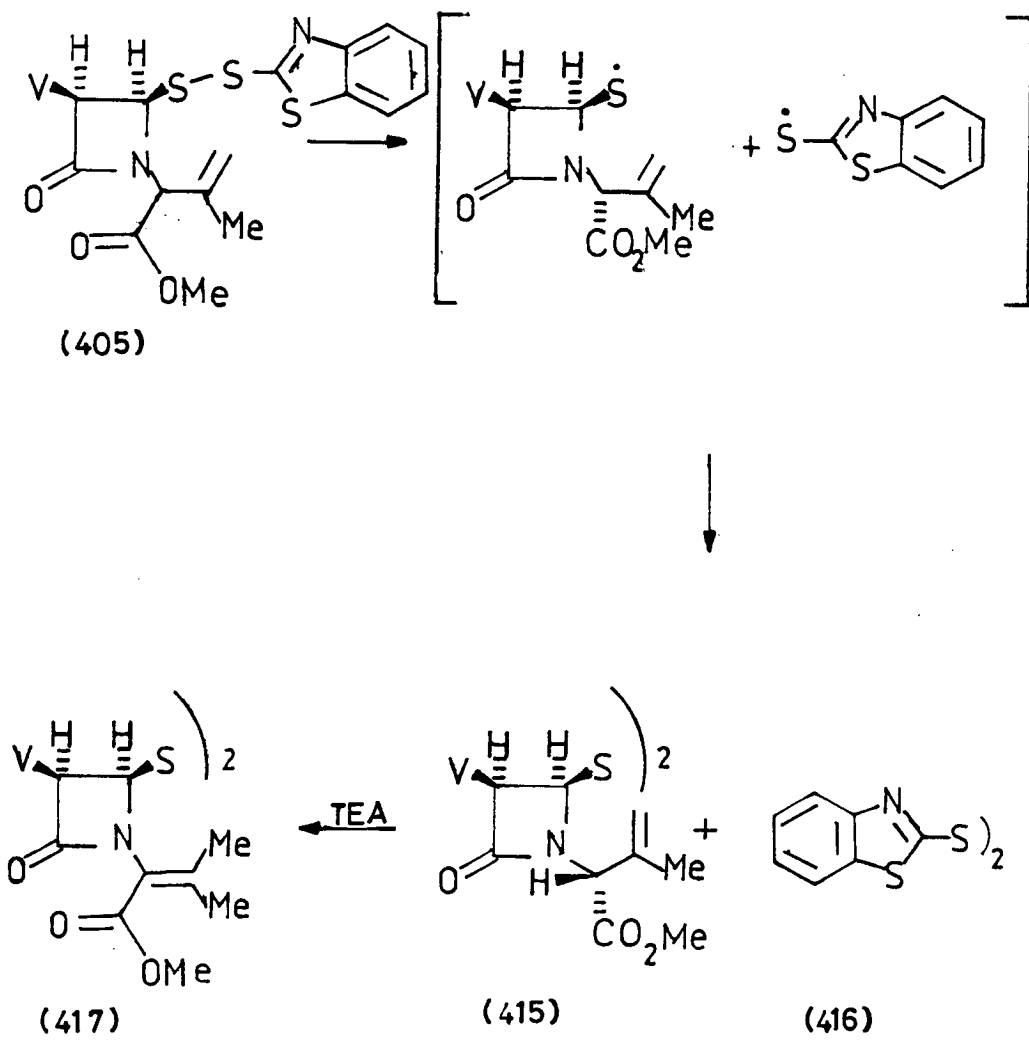
Scheme 154   Disproportionation of the disulphide
                derivative (405).

whereupon the reaction mixture was stirred and cooled to -40°C followed by the addition of triphenylphosphine (1.1 equivalents) in small portions until no more solid remained (typically 1.5 hrs). Pyridine (3 equivalents) was then added and the reaction mixture stirred at -40°C for 0.75 hr, then allowed to warm to room temperature and stirred for 4 hrs. The solvents were removed in vacuo and the residue chromatographed on silica, the eluent being ethyl acetate and petroleum ether (40-60). The yield was generally greater than 65%.

Ozonolysis of the thioester derivative (407) was carried out as described in the literature,[212] the product (408) being filtered out of the reaction mixture at 0°C, and recrystallised from ethyl acetate and petroleum ether (40-60). The yield was moderate (50-60%) and could possibly have been optimised by using the reaction mixture directly for the subsequent methanolysis.

The methanolysis step was modified in that the oxamide derivative (408) was suspended in acetone, excess methanol and 4% (v/v) of water. The suspension was then sonicated at room temperature for 18hrs. The product (409) sometimes crystallised during concentration of the solution and was merely filtered, but more often the solvents were removed in vacuo and the residue chromatographed on silica using a gradient eluent system consisting of ethyl acetate and petroleum ether (40-60). The yield varied between 60-70%.

The condensation of the azetidinone (409) and p-nitrobenzyl glyoxylate (410) was found to occur with variable yields (30-50%). Scaling up the reaction usually resulted in diminished yields and the reaction was found to be difficult to monitor by the usual methods, for example, TLC, infra-red or [1]H n.m.r. The conditions involved azeotroping the hydrate of p-nitrobenzyl glyoxylate with benzene for 1 hour, followed by addition of the dry azetidinone (409) and continued refluxing for 6-8 hours. The solution was

then cooled and the solvent removed in vacuo to give a
residue which was chromatographed on silica using ethyl
acetate/petroleum ether (40-60) as the eluent.

The characterisation was difficult since one
equivalent of each reagent was used and the R_f of both
the reagents and the product (411) was very similar, due
to the presence of polar groups. The problem was
overcome by the availability of a spray system,[214]
composed of an oxidant, (0.5% (w/v) aqueous potassium
permanganate and a staining agent (0.02% (w/v) ethanolic
bromophenol blue). This spray system could
differentiate readily the azetidinone (409), the
p-nitrobenzyl glyoxylate (410) and the product -
hemiaminal (411) by the difference in their behaviour to
the oxidant and the staining agent. This improvement in
the monitoring system enabled the yield to be improved
(65%). The infra-red analysis was not very useful
because identical functional groups existed in the
reaction mixture and in the product (411). Furthermore,
since a mixture of diastereomers resulted (in the
product), the ^{1}H n.m.r. spectrum was too complex to be
useful in monitoring the reaction.

The final diastereomeric mixture was neither separated
nor fully characterised[215] but used directly
for the next stage, in accordance with the procedures
followed previously.[212]

The chloro-derivative (412) was obtained by
treatment of the hemiaminal (411) in tetrahydrofuran with
1 equivalent of thionyl chloride (freshly distilled) and
1 equivalent of base (2,6-lutidine) at room
temperature. The 2,6-lutidinium hydrochloride was
filtered and the filtrate evaporated to dryness in
vacuo. Attempts to characterise the chloro-derivative
(412) were not very successful. The crude residue was
observed to discolour quite rapidly on standing in air at
room temperature. Further synthesis of the
chloro-derivative (412) involved the following
modifications.

a)   3 equivalents of both thionyl chloride and base were used for each equivalent of substrate.

b)   The reaction was carried out at 0°C for 0.5 hr and at room temperature for a further 0.5 hr.

c)   The work-up involved filtration under an inert atmosphere.

d)   The final crude product was used directly for the following step, due to its instability.

The phosphorane (413) synthesis was quite straightforward.   This step could readily be monitored by TLC, using the developed spray system[214], since the phosphorane (413) was quite stable and the reaction proceeded in reasonable yield (60%).   The phosphorane could be purified by chromatography on silica.   Optimal conditions were to use 1.5 equivalents of triphenyl-phosphine and an equimolar amount of base to that of the substrate in freshly distilled 1,4-dioxane.   The reaction mixture was heated to 50°C under nitrogen for 8hrs, the end-point of the reaction being indicated by the absence of a yellow spot on the TLC plate (after staining) corresponding to the chloro-derivative (412). The phosphorane (413) resulted in a purple stain after strong heating of the TLC plate.   The work-up involved filtration of the salt, adding dry toluene to the filtrate and filtering off any further 2,6-lutidinium hydrochloride, evaporating the solvent in vacuo followed by chromatography of the residue on silica using a gradient eluent system with ethyl acetate/petroleum ether (40-60).   Characterisation of the phosphorane (413) was not exhaustive but infra-red analysis and [1]H n.m.r. were in agreement with the structure (413).

The intermolecular Wittig reaction was optimised to the following conditions.   The phosphorane (413) was dissolved in dichloromethane and a mixture of gaseous

formaldehyde and dry nitrogen was bubbled into the
solution for short periods (30 seconds to 60 seconds) at
0.5 hr intervals with constant stirring. The procedure
was repeated until no phosphorane remained in the
reaction mixture as indicated by TLC. The solvent was
then evaporated in vacuo and the residue chromatographed
on silica with a gradient eluent consisting of ethyl
acetate and petroleum ether (40-60) to afford the product
as a white powder (mpt = 112°C) in good yield (80%).
The infra-red spectrum in dichloromethane indicated peaks
at 3410 cm^{-1} (N-H), 1780 cm^{-1} (β-lactam C=O str.), 1725
cm^{-1} (conjugated ester), 1700 cm^{-1} (broad, thio-ester and
amide) and 1520 cm^{-1}, 1350 cm^{-1} (due to asymmetric and
symmetric stretches of the nitro group). The ^{1}H n.m.r.
spectrum (400 MHz) in CDCl$_3$ indicated the represented
structure (414) and a ^{1}H n.m.r. spectrum (200 MHz) helped
to assign the vinylic protons by separating the two close
peaks centered around δ = 6.15 ppm which overlapped with
the C-4 proton in the 80MHz spectrum.[216] ^{1}H n.m.r.
decoupling experiments (360 MHz) confirmed the identities
of the coupling protons. Irradiation at 6.15 ppm
(vinylic proton, J=0.8 Hz) resulted in a singlet at 6.11
ppm. (vinylic proton). Irradiation at 5.65 ppm (dd,
C-3-H, J=8.8,5.2H$_z$)[216] resulted in a singlet at 7.25 ppm
(aromatic region) and the doublet at 6.02 ppm (C-4-H,
J=5.2Hz)[216] collapsed to a singlet. Furthermore,
evidence was obtained from a ^{13}C n.m.r. DEPT experiment
which showed three methylene groups to be present, one of
these being in the vinylic region. The F.A.B. mass
spectrum showed a protonated molecular ion, [MH$^+$] = 500,
and elemental analysis was in excellent agreement with
the required structure (414).

1.2.0. Alternative Approaches to Intermediate (414)

Before describing the attempts to cyclise the
target molecule (414), it may be worthwhile to outline
alternative approaches to (414), which were examined.
(Scheme 155) involved following scheme 153 to the oxamide
(408) step and then carrying out a zinc/acetic acid
reduction[217] to afford the hemiaminal (411) (R=OMe). The
crude product was taken through the subsequent three
steps to the acrylate derivative (414) (R=OMe). The
final product was too impure to characterise and at the
time it was concluded that the target molecule (414) was
perhaps unstable to silica chromatography. On checking
the [1]H n.m.r., in retrospect, it could be seen that the
required product (414) (R=OMe) had actually been
formed. This approach was abandoned on the grounds that
the methyl ester would have been no use in the final
cyclised product, since the carboxylic acid was required
for biological assay.

A further attempted modification was to make use
of the method used by Hatfield[218] as in (Scheme 156).
This would have converted the methyl penicillin V
sulphoxide (404) directly to the thioester derivative
(407) (after treatment with base), thus omitting the low
yielding disulphide step and the following step
(disulphide to thioester conversion). Unfortunately,
this method was not very successful in that, after two
separate attempts, the required product (407) could not
be isolated. The isolated product appeared to be
deacetoxycephalosporin V (418) (scheme 157). This
suggested that the molecular sieves were not very
efficient and the water that formed hydrolysed the acetic
anhydride to acetic acid which would have provided
suitable conditions, in the refluxing benzene, for the
classical penicillin sulphoxide to cephalosporin
rearrangement, first observed by R.B. Morin et al. in
1963.[61]
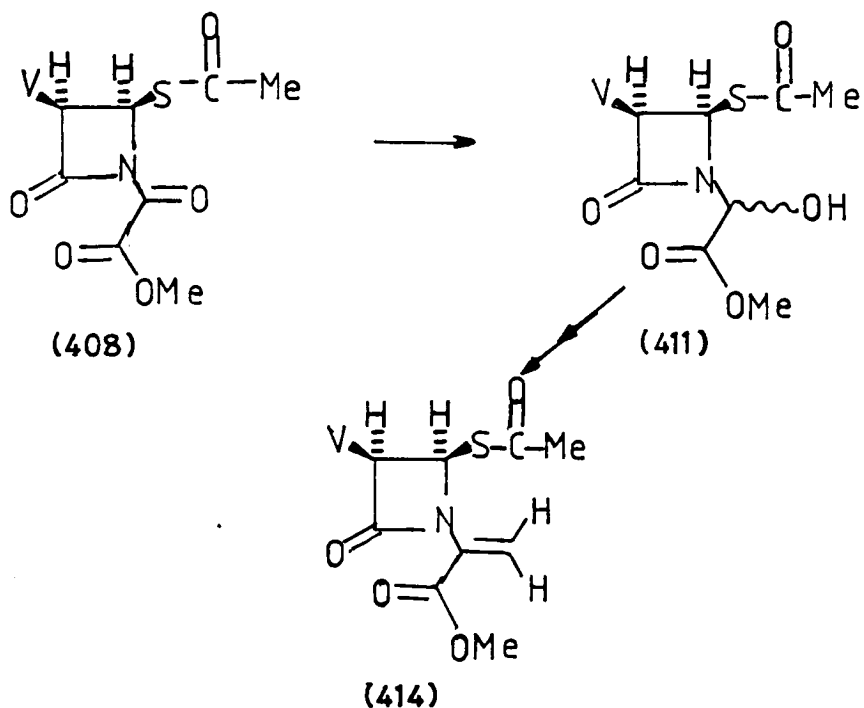
Scheme 155   Reduction of oxamide with zinc/acetic acid.
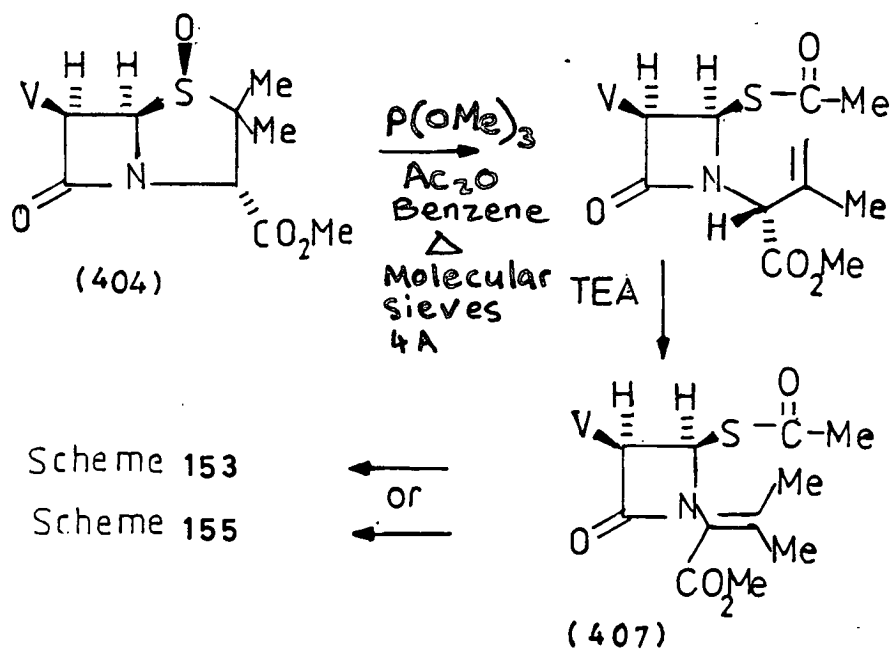
Scheme 156   Conversion of Penicillin V sulphoxide to the
thioester derivative – Hatfield's method.

(404)   (418)
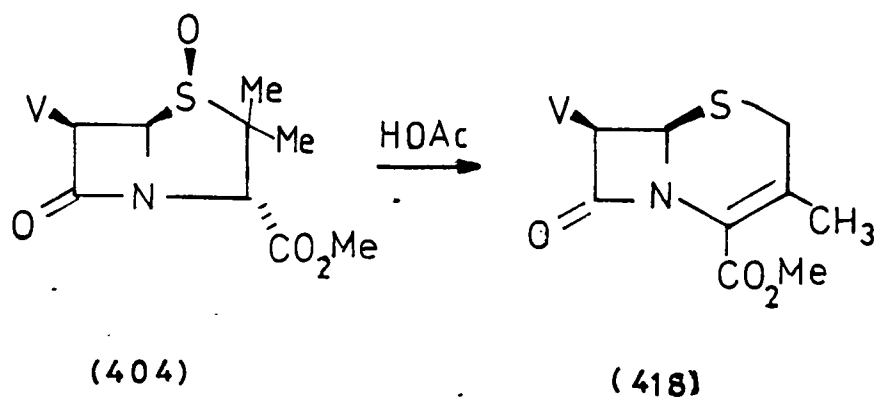
Scheme 157   Rearrangement of methyl penicillin V
              sulphoxide under the experimental
              conditions.

Further time was not spent on this route and it was abandoned for a similar reason as for the zinc/acetic acid reduction of the oxamide (408) (scheme 155). Furthermore, although the literature yields were quoted as 75%, it was believed that on a large scale, the reaction would be too sensitive to the conditions and result in quite variable yields and a mixture of products.

One final alternative approach which was attempted was as in scheme 158.[219] It was hoped to condense the thiazoline derivative (419) with the p-nitrobenzylglyoxylate (410) to generate the hemiaminal (420) and by sequential treatment with thionyl chloride, triphenylphosphine and gaseous formaldehyde to prepare the acrylate derivative (421), which could then be used for the cyclisation step. When the condensation was attempted no reaction resulted. Both starting materials could be recovered after chromatography. If more time was available it would have been quite an attractive route to the desired target molecule (394). Only a limited amount of the starting material was available, and consequently it was believed that this method would not readily give the target molecule in sufficient quantity for large scale experimentation.

One further approach which was considered, but not attempted, is shown in scheme 159. This involved the thioazetidinone (138), readily obtainable from Beecham. The sulphur could have been readily protected with the desired protecting group. The remaining steps to the target molecule (394) would have been been identical to those of Woodward.[212]
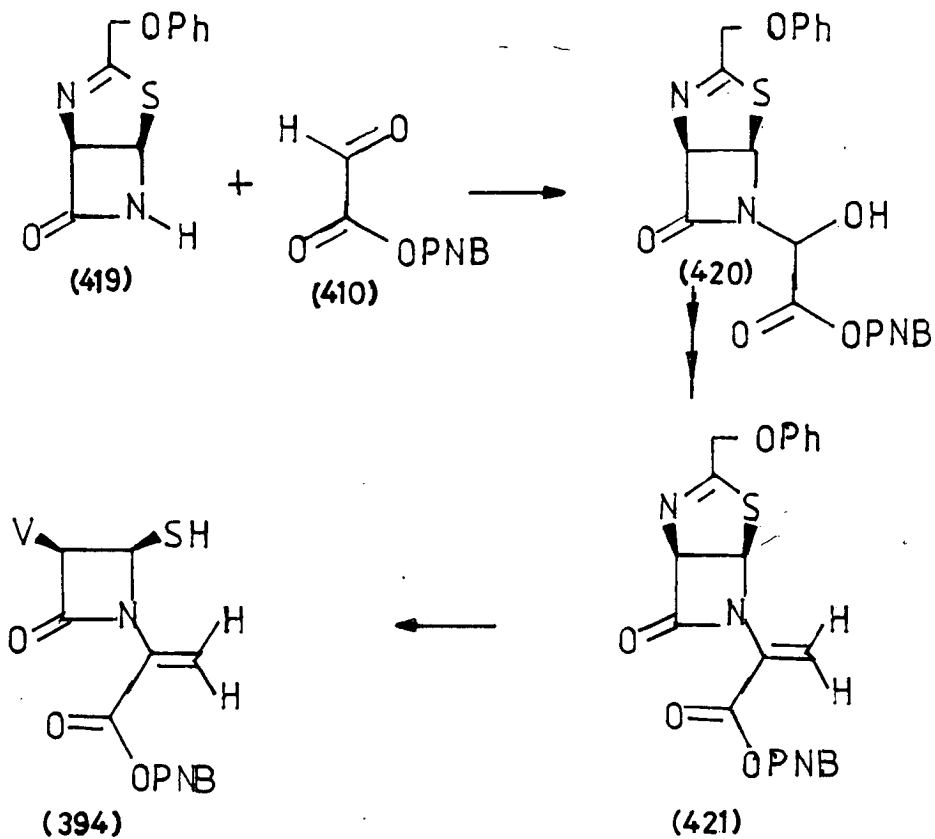
Scheme 158
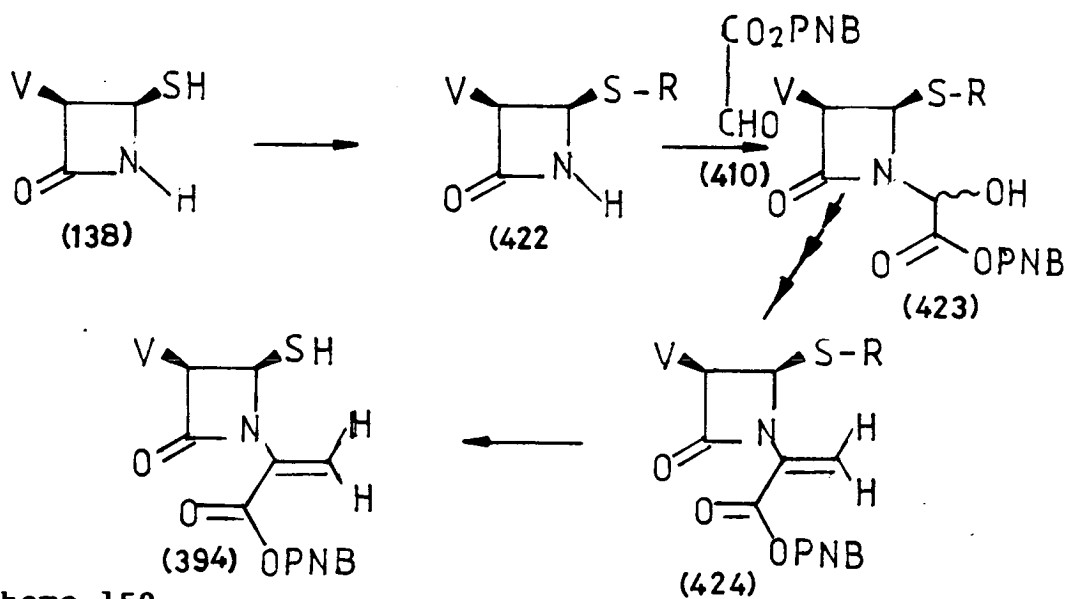
Scheme 159

1.3.0 Cyclisation attempts to bisnorpenicillin

When the target molecule (414) was first prepared, only 200mg was available. The chosen method of deprotection of the thioester was that suggested by Pearson[220] which involved the use of cyclohexylamine. This method had been used in the synthesis of isopenicillin (426) (scheme 160). This involved deprotection of the thioester (425) with cyclohexylamine, the thiol thus formed then underwent an intramolecular S_N2 reaction on the alkylhalide, at the C-4 position of the azetidinone, to generate the isothiazolidine ring (426). The second equivalent of cyclohexylamine was used to neutralise the hydrogen halide by-product.

It was decided that deprotection of the thioester derivative (414) in our system (scheme 161) would require 1 equivalent of cyclohexylamine and a second equivalent to abstract the proton from the thiol group, in order to assist addition to the acrylate. Literature reports[221] indicate that thiols readily add to acrylates in the presence of a base. The reaction was monitored by infra-red spectroscopy and TLC. The reaction conditions were as described in the experimental section, and the work-up involved washing with water and drying over magnesium sulphate (anhydrous). The aqueous washing was re-extracted with ethyl acetate in order to ensure that the required product (395) was not discarded.

The component obtained from the dichloromethane layer contained no β-lactam, according to infra-red analysis, but did contain an ester ($1740 cm^{-1}$) and a strong amide ($1670 cm^{-1}$), in addition to the nitro group stretches at $1520 cm^{-1}$ and $1350 cm^{-1}$. This disappointing result suggested that the cyclohexylamine had cleaved the β-lactam ring. The ethyl acetate extractions resulted in a component which contained traces of a β-lactam component ($1780 cm^{-1}$) which was suggested to be the symmetrical disulphide. Thus, the cyclisation appeared to have failed, since it was expected that the
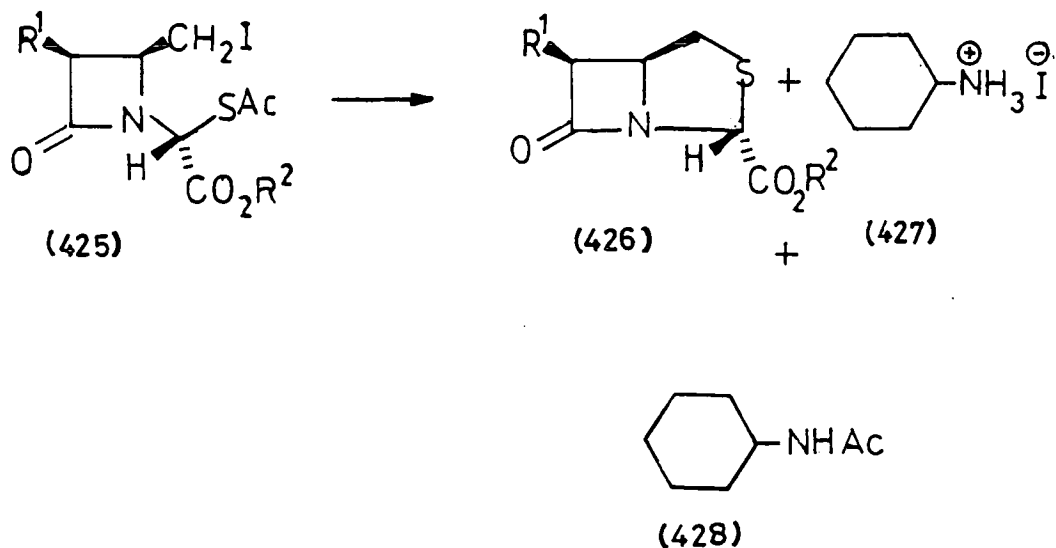
Scheme 160   Deprotection of thioester by use of
              cyclohexylamine for the synthesis of iso-
              penicillin (426).
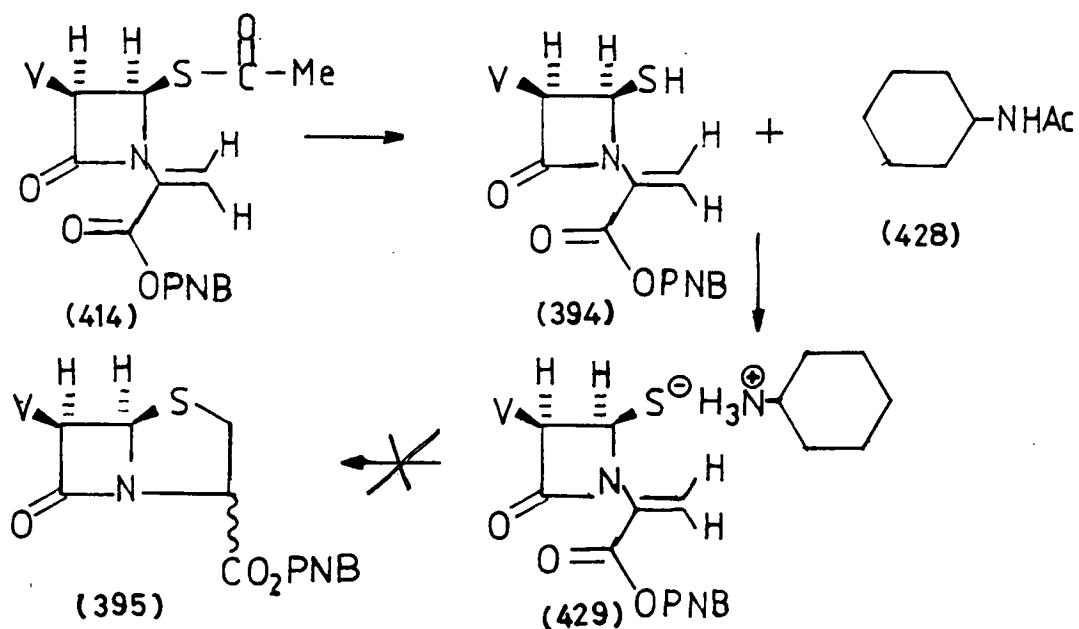
Scheme 161   Deprotection of thioester by use of
              cyclohexylanime.

infra-red spectrum would indicate an increase in the β-lactam carbonyl stretching frequency from $1780\,\text{cm}^{-1}$ to $1793\,\text{cm}^{-1}$, as observed in an authentic sample of bisnorpenicillin supplied by Beecham.   On TLC, the cyclised product (395) was expected to have a higher R_f than the monocyclic acrylothioester derivative (414) in the eluent system (70% ethyl acetate/petroleum ether (40-60)), as observed by comparison of the authentic materials.   The crude residue contained no component corresponding to the required product (395) on TLC.

The above described attempt to cyclise the acrylate was repeated when more of the acrylate derivative (414) had been obtained.   The method was modified, using only 1 equivalent of cyclohexylamine in deuteriodichloromethane and the reaction was monitored by ^1H n.m.r.   After 0.5hr a peak at $\delta = 2.25$ ppm was observed.   This was assigned as being due to the acetyl group in the by-product, N-acetylcyclohexylamide (428).   Some starting material (414) still appeared to be present but the signals corresponding to the β-lactam protons were diminishing with new peaks in the region - 3.5 ppm being observed which were expected since a doublet occurs at $\delta = 3.5$ ppm in the spectrum of bisnorpenicillin (395).   The reaction was continued for a further hour after which only a trace amount of starting material (414) was present.   A strong acetyl peak due to the by-product was clearly observed but no β-lactam proton resonances could be seen in the ^1H n.m.r.   The peaks at $\delta = 3.5$ ppm did not increase in intensity, as had been expected, but merely remained as a complex system of multiplets.   The reaction mixture was then worked-up by an aqueous wash and dried with sodium sulphate.   Infra-red analysis of the crude product indicated the presence of a weak β-lactam carbonyl stretch ($1780\,\text{cm}^{-1}$) and the material was purified by preparative TLC.   The first band obtained by washing the silica with dichloromethane comprised of 2% (w/w) of a component, having infra-red peaks at 1800,

1730, 1695cm^{-1}, but no nitro stretches were clearly observable. ^{1}H n.m.r. analysis of the dichloromethane component indicated signals at the following positions δ= 7.7-6.9 ppm (m), δ= 6.1 ppm (dd), δ= 5.22(s), δ= 5.10 (d), δ= 4.55(s), δ= 4.33 ppm (s).

