Synthesis of Nuclearly-Modified Cephalosporin Antibiotics

THESIS

presented to the University of Glasgow
in part fulfilment of the requirements
for the degree of Doctor of Philosophy

by

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1980

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To my Mum and Dad

"chance favours only the mind that is prepared"

Louis Pasteur.

ACKNOWLEDGEMENTS

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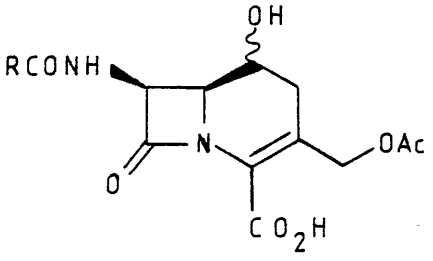
SUMMARY

Approaches are described towards synthesis of 1-hydroxy-1-carba-cephalosporin (1), a chemically interesting nuclearly modified cephalosporin analogue which may possess potent antibiotic activity.

Approaches to a bicyclic tetrahydropyridine (27), potentially convertible into carbacephem (1), are also discussed.

Oxygenated 1-carbacephems (95) and (97) have been prepared *via* a route employing the reaction between imines and a ketene precursor to give β-lactams. The 1α-isomer (95) exhibited weak antibiotic properties.



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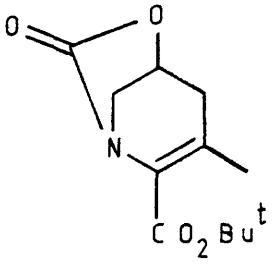

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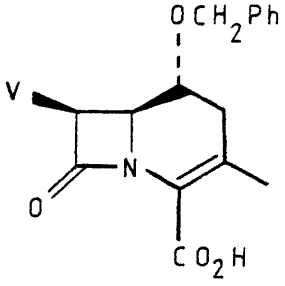

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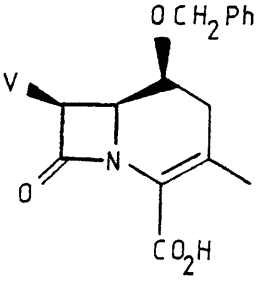

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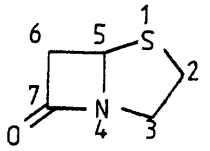
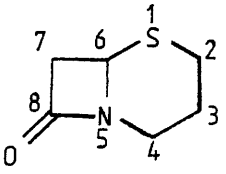
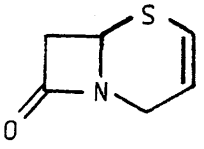
Abbreviations and Conventions

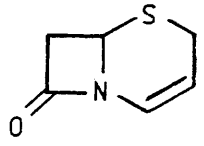
All compounds prepared during the course of this research were racemic, but for clarity only one enantiomer is represented diagramatically.

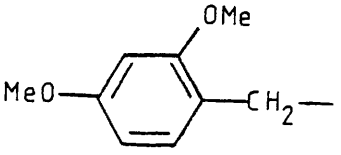
The following abbreviations have been used throughout this thesis:

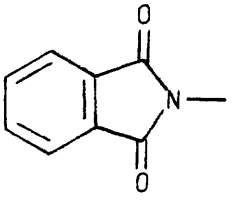
Penam 

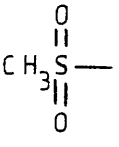
Cepham 

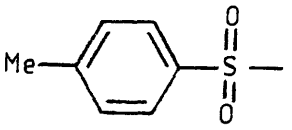
Δ^2- Cephem 

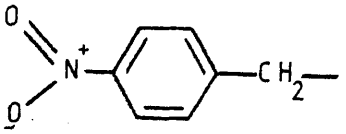
Δ^3- Cephem 

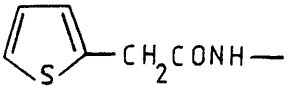
DMB 

Ft 

Ms 

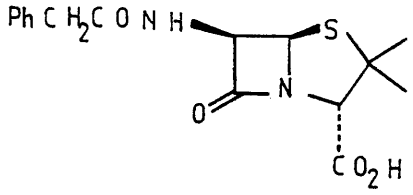
Ts 

PNB 

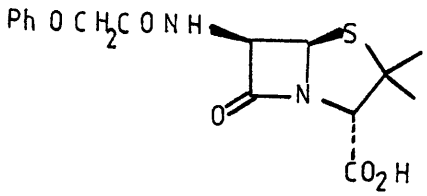
Th 

G	$PhCH_2CONH-$
V	$PhOCH_2CONH-$
Bu^t	$(CH_3)_3\ C-$
BOC	$(CH_3)_3\ C-O-\ C(O)-$
DCC	Dicyclohexylcarbodiimide
DME	Dimethoxyethane
DMSO	Dimethylsulphoxide
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran

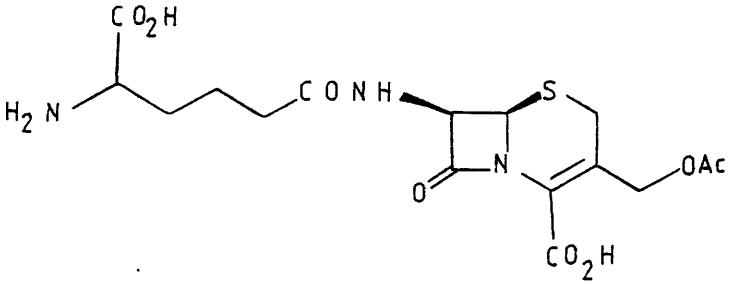
Contents



(1a) pen. G



(1b) pen. V



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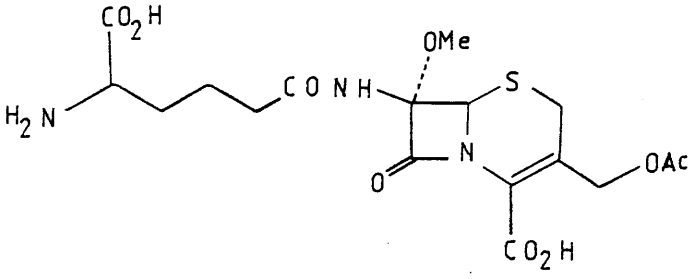

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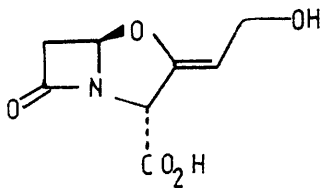
Introduction

(a) Prologue

The history of β-lactam antibiotics began in 1928 with the discovery[1] of penicillin by Alexander Fleming at St. Mary's Hospital, London. Twelve years then elapsed before Florey and Chain, in Oxford, isolated[2] and purified penicillin from the crude mould extracts; it was yet another five years before the combined results of chemical degradation and X-ray crystallography established[3] the structure (1). This delay, from discovery to structure elucidation, was due largely to the extreme lability of the molecule and the unprecedented occurrence of an azetidin-2-one unit in a natural product. The second class of β-lactam antibiotics to be discovered was the cephalosporin group. Giuseppe Brotzu, working in Sardinia, reported in 1948 that a mould, Cephalosporium acremonium, produced antibiotic material that showed activity against both Gram positive and Gram negative organisms. Brotzu believed the isolation of the active principal to be beyond his limited resources, and succeeded in having this work continued in Oxford by Abraham, Newton and their collaborators[4]. After extensive investigations cephalosporin C (2) was isolated and its structure determined[5] in 1961. Synthetic efforts to improve the biological potency and spectrum of activity of these antibiotics initially concentrated on varying the amide side chain functionality common to both penicillins and cephalosporins. This situation has changed dramatically in the last decade with the discovery of many more fundamentally modified systems, some being naturally occurring and others synthetic in origin.

In 1971 the highly active 7α-methoxycephalosporin (3), a cephamycin, was isolated from a culture of Streptomyces lipmanii at the



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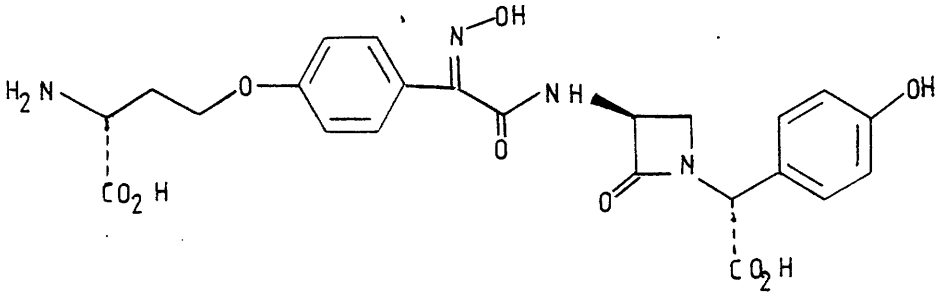

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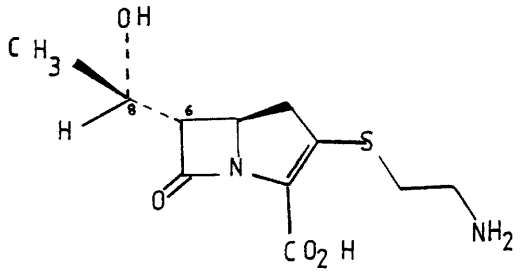

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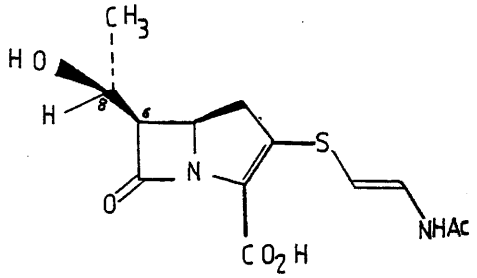

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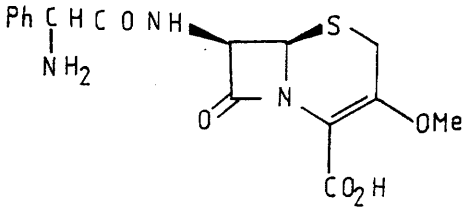

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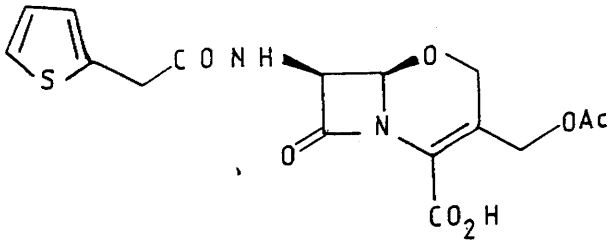
Eli Lilly laboratories[6]. In 1976 three new classes of β-lactam antibiotics were reported. A group at Beechams disclosed[7] the isolation of clavulanic acid (4), a potent β-lactamase inhibitor, from Streptomyces clavuligerus; this compound differed from all previously known β-lactam antibiotics in lacking an amide side chain and in possessing an oxygen instead of a sulphur atom at position 1. Chemists at the Fujisawa company in Japan reported[8] the isolation of a monocyclic β-lactam antibiotic, nocardicin (5), from a species of Nocardia uniforms. A team at Merck Sharp and Dohm in the U.S.A. revealed[9] their discovery of thienamycin (6) in fermentation broths of Streptomyces cattleya; almost simultaneously Beechams' chemists reported[10] the isolation of a related compound, olivanic acid (7), from Streptomyces olivaceous. In olivanic acid and thienamycin the amide side chain has been replaced by a hydroxyethyl side chain, sulphur has been replaced by a methylene group at position 1, and there is now a double bond in the fused five membered ring. Since the discovery of thienamycin and olivanic acid several other related compounds have been discovered which differ only in the stereochemistry at positions 6 and 8; these compounds are collectively known as the olivanic acids. Thienamycin and the olivanic acids function both as β-lactamase inhibitors and as broad spectrum antibiotics.

The current range of naturally occurring β-lactam antibiotics can thus be grouped into approximately six classes as indicated above. This is not so for those derived by synthesis. Chemists working in the laboratories of universities and pharmaceutical companies have produced vast numbers of β-lactam systems with novel structures, some of which show pronounced biological activity. The following is a very small selection of the more important synthetic antibiotics. In 1974, chemists at two companies, Eli Lilly[11] and Ciba Geigy[12], independently synthesised the new 3-methoxycephalosporin (8), the prototype of a



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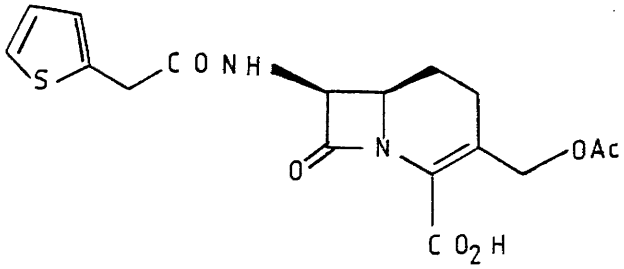

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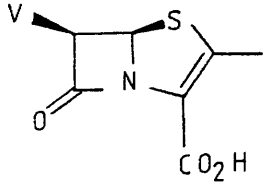
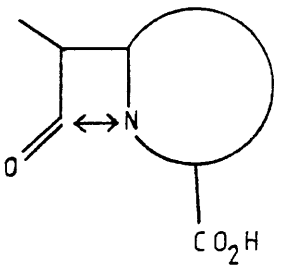