However, the sample was too dilute and still impure. Consequently, the characterisation data were ambiguous. Structure (430) (scheme 162), was tentatively suggested by the available data. This is a novel structure, but the rearrangement was known previously, as described by Wolfe[74] (scheme 163). The anhydropenicillins (431) possess a β-lactam carbonyl stretch at 1800cm^{-1} in the infra-red spectrum. The thiolactone carbonyl stretching frequency, however, was found to be at 1700cm^{-1} and not at 1730cm^{-1} as we found in our system. Our result seemed confusing when comparing the results obtained during previous anhydropenicillin preparations. It may be that in the anhydropenicillin (432) (scheme 164) the α , β -unsaturated system may be involved in a mesomeric effect with the thiolactone, hence lowering the carbonyl stretching frequency to a lower wave number. Structure (433) may be stabilised by the inductive effect due to the methyl groups. In our system (scheme 165), the resonance involving the nitrogen atom, in the ring, may be more involved with the α , β unsaturated moiety. Structure (434) may be stabilised by the protons much more readily than by the methyl groups. Attempts to synthesise this intermediate by an alternative route, for comparative purposes, was decided to be not worthwhile, in the time available. Fractional from the preparative TLC was also washed with ethyl acetate which afforded 21% (w/w) of the component which appeared to be very similar to the starting material (414), exhibiting the infra-red absorbances as follows 1780, 1748, 1690, 1520 and 1350cm^{-1}. The ^{1}H n.m.r. of the sample obtained from the ethyl acetate was found not to be that of the starting material as expected. However, it could be seen that
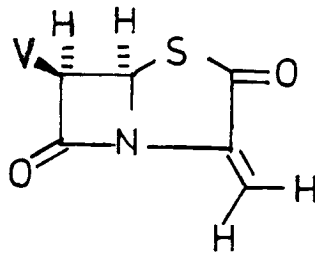
(430)

Scheme 162   Suggested structure of one of the
                by-products in the cyclisation step.
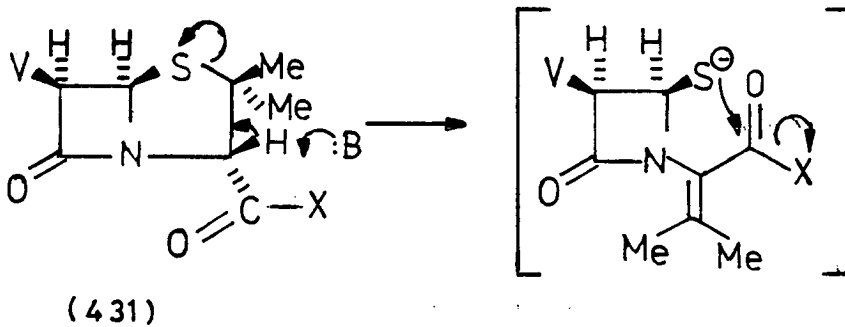
(431)

X = Cl, DCCI Anhydride
BASE = TEA
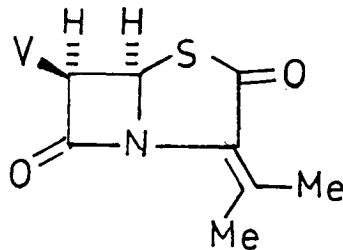
(432)

Scheme 163   Rearrangement of penicillin derivatives.

(432)   (433)
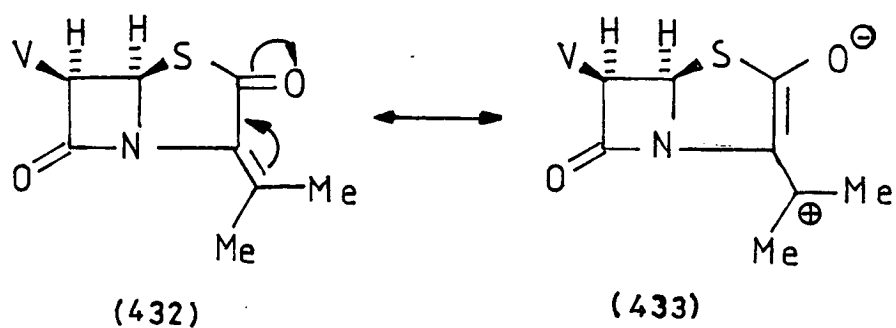
Scheme 164   Possible resonance in the anhydropenicillin.

(430)   (434)
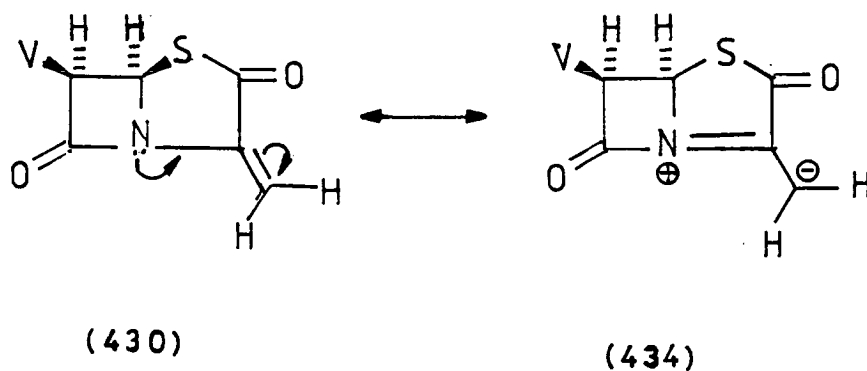
Scheme 165   Possible resonance in the product (430).

(435)
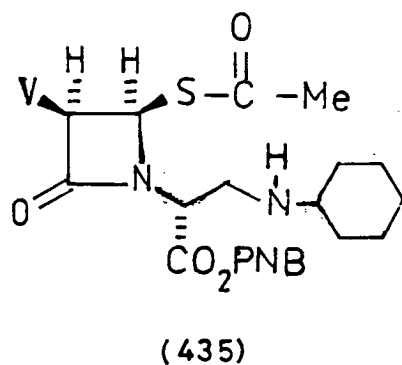
Scheme 166   Structure of by-product (435).

the vinylic protons of the acrylate were absent and that
the new multiplets in the region δ= 3.5 ppm were too
complex to be simply due to the thiazolidine methylene
protons (as in the bisnorpenicllin).   Resonances due to
the cyclohexylamino group could also be seen and the
thioester, phenoxy and p-nitrobenzyl protons were also
present.   The β-lactam protons were not clearly resolved
but multiplets in the δ=5.0 -5.6 ppm region did exist.
These results suggested the structure (435) in scheme
166.   This is reasonable in that acrylates are
susceptible to nucleophilic attack, particularly, by
thiols and primary amines.[222]   The other bands resulted
in components containing no β-lactam carbonyl stretching
frequency in the infra-red spectrum but a new strong
amide carbonyl stretching frequency was present around
$1670cm^{-1}$.   This suggested that the cyclohexylamine may
also have added to the β-lactam(436).   Scheme 167
suggests the possible transformations that resulted from
the treatment of the acrylate derivative (414) with
cyclohexylamine.   It was concluded that use of
cyclohexylamine was inappropriate for our system.
Furthermore, no evidence for the formation of
bisnorpenicillin (395) could be detected by infra-red, [1]H
n.m.r. or TLC analysis.   It was concluded that the
cyclisation via a Michael type addition would not take
place as reported by earlier workers.[223]   This
conclusion was supported by the above experimental
evidence.   It was hoped that the thiol (394) could be
isolated, since reports by two workers, namely,
Lattrell,[224] and Baldwin,[225] showed that they had managed
to obtain the dehydrovaline thiol derivative (399)
(scheme 150) and had characterised it.   Similarly, the
aim was to isolate the pure thiol (394) which,after
characterisation,would finally complete this area of
research.

Scheme 167, using cyclohexylamine as the
deprotecting reagent for the thioester, shows clearly the
problems with the use of a strongly nucleophilic

reagent. From our experience, it was suggested that a more sterically hindered base might deprotect the thioester and hence give rise to the thiol in a reasonably pure form. The base chosen was diisopropylamine which is both readily available and more sterically hindered than cyclohexylamine. This was found to undergo no reaction at all with the acrylate derivative; the starting material being recovered intact and in high yield. It was decided to add a catalytic amount of 4-dimethylaminopyridine but this resulted in complete β-lactam cleavage. A search of the literature for mild methods of deprotection of thioesters produced an article[226] in which p-chloroaniline was used to deprotect a hindered thioester by stirring the reagents at room temperature for 24 hrs to afford a 70% yield of the thiol. This was attempted with the acrylate derivative (414) (scheme 168), but again only the starting materials were recovered in high yields. It was at this point that the use of transition metals for complexing with the sulphur to labilize the thioester group was considered.[227] An article[228] illustrated the use of silver nitrate in aqueous THF or dioxane to deprotect a thioester protecting group. The yield of the silver thiolate obtained was quoted as greater than 90%.

It was concluded at the time that aqueous conditions would not be suitable for the acrylate system (414). Anhydrous conditions could be maintained by use of p-chloroaniline (438) as the nucleophile in an organic solvent. After considerable experimentation[229], the following conditions were developed (scheme 169) in which the acrylate derivative (414) was dissolved in dry THF, 2 equivalents of methanol and 2 equivalents of pyridine were added, the solution flushed with nitrogen and the vessel covered by aluminium foil. To the reaction mixture was added 2 equivalents of silver tetrafluoroborate in dry tetrahydrofuran and the solution was then warmed to 30°C for 1-1.5 hrs. The solvent was then removed in
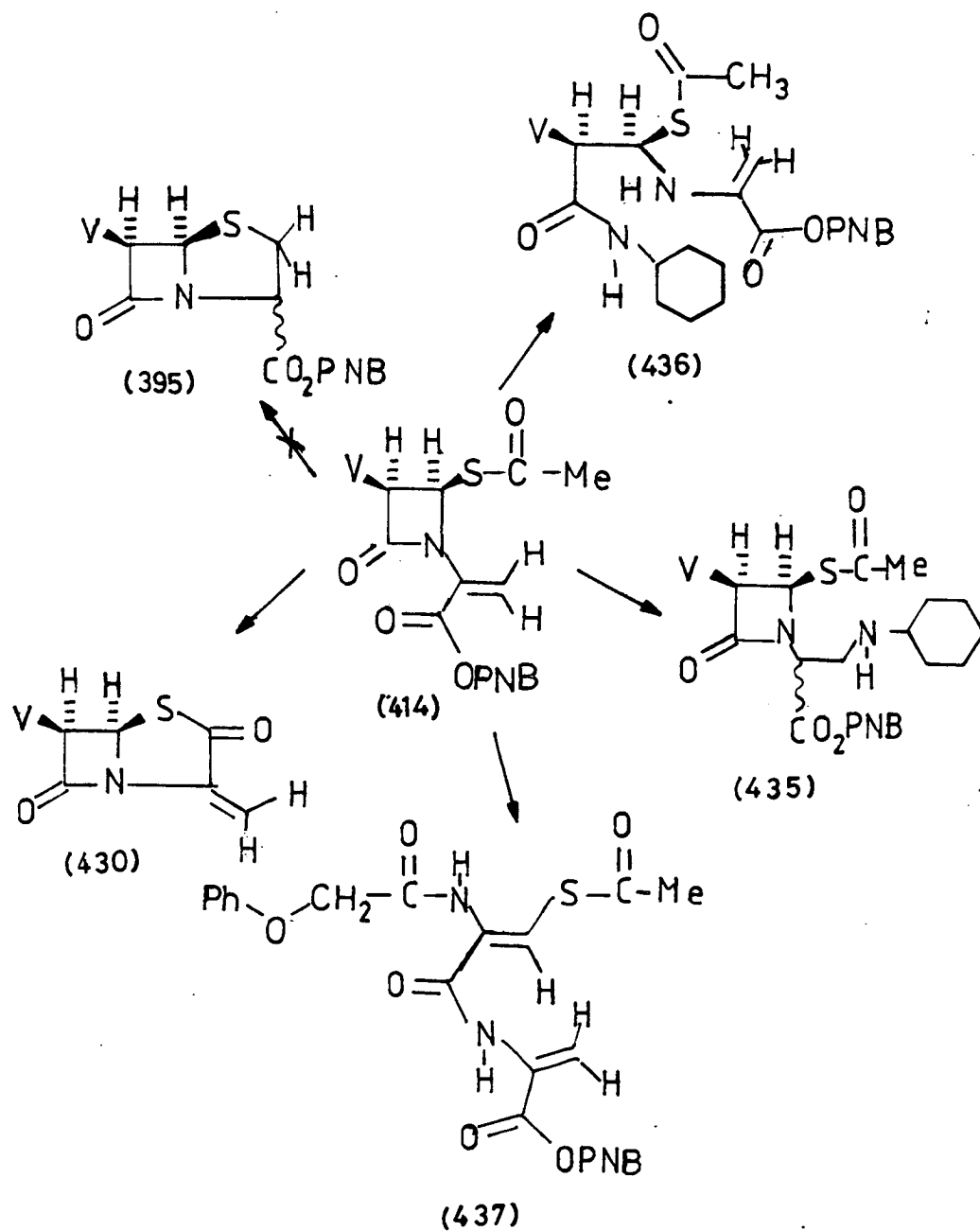
Scheme 167   Possible transformations of (414) on
              treatment with cyclohexylamine.

172
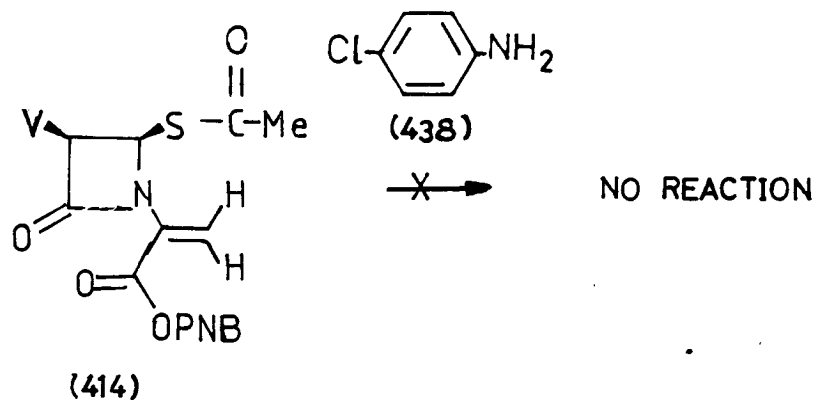
Scheme 168   Use of p-chloroaniline for deprotection of
              the thioester derivative (414).
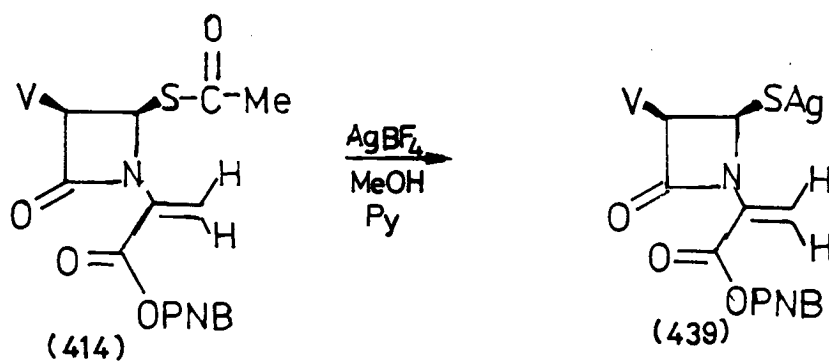
Scheme 169   Deprotection of the thioester derivative by
              use of silver salts.

Scheme 170(a)
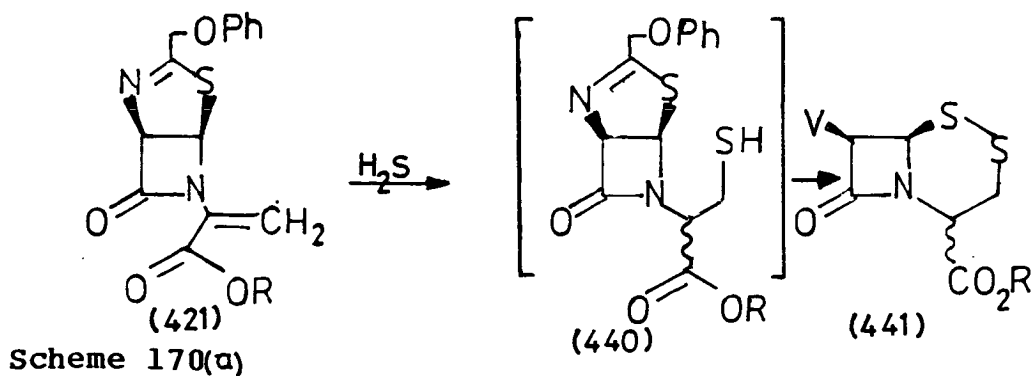
vacuo and the residue chromatographed on silica using a
(1:1) dichlormethane:ethyl acetate mixture as the
eluent. The required product was obtained in excellent
yield (70%) and was of high purity. The
characterisation data confirmed the structure represented
as (439) (scheme 169). Further confirmation was
obtained when the silver thiolate (439) was reacted with
acetyl chloride, in dichloromethane and at room
temperature, in order to regenerate the thioester
derivative (414). This proved to be identical,
according to infra-red, [1]H n.m.r. and TLC analysis, with
an authentic sample.

One of the anticipated problems in synthesising
the silver thiolate (439) was the need to use hydrogen
sulphide, which has been reported[230] to add to acrylate
(scheme 170a)
systems ∧, to generate the free thiol (394). In the
literature[231], the usual method employed for removing the
metal involved bubbling hydrogen sulphide gas through a
dichloromethane solution of the metal sulphide, the metal
being mercury or silver, at room temperature. The
yields quoted for the thiol were moderate and the
β-lactam was not attacked by hydrogen sulphide. The
problems encountered involved the instability of the
thiol to bases, and to oxygen which could result in
disulphide formation. The thiols were simply filtered
through celite or kieselghur, to remove the metal
sulphide by-product, and crystallised from cold benzene.

Despite the possibility of reaction of hydrogen
sulphide with the acrylate moiety, we attempted to
perform the reaction at low temperature (-50°C) in the
hope that reaction of hydrogen sulphide with the metal
would be highly specific and that the reaction with the
acrylate moiety would not take place. The hydrogen
sulphide, generated from ferrous sulphide and
concentrated hydrochloric acid, was passed through water
and into a dichloromethane solution of the silver
thiolate (439) at -50°C. The excess hydrogen sulphide

V=PhOCH₂CONH

$V = PhOCH_2CONH$

$PNB = CH_2$⟨benzene ring⟩NO_2

(442)

(421)

(394)

(443)

–H⊕

(445)

Hydrolysis

(444a)   (444b)

(446)
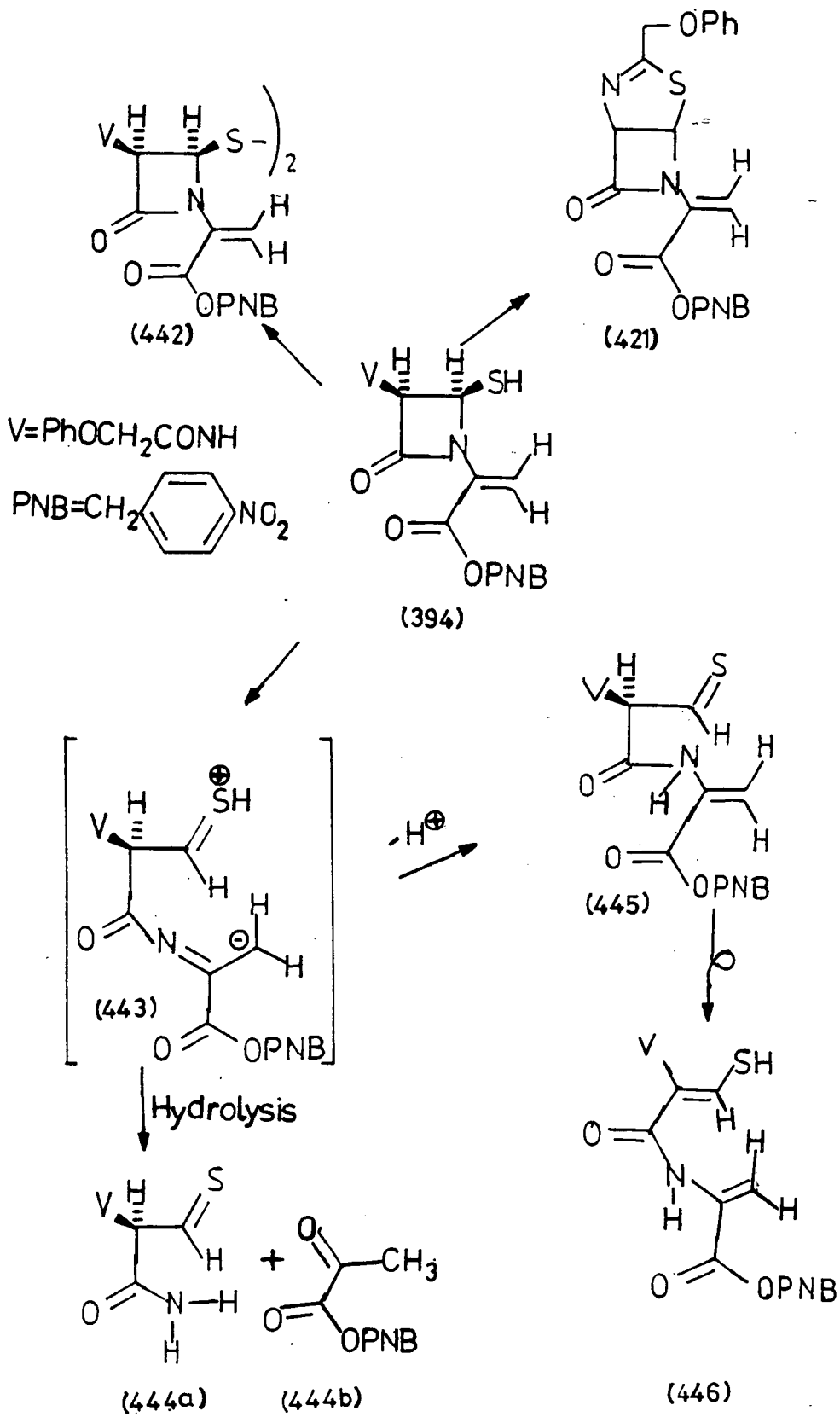
Scheme 170(b) Possible rearrangements of the target molecule (394).

was removed by flushing with nitrogen for 0.5 hr, and then the reaction was allowed to warm to room temperature. The final brown solution was passed through kieselghur affording a very pale orange filtrate. Infra-red analysis of the crude product indicated the presence of a strong amide peak ($1670 cm^{-1}$) and an ester carbonyl stretching frequency ($1750 cm^{-1}$) but no β-lactam carbonyl stretching frequency ($1770-1790 cm^{-1}$). TLC analysis of the crude reaction mixture indicated no mobile species in the eluent used (50% dichloromethane/50% ethyl acetate). Attempts to crystallise the product were unsuccessful. Chromatography of the sample on silica resulted in complete loss of sample. Scheme 170 indicates the possible rearrangements that may have taken place.

One other attempt to generate the thiol was to use excess sodium chloride in aqueous acetonitrile (scheme 171). It was hoped the equilibrium between the sodium thiolate (447) and the silver thiolate (439) may shift towards the thiolate (447) due to precipitation of silver chloride. The sodium thiolate would have a much more ionic sulphur-metal bond and might readily hydrolyse to the thiol (394), under the reaction conditions. The reaction was monitored by TLC analysis (for the disappearance of the starting material). After 12 hrs, the solution was found to have changed from colourless to yellow. Examination of an aliquot, after work-up showed very little β-lactam carbonyl stretch in the infra-red spectrum (in the region of $1770 cm^{-1}$). However, a strong amide carbonyl stretch ($1685 cm^{-1}$) was seen. This result suggested the instability of the thiol (394). Subsequently, attempts to isolate and characterise it were abandoned. It was decided to convert the silver thiolate into a suitable derivative to characterise the product and to confirm the reactivity of the silver thiolate. It was also decided to confirm the reactivity of the acrylate moiety. Using the protected acrylate derivative (414), the sample was separately treated with
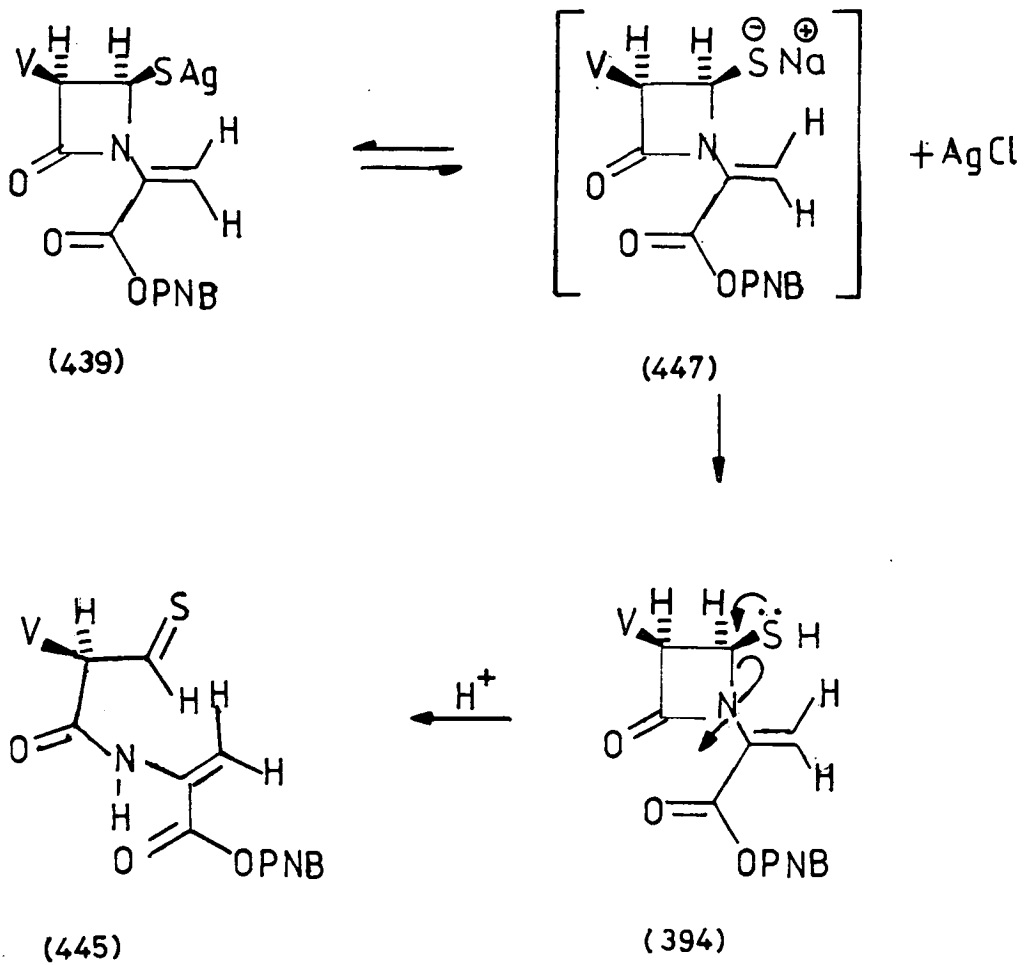
Scheme 171 Exchange of the silver thiolate to sodium thiolate.
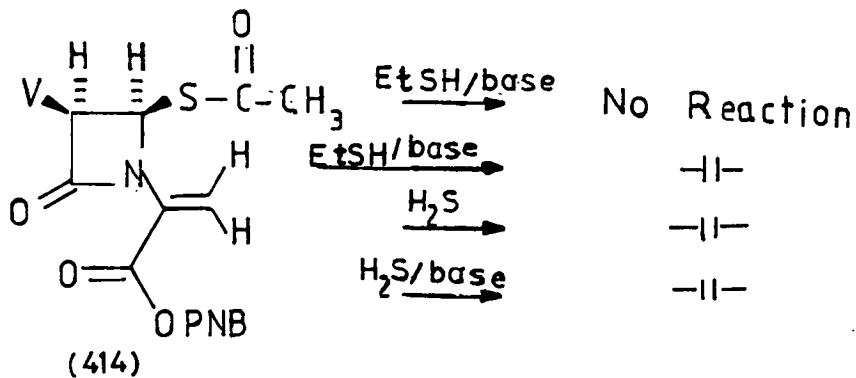
Scheme 172 Reaction of acrylate derivative (414) with other thiol reagents.

ethanethiol and hydrogen sulphide under nitrogen, both in the absence and presence of pyridine, at room temperature and also in refluxing dichloromethane. The reaction was monitored by TLC analysis (scheme 172). The starting material (414) remained unchanged and was recovered in high yield (60%) after chromatography. This proved to be identical (infra-red, ^{1}H n.m.r. and TLC analysis) to the starting material. The reaction was carried out on a 30mg scale. The conclusion from these experiments suggested that the acrylate moiety is less reactive towards thiols, and is not as susceptible to nucleophilic addition as typical α-amido acrylates.[232]

In assessing the results of our research, the following conclusions were formulated (scheme 173). The β-elimination is favoured in the thiol intermediate (394) rather than the intramolecular cyclisation, via the 5-endo-trig mode, to bisnorpenicillin V (395). Baldwin[101] has reported a rearrangement of this type, involving a thiol derivative (449) (scheme 174), pyrolysis of which at 176°C in an inert solvent afforded a γ-lactam (451). Sammes had also proposed this type of rearrangement in attempting to rationalise the unsuccessful attempts to cyclise derivatives of the type (399) (scheme 150), reported by various authors.[171]

In our system, the β-lactam nitrogen is believed to be involved in an enamine type of resonance (scheme 175). This is likely to make an intramolecular nucleophilic addition to the double bond less likely, due to the increased electron density in the π-system, and simultaneously to encourage the β-elimination process. It would seem, the electron withdrawing effect due to the p-nitrobenzyl ester plays a smaller role in the electron distribution of the π-system in (394). of Penicillins and Cephalosporins