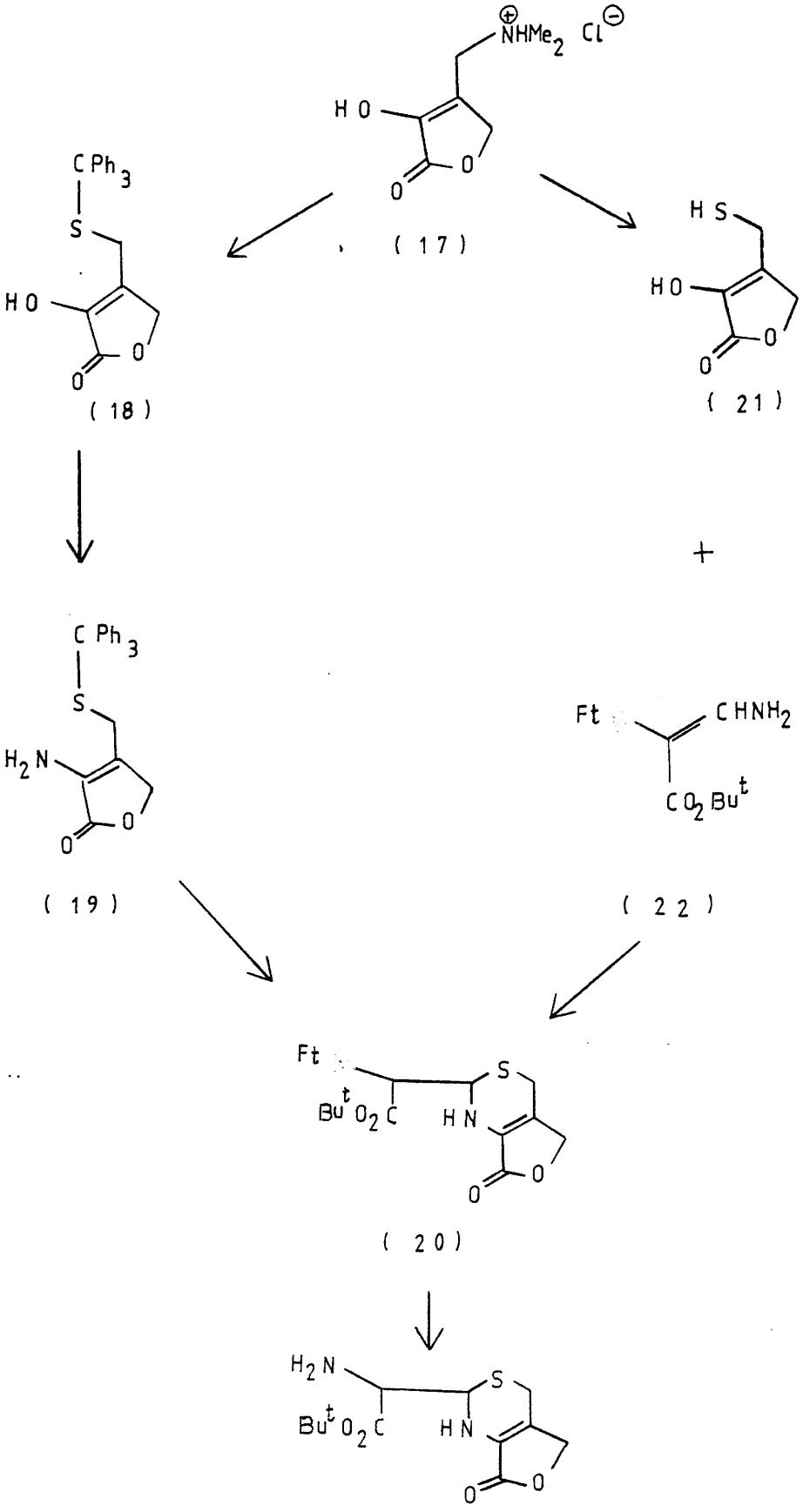
(11)

group of highly active cephalsporins with an electronegative substituent at position 3 of the cephem skeleton. In the same year, chemists in the laboratories of Merck Sharp and Dohm published syntheses of 1-oxacephalothin[13] (9) and 1-carbacephalothin[14] (10), both of which showed pronounced antimicrobial activity. In 1976 Woodward described[15] the synthesis of the penem (11), a molecule which combines the fused five membered ring of penicillin with the enamine structure of cephalosporin. Many of the β-lactam compounds cited above deviate markedly from the structural features of the original penicillins and cephalosporins while retaining potent antibiotic activity, in some cases even greater than that of the parent compounds. This has led to the synthesis of a plethora of novel β-lactam structures in the search for even more powerful antibiotics. In the following sections some of the strategies which led to syntheses of such compounds will be discussed in some detail.

(b) Sheehan's Synthesis of Penicillin V (1957)



Sheehan and Henery-Logan published[16] the first rational synthesis of a natural penicillin, penicillin V, in 1957. The synthesis was conceived at a time when it was generally believed that the instability of penicillin was due to the presence of the strained four membered lactam ring, hence the formation of this structural feature was post-poned for as long as possible in the synthetic sequence.


⊕NHMe2 Cl⊖
HO
O
O
(17)
CPh3
S
HO
O
O
(18)
HS
HO
O
O
(21)
+
CPh3
S
H2N
O
O
(19)
Ft
CHNH2
CO2But
(22)
Ft
ButO2C
HN
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O
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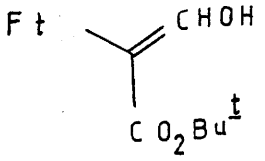

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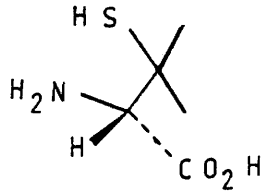

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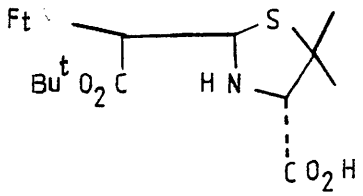

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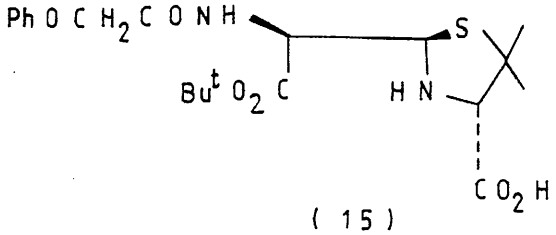

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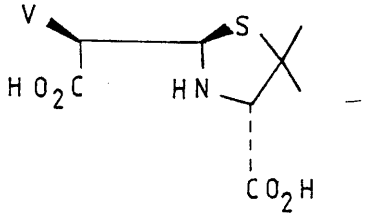

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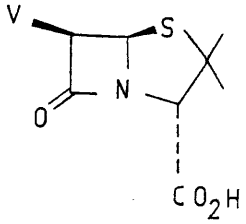
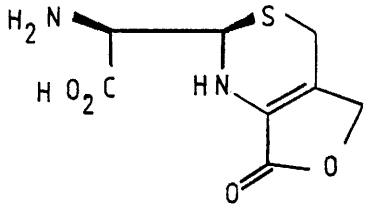


(1b)

(D)-Penicillamine[17] (13) was condensed with t-butyl phthalimido-aldehydomalonate[18] (12) to give the thiazolidine (14). This was a mixture of only two racemates of the four possible stereoisomers, one of which (the α-isomer) corresponded to the configuration found in natural penicillin; the other (the γ-isomer) could be epimerised into the α-isomer by heating in the presence of pyridine. Hydrazinolysis of the phthalimido group of the α-isomer, followed by acylation of the resulting amine with phenoxyacetyl chloride, produced the phenoxy-acetamide (15). The t-butyl ester was then cleaved with dry hydrogen chloride to give the diacid (16), thus leaving formation of the β-lactam ring as the final step in the synthesis. This was achieved using DCC, a reagent introduced by Sheehan[19] for the formation of amide bonds between amines and carboxylic acids.

Research groups working independently in the laboratories of two pharmaceutical companies, Roussel[20] and Squibb[21], have extended Sheehan's methodology to the synthesis of cephalosporin. Both groups used the lactone (17) as starting material. This compound, the condensation product of pyruvic acid, formaldehyde, and dimethylamine, was first prepared by Mannich[22] in 1924. In the Squibb laboratories the dimethylamino group was displaced by trityl mercaptan to give the sulphide (18). Replacement of the hydroxy group by an amino group gave the enamine (19). Condensation with t-butyl phthalimido-aldehydomalonate (12) was followed by deprotection of the thiol group. Spontaneous intramolecular cyclisation then gave the dihydrothiazine (20) as a mixture of stereoisomers. The Roussel group obtained this same inter-mediate from the lactone (17) by displacement of dimethylamine with thioacetic acid followed by acidic hydrolysis to the thiol (21), which was condensed with the aminomethylene-glycine ester (22). Hydrazinolysis of the intermediate (20) gave the free amino compound (23). Treatment



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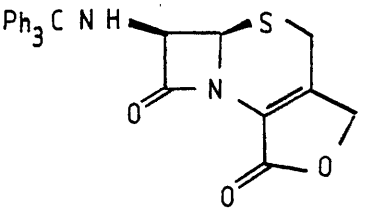