The bridge-head nitrogen has a pyramidal structure as confirmed by X-ray data.[233] Furthermore, the ultra-violet spectrum , (scheme 176), would suggest that the enamine character is quite high, from the position of λmax, for the acrylate derivative (414) compared to
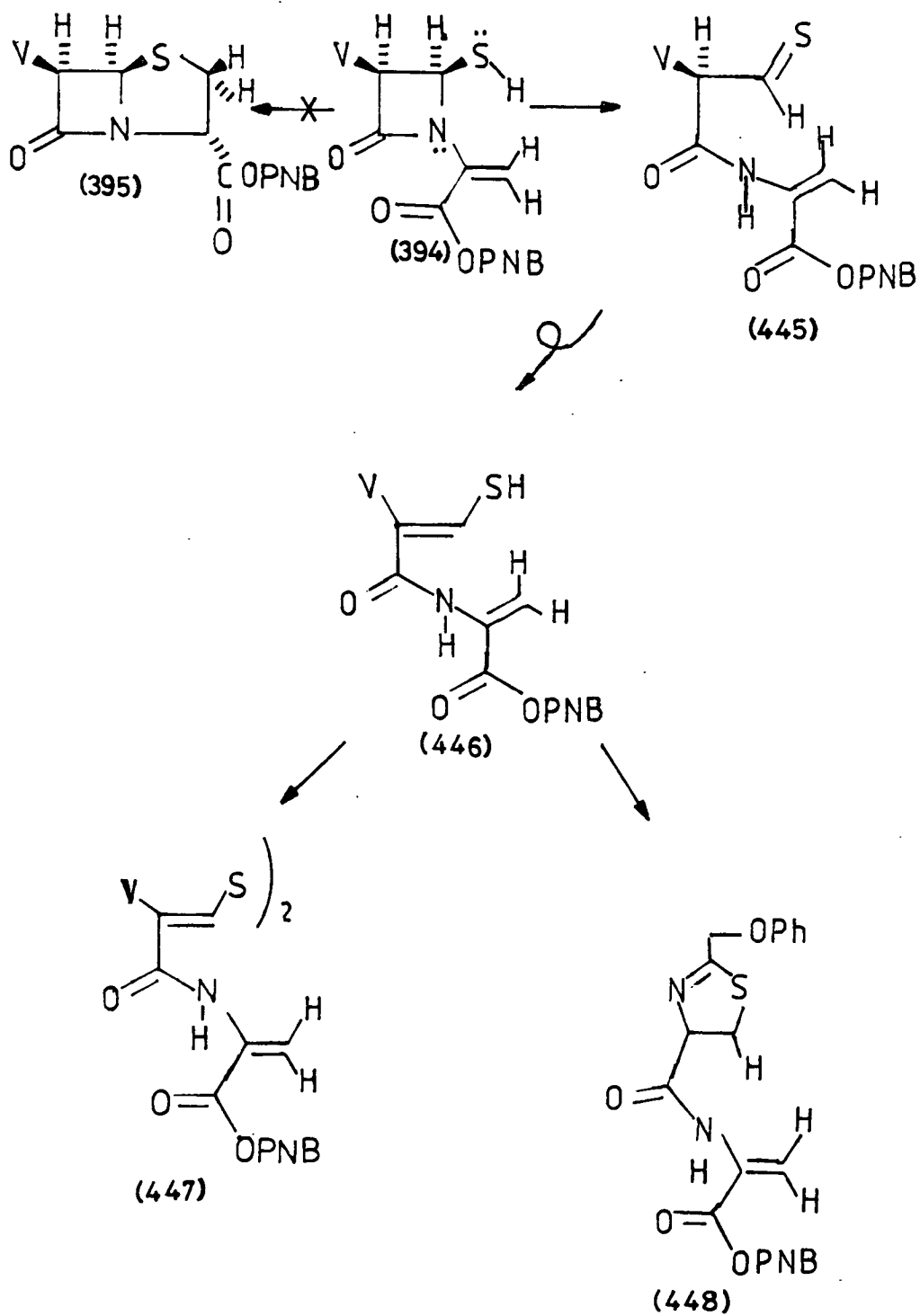
Scheme 173   Rearrangements of the thiol derivative
               (394).
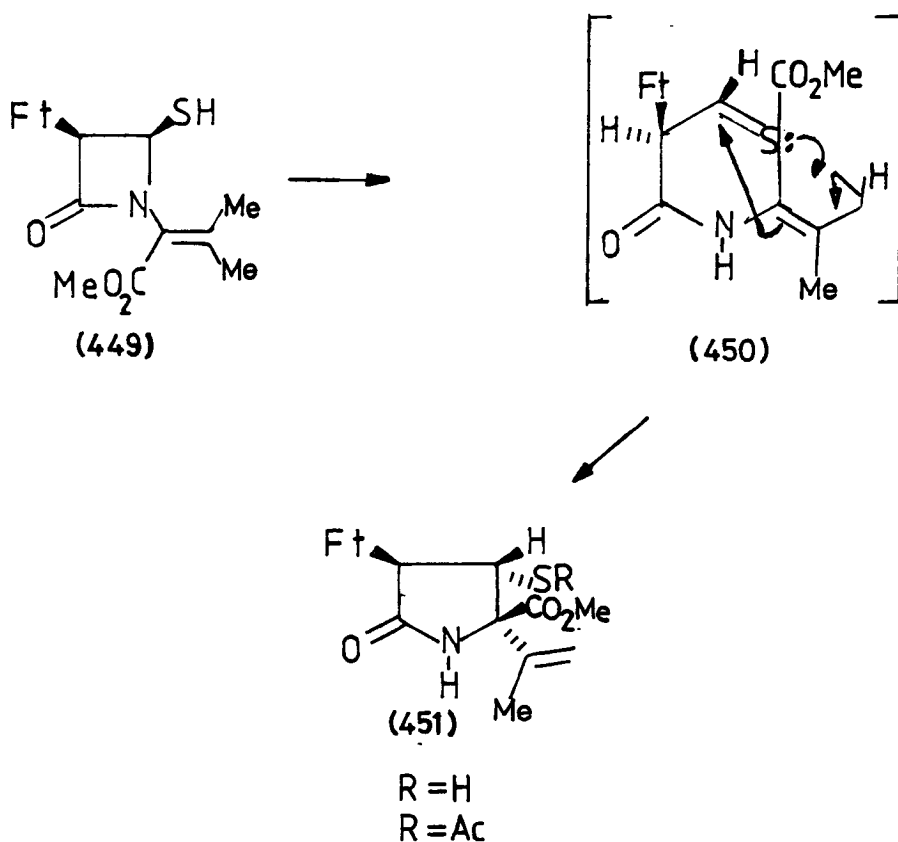
Scheme 174   Rearrangement of the thiol derivative (449)
              as reported by Baldwin and co-workers.
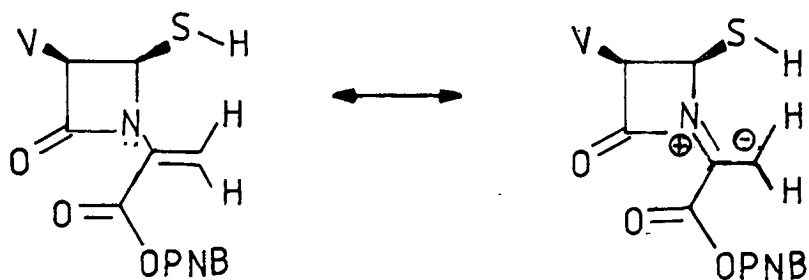
Scheme 175   Resonance involved in thiol (394).

that of α-amido acrylate derivative (452)[232], p-nitrobenzylacrylate (453) and the N,N-dialkylamino-ethene derivative (454).

Comparison of (414) with the enamine resonance form of dehydrovaline derivative (396) (scheme 177), for which the thiol has been reported to be moderately stable, suggests that the enamine-type of resonance results in a less stable canonical form, due to the postive inductive effect of the methyl groups, hence resisting the β-elimination pathway. It was thought that the ester would play a dominant role in encouraging nulceophilic addition to the double bond of (396). However, the steric effect may counter this influence. The isopropenyl moiety in secopencillins (396) is reported[234] to be relatively unreactive to most nucleophiles and electrophiles.

Consequently, the thiol (394) that was required was too unstable to isolate and the molecule underwent a β-elimination when the thiol was generated _in situ_.

Thus, we have concluded from our experiments that of the two factors, namely, steric and stereolectronic, for the 5-endo-trig cyclisation of derivative (399) (scheme 150), the latter is the important factor during cyclisation. The inability of derivative (394) (scheme 148) to undergo cyclisation to a penicillin molecule is solely due to the poor steroelectronic alignment of the thiol and the double bond. Our work has,thus, confirmed the findings of earlier workers and is also complementary to their work. Furthermore, our work suggests that an intermediate of the type (394) is not very useful in the synthesis of pencillins or related bicyclic β-lactam compounds.
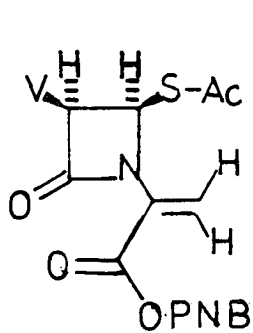
(414)

$\lambda_{max} = 261, 218$ nm
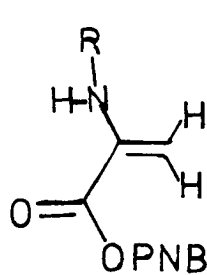
(452)

$\lambda_{max} = 271, 263, 256, 248, 223$ nm
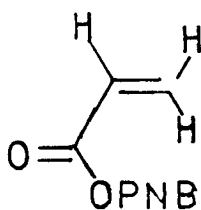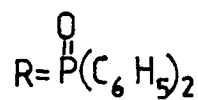
(453)

$\lambda_{max} = 261, 208$ nm
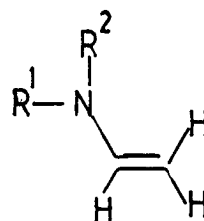
(454)

$\lambda_{max} = 220$ nm

Scheme 176   Ultra-violet absorption of model-systems.
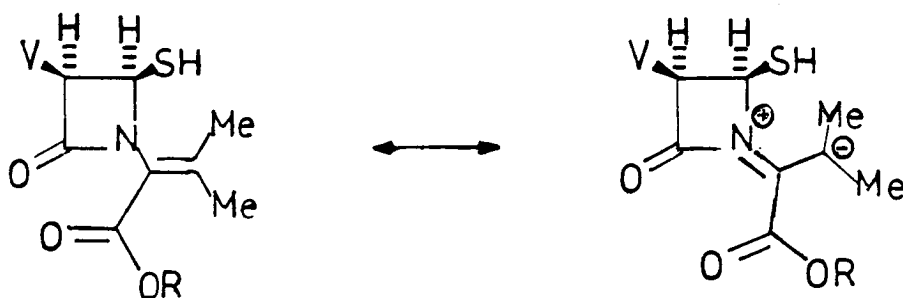
Scheme 177   Enamine resonance of the dehydrovaline
derivative (396).

1.4.0 Modification of intermediate (414) to undergo a 5-exo-tet cyclisation

A change in the strategy was considered, (scheme 178), since the acrylate (414) was not susceptible to nucleophilic attack. It was hoped that an electrophilic addition might be more successful. This would allow us to carry out a 5-exo-tet cyclisation to a penam system. This approach does not contravene Baldwin's rules[98], as did our first approach, (scheme 148), involving a 5-endo-trig cyclisation.[235] The 5-exo-tet cyclisation of the addition product (456) would be a novel means of ring formation, which to our knowledge has not been attempted previously in the synthesis of the bisnorpenicillin ring system. Consequently, approaches to the target molecules, having structure (457) (scheme 179), were investigated. Conditions for the synthesis of the acrylate derivate (414) had been optimised and it was thought that this would be a suitable starting material for the synthesis of the target molecule (457) and, furthermore, an appropriate sulphur protecting group could readily be incorporated <u>via</u> the silver thiolate (439). It was necessary to add "HX" across the double bond of the enamine system.

Ionic addition of hydrogen chloride or hydrogen bromide would have been ideal, but unfortunately, it was believed that the β-lactam ring would not survive the reaction conditions, hence, this idea was rejected. Addition of bromine (scheme 180), would give a 1,2-disubstituted derivative (458) and the cyclisation would result in a 3-bromopenam (460), in which replacement of the bromine atom by hydrogen could readily be carried out by reduction with tributyltin hydride[236]. The 3-bromopenam (460) derivative could also be treated with base in an attempt to give the penem derivative (462).

The silver thiolate (439), (scheme 181), was used first, since it was hoped that the silver ion would react

R=Ac, Ag, Trityl

(414),(439),(454)

(455)

R=H

(395)

(456)

X=Cl, Br, I, ToS
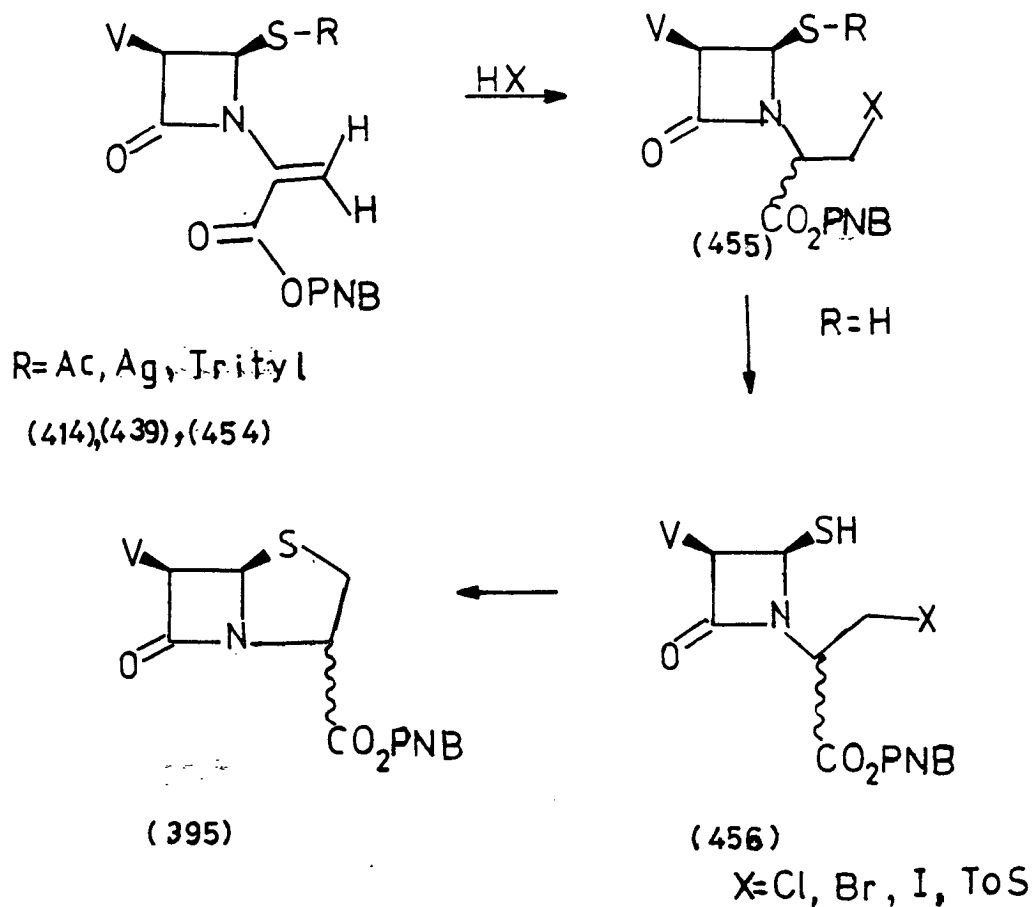
Scheme 178 Novel approach to bisnorpenicillin V <u>via</u> a 5-exo-tet cyclisation.

(457)
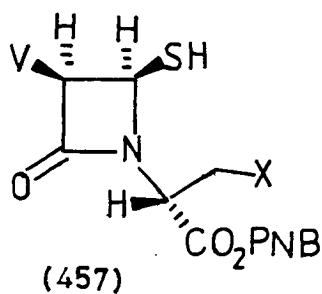
Scheme 179 Target molecule for a 5-exo-tet cyclisation.
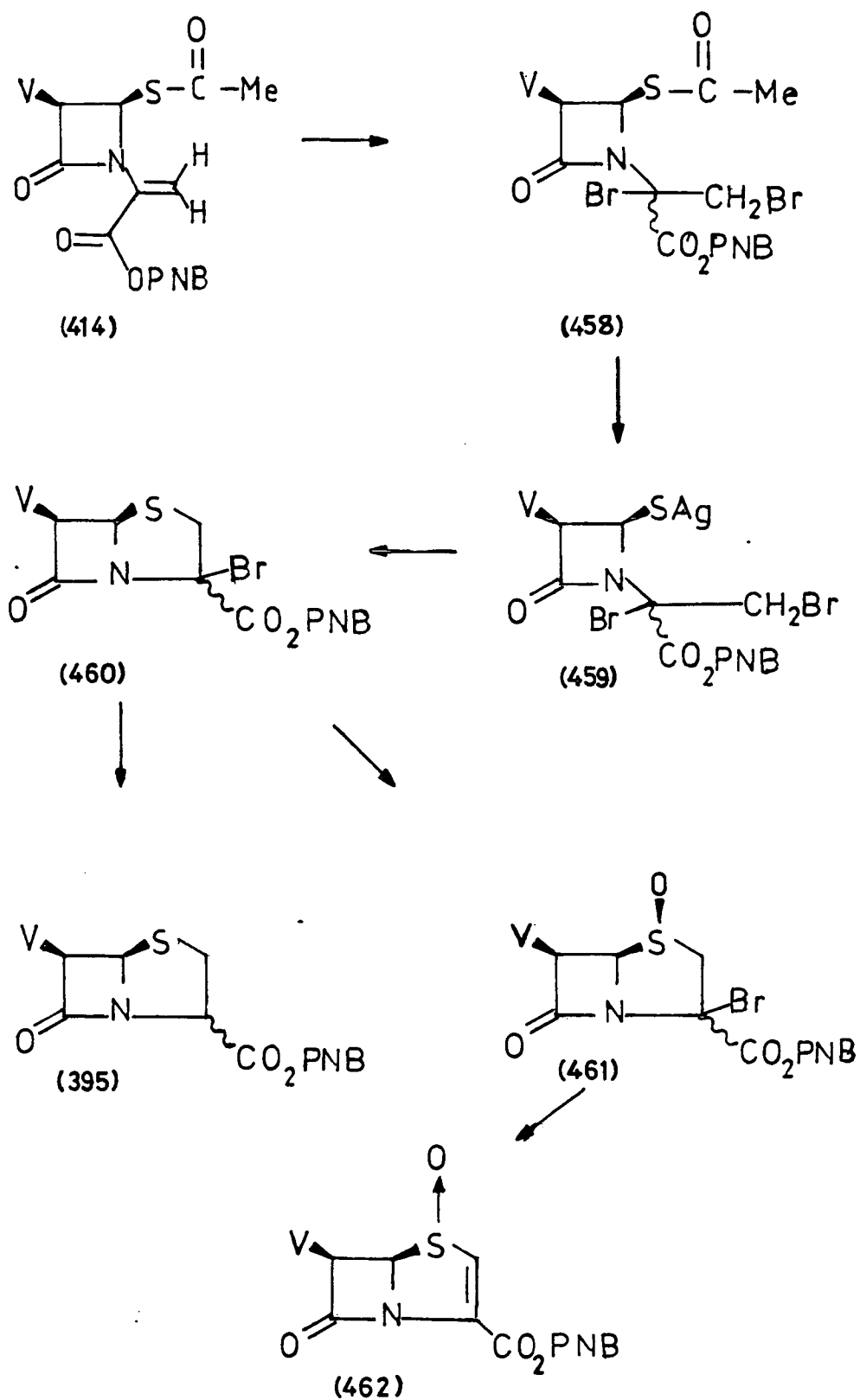
Scheme 180   Uses of the 1,2-dibromo derivative (458).
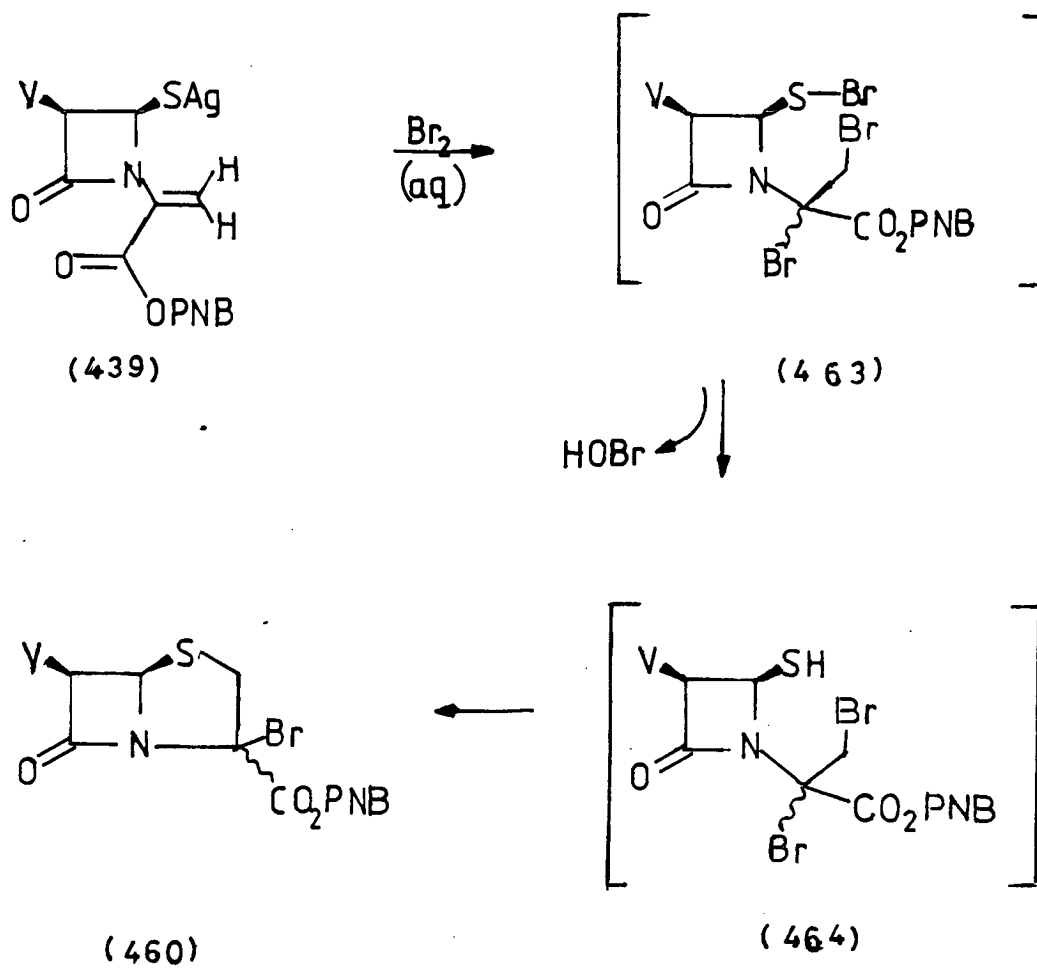
Scheme 181 Bromination of silver thiolate (439).
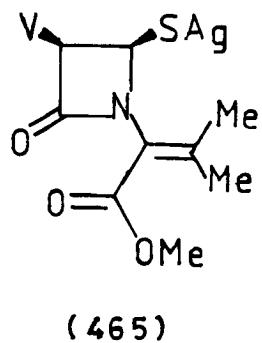
Scheme 182 Model silver thiolate from the dehydrovaline
derivative.

with bromine to result in silver bromide and the
sulphur-halide derivative (463) would hydrolyse to the
thiol (464) and hypobromous acid (HOBr) (which would be
removed into the aqueous layer and would
disproportionate). Simultaneously, the bromine would add
to the acrylate moiety resulting in the 1,2-disubstituted
derivative (464) and cyclisation might take place to form
the 3-bromo penam derivative (460).

On adding bromine water to a dichloromethane
solution of the silver thiolate (439), a precipitate
formed after a few minutes. The reaction mixture was
then quenched by addition to saturated aqueous sodium
bicarbonate and further dichloromethane washes were
employed to extract the required product. The organic
layer was washed with water and dried over sodium
sulphate. Analysis by infra-red showed that the
following absorbances were present:- 1790, 1755, 1730
cm^{-1}, 1695 cm^{-1}. These data suggested structure (460)
(scheme 181). An attempt to obtain the ^{1}H n.m.r.
spectrum was, however, unsuccessful since the sample
appeared to decompose after a short time when kept in
solution (the β-lactam was cleaved on rechecking the
sample by infra-red spectroscopy).

Before this experiment was repeated, it was
decided to carry out an analogous experiment on the
thioester derivative (414) which afforded a product,
infra-red analysis of which exhibited a β-lactam carbonyl
stretching frequency at 1785 cm^{-1} (a shift of 5 cm^{-1}).
The ester carbonyl stretch (1750 cm^{-1}) and the thioester
and amide carbonyl stretching frequencies remained
unchanged (1700 cm^{-1}). It was decided to purify the
crude product by chromatography on silica. The only
component isolated contained no β-lactam carbonyl
stretching frequency. The sample appeared to contain an
ester carbonyl (1735 cm^{-1}) and the nitro group (1520 cm^{-1}
and 1350 cm^{-1}). These results suggested that the
product obtained from the reaction was unstable to
silica. Some interesting chemistry appears to have been

taking place. Further work using other electrophiles, such as hydroboration and mercuration, to react with the acrylate would be desirable to complete this line of research. It was decided to determine whether the sulphur-halide intermediate (463) was unstable to the reaction conditions, and/or whether it reacted with the side-chain. A model compound was required to study the bromination reaction of the silver thiolate (439) and to conserve the supply of the important silver thiolate (439) while finding the optimum conditions for the reactions.

The model chosen was structure (465) (scheme 182). It has been reported[237] that the isopropenyl moiety is unreactive to bromine. Consequently, the most likely site for any reaction is the silver thiolate. The model silver thiolate derivative (465) was obtained from intermediate (407), which was prepared during the synthesis of the acrylate derivate (414) in scheme 153. The cleavage of the thioester was much more easily achieved. Excess silver nitrate was added to a methanolic solution of the thioester derivative(407) containing pyridine, and the solution stirred overnight under nitrogen, the flask being covered in aluminium foil. TLC analysis showed the reaction to have proceeded to completion. The product (465) was filtered, washed with cold methanol and dried under vacuum. The yield was good (70%) and the characterisation data corresponded to the represented structure (465) (scheme 182).

The model silver thiolate (465) was then treated with bromine water in a two phase dichloromethane/water system, the organic layer being washed with saturated sodium bicarbonate. Analysis of the residue indicated the presence of a β-lactam carbonyl stretching frequency (1785 cm^{-1}), an ester (1725 cm^{-1}) and an amide (1700 cm^{-1}) in the infra-red spectrum. The ^{1}H n.m.r. spectrum proved to be complex. However, an unusual set of signals was clearly seen. These were in the region of

δ= 7.9 ppm and were typical of an AA'BB'system similar to a para-disubstituted benzene ring. Furthermore, the β-lactam proton signals were not the typical doublet (C-4)[216] and a pair of doublets (C-3)[216] as corresponding to the cis-stereochemistry, but appeared more like a singlet (δ= 5.3 ppm) and a doublet (δ= 4.8 ppm) assignable to the trans-stereochemistry where the smaller coupling constant was not resolved. The spectrum also indicated that a mixture of products had been formed, but no starting material could be detected. A tentative structure (466) (scheme 183), was suggested. The following conclusions were derived from this experiment:-

a) bromination of the phenoxy side chain was taking place under the reaction conditions. (only at the para-position).

b) A trans-geometry of the β-lactam ring protons had resulted.

c) bromination of one of the allylic positions may also have occurred.

The crude product was too impure for the above conclusions to be definite and purification was not possible by chromatography on silica (the sample was unstable and streaked on TLC plates). A possible mechanism for the formation of the represented structure (466) is suggested in scheme 184. Further investigation of this area is desirable.

1.5.0 Development of novel sulphur-protecting groups in β-lactam compounds

Studies have also been continued concerning the sulphur protecting group, which could be removed under non-nucleophilic conditions. In addition, the group should be versatile and its derivatives obtained in high
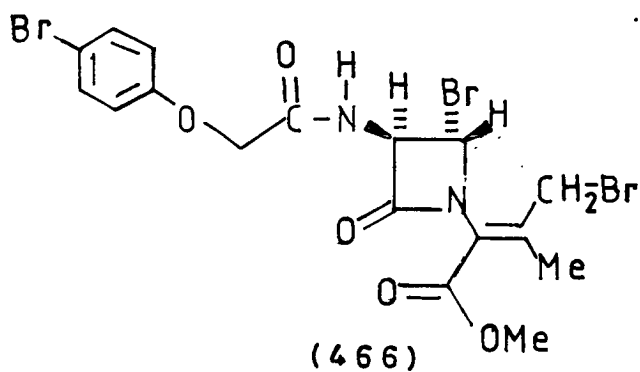
Scheme 183   Possible structure of product from
bromination of the model compound (465).
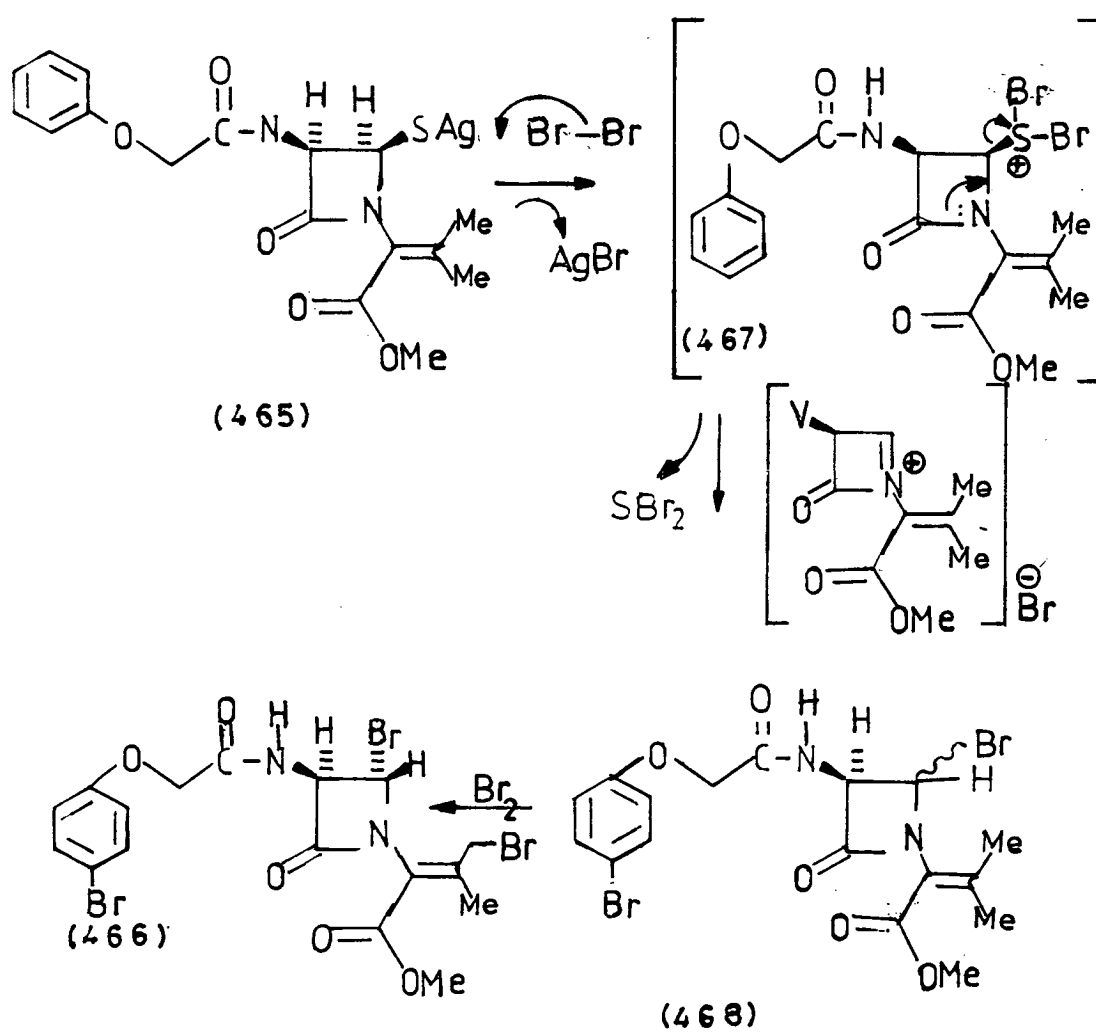
Scheme 184   Possible mechanism for the bromination
reaction.

yields. The protecting groups chosen for examination were the trimethylsilyl (SiMe$_3$), trimethyltin (SnMe$_3$), and phenylselenide (PhSe) moieties. These were chosen on the grounds that these groups had not previously been used in protecting the sulphur in β-lactam systems. In addition, the silyl group could be readily removed by treatment with flouride ion (scheme 185) and the trimethyltin group could be removed <u>via</u> a radical method. By appropriate functionalisation at the β-position of the acrylate moiety, a biradical species (473) could be generated which would potentially ring close, (scheme 186). This would be the first biomimetic synthesis of a bisnorpenam system.[238] The phenylselenide moiety was chosen for a further reason in that the selenide could be readily oxidised and by a syn-elimination of benzeneselenenic acid would afford a thione derivative (477) by a novel route (scheme 187).