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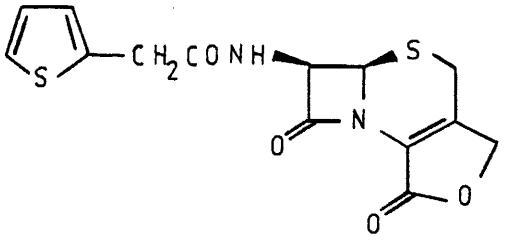

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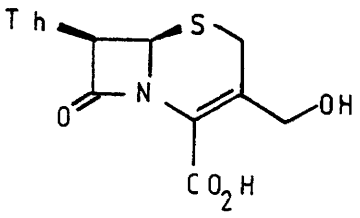

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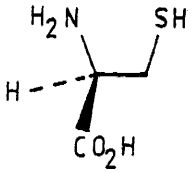

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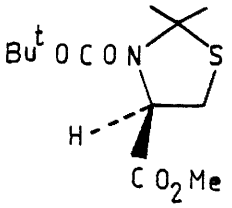

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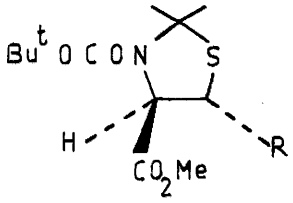


(30) R = NCO_2Me / $NHCO_2Me$

(31) R = OAc

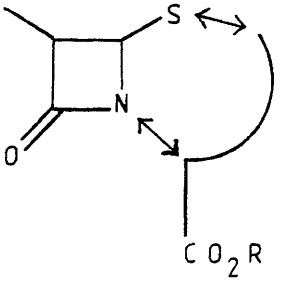
(32) R = OH

(33) R = OSO_2Me

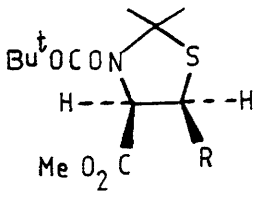
of this with acid caused both ester cleavage, and also equilibration of the stereoisomeric mixture to give mainly the racemic threo acid (24). Reprotection of the amine as its trityl derivative and β-lactam ring formation using DCC gave the cephem (25). The synthesis was completed by deprotection of the amino group and then acylation with thienylacetyl chloride to give the lactone (26). The cephem carboxylic acid (27) could be obtained in only low yield by hydrolysis of the lactone.

This synthetic strategy has not been used to any great extent for the preparation of penicillin or cephalosporin analogues. The route suffers a lack of stereospecificity and the overall yield is very poor. Neither of these criticisms can be levelled against the following cephalosporin synthesis.

(c) Woodward's Synthesis of Cephalosporin C (1965)

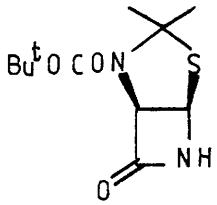


One of the outstanding features of this synthesis[23] is the complete stereochemical control achieved by use of (L)-cysteine (28), masked and activated at the methylene position as the cyclic thiazolidine (29). This chiral building-block reacted stereoselectively with dimethyl azodicarboxylate to form compound (30); oxidative cleavage with lead tetraacetate gave the acetate (31) accompanied with a small amount of the cis epimer. Transesterification liberated the alcohol (32). This



(34) R = N_3

(35) R = NH_2



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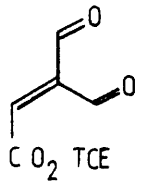

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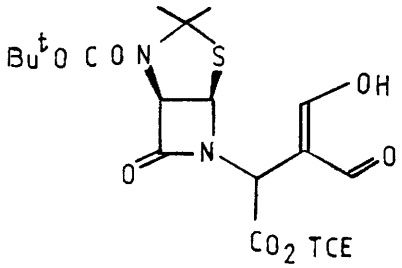

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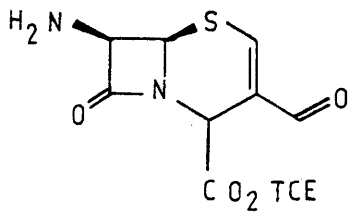

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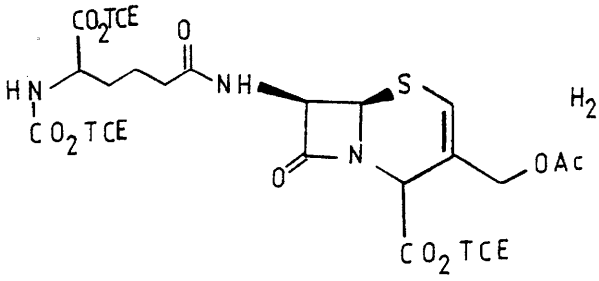

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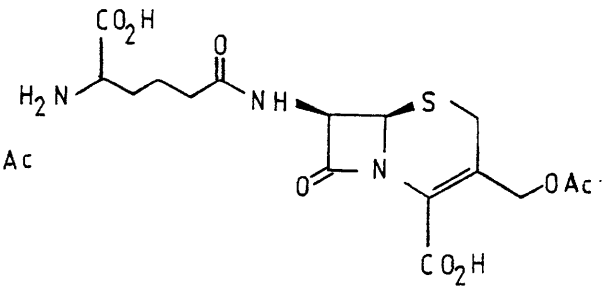

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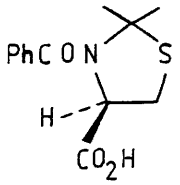

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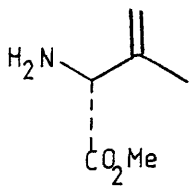

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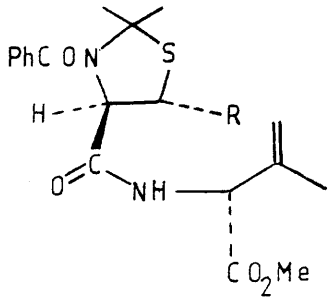


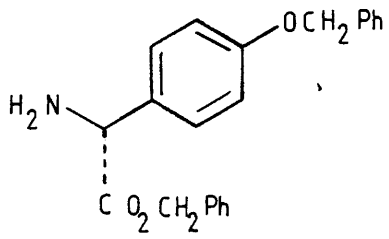
(43) R = H

(44) R = OCOPh

(45) R = Cl

was transformed into the amine (35) by conversion into the mesylate (33), displacement with inversion with azide ion to give azide (34), and finally reduction with aluminium amalgam. Tri-isobutylaluminium effected smooth cyclisation of the amino-ester (35) to the β-lactam (36). This is a key intermediate, a corner stone in penam and cepham synthesis, containing as it does the basic structural elements common to both penicillins and cephalosporins. It has been synthesised[24] from penicillin itself and has been used further in the preparation of nuclearly modified cephalosporin analogues, as will be discussed in more detail in the next section. One final point to note here is that the β-lactam ring has been created at a relatively early stage. To return to the synthesis under discussion, conjugate addition of the lactam nitrogen function to the dialdehyde (37) (the condensation product of malondialdehyde and trichloroethyl glyoxylate) gave the enolised dialdehyde (38). This species now contains all the elements necessary for formation of the cephem skeleton. Treatment with TFA liberated the amino and mercapto groups and caused cyclisation to the Δ^2-cephem (39). Acylation with a protected (D)-α-amino-adipic acid followed by reduction and acetylation gave cephem (40). The synthesis of cephalosporin C was completed by base catalysed isomerisation of the double bond followed by reductive removal of the ester protection.

This general methodology has been extended to the synthesis of other β-lactam antibiotics. In 1976, Baldwin described[25] the first stereocontrolled total synthesis of a penicillin system. Although this employed a similar activation of the methylene group of (L)-cysteine, it differed from Woodward's cephalosporin synthesis in that it involved a peptide-to-β-lactam conversion and was therefore more biogenetically patterned. The peptide (43) was created by condensation of the protected (L)-cysteine (41) with (D)-isodehydrovaline methyl ester (42).



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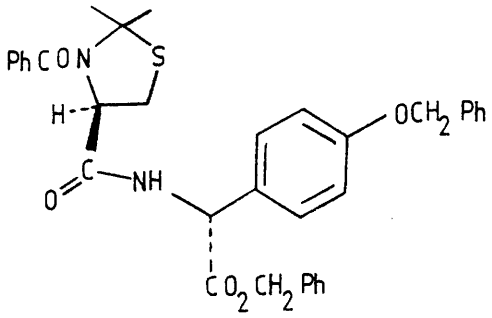

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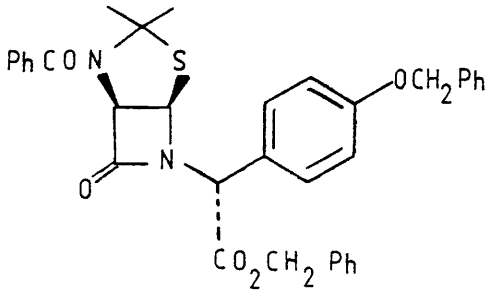

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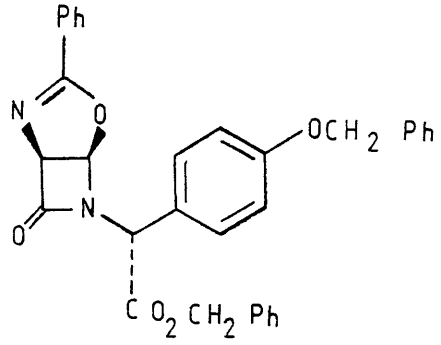

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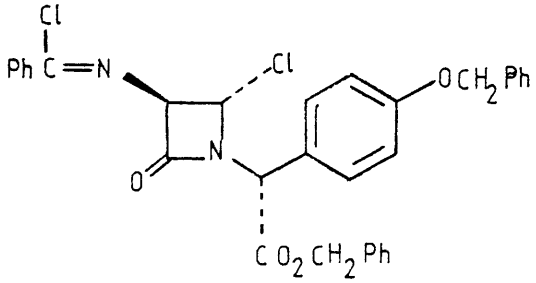
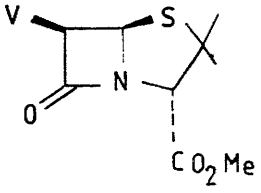


(58)

Functionalisation of the methylene group of this cysteine derivative was achieved by treatment with benzoyl peroxide to give the benzoate (44). Exposure to hydrogen chloride formed the chloride (45), which was then cyclised to the β-lactam (46), using sodium hydride to deprotonate the amide function. This compound now contains all the structural features necessary for penicillin formation. The necessary further transformations are removal of the acetonide protection and cyclisation. These were achieved by the following sequence of reactions. Oxidation with m-chloroperbenzoic acid formed the sulphoxide (47), which was rearranged to the β-keto-sulphide (48). Formation of the epimeric oxiranes (49) and rearrangement then gave the epimeric aldehydes (50) m-Chloroperbenzoic acid oxidation produced a diastereoisomeric mixture of sulphoxides; thermal syn-elimination of either of these isomeric sulphoxides destroys the chirality at sulphur, to form a single sulphenic acid (51). This acid was not isolated, electrophilic attack on the double bond resulting in formation of the sulphoxide (52). Reduction then gave the penicillin (53).

A similar strategy can be seen in a stereocontrolled synthesis[26] of nocardicin A carried out in the laboratories of Eli Lilly and published in 1978. The synthesis parallels that of Baldwin with a protected (D)-isodehydrovaline (42) being replaced by a protected (D)-p-hydroxyphenylglycine (54). DCC-Mediated coupling of the amine (54) with the protected (L)-cysteine (41) furnished the peptide (55). Sequential treatment with benzoyl peroxide, hydrogen chloride, and sodium hydride formed the β-lactam (56), obtained as an epimeric mixture due to epimerisation α to the ester group; recrystallisation under equilibrating conditions gave the pure isomer (56). Treatment of this with aqueous mercuric acetate gave the oxazolidone (57) which underwent cleavage with phosphorus pentachloride to produce the chloro derivative (58).



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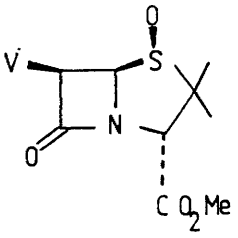

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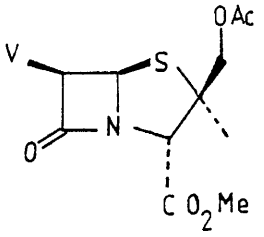

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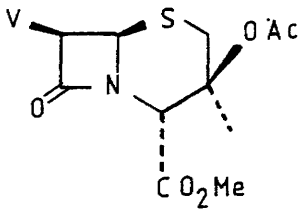

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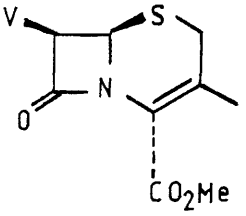

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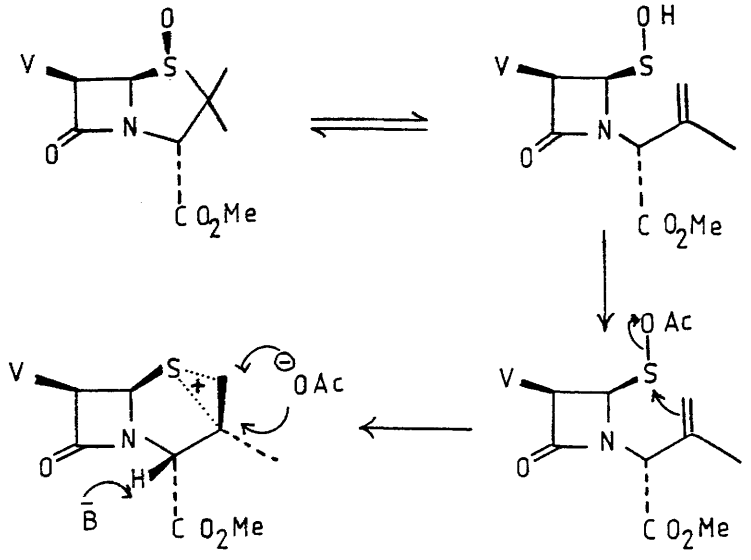
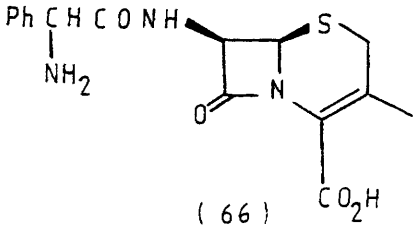


SCHEME 2.