It was decided that since only a limited supply of the silver thiolate (439) was available, model experiments should be carried out using the silver thiolate of the isopropenyl derivative (465).

1.5.1   Synthesis of Sulphur-Seleno derivatives and their uses

Scheme 187 was first attempted, since many synthetic reports[237-242] were available for the thione derivative (477).

The thione derivative (483) was first reported in 1976 by Brandt[239] <u>et al</u>. (scheme 188). This method involved a Norrish type II photoelimination reaction of the corresponding 4-acylmethylthio-2-azetidinone (482) on irradiation with UV light. Other reports[240] involved thiosulphinate derivative (488) (scheme 190). On heating (488) in refluxing benzene, a thione derivative (489) resulted. A later report[241] involved a total synthetic
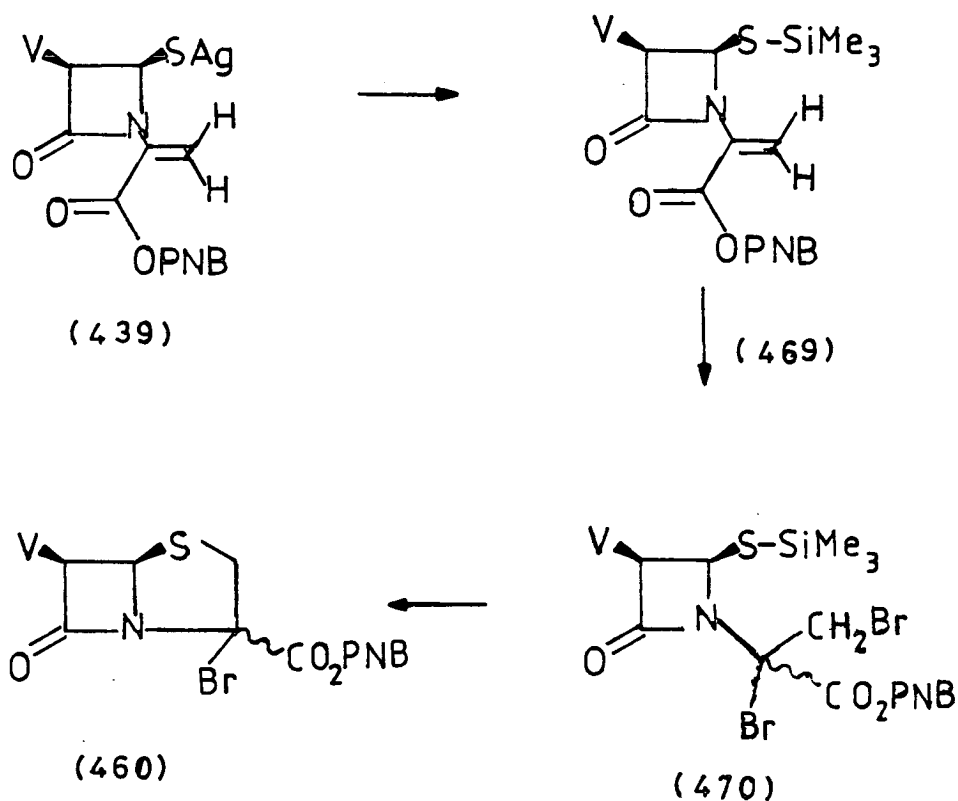
Scheme 185   Use of trimethylsilyl (TMS) group as a
protecting group for sulphur.
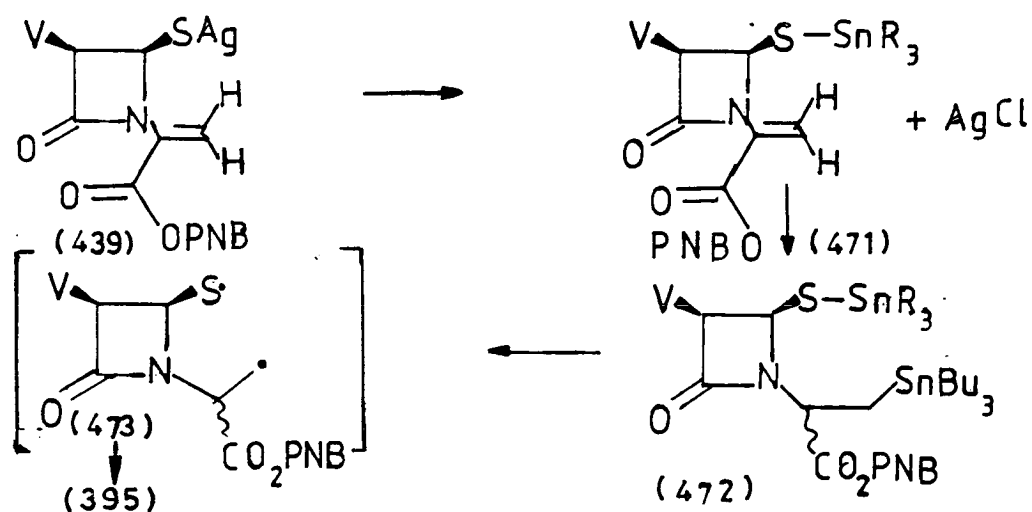
Scheme 186   Use of trimethyltin (TMT) group as a
protecting group for sulphur.

Scheme 187 Use of phenylseleno group as a protecting group for sulphur.
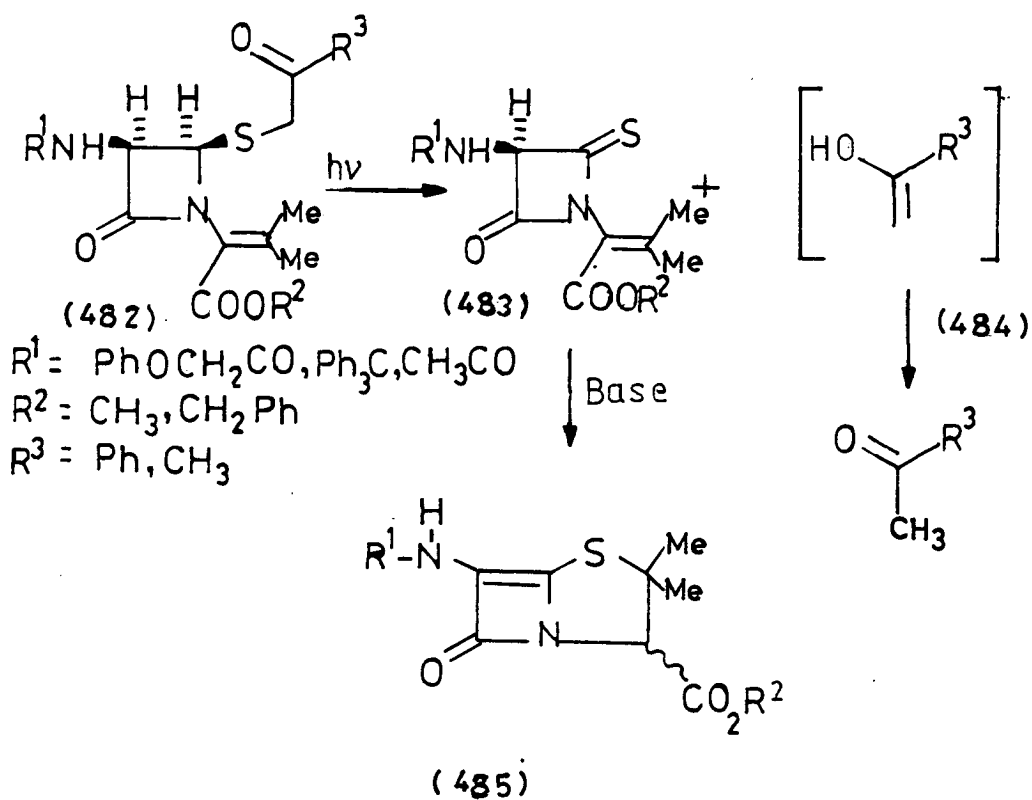
Scheme 188   Norrish type II photoelimination reaction for the generation of a thione.
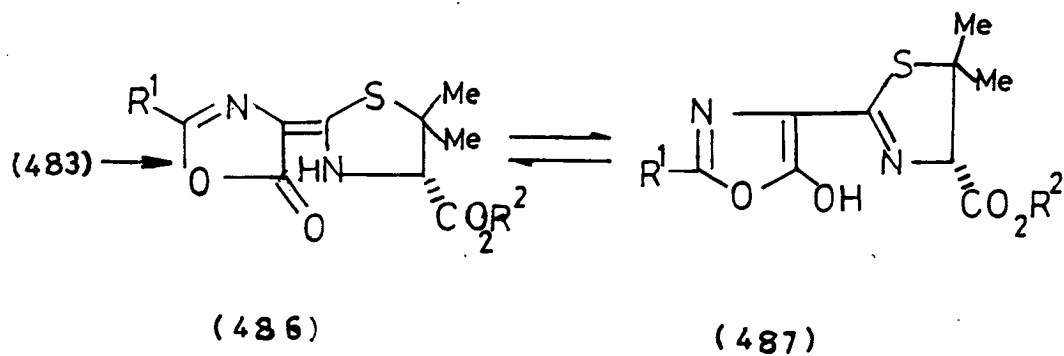
Scheme 189   Base treatment of a thione derivative.

method (scheme 191) involving a cycloaddition of thioketenes (490) with isocyanates (491) to result in an N-unsubstituted 4-thioxo-2-azetidinone (493). A recent report[242] (scheme 192) which illustrated both total synthetic and semi-synthetic approaches to the thione derivative (499), and its properties, has been published. It can be seen that in each of these reports, the N-substituent of the β-lactam, is an isopropenyl moiety. This was partly to confirm the report by Brandt et al.[239] (scheme 188) that base treatment of the thione derivative (483) resulted in a bicyclic system (485). This report[239] was disputed by the authors.[243] A rearrangement of the thione (483) to an oxazolinone derivative (486) (scheme 189) had taken place. The infra-red absorption of the oxazalactone carbonyl stretching frequency could easily be misinterpreted as an intact β-lactam carbonyl stretching frequency. From our experience with the acrylate derivative (414), it was predicted that the thione derivatives (477a) (scheme 187) would not undergo cyclisation, as correctly reported by Bachi and co-workers.[243] This failure was attributed to incorrect orbital alignment and also to the steric factors, due to the methyl groups, as stated earlier. We envisaged that by appropriate functionalisation of the thione derivative (480),it may be possible to cyclise it by a novel route and simultaneously to substitute the C-5 position (481), as illustrated in scheme 187.

Reaction of a slight excess of phenylselenyl chloride with the model silver thiolate (465) in dichloromethane resulted in formation of the phenylseleno derivative (474b) within 10 minutes, as indicated by TLC analysis. The crude product was purified by chromatography on silica using two isocratic eluent systems, firstly, 100% petroleum ether (40-60) to remove diphenyldiselenide (475), and secondly, (1:1) dichloromethane/ethyl acetate to isolate the phenyl-seleno derivative (474b) in high yield (90%). The pure
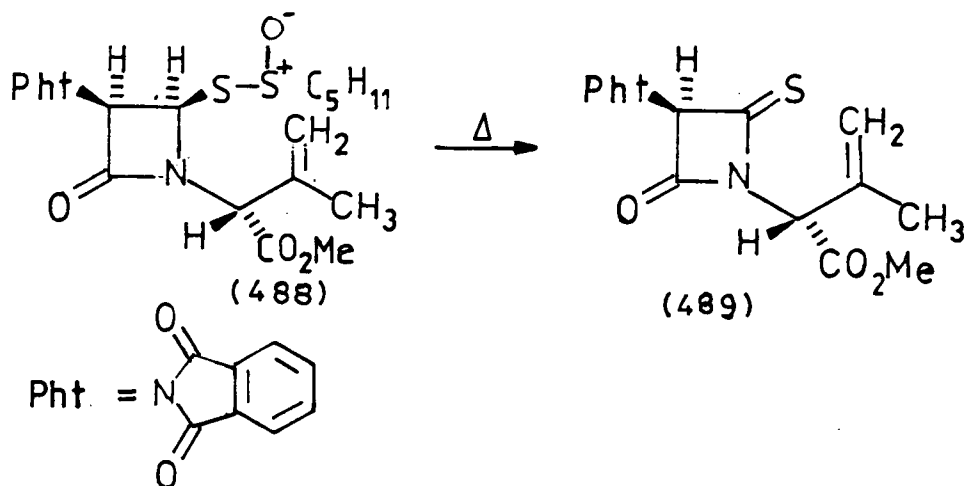
Scheme 190   Generation of a thione from thiosulphinates derived from penicillin sulphoxides.

$R^1 = {}^iPr, {}^tBu$

$R^2 = {}^tBu$

$R^3 = OPh, Ph, 4-Me\,C_6H_4, Me, Cl$
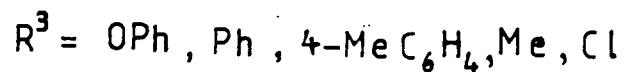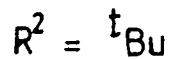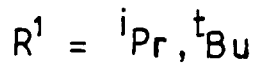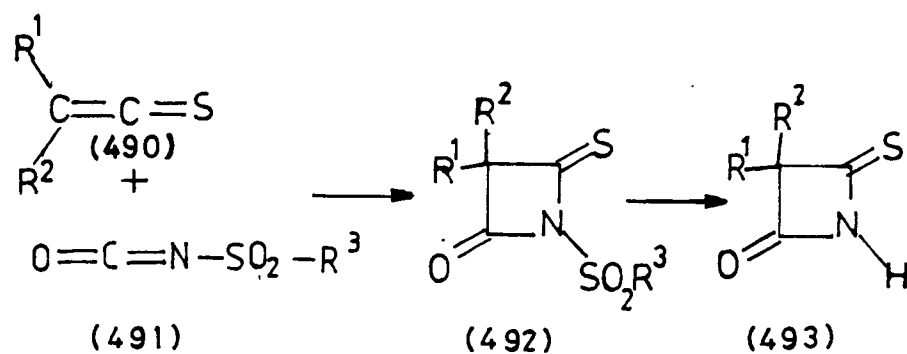
Scheme 191   Generation of a thione by total synthesis.

(494)

R = CH₂CH₂CO₂Me

$R = CH_2CH_2CO_2Me$

Phth =

(495)

(496)

TEA

(497)

[O]

(499)

Δ

(498)
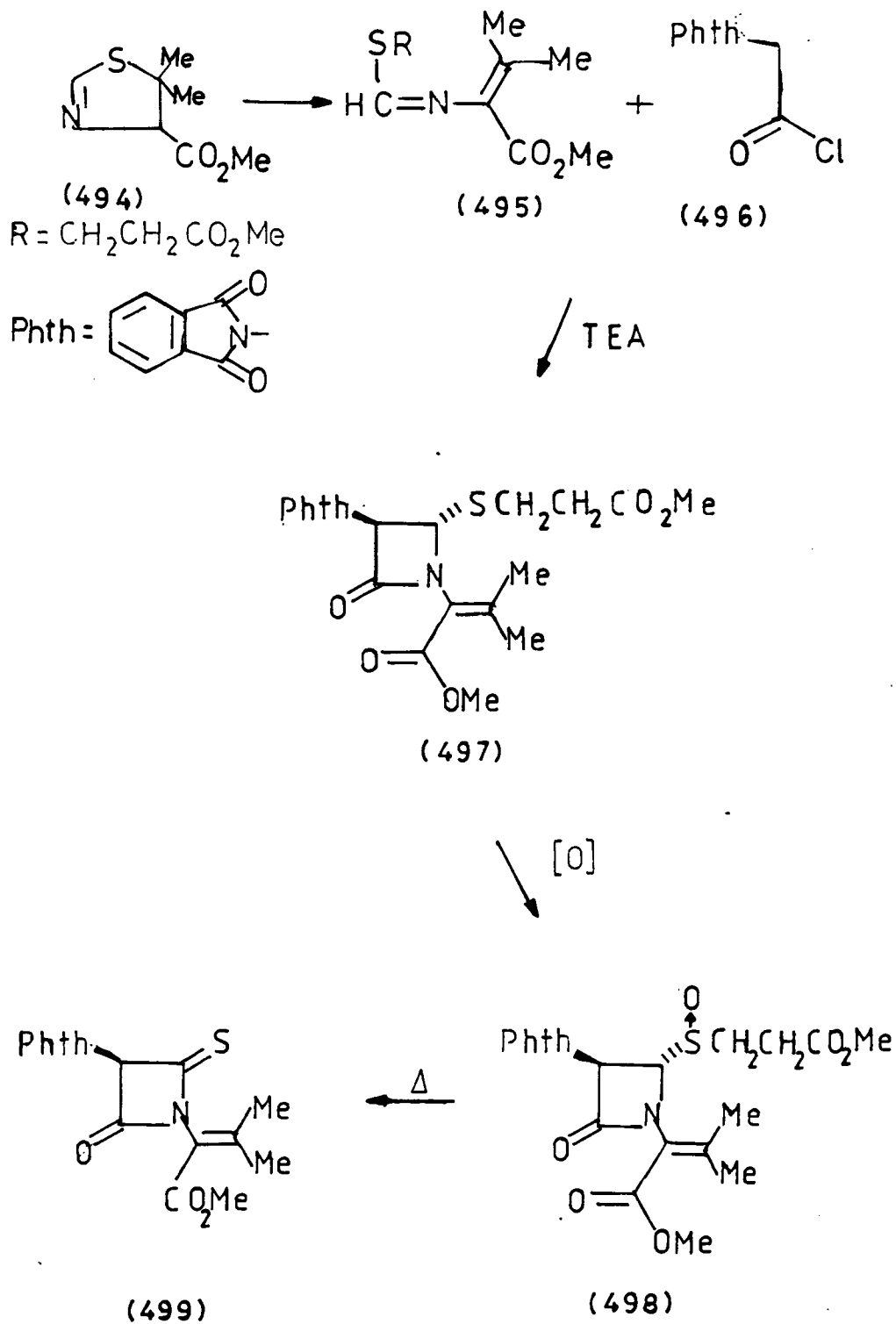
Scheme 192   Generation of a thione by total synthesis,
involving a sulphenic acid intermediate.

product (474b) had a β-lactam carbonyl stretching frequency at 1775 cm^{-1}, the ester at 1725 cm^{-1} and the amide at 1695 cm^{-1} in the infra-red spectrum.   The ^{1}H n.m.r. was unchanged compared to (465),except for the appearance of the 5-proton phenyl signal in the aromatic region.   The elemental analysis was in agreement with the represented structure (474b).   The reaction was repeated using the silver thiolate (439), and after purification by chromatography on silica, the product (474a) was obtained in 92% yield as a yellow foam. Characterisation data were in excellent agreement with the represented structure (474a).

The model phenylseleno derivative (474b) was first used for the oxidative - β-syn-elimination reaction, using metachloroperbenzoic acid (MCPBA) in dichloromethane at 0°C under nitrogen.   After 18 hrs, the reaction was worked—up by evaporating off the solvent and chromatographing the residue on silica.   Only a small amount of the thione derivative (477b) was isolated (14% yield).   However, a high yield (72%) of the starting material (474b) was re-isolated, this was treated with excess MCPBA overnight but no β-lactam containing product was isolated.   The infra-red spectrum of the thione derivative (477b), isolated by chromatography[244] on silica, contained an unusually high β-lactam carbonyl stretching frequency (1830 cm^{-1}), as reported in the literature.[245]   The sample subjected to ^{1}H n.m.r. analysis was impure, but the doublet J=10Hz at δ= 5.0 ppm due to C-3 proton of the azetidinone was evident and the structure (477b) (scheme 187) was indicated.   In addition, the remaining signals were in agreement with the reported characterisation data for the thione derivative (477b).   It was concluded that the strategy was viable and the reaction was thus attempted with the phenylseleno derivative (474a).   The low yield of the thione derivative (477b) prompted the need to modify the work-up procedure.   This involved a sodium bicarbonate wash to remove the m-chlorobenzoic acid and benzene

selenenic acid by-products, and carrying out the
reaction in dichloromethane and monitoring the reaction
by infra-red analysis, for the appearance of the new peak
at 1830 cm^{-1}, resulted in a very weak peak in the region
of interest. This peak did not increase in intensity
with addition of a further 1.1 equivalents metachloroper-
benzoic acid, the reaction appearing to have stopped.
The β-lactam remained intact (broad peak 1770 cm^{-1},246
plus a weak peak at 1830 cm^{-1}). Purification of the
sample, on preparative TLC, (silica as stationary phase),
failed to produce the thione derivative (477a). The
starting material (474a) was isolated in 29% yield, and
exhibited a β-lactam carbonyl stretching frequency (1775
cm^{-1}) in the infra-red spectrum and was obtained in
sufficient quantity to be identified as (474a).

Reports[239-242] by various workers have indicated
the unstable nature of thione derivatives, for example
(483) (scheme 188), to silica and acidic conditions. The
by-products produced in our approach (scheme 187)
necessitated the need for chromatography on silica for
purification. It was found that the model thione
derivative (477b) could be isolated after chromatography,
although low yields were obtained. Consequently, it was
believed that the literature reports had over-emphasised
the instability of the thiones to silica. However, it
seems that our thione derivative (477a) was much more
·sensitive to silica, and possibly to the reaction
conditions. It is, thus, necessary to use a different
oxidant in non-acidic conditions, perhaps with the aid of
a buffer system, and a method that circumvents the
silica-gel chromatography for purification. Work on
scheme 187 was abandoned.

1.5.2 Synthesis of Sulphur-tin derivatives and their uses

A recent report by Baldwin[238] illustrated a biomimetic synthesis of penicillin-V (scheme 193). This involved the unstable thiol (501) which was treated with Udenfriends reagent[247] [iron(ii) sulphate, asorbic acid and ethylenediamine tetra-acetic acid] at pH 4.4 and shaken in the presence of oxygen. Acidification to pH 3 resulted in the appearance of a bioactive material in the dichloromethane extract. This active compound had identical characterisation data to penicillin V (2). From a detailed analysis, using a dideuteriated species (502), the authors had concluded that their biomimetic route lacked the stereospecificity and regiospecificity of the enzyme catalysed process, and that their work supported a free-radical intermediate for the penicillin biosynthesis.

This report then provided confidence for our approach (scheme 186) which would have complemented and supported the published work. It was decided to functionalise the acrylate derivative (414) to a structure of the type (504) as represented in scheme (194). This involved a radical addition of tributyltinhydride across the double bond. Typical conditions in the literature[248] involved refluxing the unsaturated system and tributyltinhydride in benzene with a radical initiator such as α, α' azabisisobutyro-nitrile for 1-2 hours and isolating the organotin compound by chromatography. Carrying out the reaction, as reported, resulted in an unexpected product. Together with some starting material (414), an additional component was isolated, which after a further preparative chromatography, was concluded to have structure (505) (scheme 195), as indicated by ^{1}H n.m.r. and infra-red analysis. A diastereomeric mixture had resulted which was difficult to separate and this made the ^{1}H n.m.r. difficult to assign unambiguously. However, the
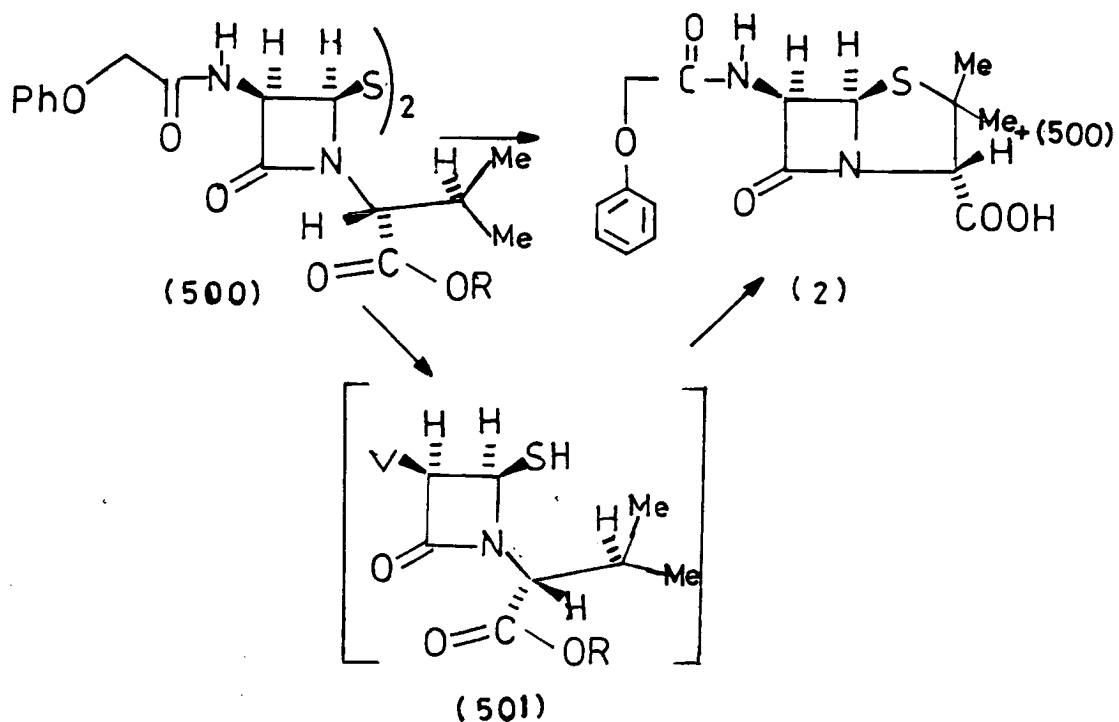
Stereospecificity of the cyclisation

(502)   (503)
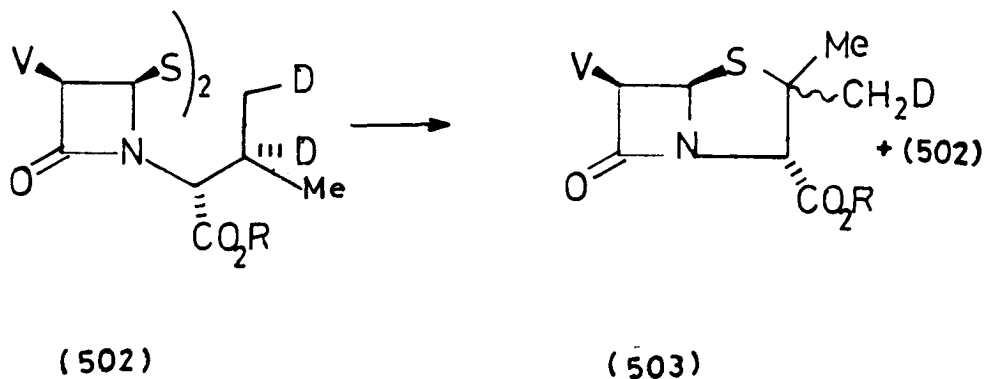
Scheme 193   Biomimetic synthesis of penicillin.

(414)   (504)

a=Bu₃SnH/AIBN/Δ
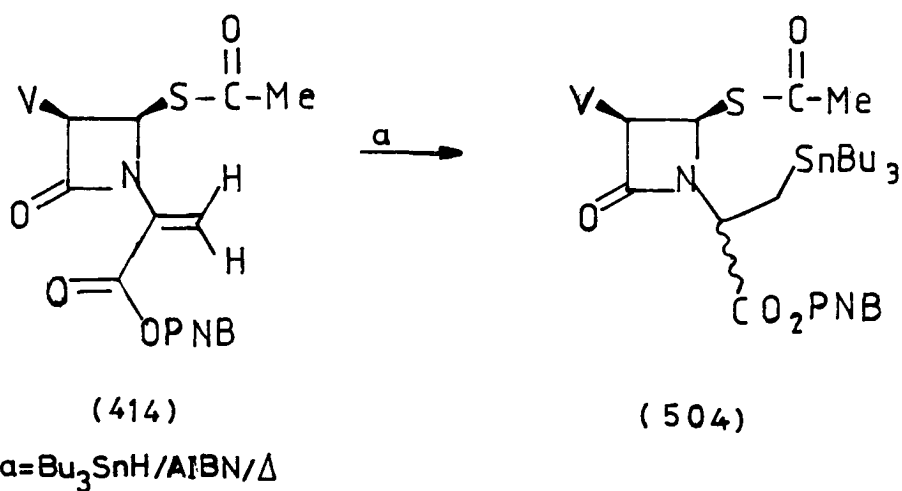
Scheme 194   Functionalisation of the acrylate.