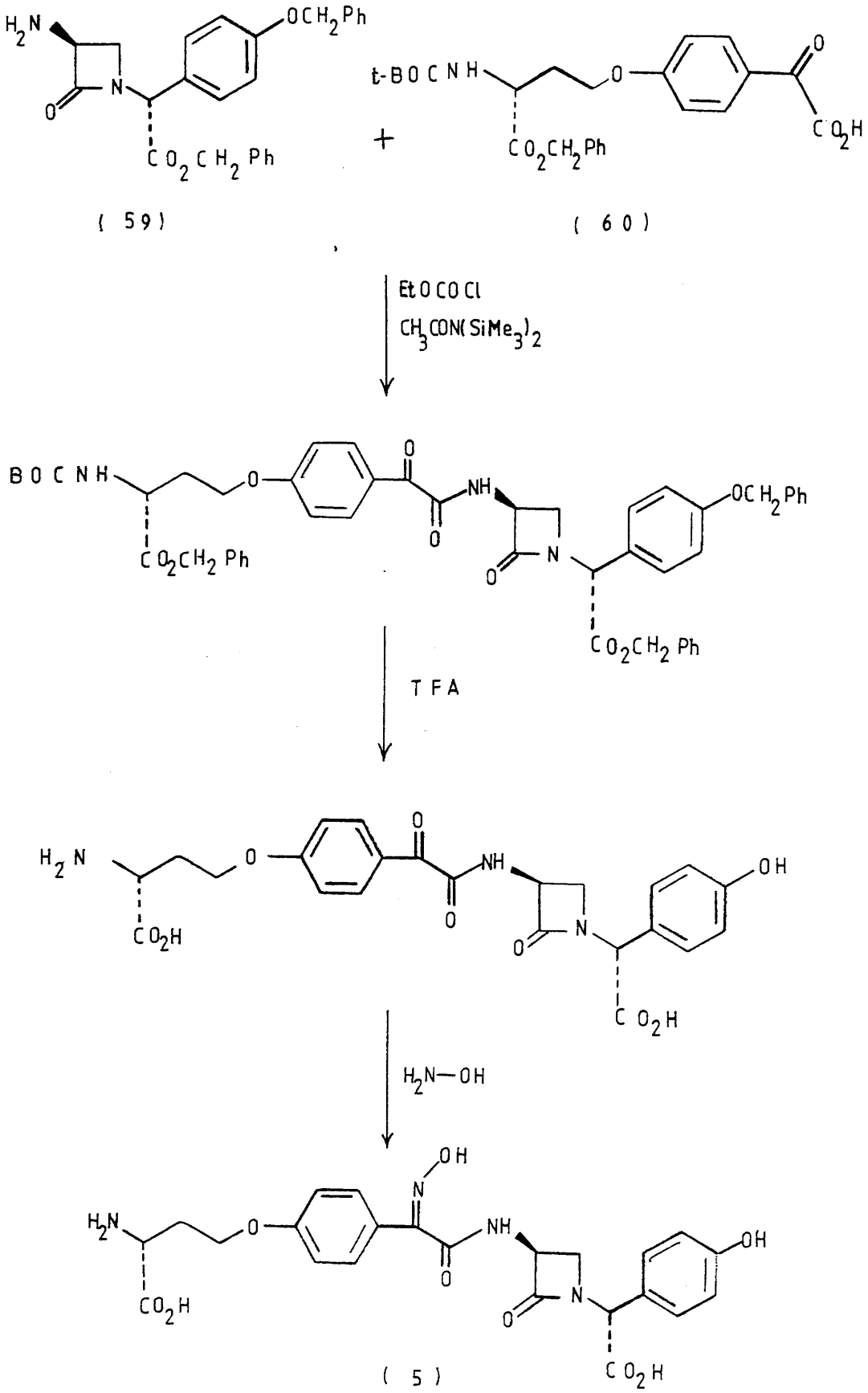
(66)

Reductive dechlorination followed by hydrolysis afforded the protected 3-aminonocardicinic acid (3-ANA) (59). The synthesis of nocardicin A was completed by acylation with the glyoxylic acid[27] (60) as outlined in Scheme 1.

(d) The Conversion of Penicillins into Cephalosporins

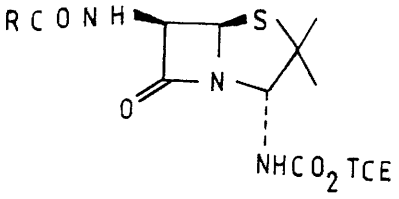
6-Aminopenicillanic acid (6-APA) is produced in very large quantities by fermentation and is therefore quite inexpensive. This is not true of 7-aminocephalosporanic acid (7-ACA), so considerable effort has been expended in the search for methods of conversion of penicillins into cephalosporins. Success was first achieved by Morin [28,29] and co-workers at the Eli Lilly laboratories in 1963. Oxidation of the penicillin ester (61) with sodium metaperiodate gave the β-sulphoxide (62). When heated in acetic anhydride this was converted into a 2:1 mixture of penam (63) and cepham (64). Cepham (64), on treatment with triethylamine, eliminated acetic acid to form 3′-deacetoxycephalosporin (65). Alternatively, the penicillin sulphoxide (62) could be converted directly into the cephalosporin (65) by heating in xylene containing a catalytic amount of TsOH. The mechanism of this reaction involves displacement of a sulphoxide-sulphenic acid equilibrium by formation of an episulphonium ion from the sulphenic acid under the reaction conditions. The various products observed can be explained by alternative fates of the episulphonium ion (Scheme 2). This ring expansion reaction of penicillin sulphoxides is now the basis of a commercial production[30] of the orally active cephalexin (66) from a penicillin.

In 1972 Woodward described[31] the synthesis of a nuclearly modified cephalosporin (74) from a penicillin, which involved a new method for the construction of a bicyclic skeleton from a monocyclic β-lactam.



(59) (60) (5)

SCHEME 1.



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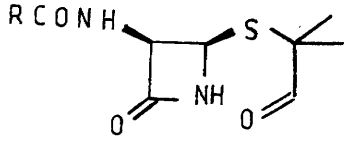
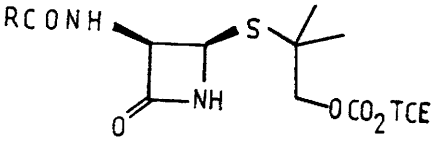


(68)

a. R = $PhCH_2$

b. R = $PhOCH_2$

c. R = Bu^tO



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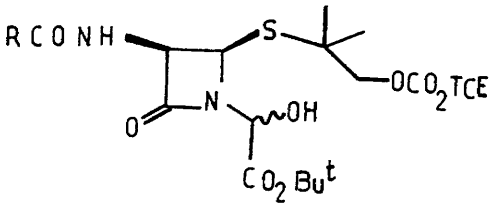

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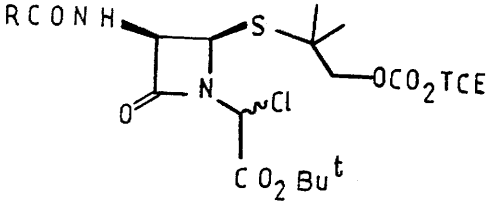

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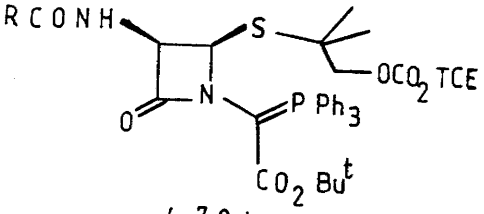

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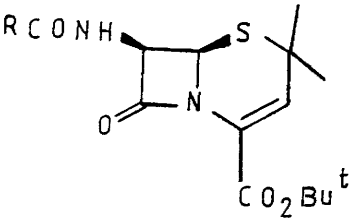