(505)

Scheme 195

(506)   (507)   (460)
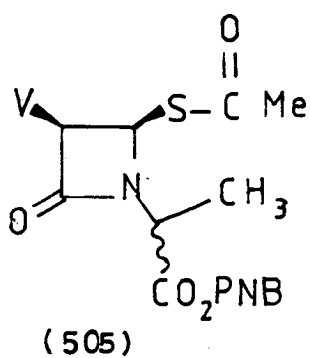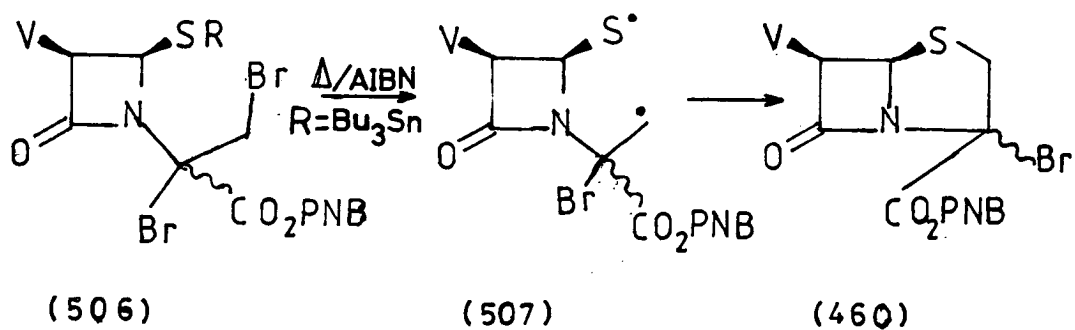
Scheme 196

acrylate protons at δ = 6.1 ppm were absent and a new peak had developed at δ = 1.6 ppm (this existed as a doublet, J = 7.5 Hz, (220 MHz [1]H n.m.r.)).   The acrylate double bond had been reduced and no tin-addition product   was detected.   This disappointing result indicated that radical addition to the acrylate would not be very successful.   Use of a 1,2-dibromo acrylate derivative (506) (scheme 196) may be more successful in generating the bi-radical intermediate (507) for a successful biomimetic synthesis of the bisnorpenam (460).

The biomimetic approach was still feasible, hence the synthesis of the trialkylstannylthio derivative (471) was attempted (scheme 186).   Literature reports[249] indicated that these types of derivatives are quite stable.   A typical synthesis was as shown in scheme 197.   The use of sodium hydroxide would have been too vigorous for our purpose, hence, the following method was adopted using the model silver thiolate (465) (scheme 198).

The reaction was carried out on a 100 mg scale in dichloromethane under nitrogen, but no reaction resulted.   Changing the solvent to tetrahydrofuran had no effect on the result, and after consideration it was concluded that the tributyltin group may have been too large, and was too sterically hindered to react with the silver thiolate (465).   Repeating the reaction with trimethyltin chloride in dichloromethane under nitrogen, resulted in the absence of any starting material   (TLC), within 10 minutes from the start of the reaction.

The β-lactam remained intact as seen in the infra-red spectrum (1765 cm^{-1}) and the crude product was chromatographed on silica with two isocratic eluent systems

      a)   50% dichloromethane/ethyl acetate

      b)   100% ethyl acetate.

$$Et_3SnCl \ + \ PhSH \ \xrightarrow{NaOH} \ Et_3Sn-SPh$$

(508)   (509)   (510)
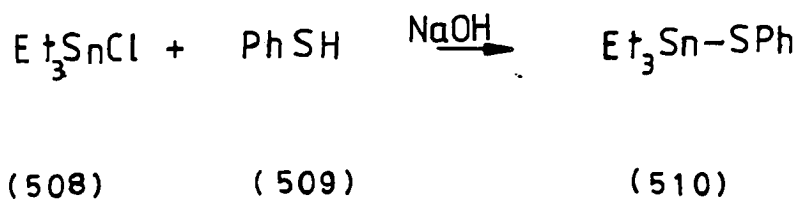
Scheme 197   Synthesis of alkylstannyl thio derivative.

(465)   (511)
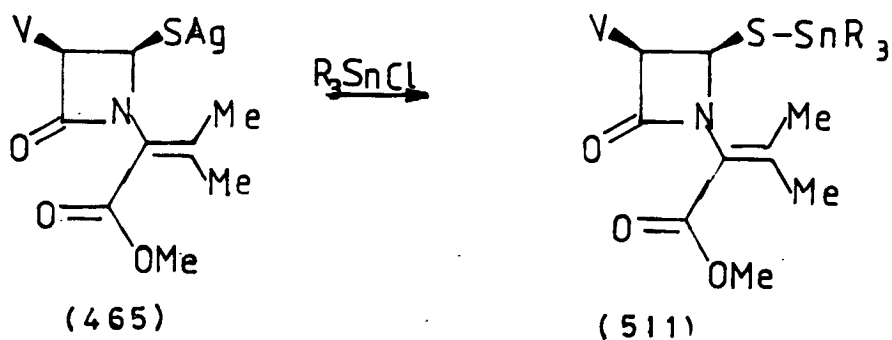
Scheme 198

The expected β-lactam containing component was isolated from the 100% ethyl acetate fractions as a white solid. Infra-red analysis indicated a new peak at 3290 cm^{-1}) (broad), and the amide carbonyl stretch was at a higher frequency (1700 cm^{-1}) than expected. The β-lactam carbonyl stretch appeared at 1765 cm^{-1} and the conjugated ester carbonyl stretch remained at 1720 cm^{-1}. One further point was that the carbon-carbon double bond appeared to have a stronger absorption band at 1655 cm^{-1}. The ^{1}H n.m.r. of the white solid in deuteriochloroform showed a broad signal at δ = 8.3 ppm (N-H), a complex set of multiplets δ = 7.3-6.8 ppm (aromatic protons), a set of broad peaks at δ = 5.4-5.1 ppm (β- lactam protons) and the AB system of the phenoxymethylene protons at δ = 4.6-4.1 ppm. The remaining peaks, due to the methyl group (δ = 3.7 ppm) and the isopropenyl group (δ= 2.1 ppm and δ= 2.0 ppm) were also seen, but the important peak due to the trimethyltin protons at δ= 0.7 ppm was too weak to be attributable to the structure (511) (scheme 198) and may have simply been hexamethyldistannane (512). The data suggested that the starting material (465) was recovered in addition to a small amount of a by-product (513) which may have resulted from a rearrangement (scheme 199).

The TLC (EtOAc) of the β-lactam containing fraction indicated the presence of 3 spots. A minor high running spot (Rf = 0.65) was attributed to the trimethyl-tin dimer (512) and the major spot (Rf = 0.45) was possibly (513). Literature data for (513) reported the I.R. spectrum as follows:- 1720, 1690, 1655 cm^{-1}. Many of these peaks would have overlapped the peaks in the silver thiolate infra-red spectrum, except for the peak at 1655 cm^{-1}. The base-line spot was the starting material (465). The recovery of the starting material (465) suggested that incomplete reaction had taken place. However, a TLC (50% EtOAc in DCM) of the reaction mixture, after 10 minutes from start of the reaction indicated that no starting material (465) was

(511)

(514)

Me₃Sn-O-SnMe₃

(512)

(513)
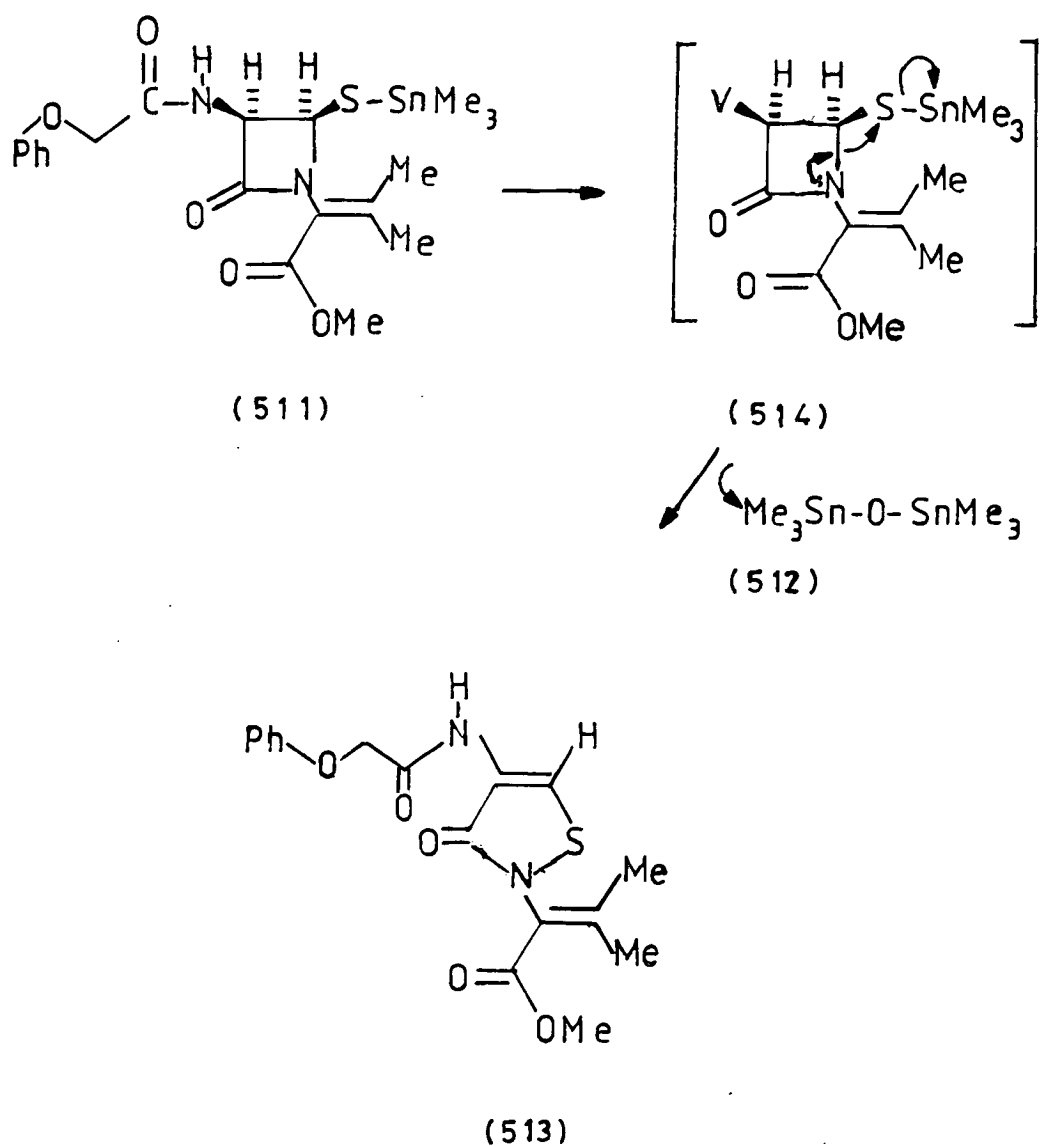
Scheme 199   Rearrangement of sulphur-tin derivative
(511).

present. In retrospect, it would seem that a complex involving the silver thiolate and the trimethyltin chloride, may have been formed which decomposed either to the starting materials, or to the products, when placed in contact with silica.

At the time of the experiment, it was concluded that the sulphur-tin bond was cleaved during chromatography on silica. Consequently, it was decided that purification of the trialkyltin derivative (511) would not be possible by chromatography, and therefore purification of derivatives of this type would have to be carried out by crystallisation in future syntheses. The above reaction was repeated with the reaction time increased to 15 hrs, using the acrylo-silver thiolate derivative (439). The resulting precipitate was filtered over anhydrous sodium sulphate and washed with dichloromethane. Concentration of the filtrate under reduced pressure resulted in the recovery of the starting material (439) (63mg, 37%), as indicated by T L C , I.R. and ^{1}H n.m.r. analysis. Further washings of the precipitate with acetone resulted, on evaporating the solvent, in a white solid (mpt 104°C), which contained a β-lactam carbonyl stretch (1765 cm^{-1} (broad))[250]. The ^{1}H n.m.r. in deuterioacetone resulted in extremely broad peaks but a peak due to the trimethyltin protons was seen at δ= 1.2 ppm. However, the integral did appear quite low. The work was halted at this stage. Further work is desirable in this area (scheme 180).

1.5.3 Synthesis of Sulphur-Silicon derivatives and their use

Work had also been carried out as regards to scheme 185. Initially, the tert-butyldimethylsilyl chloride[251] was used to produce the thio-silane derivative (515) (scheme 200). However, after

chromatography, no product was isolated which contained a β-lactam intact product. The experiment yielded the following conclusions:-

a)   The thio-silane derivative (515) was unstable to hydrolytic conditions.

b)   Purification was not possible by chromatography, hence, it may be necessary to use the product as a crude sample.

Literature reports[252] have indicated the use of thio-silane derivatives (517) (scheme 201), for synthesis of thioesters (518) from carboxylic esters (516) in high yields and regiospecificaly, even in the presence of α, β unsaturated systems. Typical conditions[253] involved reacting a magnesium thiolate (519) with trimethylsilyl chloride (520) in anhydrous ether (scheme 202). Our reaction involved the use of a silver thiolate (439) and consequently, by analogy it was decided to carry out the reaction described above with a few modifications. The silver thiolate (439) was insoluble in ether, hence, dry dichloromethane was the solvent of choice. Addition of the reagents was carried out at 0°C, excess trimethylsilyl chloride was used and the reaction mixture was then allowed to warm up to room temperature and stirred overnight. The white precipitate was filtered off through celite and the filtrate evaporated _in vacuo_. The model silver thiolate (465) afforded a white powder on evaporating the solvent. Infra-red analysis of the crude material indicated the presence of a β-lactam carbonyl stretch (1765 cm^{-1}), in addition to one new peak at 3300 cm^{-1} and the sharp peak at 3410 cm^{-1} (N-H). Furthermore, the ester carbonyl stretch remained at 1720 cm^{-1} and the amide carbonyl stretch appeared at 1685 cm^{-1} plus a further peak at 1670 cm^{-1}. The ^{1}H n.m.r. of the crude product was confusing, but no signal due to trimethyl silyl protons was seen. It was concluded that the thio-

(439) (515)

Scheme 200

(516) (517) (518)
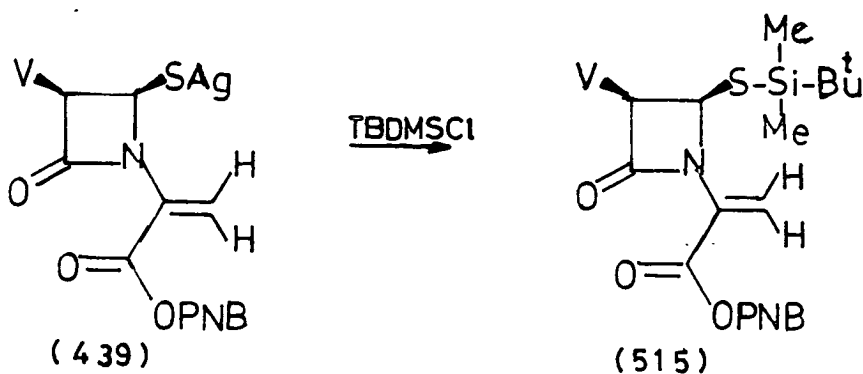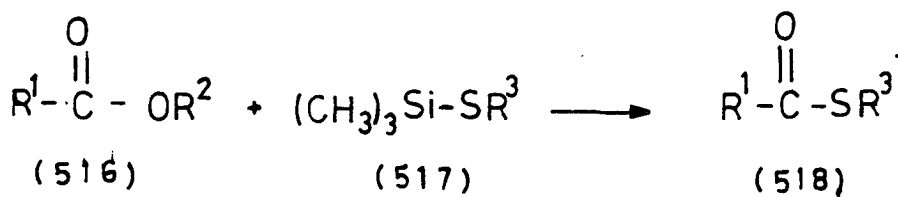
$R^1 = Ph-, \ o\text{-}OMe\text{-}Ph-, \ Ph\text{-}CH=CH-, \ PhCH_2-$

$R^2 = -CH_2CH_3$

$R^3 = -CH_2CH_3 , -Ph$

Scheme 201   Use of thio-silane derivatives.

MeSMgI + Me$_3$SiCl $\xrightarrow{Et_2O}$ MeS-SiMe$_3$ + MgICl

(519)   (520)   (521)   (522)
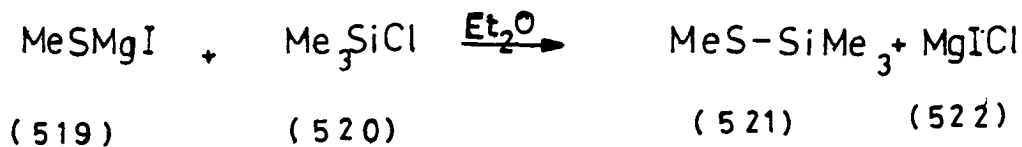
Scheme 202   Typical conditions for synthesis of thio-
silane derivatives.

silane derivative (523) (scheme 203) may have been too unstable and might have rearranged, similarly to the thio-stannane derivative (511) (scheme 199), to (513), the hexamethyldisilane (524) being readily lost on evaporating the solvent <u>in vacuo</u>. Further work with the model silver thiolate (465) was abandoned and the above reaction was repeated with the acrylo silver thiolate (439). The resulting crude product was found to be a yellow gum. ^{1}H n.m.r. of the crude material indicated broad peaks (possibly due to some paramagnetic material being present). However, the TMS peak was sharp but the integral showed that this was smaller than expected. As the peaks were not well resolved it was not possible to draw any conclusion. The infra-red spectrum indicated the presence of the N-H stretch (3400 cm^{-1}), the β-lactam carbonyl stretch (1775 cm^{-1}), the ester carbonyl stretch (1730 cm^{-1}) and the amide carbonyl stretch (1695 cm^{-1}). Work with this derivative was halted at this stage due to insufficient time.

1.6.0 <u>Penicillin to cephalosporin conversion</u>

We had also undertaken work as regards to a novel conversion of a penicillin to a cephalosporin nucleus as outlined in scheme 206. Earlier reports make use of the classical penicillin sulphoxide to cephalosporin rearrangement first reported by Morin in 1963.[61] Other reports make use of activated secopenicillins (525) (scheme 204) which ring close by a 6-exo-tet type of cyclisation. More recently, intramolecular Wittig reactions (scheme 205)[254] have readily been used to make highly sensitive cephem nuclei. Many other methods have been reported and an excellent review has appeared.[255]

Our proposed method (scheme 206) involved reacting a very reactive ketone (529), (i.e. readily hydrates if
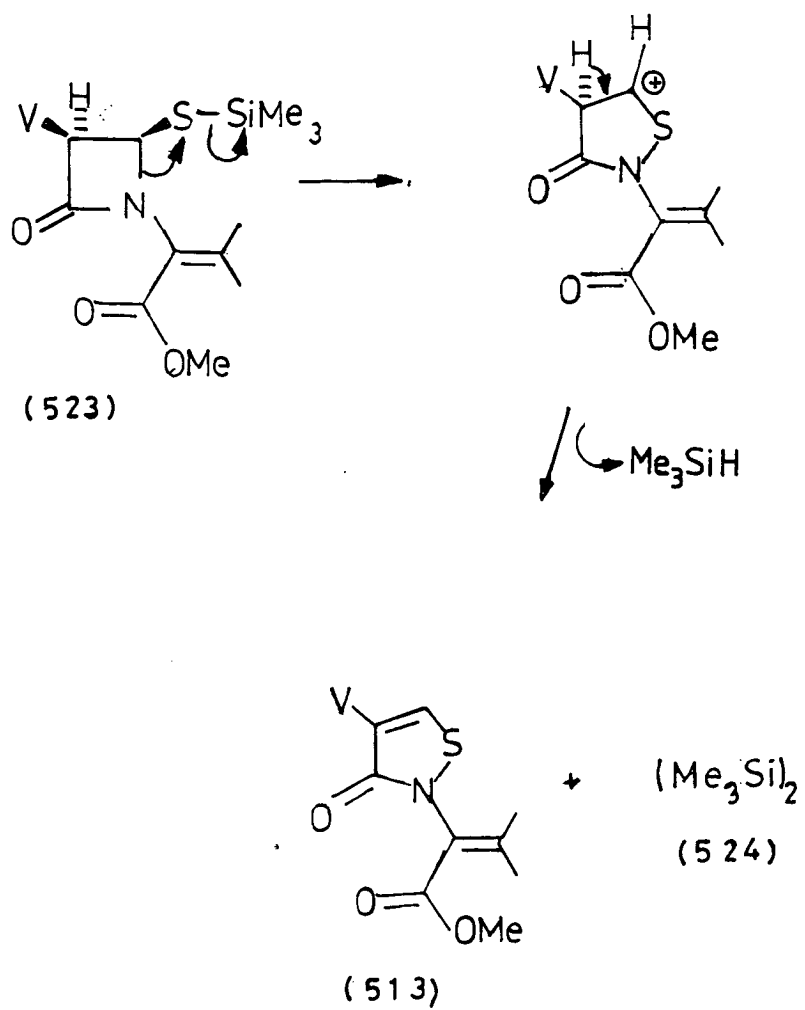
Scheme 203   Rearrangement of thio-silane derivative.

exposed to moisture), with the phosphorane⁻(413) (scheme 153). Activation of the methine position using N-bromo-succinimide might result in the bromo derivative (531). Cleavage of the thioester (531), using the method described earlier, would result in the silver thiolate (532), which may be induced to cyclise to the cephalosporin (533) with silver bromide precipitation as the driving force of the reaction.

The ketone (529) was obtained by an excellent method described by Raphael,[256] who showed that the ketone was stable only as the hydrate (534) (scheme 207) and required distillation to remove water prior to use. The reaction between (413) and (529) was carried out in dichloromethane, under nitrogen. However, it was found that no reaction took place at room temperature or in refluxing dichloromethane. Both starting materials were recovered by chromatography in very good yields and were identical to the authentic samples by infra-red and ¹H n.m.r. analysis. Changing the solvent to toluene resulted in no reaction at room temperature. Gradual increase in temperature to 90°C over 4 hrs resulted in some reaction taking place as indicated by the development of a new spot in the TLC plate. The reaction was continued for 16 hrs, worked up and chromatographed on silica. Some starting material was isolated, in addition to a further component, which was attributed

structure (535), which was probably formed by an intramolecular Wittig reaction followed by fragmentation of the penem system (536) (scheme 209) under acidic conditions.[257] No trace of any intermolecular Wittig product was isolated, hence showing that no reaction had taken place between the phosphorane (413) and the 5-oxo-2-phenyl-1,3-dioxane (529). Thus, the intramolecular Wittig reaction was favoured compared to the intermolecular Wittig reaction. Consideration of the problems[258] led to the conclusion that this approach (scheme 206) should be abandoned. Changing the thiol protecting group to one containing no carbonyl group may

212
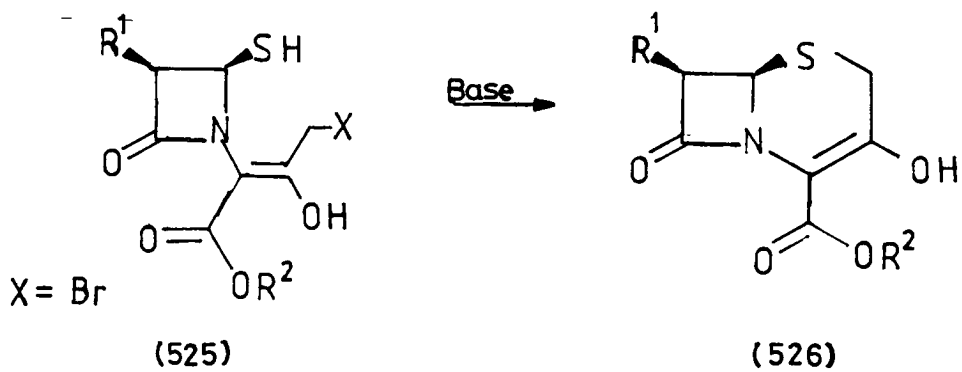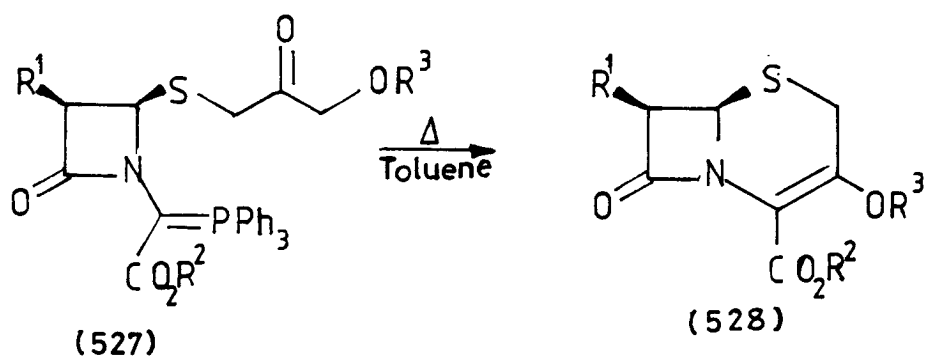
Scheme 204

Scheme 205
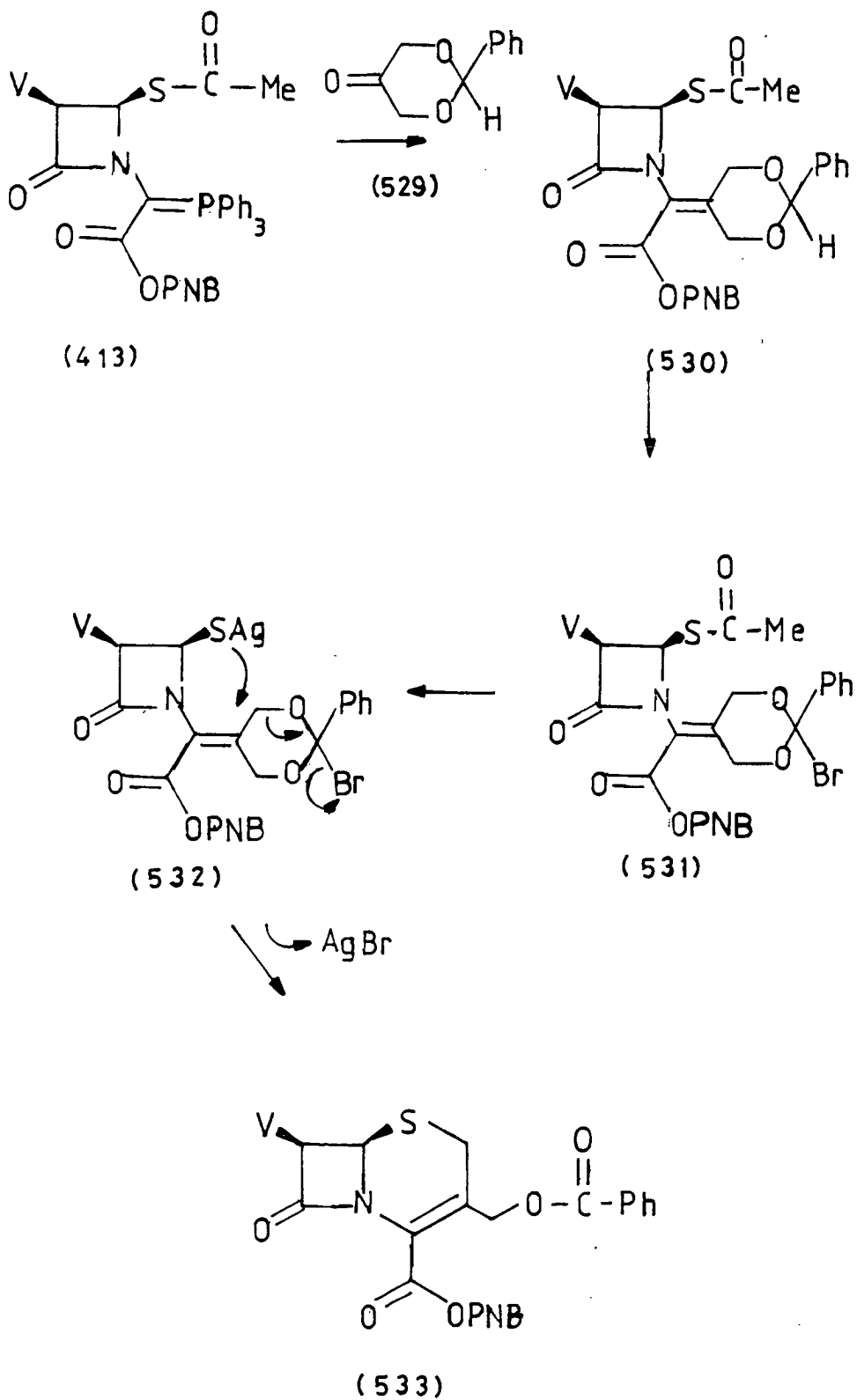
Scheme 206   Novel penicillin to cephalosporin
               conversion.

have allowed more forcing conditions to be used and, thus, possibly lead to a successful conclusion.

1.6.1 A modified penicillin to cephalosporin conversion involving a carbanion in a 6-endo-trig cyclisation

A recent report was found which used α-bromo-nitromethane for the synthesis of thio-ethers. This reagent suggested an alternative approach to the synthesis of a cephem nucleus (scheme 210). Alkylation of silver thiolates of the type (465) with alkyl halides are well known.[259] It was hoped that the nitro group would promote a nucleophilic displacement of the bromide and precipitation of silver bromide would drive the reaction to completion. The methylene protons α to the nitro group would be sufficiently acidic in the sulphoxide derivative (541) to be removed using a mild base, such as triethylamine, and the carbanion (542) generated might undergo an intramolecular Michael addition to result in the cepham derivative (543). Reduction of the sulphoxide (543) to the sulphide (544) could readily be accomplished using a trihalophosphine in dimethylform-amide. Substitution of the nitro group by a phenylseleno group affords the derivative (546) which could give rise to a double bond in the Δ^2 position (547) via oxidative elimination. This would readily be converted to the Δ^3 structure (548). The reaction between (439) and (539) was carried out in dichlormethane at 0°C, room temperature and refluxing dichloromethane. No reaction occurred and only the starting material (439) was isolated after chromatography. A survey of the literature[260] on the reactions of α-bromo nitromethane indicated that this reagent promoted radical reactions, thiolates normally affording disulphides. This approach was abandoned.

(534) → (529)

Scheme 207

(535)

Scheme 208

(536) → (537) + (538)

Scheme 209

Scheme 210

$$R-\overset{O}{\overset{\|}{C}}-O-CH_2 \overset{\frown}{\bigcirc} CH-CH_2-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-Resin$$

Me – Si – Me
|
Me $F^{\ominus}$

Handle (549)

↓ TBAF

$$R-\overset{O}{\overset{\|}{C}}-O^{\ominus} \overset{n}{\underset{}{N}}Bu_4^{\oplus} + CH_2= \bigcirc =CH-CH_2-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-Resin$$

(550)　　　　(551)

$$CH_3-\bigcirc-CH=CH-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-Resin$$

(552)

$$R-\overset{O}{\overset{\|}{C}}-O^{\ominus}\overset{n}{N}Bu_4^{\oplus}$$

(553)

$$R-\overset{O}{\overset{\|}{C}}-OH$$

(554)
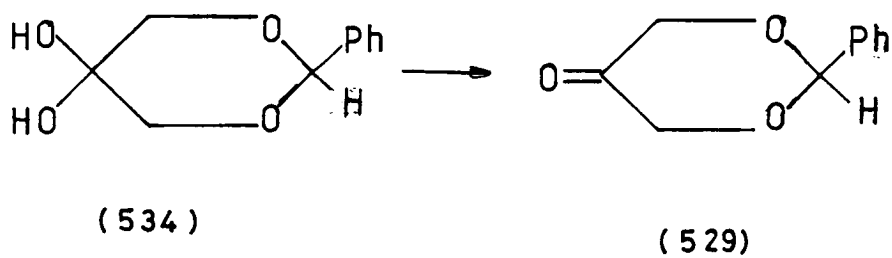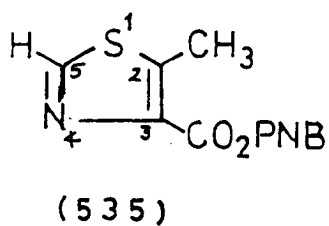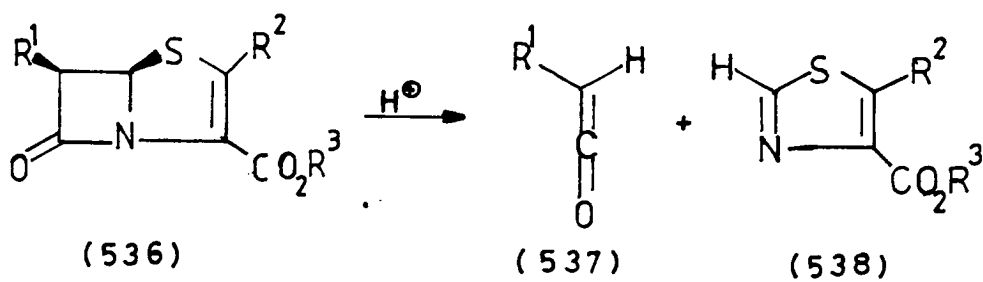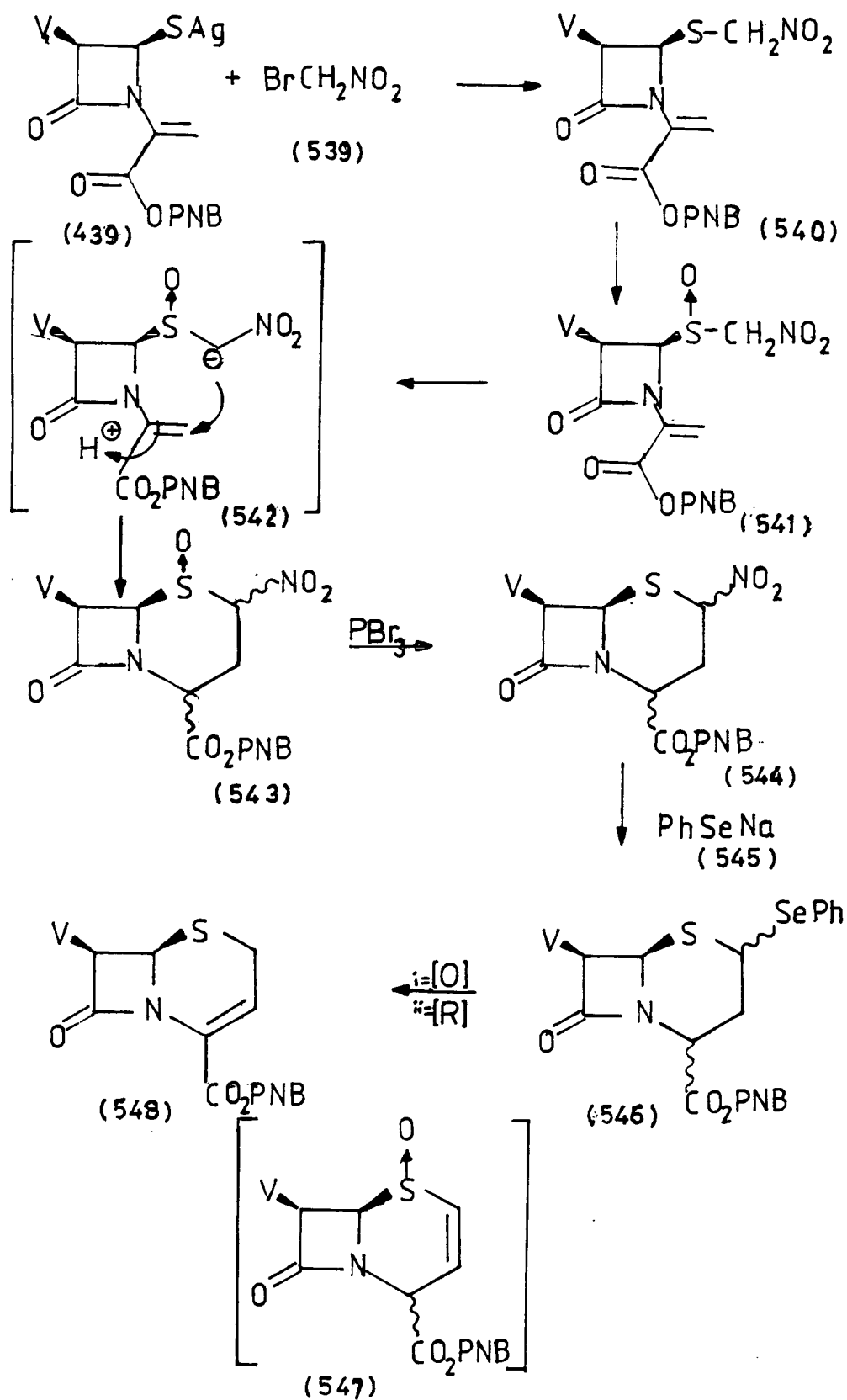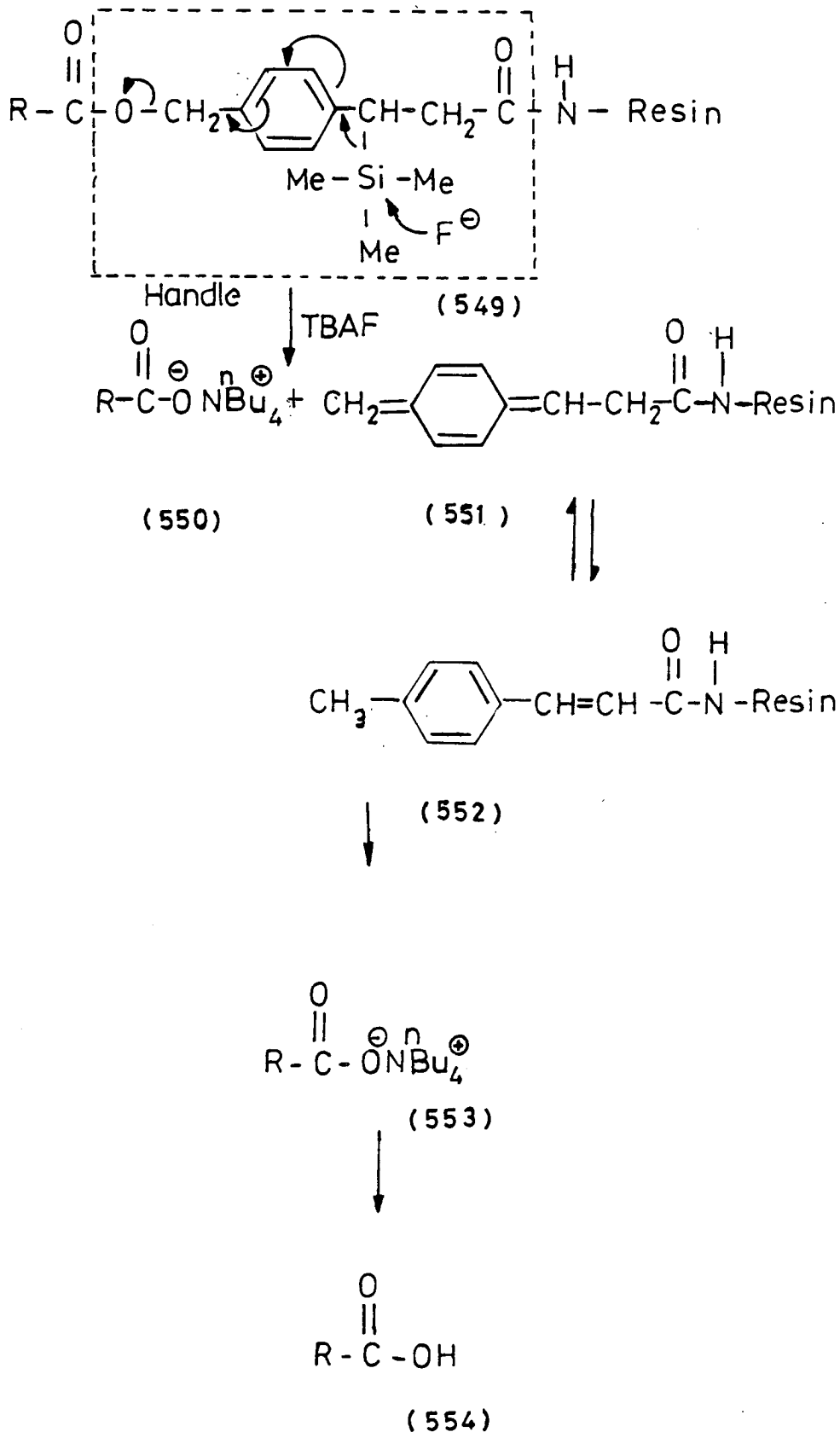
Scheme 211　Use of a novel handle in solid-phase peptide synthesis.

1.7.0. Novel strategy for protection and deprotection of esters of β-lactam derivatives

Work was commenced on new strategies for deprotection of the ester group of β-lactam compounds. This involved the use of solid-phase methodology.[261] In the course of recent work carried out in these laboratories, a novel "handle" (549) was developed which could be used to remove the peptide from the solid support by use of fluoride ions (scheme 211).

In the β-lactam field, the common protecting ester groups for the carboxylate are as follows:-

a) 2,2,2-trichloroethyl (555) (TCE) (scheme 212). This is readily removed by zinc and acetic acid. Yields are moderate (60%), and the group can only be used for acid stable derivatives.

b) p-nitrobenzyl (556) (PNB) (scheme 212). This is readily removed by hydrogenation over palladium on charcoal in a variety of solvents. Poisoning of the catalyst due to the presence of sulphur in the β-lactam derivative makes the process expensive.

c) benzhydryl (557) Bzh) (scheme 212). This is commonly used for cephalosporin derivatives due to their greater acid stability. Deprotection involves the use of trifluoro acetic acid (TFA) which results in serious losses due to β-lactam cleavage and, hence, low yields.

It was envisaged that our strategy, (scheme 213), would readily afford the ester (560) on the solid support by use of a suitable method (acyl halide, mixed anhydride or DCCI coupling) and after the transformation of the β-lactam derivative (R^1) had been completed the ester would be deprotected using anhydrous tetrabutylammonium fluoride. The tetrabutylammonium salt (562) could readily be washed off the support and the solution passed through an ion-exchange column (pH 3, carboxylic

acid-type) which should afford the required acid (563) in high yield.   The advantages for the use of solid phase are as follows:-

a)   The method of deprotection of the ester is very mild, due to the use of fluoride ion.

b)   No expensive catalysts are required.

c)   Yields are expected to be near quantitative.

d)   Very pure samples can be obtained, which can be used directly for biological testing.

However, there are disadvantages, as follows:-

a)   Only small quantities of sample can be obtained (depending on weight of support used, size of reaction vessel and efficiency of protecting/deprotecting steps).   In peptide synthesis, the small amount of material obtained from solid-phase synthesis, usually has high activity and the effect is identified quite readily.   However, for β-lactam antibiotics, reasonable quantities are needed in order to check the activity against the many gram-negative and gram-positive bacteria.

b)   Esterification must be quantitative.   Prior to carrying out the reactions on the support,it was necessary to determine whether the strategy worked in solution (scheme 214).

The synthesis of the handle (565) is shown in scheme 215,[262] and the crude material was chromatographed on silica to afford the desired product in yields of 50-60% (unoptimised).   Characterisation data were in excellent agreement with the assigned structure (565) (scheme 215).   Small scale reaction of the potassium salt of penicillin-V (564a) with the handle (565) in dimethylformamide (DMF) at room temperature was unsuccessful after 19 hrs.   The reaction conditions were then modified by addition of one equivalent of silver nitrate.   After stirring the reaction mixture for a further hour,the TLC indicated that no reaction had taken place.   On checking the reaction mixture,it was noticed
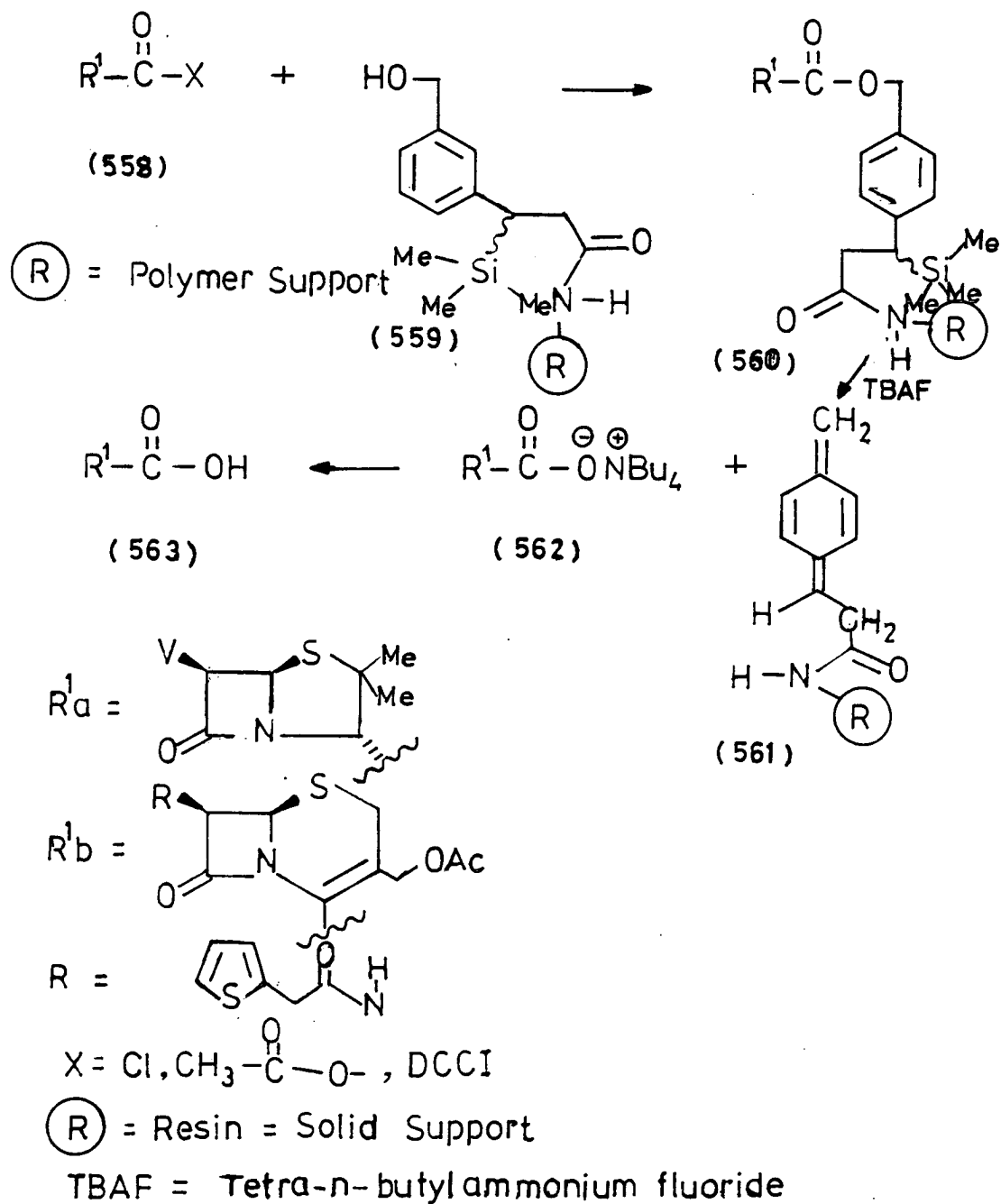
Scheme 213   Application of solid phase synthesis to
β-lactam derivatives.
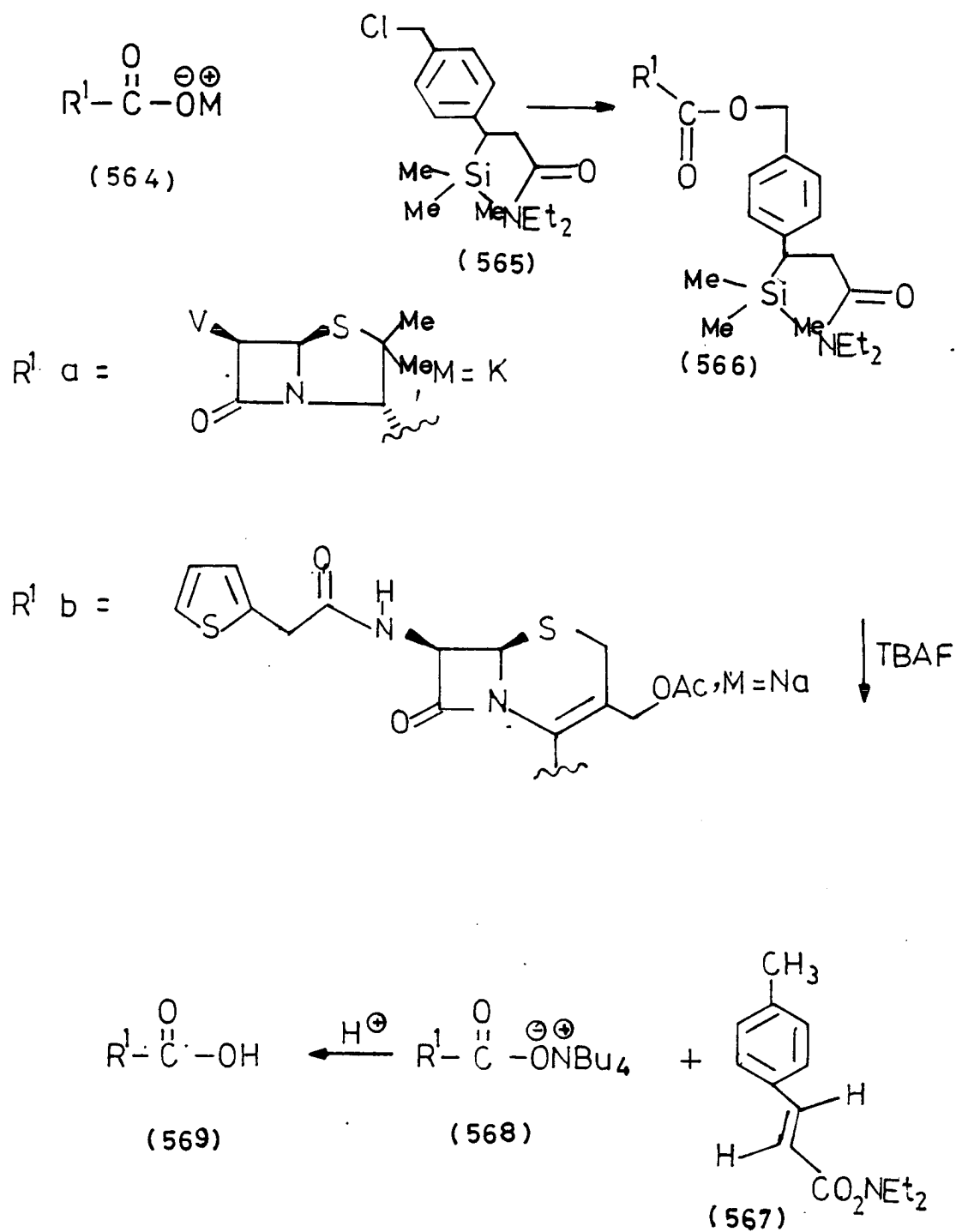
Scheme 214 _ Use of solid-phase methodology.

that the silver nitrate had not dissolved. However, addition of a little water resulted in a homogeneous solution. After stirring for 0.5 hr, the TLC indicated some reaction was taking place. The reaction was continued for a further hour and then worked-up. Chromatography on silica of the crude sample resulted in the isolation of some starting material (the handle (565)) and also a further β-lactam containing component, which required a further chromatography for purification. The ^{1}H n.m.r. of the sample confirmed that the required product (566a) had been obtained. A new peak was observed at $\delta = 5.2$ ppm which was a singlet and had an integral corresponding to two protons. In addition, the signal due to the chloromethyl group ($\delta =$ 4.7 ppm) had disappeared. Furthermore, the signal due to the trimethylsilyl (TMS) group was in the correct ratio to that of the phenoxymethylene protons ($\delta = 4.5$ ppm) of the penicillin-V. The yield, however, was only 8%. It was concluded at the time that insufficient silver nitrate was added,[263] and the reaction time should be increased.

Repeating the aqueous reaction conditions on a larger scale and with longer reaction times, for both potassium penicillin V (564a) and sodium cephalothin (564b), resulted in either very low yields or no product, respectively.

The reaction was then modified to employ the following conditions (scheme 216):- potassium iodide was added to the dimethylformamide solution of the reagents and the reaction mixture stirred and monitored by TLC analysis until no starting materials remained. This method improved the yields and made the strategy feasible. It was found that potassium penicillin V (564a) required longer reaction times (22 hrs - 24 hrs) compared to the sodium cephalothin (564b) (8 hrs). In general, higher yields were obtained with the cephalosporin derivative (60%) compared to the penicillin derivative (30%). The yields were not optimised.
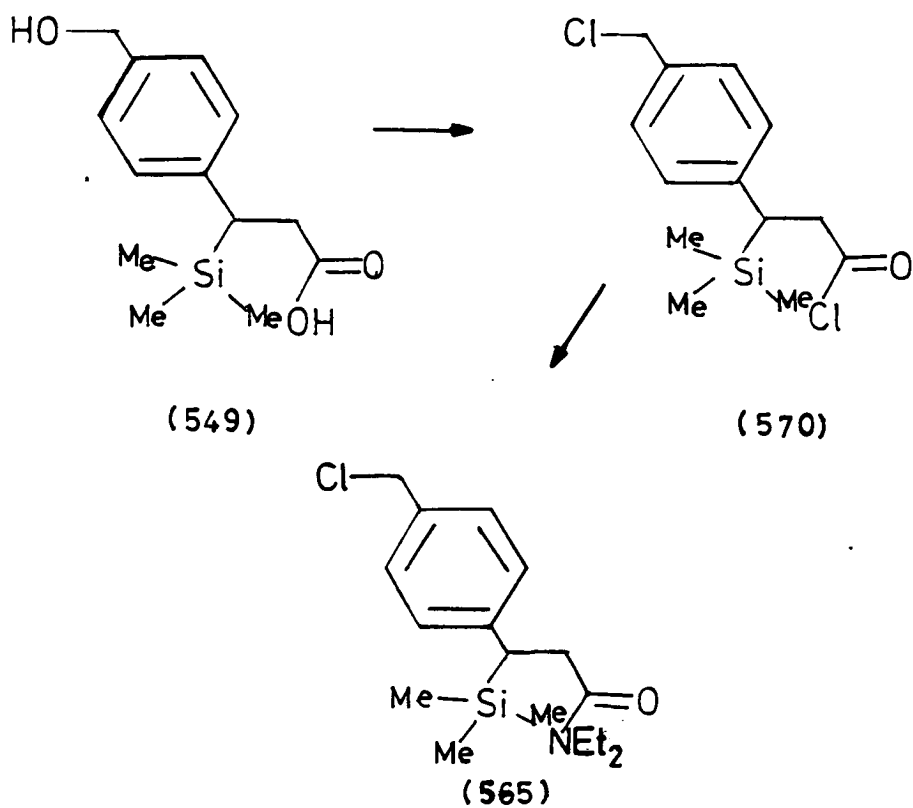
Scheme 215   Synthesis of modified handle (565).
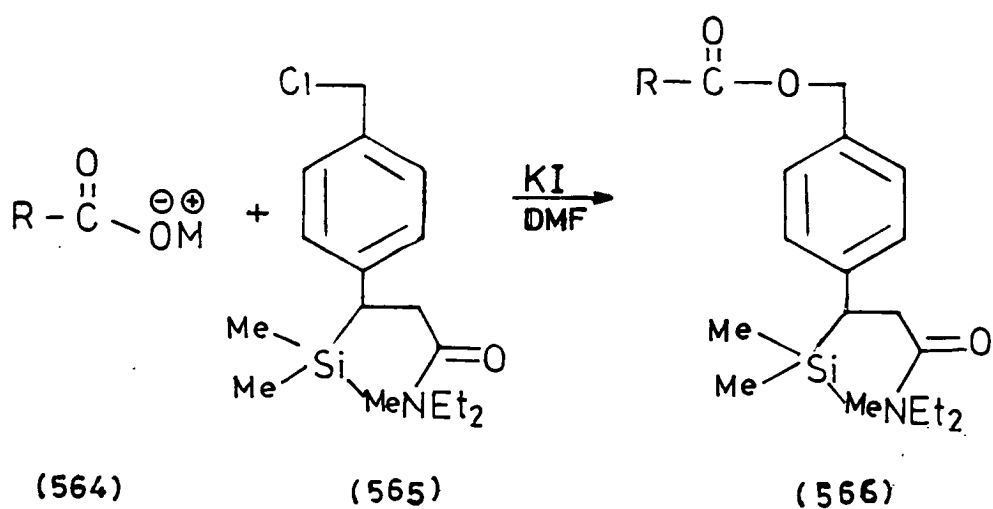
Scheme 216

Having obtained the required esters in a pure form, small scale attempts were made to cleave the β-lactam moiety from the handle by use of anhydrous tetrabutylammonium fluoride (TBAF). The reaction was carried out in acetonitrile using 1 equivalent of ester and 1 equivalent of TBAF, whereupon the reaction mixture changed colour (yellow-orange) within 5 minutes. The reported[264] procedure involved treating the resin with TBAF for 5-10 min followed by working-up the product. In our case, it was found that longer reaction times were needed for the reaction to complete. The penicillin V derivative (566a) was found to deprotect faster (5 min) than the cephalothin derivative (566b) (20 min). The reaction was also modified by the use of triethylamine (TEA), which would have neutralised any traces of HF generated in the reaction mixture. The work-up involved evaporation of the reaction solvent in vacuo. To the crude residue diethyl ether was added, the solvent was decanted (this removed the soluble cinnamide derivative (567)), and to the remaining residue was added water and ethyl acetate. The two-phase system was stirred, cooled to 0°C, and acidified with 2N hydrochloric acid to pH 2.5. The organic layer was separated, washed with brine and dried with sodium sulphate. Furthermore, it was found that the cephalosporin derivative (568b) required a basification (5% sodium bicarbonate solution) followed by an acidification (2N hydrochloric acid) and extraction into an organic solvent. The results were ambiguous in that the β-lactam containing products were not readily characterised due to the problems in purification. It was concluded that the problems had arisen due to the tetrabutylammonium salt of the penicillin V (568a) and cephalothin (568b). This salt would give rise to solubility of the products (568a) and (568b) in both the aqueous and the organic phases. Consequently, the organic phase would contain the tetrabutylammonium salt of the β-lactam component (568), the penicillin V acid (569a) or the cephalothin acid (569b), and the by-product

(567). It was not possible to chromatograph either penicillin V acid (569a) or cephalothin acid (569b) on silica, as this would result in β-lactam cleavage. Ion exchange[265] columns also resulted in β-lactam cleaved products. It was decided to use a different means of providing the fluoride ion, for deprotecting the handle and simultaneously providing a suitable salt of penicillin V or cephalothin. The modified method involved the use of potassium fluoride in methanol at 0°C. The required products would have been readily obtained in the organic phase on acidification of the aqueous/organic two phase system. The deprotection reaction was carried out at 0°C for 0.5 hr and the reaction worked-up. This resulted in isolation of only the starting material (566). The reaction was repeated at room temperature, with the reaction time increased to 24 hrs, again only the starting material (566) was isolated. It was concluded that the fluoride ion was strongly solvated and thus, unavailable for reaction with the silyl group. The work was stopped at this stage. It was concluded that the strategy worked, but difficulties in purification of the product made it impossible to determine the efficiency of the process. The purification problem would be minimised when using the solid support, since the cinnamide by-product (567) would remain on the resin. Further work is, thus, desirable in this area.

1.8.0. Summary of work completed

Achievements

This work has confirmed and supported previous failures in the attempts to form the thiazolidine ring of the penicillin, by an intramolecular Michael addition, as represented in scheme 148. Furthermore, a novel route

to the thione derivative (477a) was developed.   The
novel solid phase strategy for carboxylate protection and
deprotection in the β-lactam area has been shown to have
some success.