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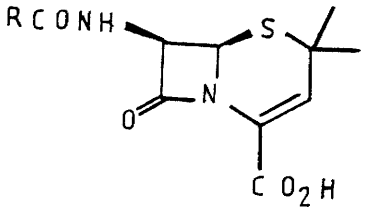
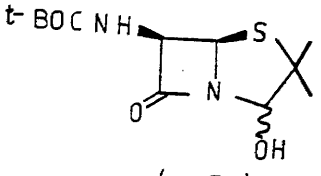


(74) a & b



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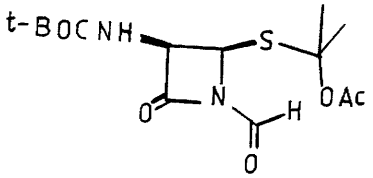

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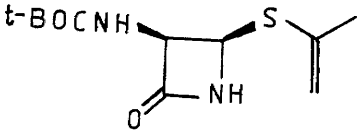

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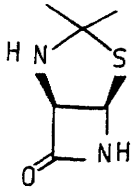

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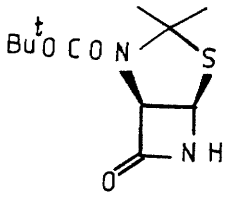
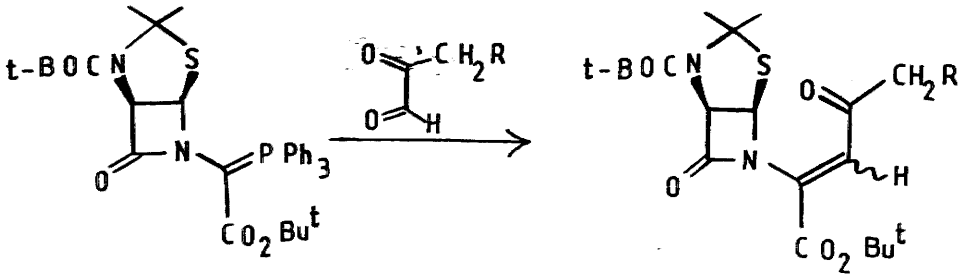


(36)

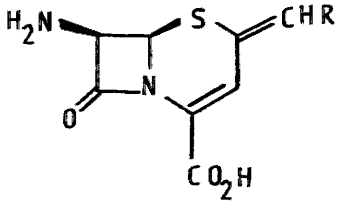
Curtius degradation of a penicillin, followed by treatment of the resulting isocyanate with 2,2,2,-trichloroethanol gave the penicillin derivative (67). Reductive removal of the trichloroethyl group with zinc in the presence of water led to the aldehyde (68). Reduction to the alcohol was followed by reprotection as the 2,2,2,-trichloroethyl carbonate (69). This monocyclic β-lactam condensed with t-butyl glyoxylate to produce an epimeric mixture of hydroxyamides (70), which were converted into the corresponding chlorides (71) using thionyl chloride. Reaction of the chlorides with triphenylphosphine in the presence of base gave the stable phosphorane (72). The sequence (69) to (72) permits a new procedure for construction of a bicyclic skeleton from a monocyclic β-lactam. Two new carbon atoms are introduced, one of which will become the carboxyl group common to both penicillins and cephalosporins and the other is part of a phosphonium ylide which will be used in a Wittig reaction to complete the bicyclic structure. This general method of annelating β-lactams has been used in many laboratories for the synthesis of analogues of penicillin and cephalosporin; some examples of these will be seen in the following sections. The synthesis was completed by deprotection of the alcohol and oxidation to the corresponding aldehyde, which underwent spontaneous cyclisation to the protected cephem (73). Removal of the ester protection gave the free acid (73), which Woodward called a "cephalocillin" because it had features common to both cephalosporin (an enamine group) and penicillin (a gem-dimethyl group).

In the same year, the previously discussed intermediate (36) was synthesised from penicillin by Heusler[24]. The synthesis proceeded via the aldehyde (68c) which exists as the hemiaminal (75). Photo-induced oxidation with lead tetraacetate gave the N-formyl β-lactam (76). Thermal elimination of acetic acid followed by selective cleavage of the formyl group with aqueous ammonia produced the thio-alkene (77).



(79)

(80)



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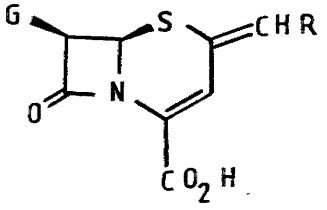
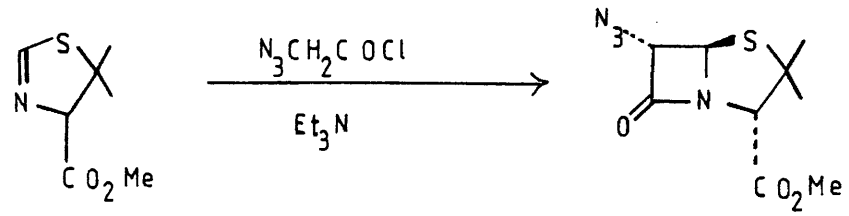


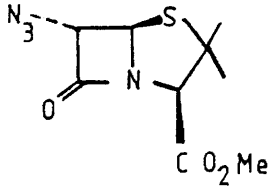
(82)

R= (eg) Me, Et, Ph, PNB.



(87)

(88)



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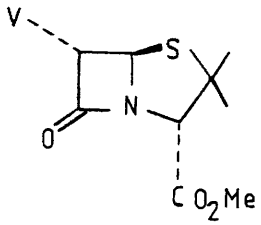
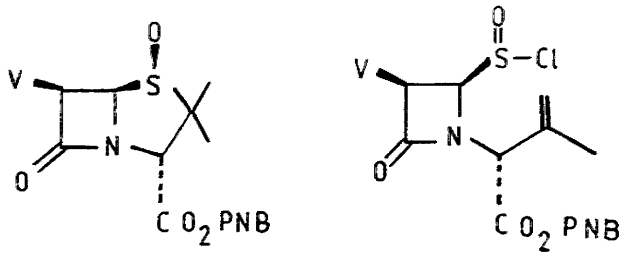


(90)

Liberation of the free amine by treatment with TFA was accompanied by intramolecular cyclisation to give the thiazolidine (78), and thence the key intermediate (36). This represents a formal synthesis of a cephalosporin from a penicillin. However, this is not commercially viable due to the large number of steps involved. Its importance lies in the variety of nuclearly modified cephalosporin antibiotics which have been prepared from the intermediate (36). A comprehensive account of these analogues is outwith the scope of this review, but one series of compounds synthesised[32] from this building block which possess interesting antimicrobial activity is outlined below.

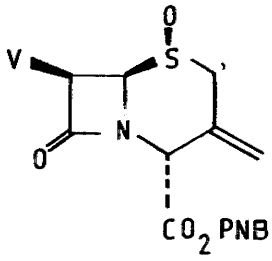
The phosphorane (79) was prepared from (36) by application of the glyoxylate condensation sequence detailed above. Wittig reaction with a series of α-keto aldehydes gave the condensation products (80) as mixtures of geometric isomers. These could be separated chromatographically and additional (Z)-isomer obtained by photochemical isomerisation of the undesired (E)-isomer. TFA-Mediated cleavage of the t-BOC group was followed by intramolecular cyclisation and dehydration; the t-butyl ester was also cleaved, although more slowly, and the ultimate product was the amino acid (81). Acylation gave the cephalosporin analogues (82), which exhibited good antibacterial activity against both normal and penicillin resistant strains of S. aureus and other microorganisms.