1.9.0. Future work in this area of research

It is hoped that future work would involve an
electrophilic addition to the acrylate moiety in (570)
(scheme 217).   This would allow an intramolecular
nucleophilic substitution, via a 5-exo-tet mode of
cyclisation - to afford a bisnorpenicillin type system
(395).   Furthermore, by appropriate functionalisation of
the addition product (571), it may be possible to obtain
the penem derivatives (574).   Work involving the sulphur
protecting groups such as silyl, stannyl or seleno, still
requires further investigation in order to develop
conditions which would readily generate a thiolate, a
thyl or a thione species respectively and which may
undergo annelation to form a bicyclic ring system.
Finally, work on the novel solid-phase strategy (scheme
213) could give rise to the synthesis of some very
sensitive β-lactam systems.   Furthermore, manipulation
of the resin bound β-lactam derivative may give rise to
high yields of the required products with little or no
β-lactam cleavage, due to steric inhibition around the
β-lactam bond.
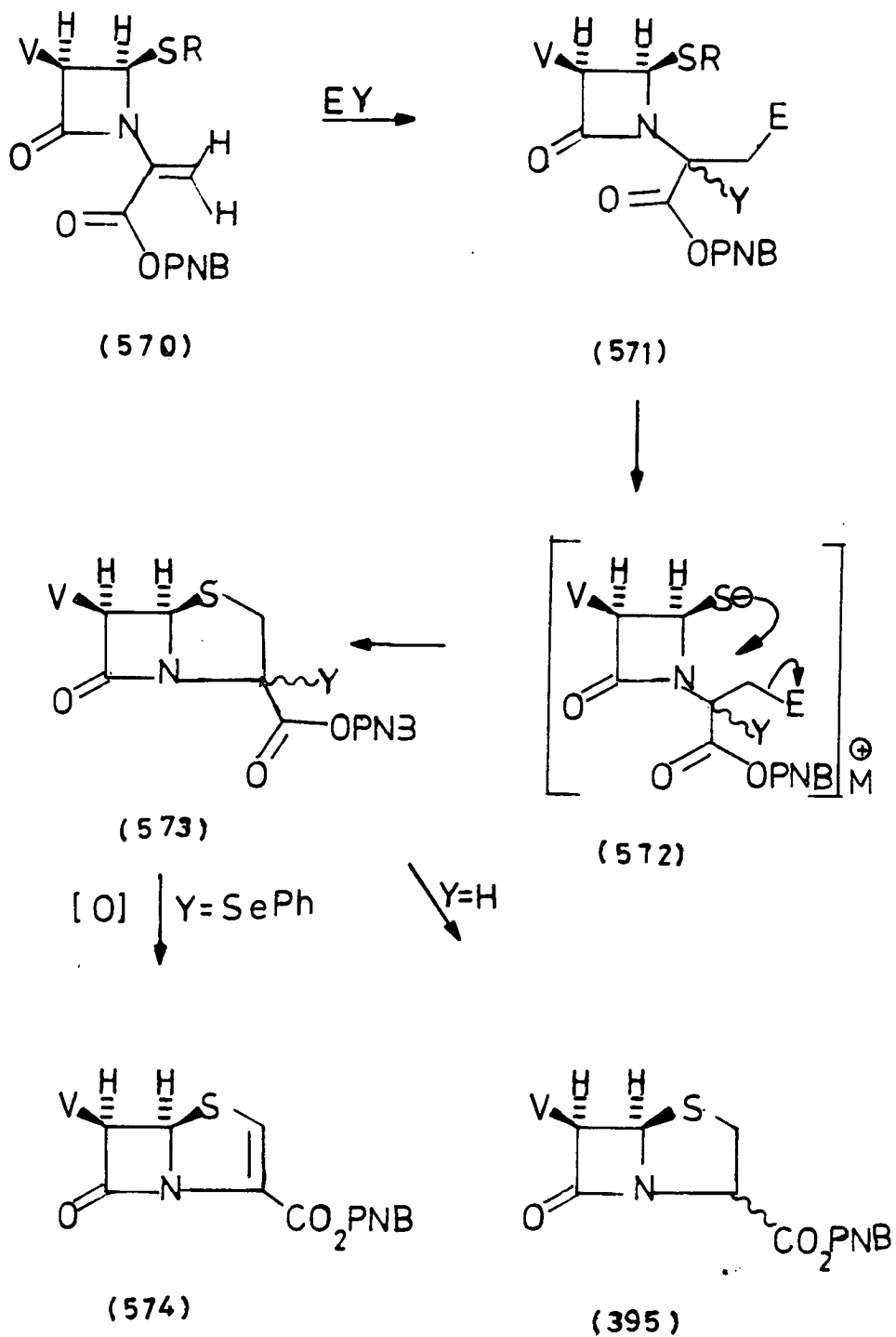
Scheme 217   Future investigations involving acrylate
derivative (570).

CHAPTER THREE -EXPERIMENTAL

1.0.0 EXPERIMENTAL SECTION

1.1.0 Purification of Solvents

Dimethylformamide (DMF) was distilled over calcium hydride under reduced pressure.

Acetonitrile, dichloromethane (DCM) and 1,4-dioxane were refluxed over, and distilled from, calcium hydride.

Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen.

Benzene and toluene were washed with cold concentrated sulphuric acid, aqueous sodium bicarbonate and water, dried over magnesium sulphate, decanted and distilled from sodium under nitrogen.

Ethyl acetate and petroleum ether (bpt 40-60°C) were redistilled.

Acetic Anhydride was dried over phosphorus pentoxide followed by reflux and distillation over magnesium turnings.

Diethyl ether was dried over sodium wire.

Chloroform was passed through an alumina column.

1.1.1 Analytical procedures

Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates. The major eluent systems employed were as follows:-

A = 75% ethyl acetate/25% petroleum ether (40-60)

B = 50% ethyl acetate/50% petroleum ether (40-60)

C = 50% ethyl acetate/50% dichloromethane.

Some minor eluent systems were also used. These are indicated when utilised.

The spots were identified by three methods:

a)   ultra-violet irradiation at $\lambda = 254$ nm.

b)   potassium permanganate spray:- 0.5% w/v aqueous solution.

c)   bromophenol blue:- 0.02% w/v ethanolic solution.

Preparative column chromatography was performed as follows:-

Dry flash chromatography[266] using TLC grade Merck Silica - Kieselgel 60H.

Flash chromatography using Merck silica, mesh size 230-400.

The sample was either pre-adsorbed on the silica or applied as a concentrated solution to a prepared chromatography column depending on the stability of the sample. The eluent employed was variable and will be indicated in the appropriate section.

Melting points were determined using a Buchi S10 apparatus and are uncorrected.

Infra-red (I.R.) spectra were recorded on a Perkin Elmer-781 spectrophotometer, using dichloromethane as the solvent, unless stated otherwise.

^{1}H nuclear magnetic resonance spectra were determined on varian EM-360 (60 MHz), Bruker FT WP 80 MHz and FT WP 200 MHz instruments. Chemical shifts measured in ppm using tetramethylsilane (δ = 0) as an external standard, with deuteriochloroform as the solvent unless otherwise stated. Ultraviolet spectra were recorded on Varian/Cary 210 spectrophotometer. Mass spectroscopy was performed on a KRATOS Ms 50 TC machine. Fast atom bombardment (FAB) spectra were obtained using thioglycerol as the matrix. Microanalyses were performed by Mrs. E. McDougall of the Edinburgh University analytical service.

All reactions were performed under dry nitrogen unless stated otherwise.

1.1.2   Characterisation data of derivatives from scheme 153

Potassium penicillin V (402)[270]

νmax/cm^{-1} (nujol):-   3370, 1775, 1726, 1660.

Penicillin V sulphoxide (403)[62]

mpt = 162°C (dec.) (acetone/water);

ν_{max}/cm^{-1} (nujol):- 3370, 1788, 1725, 1660, 1020.

Methyl penicillin V sulphoxide (404)[62]

mpt = 116°C (methanol)

Rf = 0.21 (A) ν_{max}/cm^{-1}:- 3390, 1805, 1750, 1685, 1160;

^1H n.m.r./ppm:- 1.19 (s, 3H), 1.70 (s, 3H), 3.81 (s,3H), 4.54 (s, 2H), 4.68 (s, 1H), 5.06 (d, J=6Hz, 1H), 6.09 (dd, J = 6, 12Hz, 1H), 6.92-7.38 (m, 5H), 8.25 (d, J=12 Hz, 1H). Found C, 53.4; H, 5.0; N, 7.4: $C_{17}H_{20}N_2O_6S$ requires C, 53.7; H, 5.3; N, 7.4%.

Methyl 3-methyl 2(S)-[3(4)-Penoxyacetamido-4(R)-S-(2-mer-captobenzothiazolyl()thioazetidin-2-on-1-yl]but-3-enoate (405)[57]

mpt = 147°C (ethyl acetate/petroleum ether (40-60);

Rf = 0.48 (A);

ν_{max}/cm^{-1}:- 3405. 1780, 1745, 1695;

^1H n.m.r./ppm:- 1.96 (s, 3H), 3.72 (s, 3H), 4.56 (m. 2H), 4.96 (s, 1H), 5.20 (s, 1H), 5.21 (s, 1H), 5.49 (dd, J = 6, 12 Hz, 1H), 5.61 (d, J = 6Hz, 1H), 6.9-7.9 (m, 6H); Found C, 53.7; H, 4.3; N, 7.5; $C_{24}H_{23}N_3O_4S_3$ requires C, 54.4; H, 4.3; N, 7.5%.

Methyl 3-methyl-2-(3 (R)-phenoxyacetamido-4(R)-(S-(2-mercaptobenzothiazolyl))thio azetidin-2-on-1-yl] but-2-enoate (406)[67]

Rf = 0.48 (A);

ν_{max}/cm^{-1}:- 3425, 1780, 1728, 1695, 1633;

^1H n.m.r./ppm:- 2.15 (s, 3H), 2.17 (s, 3H), 3.58 (s, 3H), 4.58 (m, 2H), 5.25 (dd, J = 5,10 Hz, 1H), 5.60 (d, J = 5Hz, 1H), 6.91-7.85 (m, 10H); Found C, 54.6; H, 4.3;

N, 7.8; $C_{24}H_{23}N_3O_5S_3$ requires C, 54.4; H, 4.4; N, 7.5%.

Methyl 3-methyl-2-(3(R)-phenoxyacetamido-4(R)-acetylthio-azetidin-2-on-1-yl) but-2-enoate (407)[123]

Rf = 0.48 (A);

ν_{max}/cm^{-1}:- 3400, 1780, 1725, 1700, 1690, 1635;

^1H n.m.r./ppm:- 2.06 (s, 3H), 2.22 (s, 3H), 2.25 (s, 3H), 3.79 (s, 3H), 4.55 (s, 2H), 5.37 (dd, J = 5, 8Hz, 1H), 6.02 (d, J = 5Hz, 1H); 6.7-7.4 (m, 5H), 7.92 (d, J = 8Hz, 1H).

1-(methyloxalyl)-(3R)-phenoxyacetamido-(4R)-acetylthio-azetidin-2-one (408).[123]

mpt = 145°C (ethyl acetate/petroleum ether (40-60);

ν_{max}/cm^{-1}:- 3415, 1823, 1754, 1705; ^1H n.m.r./ppm:- 2.33 (s, 3H), 3.99 (s, 3H), 4.49 (s, 2H), 5.54 (dd, J = 5, 10Hz, 1H), 6.02 (d, J = 5Hz, 1H); 6.95-7.4 (m, 5H), 8.76 (d, J = 10Hz, 1H).   Found C, 50.5; H, 4.3; N, 7.4; $C_{16}H_{16}N_2O_7S$ requires C, 50.5; H, 4.2; N, 7.4%.

(3R)-Phenoxyacetamido-(4R)-acetylthioazetidin-2-one (409).[123]

mpt = 138°C (ethyl acetate/petroleum ether (40-60);

Rf = 0.30 (A); ν_{max}/cm^{-1}:- 3410, 1790, 1697;

^1H n.m.r./ppm:- 2.24 (s, 3H), 4.52 (s, 2H), 5.45 - 5.57 (m, 2H, ABX system), 6.92-7.14 (m, 3H), 7.25 - 7.37 (m, 2H, 7.46 (d, J = 10Hz, 1H);   Found C, 52.8 ; H, 4.9 ; N, 9.2 ; $C_{13}H_{14}N_2O_4S$ requires C, 53.1 ; H, 4.8 ; N, 9.5 .

4-Nitrobenzyl ((3R)-phenoxyacetamido-(4R)-acetylthio-azetidin-2-on-1-yl)-triphenylphosphoranylidenacetate (413).[123]

Rf = 0.27 (B); ν_{max}/cm^{-1}:- 3400, 1770, 1725, 1690, 1625, 1520, 1350; ^1H n.m.r./ppm:- 2.25 (s, 3H), 4.53 (s, 2H), 5.25 (s, 2H), 5.45-5.57 (m, 2H), 6.92-8.34 (m, 24H.

di[1-(1-methoxy-3-methyl-1-oxobut-2-en-2-yl)-3(R)-Phen-
oxyacetamido azetidin-2-on-4(R)-yl]disulphide (417).[67]

mpt = 123°C (diethyl ether); Rf = 0.48 (A); Vmax/cm^{-1}:-
3400, 1780, 1725, 1698, 1635; ^{1}H n.m.r./ppm:- 2.0 (s,
3H), 2.3 (s, 3H), 3.54 (s, 3H), 4.56 (m, 2H), 5.25 (dd, J
= 5, 10Hz, 1H); 5.61 (d, J = 5 Hz, 1H), 6.91-7.85 (m,
6H). FAB MS [MH]$^+$ = 704.

1.2.0 Synthesis and Chemistry of 4-Nitrobenzyl 2-((3R)-
Phenoxyacetamido-(4R)-acetylthio-2-azetidinon
-1-yl) propenoate (414)[212]

The phosphorane[212] (4.0g, 5.35 mmol) was dissolved
in dichloromethane (200ml). Dry formaldehyde[266] was
bubbled into the reaction mixture for short periods
(2 min) every fifteen to twenty minutes until no more
starting material (413) remained (1.5h) by TLC (A).
The solution was filtered through celite and the filtrate
concentrated under reduced pressure[268] whereupon the
residue was purified by flash chromatography, using
gradient elution with ethyl acetate and petroleum ether
(40-60), affording (414) (1.3g, 49%). This solidified
on trituration with diethylether. Mpt = 112°C, Rf = 0.7
(system C); Vmax/cm^{-1}:- 3410, 1782, 1735, 1700, 1525,
1350; ^{1}H n.m.r./ppm:- 2.3 (s, 3H), 4.6 (s, 2H), 5.35 (s,
2H), 5.65 (dd, J = 5.2 Hz, 8.8 Hz, 1H), 6.05 (d, J = 5.2
Hz, 1H), 6.15 (d, J = 0.8 Hz, 1H), 6.19 (d, J = 0.8 Hz,
1H), 6.9 (d, J = 7.9 Hz, 2H), 7.05 (m, 1H), 2.28-7.4 (m,
3H), 7.58 (d, J = 8.7 Hz, 2H), 8.25 (d, J = 8.7 Hz, 2H);
^{13}C n.m.r., δ/ppm[269] 30.5 (a), 59.9 (b), 64.5 (c), 65.5
(d), 66.9 (e), 114.5 (f), 118.1 (g), 121.9 (h), 123.4
(i), 128.2 (j), 129.4 (k), 130.0 (1), 142.0 (m), 147.5
(n), 156.9 (o), 161.2 (p). 163.5 (q), 168.6
(r); ^{13}C n.m.r., DEPT P$\emptyset$ = $\frac{3}{4}$, δ/ppm 30.8 (+ve),
60.1 (+ve) 64.6 (+ve), 65.8 (-ve), 67.0 (-ve), 114.6

(+ve), 118.4 (-ve), 122.1 (+ve), 123.3 (+ve), 123.6 (+ve), 128.5 (+ve), 129.6 (+ve); UV(MeCN)λ_{max} 218,261nm;

Found C, 55.1; H, 4.2; N, 8.0; $C_{23}H_{21}N_3O_8S$ requires C, 55.3; H, 4.2; N,8.4%; M.S (FAB, Thioglycerol) m/e 500 (MH$^+$).

1.2.1   Attempted cyclisation of (414)

a)   Using 2 equivalents of cyclohexylamine

The acrylate derivative (414) (200 mg, 0.4 mmol) was dissolved in dichloromethane (5ml).   The solution was flushed with nitrogen and freshly dried cyclohexylamine (0.8 mmol) in dichloromethane (5ml) was added.   The reaction was monitored by infra-red analysis and it was found that the β-lactam carbonyl stretching frequency (1780 cm^{-1}) diminished with time and became negligible after 1.5 h.   The solvent was removed in vacuo.   Thin layer chromatographic analysis indicated a complex mixture and the infra-red spectrum of the crude product contained no carbonyl stretching frequency above 1740 cm^{-1}.

b)   Using 1 equivalent of cyclohexylamine

The acrylate derivative (414) (60.5 mg, 0.12 mmol) was dissolved in CD$_2$Cl$_2$ (0.5 ml).   To the solution,dry cyclohexylamine (0.014 ml, 0.12 mmol) was added and the reaction was then monitored by ^{1}H n.m.r. spectroscopy (80 MHz) at 15 minute intervals for 45 minutes.   The olefinic protons had disappeared after 15 min, as had the β-lactam protons.   After 30 min, a new peak at δ = 2.4 ppm had developed (N-acetyl cyclohexylamide).   The reaction mixture was then diluted with dichloromethane

(10ml) and washed with distilled water.   The dichloromethane layer was dried (sodium sulphate) and the solvent evaporated _in vacuo_.   The aqueous washing was re-extracted with ethyl acetate and then discarded.   The ethyl acetate was dried (sodium sulphate) and evaporated _in vacuo_.   Thin layer chromatographic analysis of the dichloromethane fraction indicated only components with lower Rf values than the starting material, together with a trace amount of starting material.   TLC analysis of the ethyl acetate fraction indicated a component with a similar Rf value to the starting material.   Infra-red analysis of the ethyl acetate fraction indicated the presence of a β-lactam carbonyl stretch (1780 cm^{-1}) but only a trace amount was available.   The crude product from the dichloromethane fraction was purified by preparative thin layer chromatography, the eluent being ethyl acetate.   Three fractions were observed, each was extracted from the silica with ethyl acetate and dichloromethane.   Fraction 1 (dichloromethane), 1 mg, (430), νmax/cm^{-1} 1800, 1730, 1695; δ/ppm 4.33 (s, 2H), 4.65 (s, 1H), 5.10 (d, J = 5Hz, 1H), 5.22 (s, 1H), 6.1 (dd, J = 5 Hz, 12Hz, 1H), 7.4 (M,);   Fraction 1 (ethyl acetate), (435), 10.8 mg, νmax/cm^{-1} 3410, 1780, 1748, ·1690, 1520, 1350; ^{1}H n.m.r./ppm 1.5 (m, 11H), 2.3 (S, 3H), 3.5 (m, H) 4.6 (s, 2H), 5.4 (s, 2H), 5.6 (m, 2H), 6.9-8.3 (m, 10H);   Fraction 2 (dichloromethane) (430), 0.7 mg,   max/cm^{-1} 1800, 1755, 1695;   Fraction 2 (ethyl acetate) (436), 1.5 mg, νmax/cm^{-1} 1730, 1670, 1520, 1350;   Fraction 3 (dichloromethane + ethyl acetate) (436), 1.7 mg, νmax/cm^{-1} 1730, 1670, 1520, 1350.

c)   Using 1 equivalent of diisopropylamine

The acrylate derivative (414) (31.2 mg, 6.3 x 10^{-5} mol) was dissolved in dichloromethane (5 ml) under nitrogen.   The solution was cooled to -20°C.   To the

reaction mixture was added a solution of diisopropylamine (6.3×10^{-5} mol, 6.4 mg) in dichloromethane (0.5 ml) and the reaction was monitored by infra-red and TLC analysis. The reaction mixture was allowed to warm to room temperature over 0.5 h and then stirred overnight. After 20 h, no reaction had taken place as indicated by infra-red and TLC analysis. ν_{max}/cm^{-1} 3410, 1780, 1730, 1700, 1420, 1350. A catalytic amount (2.5% w/w (414)) of N,N-dimethylaminopyridine was added. The β-lactam cleaved within two minutes as indicated by infra red analysis. ν_{max}/cm^{-1} 3400, 1730, 1700 (br), 1520, 1350.

d)   Using 1 equivalent of 4-chloroaniline

4-chloroaniline (4.4 mg, 3.5×10^{-5} mol) was added to a solution of the acrylate derivative (414) (17.7 mg 3.5×10^{-5} mol) in sodium dried benzene (5 ml). The reaction mixture was stirred for 25h at room temperature under nitrogen. The solvent was then evaporated in vacuo and the residue dissolved in dichloromethane. An infra-red spectrum was obtained which indicated peaks identical to the starting materials. This was confirmed by TLC analysis. The residue was re-dissolved in benzene (5ml) and heated to 35°C for 1h and to 40°C for 2h. In both cases, no reaction occurred and infra-red and TLC analysis confirmed the presence of only the starting materials.

e)   Using 1 equivalent of mercuric acetate

The acrylate derivative (414) (24 mg, 4.7×10^{-5} mol) was dissolved in dimethylformamide (DMF) (5ml) under nitrogen. The reaction mixture was cooled to -20°C. To the stirred solution, a solution of mercuric acetate (16 mg, 5.08×10^{-5} mol) in DMF (5 ml) was added

dropwise. The reaction mixture was allowed to warm to
room temperature and stirred for a further 50 min. No
precipitate formed, as was expected. TLC analysis
indicated the presence of the starting material (414) (Rf
= 0.7, A). The reaction mixture was warmed to 35°C.
After 1h,TLC analysis indicated presence of mainly
starting material in addition to a few minor spots close
to the base line. DMF was removed in vacuo and the
residue was taken up in dichloromethane, with a precipitate
remaining. Infra-red analysis of the dichloromethane
layer indicated that the starting material was the main
component. ν_{max}/cm^{-1} 3410, 1780, 1730, 1700, 1520,
1350. Infra-red analysis (nujol mull) of the
precipitate indicated no β-lactam containing component,
to be present.

f) Using 1 equivalent of Lithium Hydride

The acrylate derivative (414) (18.6 mg, 3.73 x
10^{-5} mol) was dissolved in dry benzene (10 ml) to which
lithium hydride (0.3 mg, 3.73 x 10^{-5} mol) was added.
The suspension was heated to 65°C and stirred for 2 h.
TLC analysis (A) indicated only the presence of
the starting material (414).
Removal of the lithium hydride and evaporating the
filtrate to dryness in vacuo afforded a clear gum. ^{1}H
n.m.r. analysis indicated only the starting material to
be present. ^{1}H n.m.r, (200 M Hz) δ/ppm 2.2 (s, 3H),
4.55 (s, 2H), 5.35 (s, 2H), 5.65 (dd, J = 5.2, 8.8 Hz,
1H), 6.03 (d, J = 5.2 Hz, 1H), 6.09 (s, 1H), 6.14 (s,
1H), 6.8-8.2 (m, 10H).

g) Using 1 equivalent of lithium hydroxide

Treatment of the acrylate derivative (414) (20mg,
4 x 10^{-5} mol) in 50% aqueous tert-butanol (5 ml) with an

aqueous solution of lithium hydroxide (0.05M) by a titrimetric method resulted in complete decomposition of the starting material (414) as indicated by TLC analysis, by a U.V. active spot on the base-line. Infra-red analysis of the dried residue indicated no carbonyl peak above 1730 cm^{-1}.

1.2.2 Synthesis of 4-Nitrobenzyl 2-((3R)-phenoxyacet- amido-(4R)-Silver thiolato-2-azetidinon-1-yl) propenoate (439)

The acrylate derivative (414) (470 mg, 9.4 x 10^{-4} mol) was dissolved in freshly dried and distilled tetrahydrofuran (10 ml) under nitrogen and the reaction vessel was covered by aluminium foil. To the stirred reaction mixture was added,dropwise,a solution of silver tetrafluoroborate (367 mg, 18.8 x 10^{-4} mol) in tetrahydrofuran (5 ml), containing methanol (1 ml) at room temperature. Excess dry pyridine (0.05 ml) was added, and the reaction mixture was then heated at 30°C for 1.5 h. The end point of the reaction was determined by TLC analysis (C). The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was taken up in dichloromethane and washed twice with distilled water (10 ml) and once with brine (10 ml). The organic layer was dried with anhydrous sodium sulphate, filtered, and the solvent evaporated in vacuo. The crude product was chromatographed by flash chromatography.

Two isocratic eluent systems were used

a)   70% ethyl acetate/30% petroleum ether (40-60)

b)   50% ethyl acetate/50% dichloromethane

The silver thiolate (439) (379 mg, 70%) was isolated from the fractions of (b). The product (439) solidified on trituration with diethyl ether and was stored at 0°C and in the dark. Mpt 113-115°C. Rf = 0.64 (C), νmax/cm^{-1}

3405, 1770, 1710, 1675, 1525, 1350, ^{1}H n.m.r. (CD$_2$Cl$_2$, 80 MHz) δ/ppm 4.61 (m, 2H), 5.35 (m, 3H), 6.13 (S, 1H), 6.19 (S, 2H), 6.97 - 8.18 (m, 10H); Found C, 44.1; H, 3.1; N, 7.5; AgC$_{21}$H$_{18}$N$_3$O$_7$S (1/2 H$_2$O) requires C, 44.0; H, 3.3; N, 7.3%; M.S. (FAB, Thioglycerol) m/e 565 (MH$^+$), 587 (MNa$^+$), 458 (MH$^+$ - Ag).

1.2.3. Reaction of the Silver thiolate (439) with acetyl chloride

The silver thiolate (439) (45.2 mg, 8.0 x 10^{-5} mol) was dissovled in acetonitrile (5 ml) under nitrogen. The reaction vessel was covered with aluminium foil and cooled to 0°C. To the stirred solution was slowly added acetyl chloride (0.14M in MeCN, 0.6 ml). After five minutes, the white precipitate was removed by filtration through celite. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography on silica using a gradient eluent system of ethyl acetate and petroleum ether (40-60), affording the acrylate derivative (414) (11mg, 30%) as a colourless gum. Rf = 0.7 (C); νmax/cm^{-1} 3410, 1780, 1732, 1700, 1525, 1350; ^{1}H n.m.r. (80 MHz) δ/ppm 2.2 (s, 3H), 4.6 (s, 2H), 5.3 (s, 2H), 5.6 (dd, J = 5, 9Hz, 1H), 6.0 - 6.1 (d, J = 5Hz, 3H), 6.8-8.3 (m, 10H); Found C, 55.1; H, 4.5; N, 8.0; C$_{23}$H$_{21}$N$_3$O$_8$S requires C, 55.3; H, 4.2; N, 8.4%; M.S. (FAB, Thioglycerol) m/e 500 (MH$^+$).

1.2.4. Attempted generation of the thiol (394) from (439)

a)   Reaction with hydrogen sulphide

The silver thiolate (439) (35 mg, 6.2 x 10^{-5} mol) was dissolved in dichloromethane (10 ml) under nitrogen

and the reaction vessel covered with aluminium foil. The reaction mixture was then cooled to -50°C.   A mixture of hydrogen sulphide and nitrogen was bubbled slowly through the reaction mixture for 30 seconds.   The solution was kept at -50°C, flushed with nitrogen for 0.5h and then allowed to warm to room temperature.   The black precipitate was removed by filtration through kieselguhr and washed with dichloromethane.   The filtrate was concentrated <u>in vacuo</u>.   TLC analysis of the crude product in systems A and C indicated no mobile components, the spot remaining on the baseline.   Infra red analysis indicated that the β-lactam had cleaved; V_{max} 3390 (br), 1750, 1690-1670 (br), 1525, 1350 cm^{-1}. Attempts to purify sample by crystallisation or chromatography resulted in complete loss of material.

b)   <u>Reaction with sodium chloride</u>

The silver thiolate (439) (10mg, 1.75 x 10^{-5} mol) was dissolved in (1:10) water/acetonitrile mixture, (5 ml), to which was added excess sodium chloride (500 mg).   The reaction mixture was then stirred for 12h   TLC analysis (C) indicated a new spot (Rf = 0.71) and a spot on the base line.   No spot corresponding to the starting material (439) (Rf = 0.64) was present.   Infra-red analysis of the crude product indicated that the β-lactam had cleaved.   Preparative thin layer chromatography resulted in a solid (1 mg); V_{max}/cm^{-1} 3375, 1723, 1685, 1590, 1522, 1350.

1.2.5 Reactions of silver thiolate (439)

a) With bromine water

The silver thiolate (439) (51 mg, 9.10 x 10^{-5} mol) was dissolved in dichloromethane (15 ml) and to this solution was added distilled water (15 ml) and a 1% (w/v) solution of aqueous bromine (0.5 ml). The reaction mixture was stirred for 5 minutes and then diluted with saturated aqueous sodium bicarbonate solution (10 ml). The organic layer was removed, washed with distilled water and dried over anhydrous sodium sulphate. The dried solution was filtered and concentrated in vacuo to afford a clear gum (58 mg). νmax/cm^{-1} 3405, 1790, 1755, 1730, 1695; Attempts to obtain a ^{1}H n.m.r. spectrum resulted in the formation of a precipitate in CDCl$_3$ and no β-lactam containing component. Work was stopped at this stage with this sample.

b) With phenylselenenyl chloride

The silver thiolate (439) (97 mg (1.71 x 10^{-4} mol) was dissolved in dichloromethane (10 ml), the solution was flushed with nitrogen and the reaction vessel was covered with aluminium foil. To the stirred solution phenylselenenyl chloride (33.7 mg, 1.76 x 10^{-4} mol) in dichloromethane (10 ml) was added dropwise. The reaction mixture was stirred for 2h. The reaction mixture was filtered through celite and the filtrate concentrated in vacuo. The residue was purified by flash chromatography on silica using two isocratic eluent systems: (i) petroleum ether (40-60), (ii) 50% ethyl acetate/50% dichloromethane. This afforded 96 mg (92%) of a pale yellow foam. Rf 0.75 (C); ^{1}H n.m.r. (60 MHz) δ/ppm 4.54 (s, 2H), 5.27 (s, 2H), 5.6-6.2 (m, 4H), 6.95-8.6 (m, 15H); νmax/cm^{-1} 3410, 1775, 1730, 1695, 1525, 1350; M.S. (FAB, Thioglycerol) m/e 613 (MH^{+});

Found C, 52.3; H, 3.7; N, 6.7 $C_{27}H_{23}N_3O_1S$ Se requires C, 52.9; H, 3.8; N, 6.9%.   These data suggested that the product had the structure (474a)..

Oxidation of the sulphur-seleno derivative (474a) with meta-chloroperbenzoic acid (mCPBA)

The thio-seleno derivative (474a) (90.7 mg, 1.48 x 10^{-4} mol) was dissolved in dichloromethane (5 ml) and the solution flushed with nitrogen. The stirred reaction mixture was cooled to 0°C and a solution of mCPBA (26 mg, 1.5 x 10^{-4} mol) in dichloromethane (1 ml) was added dropwise. The reaction mixture was stirred, allowed to warm to room temperature and the reaction was monitored by TLC analysis (B).   After 55h, the reaction appeared to have stopped although some starting material (474a) was still present (TLC).   An aliquot (1 ml) of the reaction mixture was removed and worked-up via a saturated sodium bicarbonate wash and dried with anhydrous sodium sulphate.   Infra-red analysis indicated the following: ν_{max}/cm^{-1} 3360, 1825 (w), 1772, 1730, 1685, 1520, 1348. The major component appeared to be the starting material (474a) (TLC and IR) but some required product (477a) seemed to be present.   The crude mixture was treated with further mCPBA (4 mg, 2.3 x 10^{-5} mol) in dichloromethane (2 ml) and the solution was stirred for 10 minutes.   Usual work-up resulted in a gum, infra-red analysis of which indicated the following: ν_{max}/cm^{-1} 3405 (br), 1790, 1760, 1735, 1700, 1590, 1525, 1350, which was not compatible with the required product (477a) and, hence, was not pursued further.   The remaining mixture was worked up in the usual way.   Infra-red analysis of the crude sample indicated the following: ν_{max}/cm^{-1} 3410, 1825 (w), 1775, 1730, 1695, 1522, 1350. Attempt to purify the sample by preparative TLC (B) resulted in the isolation of a solid which was identified

as (474a) (19 mg, 30%) (TLC and IR analysis).   No thione
derivative (477a) could be isolated.   Work was stopped
at this stage with this sample.

c)   <u>With trimethyltin chloride</u>

        The silver thiolate (439) (99 mg, 1.76×10^{-4} mol)
was dissolved in dichloromethane (2 ml) with stirring
under nitrogen.   The reaction vessel was covered with
aluminium foil and a solution of trimethyltin chloride
(40.6 mg, 2×10^{-4} mol) in dichloromethane (1 ml) was
added at 0°C.   The reaction mixture was stirred for
15h.   The white precipitate was removed by filtration
through anhydrous sodium sulphate and washed with
dichloromethane (3 x 10 ml) and the filtrate concentrated
<u>in vacuo</u>.   37.4 mg of a yellow gum resulted.   This was
identified as the starting material (439) (37%
recovery);   Rf = 0.64 (C); ν_{max}/cm^{-1} 3405, 1770, 1710,
1675, 1525, 1350.   The residue was further washed with
acetone (5 x 10 ml) and the filtrate resulted in a white
solid (57 mg) on concentrating <u>in vacuo</u>.   Mpt 104°C;
ν_{max} (Nujol)/cm^{-1} 1765, 1715, 1675, 1522, 1350; ^{1}H n.m.r.
(80 MHz) (d6- acetone) δ/ppm 1.2 (s, 3H), 4.7 (s, 2H),
5.5 (s, 2H), 6.0-8.3 (m, 4H), 6.9-8.2 (m, 10H): (peaks
were generally broad).   Work was stopped at this stage
with this sample.

d)   <u>With chlorotrimethylsilane</u>

        The silver thiolate (439) (158 mg, 2.8×10^{-4} mol)
was dissolved in dichloromethane (3 ml) and the reaction
vessel covered with aluminium foil and flushed with
nitrogen.   The stirred reaction mixture was cooled to
0°C and chlorotrimethylsilane (0.3 ml, 3.2 mmol) was
added.   The reaction mixture was stirred for 18 hours
resulting in a white precipitate.   The reaction mixture

was filtered through kieselguhr under nitrogen and the filtrate was evaporated <u>in vacuo</u> resulting in a yellow foam (126 mg). V_{max}/cm^{-1} 3400 (br), 1774, 1725, 1688, 1525, 1350; A satisfactory ^1H n.m.r. spectrum could not be obtained. Work was stopped at this stage with this sample.

e) <u>With bromonitromethane</u>

The silver thiolate (439) (96 mg, 1.7×10^{-4} mol) was dissolved in dichloromethane (5 ml). The reaction vessel was covered with aluminium foil, flushed with nitrogen and cooled to 0°C. To the stirred solution was added a solution of bromonitromethane (26 mg, 1.71×10^{-4} mol) in dichloromethane (2 ml). The reaction was monitored by TLC analysis (C). After 6h at 0°C, no reaction had occurred. The reaction was allowed to warm to room temperature and stirred for 1h. No reaction was detectable. The reaction mixture was heated to 36°C for 1 h. No reaction was detected. The solvent was removed <u>in vacuo</u> and replaced by dimethylformamide (5 ml). The reaction was continued at room temperature for 6h. No reaction was detectable by TLC analysis, only the starting materials being present. Work was abandoned on this line of research.

1.2.6. <u>Attempted synthesis of 4-Nitrobenzyl 2-((3R)-Pheno-xyacetamido-(4R)-thioacetyl-azetidin-2-one-1-yl)-3-tri^{n}butylstannyl propanoate (504)</u>

The acrylate derivative (414) (180 mg, 0.36 mmol) was dissolved in benzene (10 ml) under nitrogen. Azobisisobutyronitrile (5mg, 3% w/w) was added to the reaction mixture followed by tri-n-butylstannane (215 mg, 0.74 mmol). The reaction mixture was heated to 80°C for

2h .   The cooled solution was concentrated <u>in vacuo</u> and the residue subjected to flash chromatography on silica using an isocratic eluent system (70% ethyl acetate/30% petroleum ether (40-60)).   The β-lactam containing fractions were combined and preparative TLC using an eluent system of 30% ethyl acetate/70% petroleum ether (40-60) was carried out.   Three components were isolated.   Fraction 1 (20mg); Rf = 0.8 (A), νmax/cm^{-1} 1733, 1610, 1520, 1349, ^{1}H n.m.r. (80 MHz) δ/ppm 0.9-2.0 (m, 5H), 4.81 - 4.84 (m, 2H), 7.46-8.25 (AB, J = , 4H); sample not identified: Fraction 2 (80 mg) Rf = 0.67 (A) identical to acrylate derivative (414) in system A; νmax/cm^{-1} 3410, 1783, 1733, 1700, 1525, 1350;   sample identified as starting material (414):   Fraction 3 (25 mg); νmax/cm^{-1} 3408, 1780, 1748, 1700, 1685, 1522, 1350; TLC analysis indicated two close spots, a further chromatography of this fraction resulted in 6.3 mg of a β-lactam containing component.   Rf = 0.46 (A) (c.f starting material (414) Rf = 0.67 (A)); νmax/cm^{-1} 3410, 1780, 1745, 1700, 1685 (sh), 1523, 1350; ^{1}H n.m.r. (200 MHz) δ/ppm 1.58 (d, J = 7.5 Hz, 3H), 2.2 (s, 3H), 4.6 (m, 2H), 5.2 (m, 1H), 5.5 (m, 1H), 5.6-5.8 (m, 1H), 6.9-8.2 (m, 10H);   M.S. (FAB, Thioglycerol) m/e 500 (m$^+$), 426.   The structure of sample was assigned as (505).

1.2.7   <u>Reactions of 4-nitrobenzyl 2-((3R)-phenoxyacet-amido-(4R)- acetylthio azetidin-2-on-1-yl)-propenoate (414)</u>

a)   <u>With ethanethiol</u>

The acrylate derivative (414) (30 mg, 6.0 x 10^{-5} mol) was dissolved in dichloromethane (5 ml) to which was added an excess of ethanethiol (11 mg, 1.8 x 10^{-4} mol) in dichloromethane under nitrogen.   The reaction was monitored by TLC analysis (A).   After 1.5h at room temperature, no reaction was detectable.   The reaction

mixture was then refluxed for 2h and no change was detected. The reaction mixture was cooled and excess pyridine (0.2 ml) was added and then refluxed for 10 minutes. No reaction was detected by TLC analysis. Flash chromatography of the reaction mixture using a gradient eluent of ethyl acetate and petroleum ether (40-60) resulted in isolation of the starting material (414) (18mg, 60%) as a white foam. ν_{max}/cm^{-1} 3410, 1783, 1734, 1700, 1525, 1350; ^{1}H n.m.r. (80 MHz) δ/ppm 2.2 (s, 3H), 4.6 (s, 2H), 5.3 (s, 2H), 5.6 (dd, J = 5, 9Hz, 1H), 6.6-6.1 (d, J = 5Hz, 3H), 6.8-8.3 (m, 10H).

b) With Hydrogen Sulphide

The acrylate derivative (414) (18 mg, 3.6×10^{-5} mol) was dissolved in dichloromethane (10 ml) at room temperature under nitrogen. Hydrogen sulphide was bubbled through the reaction mixture for 5 minutes. The reaction mixture was flushed with nitrogen for 15 minutes. TLC analysis indicated only the presence of the starting material (414), suggesting that no reaction had taken place. Flash chromatography using 50% ethyl acetate and 50% dichloromethane as eluent resulted in 15 mg (70%) of the starting material (414). ν_{max}/cm^{-1} 3410, 1780, 1730, 1700, 1525, 1350.

c) Reaction with Bromine

The acrylate derivative (414) (20 mg, 4.0×10^{-5} mol) was dissolved in dichloromethane (10 ml) and cooled to 0°C. A solution of aqueous bromine (10 ml, 1% w/v) was added and the solution stirred for 10 minutes. After the aqueous layer had decolourised, the organic layer was separated and washed with distilled water and then dried over anhydrous sodium sulphate. The dried organic layer was filtered and the solvent removed in

<u>vacuo</u>. Infra-red analysis of the crude product
indicated the following peaks: Vmax 1785, 1750, 1700,
1520 and 1350 cm^{-1}. The sample was purified by
preparative TLC (A) resulting in 12 mg of a solid.
Infra red analysis indicated that the β-lactam carbonyl
stretching frequency was absent. Vmax/cm^{-1} 1733, 1700
(br), 1520, 1350.

1.2.8 Synthesis of Methyl 2-((3R)-Phenoxyacetamido-(4R)-Silver thiolatoazetidin-2-on-1-yl) 3-methyl-but-2-enoate (465)

The β-lactam (407) (0.52 g, 1.27 mmol) was
dissolved in methanol (10 ml) and cooled to 0°C under
nitrogen. Silver nitrate (0.22g, 1.27 mmol) and
pyridine (0.1g, 1.27 mmol) were added to the reaction
mixture. The reaction vessel was covered by aluminium
foil and stirred for 18h. The white precipitate was
filtered under nitrogen, washed with cold methanol (2 x
10 ml), diethyl ether (20 ml), and dried under vacuum.
The pale yellow solid was stored at 0°C in the dark. Rf
0.36 (C), Mpt 130°C (decomposed); Vmax/cm^{-1} 3405, 1765,
1720, 1700, 1650; ^{1}H n.m.r. (80 MHz) δ/ppm 2.1- 2.15 (s,
6H), 3.75 (s, 3H), 4.5 (m, 2H), 5.3 (m, 2H), 6.8-7.3 (m,
5H), 8.15 (d, J = 9Hz, 1H).

1.2.9 Reactions of (465)

a) With acetyl chloride

The silver thiolate (465) (10mg, 2.1 x 10^{-5} mol)
was dissolved in acetonitrile (10 ml) under nitrogen.
The reaction vessel was covered with aluminium foil and
cooled to 0°C. A solution of acetyl chloride (1.6 mg,

2.1 x 10^{-5} mol) in acetonitrile (2 ml) was added dropwise to the reaction mixture and stirred for 5 minutes. The white precipitate was filtered under nitrogen (this darkened on exposure to light for 10 minutes). The filtrate was concentrated <u>in vacuo</u>. The residue was purified by flash chromatography using a gradient elution with ethyl acetate and petroleum ether (40-60), resulting in a foam (3 mg). Rf 0.55 (C); νmax/cm^{-1} 3400, 1780, 1725, 1700, 1690, 1635; Comparison with the literature[218] confirmed the β-lactam (407) had been obtained in 35% yield.

b)   <u>With bromine water</u>

The silver thiolate (465) (50 mg, 8.4 x 10^{-4} mol) was dissolved in dichloromethane (10 ml), with stirring. The solution was cooled to 0°C and then a solution of aqueous bromine (20 ml, 1% w/v) was added dropwise. After stirring for 5 minutes, the solution was diluted with a solution of saturated sodium bicarbonate (10 ml) and the organic layer was removed and washed with distilled water (2 x 5 ml) and brine (5 ml), and dried over anhydrous sodium sulphate. The filtrate was concentrated <u>in vacuo</u>. Infra-red analysis of the crude product indicated the following: νmax/cm^{-1} 1785, 1725 and 1700, suggesting that the β-lactam bond was intact. ^{1}H n.m.r. (80 MHz) of the crude product indicated an AA' BB' proton resonance pattern typical of a paradi-substituted aromatic ring (δ= 8.2 - 7.7 ppm). TLC analysis indicated that the sample was unstable to silica. Work was stopped at this stage with this sample.

c)   <u>With phenylselenenyl chloride</u>

The silver thiolate (465) (145 mg, 3.1 x 10^{-4} mol)

was dissolved in dichloromethane (10 ml) under nitrogen. The reaction vessel was covered with aluminium foil. A solution of phenylselenenyl chloride (62 mg, 3.2×10^{-4} mol) in dichloromethane (10 cm^3) was added dropwise and the reaction mixture stirred at room temperature for 10 minutes. The white suspension was filtered through celite. The solids were washed with dichloromethane and the solvent evaporated <u>in vacuo</u>. The residue was purified by flash chromatography on silica, using a dual eluent system consisting of a) petroleum ether (40-60) and b) 50% ethyl acetate/50% dichloromethane. This resulted in isolation of a yellow solid (474b) (124 mg). Rf 0.64 (C), νmax/cm^{-1} 3415, 1775, 1725, 1695; ^{1}H n.m.r. (80 MHz) δ/ppm 1.9 (s, 3H), 2.1 (s, 3H), 3.69 (s, 3H), 4.45 (s, 2H), 5.4 (m, 2H), 7.28 (m, 11H); M.S (Thioglycerol, FAB) m/e 519 (M$^+$); accurate mass found 519.071676 $C_{23}H_{24}N_2O_5S$ Se requires 519.0717. The structure was assigned as (474b).

Reaction of sulphur-seleno derivative (474b) with meta-chloroperbenzoic acid (mCPBA)

The sulphur-seleno derivative (474b) (77mg, 1.5×10^{-4} mol) was dissolved in dichloromethane (5 ml) and cooled to 0°C under nitrogen. To the stirred solution, a solution of mCPBA (26 mg, 1.5×10^{-4} mol) in dichloromethane (1 ml) was added dropwise. The reaction mixture was stirred at 0°C and monitored by TLC (C). After 18h, some starting material (474b) was still present (TLC) but a new spot had developed at Rf = 0.76. The solvent was evaporated <u>in vacuo</u>. Infra-red analysis of the crude product indicated the following peaks, νmax/cm^{-1} 3600-2700 (br), 1830, 1720 (br). The residue was chromatographed on silica, using a gradient elution consisting of ethyl acetate and petroleum ether (40-60). Three components were isolated. Component 1

(4.9 mg), Rf 0.74 (B); νmax/cm^{-1} 3040, 2980, 1570, 1475; structure unidentified. Component 2 (9.6 mg), Rf = 0.46 (B); νmax/cm^{-1} 3410, 1830, 1733, 1690,; ^{1}H n.m.r. (200 MHz) δ/ppm 2.10 (s, 3H), 2.30 (s, 3H), 3.80 (s, 3H), 4.53 (s, 2H), 5.1 (d, J = 7.6 Hz, 1H), 6.9 - 7.7 (complex, 6H); Structure identified as the thione derivative (477b). Component 3 (56mg), Rf = 0.26 (B); νmax/cm^{-1} 3415, 1775, 1725, 1695; structure was identified as the starting material (474b) by comparison of infra-red and TLC analysis. Attempts to improve the yield of the thione derivative (477b), by use of excess metachloroperbenzoic acid, resulted in no β-lactam containing component (IR) being isolated.

d)   With trimethyltin chloride

Trimethyltin chloride (88.6 mg, 4.5 x 10^{-4} mol) in dichloromethane (2 ml) was added to a solution of the silver thiolate (465) (214 mg, 3.8 x 10^{-4} mol) in dichloromethane (4 ml). The reaction vessel was covered with aluminium foil and stirred under nitrogen at room temperature and monitored by TLC analysis. After 10 minutes, no starting material remained . Infra red analysis of the crude reaction indicated the following peaks: νmax/cm^{-1} 3400 (br), 1760 (br), 1720, 1650. The residue was purified by flash chromatography using two isocratic eluent systems, ethyl acetate/dichloromethane (1:1) and 100% ethyl acetate. A white solid was isolated (116 mg) from the first eluent system, Rf = 0.45 , (ethyl acetate); νmax/cm^{-1} 3410, 3290, 1765, 1720, 1700, 1665; ^{1}H n.m.r. (200 MHz) δ/ppm 0.63 (S, 0.25H), 2.02 (s, 3H), 2.14 (s, 3H), 3.71 (s, 3H), 4.2-4.7 (m, 2H), 5.01-5.44 (m, 2H), 6.8- 7.3 (m, 5H), 8.4 (s, 1H); structure was tentatively suggested as (513). Work was stopped at this stage with this sample.

e)   With chlorotrimethylsilane

The silver thiolate (465) (126 mg, 2.68 x 10^{-4} mol) was dissolved in dichlormethane (5 ml) and stirred under nitrogen.   The reaction mixture was then cooled to 0°C, excess chlorotrimethylsilane (0.3 ml, 2.4 x 10^{-3} mol) was added and the reaction mixture was stirred for 24h.   The reaction mixture was filtered through kieselguhr and the residue washed with dichloromethane. The solvent was evaporated in vacuo.   Infra-red analysis of the crude product indicated the following peaks:- Vmax/cm^{-1} 34.0, 3300 (brd), 176$\mathfrak{5}$, 1720, 1685, 1670. Attempts to obtain a reasonable ^{1}H n.m.r. spectrum were unsuccessful.   The peaks were found to be too broad. Work was stopped at this stage with this sample.

1.3.0. Novel route for the Conversion of Penicillin V to a cephalosporin nucleus

1.3.1. Atempted reaction of 4-Nitrobenzyl (C3R)-phenoxyacetamido-(4R)-acetylthio-zetidin-2-one-1-yl) triphenylphosphoranylidenacetate (413) with 5-oxo-2-phenyl-1,3-diozane (529)

METHOD A

The ketone (529) (324 mg, 1.2 x 10^{-3} mol), obtained by vacuum distillation of the hydrated form (534)[256] was dissolved in dichloromethane (5 ml) under nitrogen.   To the reaction mixture was added a solution of the phosphorane (413) (150 mg, 2 x 10^{-4} mol) in dichloromethane (10 ml).   The reaction mixture was stirred at room temperature and monitored by TLC and I.R. analysis.   After 23h, no reaction was detectable (TLC

(A)). The reaction mixture was heated at 45°C for 24h. Analysis indicated only the presence of starting materials (413) and (529). These were isolated by flash chromatogrphy using a gradient elution of ethyl acetate and petroleum ether (40-60). The first sample was a white solid (207 mg); νmax/cm^{-1} 1745; ^{1}H n.m.r. (60 MHz) δ/ppm 4.6 (s, 4H), 5.9 (s, 1H), 7.5 (s, 5H); This was identified as the ketone (529). Further proof of its identity was obtained by reacting with ethyltri-phenylphosphoranylidenacetate in dichloromethane to result in the condensation product. Chromatography resulted in a clear oil (350 mg). νmax/cm^{-1} 2820, 1710, 1665; ^{1}H n.m.r. (80 MHz) δ/ppm 1.2 (t, 7Hz, 3H), 4.2 (q, 7Hz, 2H), 4.5 (s, 2H), 4.7 (s, 1H), 5.6 (s, 2H), 5.8 (m, 1H), 7.3 (m, 5H).

The second sample was a yellow foam (140 mg, 93% recovery) νmax/cm^{-1} 3410, 1770, 1700, 1630, 1520, 1350; Rf 0.38 (ethyl acetate). This was identified as the phosphorane (413). Further proof of its identity was obtained by reaction with formaldehyde to afford the condensation product, the acrylate derivative (414) Rf 0.51 (A), νmax/cm^{-1} 3410, 1780, 1730, 1700, 1520, 1350; ^{1}H n.m.r. (60 MHz) δ/ppm 2.3 (s, 3H), 4.7 (s, 2H), 5.00 (d, J - 10Hz, 1H), 5.4 (s, 2H), 5.7 (dd, J = 10, 4Hz, 1H), 6.1 (m, 3H), 6.9 - 8.4 (m, 9H).

METHOD B

The ketone (529) (320 mg, 1.79×10^{-3} mol) was dissolved in dry toluene (30 ml) and flushed with nitrogen. A solution of the phosphorane (413) (1.34g, 1.8 mmol) in dry toluene (10 ml) was added to the stirred reaction mixture at room temperature. The solution was refluxed for 24 hrs. The reaction solvent was removed in vacuo and the residue chromatographed on silica using eluent system B. Three components were isolated. Component 1 was a clear gum (360 mg), Rf 0.63 (B);

ν_{max}/cm^{-1} 1745, 1130; ^{1}H n.m.r. (60 MHz) δ/ppm 4.5 (s, 4H), 5.9 (s, 1H), 7.55 (s, 5H). This component was identified as ketone (529). Component 2 was a beige solid (360 mg). Rf 0.41 (B), Mpt 134-135°C, ν_{max}/cm^{-1} 1720, 1525, 1350; ^{1}H n.m.r. (80 MHz) δ/ppm 2.8 (s, 3H), 5.5 (s, 2H), 8.0 (AB, 4H), 8.7 (s, 1H); M.S. (FAB, Thioglycerol) m/e 278 (M$^+$), $C_{12}H_{10}N_2O_4S$ (M.W 278.0361); Found C,51.2; H, 3.6; N, 9.8 requires C,51.7; H,3.6; N,10.1%. This component was identified as 2-methyl-3-(4-nitro benzyloxy carbonyl)-thiazole (535) (93% yield). Component 3 was a yellow foam (300 mg), Rf 0.21 (A), ν_{max}/cm^{-1} 3410, 1765, 1695, 1630, 1525, 1350; ^{1}H n.m.r. (60 MHz) δ/ppm 2.3 (s, 3H), 4.7 (s, 2H), 5.4 (s, 4H), 7.0 - 8.5 (m, 25H); this was identified as the phosphorane (413) (22% recovery). Work was stopped at this stage with this synthetic approach.

1.4.0   β-Lactam Derivatives on Solid Supports

1.4.1   Synthesis of (±) 3-(4-chloromethylphenyl)-3-trimethylsilylpropanoyl chloride (570)[252]

Thionyl chloride (7.14g, 0.06 moles) was added to the hydroxy-acid derivative (549) (3g, 0.012 mol)[264] and stirred for 1.5 h. in DMF (10 ml). The solvent and excess $SOCl_2$ were removed <u>in vacuo</u> to afford the crude acyl chloride (570) (3.1g, 90%) of sufficient purity for use in the subsequent step. ν_{max}/cm^{-1} 2960, 1800, 825; ^{1}H n.m.r. (80 MHz) δ/ppm 0.0(s, 9H), 2.7 (dd, J = 5.1, 10.5 Hz, 1H), 3.2 (complex, 2H), 4.6 (s, 2H), 7.0 - 7.3 (ABq, 4H).

1.4.2 Synthesis of (+) N,N-Diethyl 3-(4-chloromethyl-phenyl)-3-trimethylsilylpropanoamide (565)

The acid chloride (570) (0.5g, 0.002 mol) was dissolved in dichloromethane (5 ml), diethylamine (0.15g, 0.002 mol) , saturated sodium bicarbonate (5 ml) and brine (5 ml) added, and the mixture stirred until reaction was complete (TLC). The organic layer was then separated, washed with sat. sodium bicarbonate (3 x 10 ml), citric acid (3 x 10 ml), brine (2 x 10 ml) and water (2 x 10 ml). The organic layer was dried over magnesium sulphate, filtered and the solvent removed in vacuo. The residue was chromatographed on silica using a gradient eluent system with ethyl acetate and petroleum ether (40-60). The required product (565) was obtained as a yellow viscous oil (0.36g) in 62% yield. Vmax/cm^{-1} 2970, 1633, 864, 840; ^{1}H n.m.r. (80 MHz) δ/ppm 0.0 (s, 9H), 1.0 (t, J - 9.9 Hz, 3H), 1.1 (t, J = 9.9 Hz, 3H), 2.7 (complex, 3H), 3.2 (q, J = 9.9 Hz, 4H), 4.5 (s, 2H), 7.1 - 7.3 (ABq, 4H); Found C, 62, 4; H, 8.5; N; 4.2; $C_{17}H_{28}NOClSi$ Requires C, 62. 7; H, 8.6; N, 4.3%.

1.4.3 Reaction of N,N-diethyl (3)-(4-chromomethyl-phenyl)-(3)-Trimethylsilylpropanoamide (565) with potassium salt of penicillin-V (564a)

To a stirred suspension of potassium penicillin V (564a) (290 mg, 7.47 x 10^{-4} mol) in dimethylformamide (6 ml) was added a solution of the chloro-amide derivative (565) (240 mg, 7.5 x 10^{-4} mol) in dimethylformamide (5 ml). Potassium iodide (23 mg, 1.4 10^{-4} mol) was added, and the reaction mixture was stirred under nitrogen for 24h. The reaction was monitored by TLC analysis (C). The suspension was filtered through kieselguhr and washed with dichloromethane (4 x 20 ml). The solvent was

evaporated <u>in vacuo</u> and the residue was chromatographed on silica, using a gradient eluent system with dichloromethane and ethyl acetate. The product (566a) was isolated as a gum (101 mg) in 30% yield (unoptimised). Rf = 0.65 (C); νmax/cm^{-1} 3405, 1790, 1745, 1695, 1635; ^{1}H n.m.r. (80 MHz) δ/ppm 0.0 (s, 9H), 1.1 (M, J = 6Hz, 6H), 1.3 (s, 3H), 1.6 (s, 3H), 2.8 (m, 3H), 3.3 (q, J = 6Hz, 4H), 4.5 (s, 1H), 4.6 (s, 2H), 5.2 (s, 2H), 5.6 (m, J = 4,12 Hz, 2H), 6.9 - 7.4 (m, 10H).

1.4.4 <u>Reaction of N,N—diethyl 3-(4-chloromethyl phenyl)-3-Trimethyl silyl propanoamide (565) with sodium 2-thienyl acetamido cephalosporinate (564b).</u>

To a stirred suspension of (564b) (305 mg, 7.6 x 10^{-4}) mol) in dimethylformamide (7 ml) was added a solution of the chloro—amide (565) (243 mg, 7.6 x 10^{-4} mol) in dimethylformamide (5 ml) and potassium iodide (24mg). The reaction mixture was stirred under nitrogen for 8h, the reaction being monitored by TLC analysis (C). The reaction solvent was evaporated <u>in vacuo</u>. The residue was dissolved in dichloromethane and the suspension filtered through kieselguhr. The kieselguhr was washed several times with dichloromethane (4 x 20 ml), the solvent removed <u>in vacuo</u>, and the residue chromatographed on silica using a gradient eluent system with dichloromethane and ethyl acetate. The product (566b) was isolated as a gum (3005 mg) in 60% yield (unoptimised). Rf 0.49 (C); νmax/cm^{-1} 3410, 1785, 1735, 1670, 1630, 1223; ^{1}H n.m.r. (80MHz) δ/ppm 0.0 (s, 9H), 1.1 (m, J = 7Hz, 6H), 2.1 (s, 3H), 2.8 (m, 3H), 3.4 (m, J = 7Hz, 5H), 3.8 (s, 2H), 4.5 - 5.2 (complex, 5H), 5.8 (dd, J = 5, 9Hz, 1H), 6.4 (d, J = 9Hz, 1H), 6.9-7.3 (complex, 8H).

1.4.5 Cleavage of the (R) phenyoxyacetamido-penicillinanic acid (569a) from the "handle" derivative (566a) with tetra-n-butylaminonium fluoride (TBAF)

The "handle" derivative (566a) (40 mg, 6.3×10^{-5} mol) was dissolved in dry acetonitrile (5 ml) and flushed with nitrogen. The reaction mixture was stirred and cooled to 0°C, and a solution of TBAF in acetonitrile (0.022 M, 3 ml) was added dropwise. The reaction mixture was stirred for 5 min at 0°C and the solvent removed _in vacuo_. The residue was triturated with diethyl ether (3 x 10 ml), and then dissolved in distilled water (10 ml). The solution was washed with ethyl acetate (3 x 5 ml), and then cooled to 0°C. Ethyl acetate (10 ml) was added and the vigorously stirred solution was acidified carefully to pH 2.5 with 2N hydrochloric acid. The organic layer was removed and dried with sodium sulphate. TLC analysis indicated that no starting material was present (Rf = 0.65 (C)) and the solvent was removed _in vacuo_. A gum resulted (21 mg); ν_{max}/cm^{-1} 3405, 1788, 1730, 1690. Comparison with an authentic sample of (6R), 5R-Phenoxy- acetamido penicillanic acid (569a) resulted in excellent agreement ν_{max}/cm^{-1} 3405, 1786, 1728, 1690. The sample from diethyl ether washing had the following data:- ν_{max}/cm^{-1} 1650; ^{1}H n.m.r. (80 MHz) δ/ppm 1.1 (t, J = 9Hz, 6H), 2.3 (s, 3H), 3.5 (q, J = 9Hz, 4H), 6.7 - 7.8 (complex, 6H); these data suggested that the structure was a cinnamide derivative (567).

Cleavage of (7R,6R) 2-thienylacetamidocephalosporanic acid (569b) from the "handle" derivative (566b) with tetra-n-butyl ammonium fluoride (TBAF)

The "handle" derivative (566b) (60 mg, 9.21×10^{-5} mol) was dissolved in acetonitrile (10 ml) and flushed

with nitrogen. The solution was cooled to 0°C and triethylamine in acetonitrile (0.2M, 0.5ml) was added. To the stirred reaction mixture a solution of TBAF (0.28 mM, 3.30ml) was added dropwise.

After 20 min., all volatile materials were removed in vacuo. To the residue, distilled water (30 ml) was added and a diethyl ether (3 x 20ml) extraction carried out. The aqueous layer was cooled to 0°C and ethyl acetate (20 ml) was added. The two phase system was vigorously stirred and adjusted to pH 8 with 5% w/v sodium carbonate. The organic layer was separated. The aqueous layer was diluted with ethyl acetate, cooled to 0°C and acidified to pH 2.5 with 2N hydrochloric acid. The organic layer was separated, washed with brine, dried over sodium sulphate and filtered. The filtrate was concentrated in vacuo. A yellow gum resulted (20 mg) which was not crystallisable. νmax/cm^{-1} 3405 (br), 1783, 1740, 1685, 1604; This proved to be similar to an authentic sample of keflin acid (569b): νmax cm^{-1} 3405 (br), 1785, 1742, 1685, 1605. The ethyl acetate extraction of the basified solution resulted in a mixture of the cinnamide derivative (567) and the tetra-n-butylammonium keflin salt (568b). The diethyl ether extraction resulted in the cinnamide derivative (567); νmax/cm^{-1} 1650.

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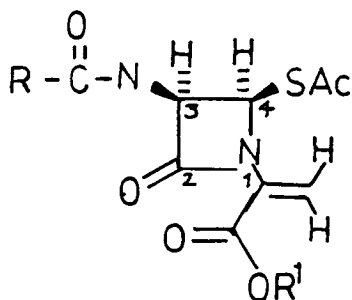
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269.
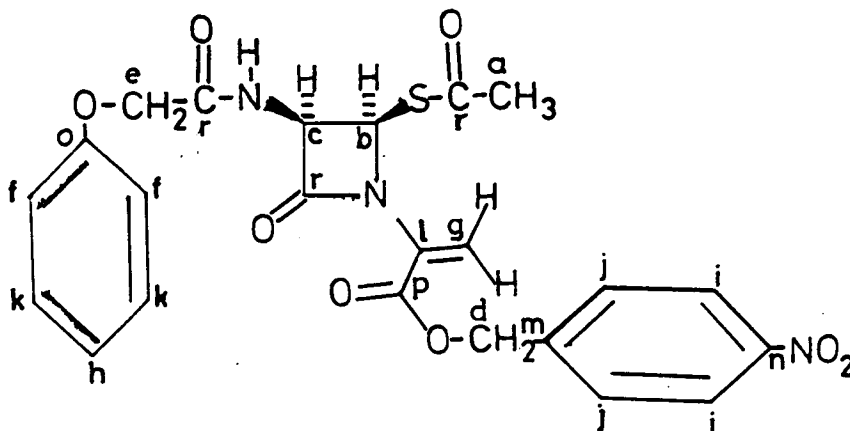
270. Sample supplied by Beecham Pharmaceuticals PLC.