The abnormal Pummerer ring expansion is an important commercial route to a number of cephalosporins. The transformation of penicillins into cephalosporins carried out in Woodward's laboratories are not commercially viable but they do allow the preparation of many different analogues. The following method, reported[33] by Kukolja in 1976, offers a penicillin to cephalosporin conversion which is both commercially feasible and can potentially lead to a wide range of cephalosporin



(83)

(84)



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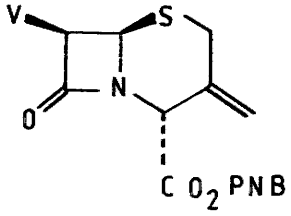

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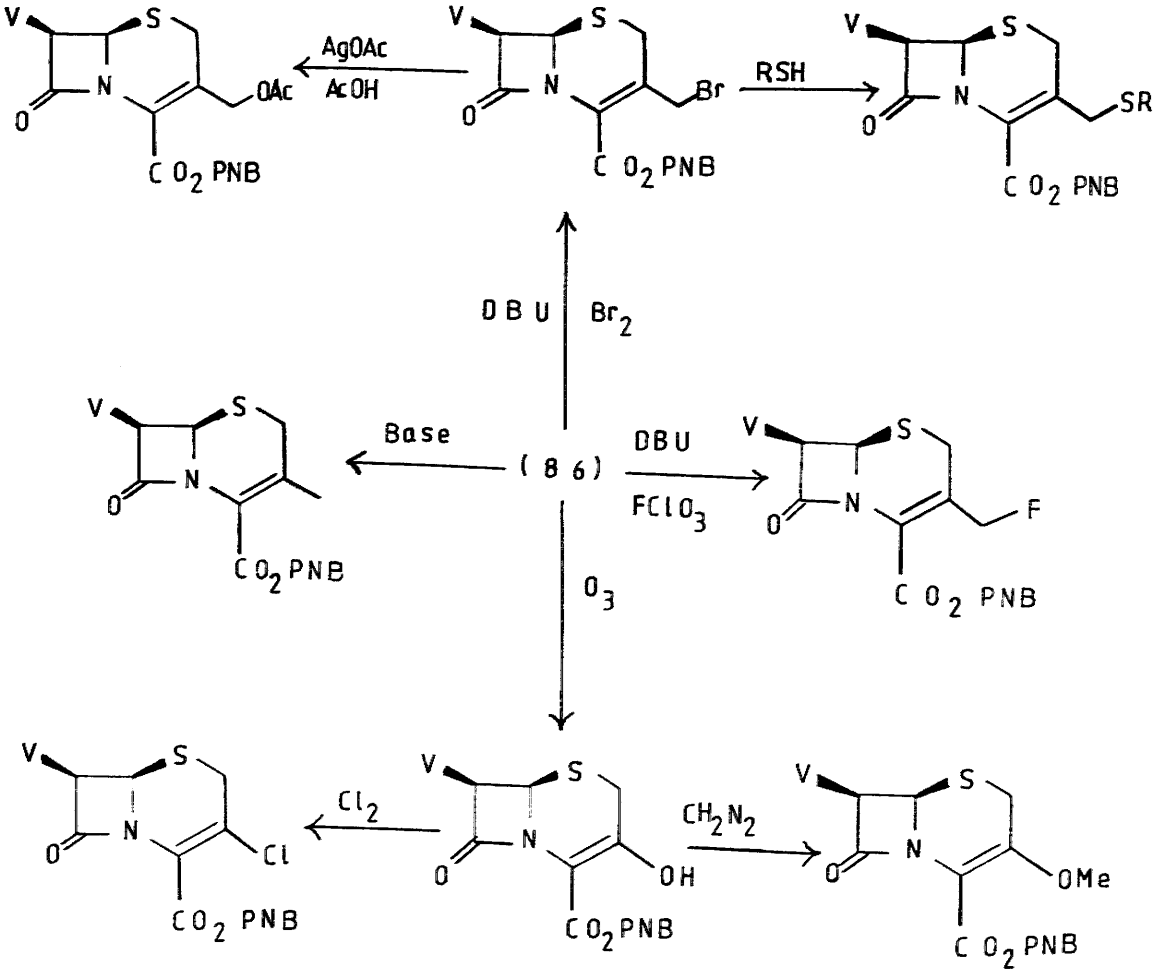
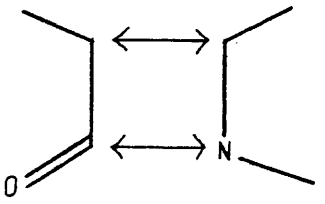


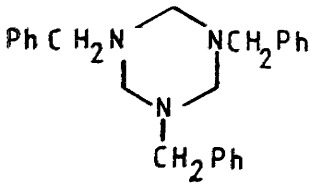
SCHEME 3.

analogues. The exomethylene cepham (86) was synthesised from the penicillin sulphoxide (83) by the following route. Oxidation with N-chlorosuccinimide, or another source of positive halogen, gave the sulphinyl chloride (84), which cyclised to the exomethylene cepham sulphoxide (85) on treatment with Lewis acid. The sulphoxide function was then reduced to give the cepham (86). This constitutes a very short, high yielding sequence of reactions which uses relatively inexpensive reagents. The exomethylene cepham (86) is a versatile intermediate for the synthesis[34] of a wide range of cephalosporins, as outlined in Scheme 3.

(e) The Merck Synthesis (1973)



The synthetic approach adopted by chemists in the laboratories of Merck Sharp and Dohm involves formation of β-lactams by cycloaddition of imines with ketenes or ketene equivalents. This reaction was first observed by Staudinger[35] in 1907 and has been extensively studied by Bose[36]. Indeed, Bose synthesised[37] methyl ($\pm$)-6-epipenicillanate (90) <u>via</u> such a cycloaddition reaction. The thiazoline (87), prepared from N-formyl ($\pm$)-penicillamine, was treated with azidoacetyl chloride and triethylamine to give the <u>trans</u> β-lactam (88) in low yield. Surprisingly, the epimeric β-lactam (89), another possible product



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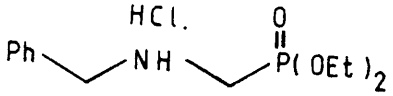

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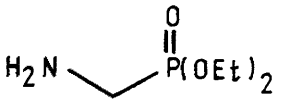

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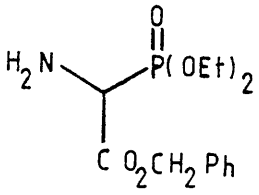
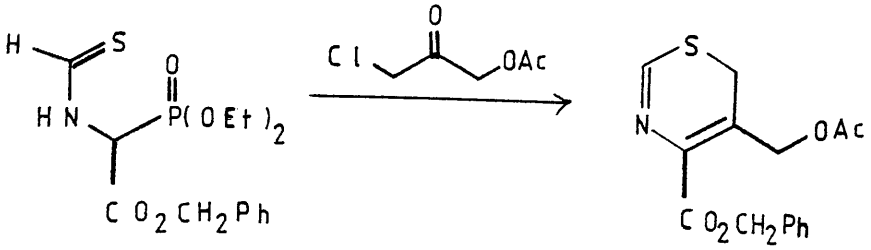
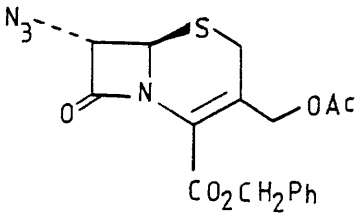


(94)



(95)

(96)



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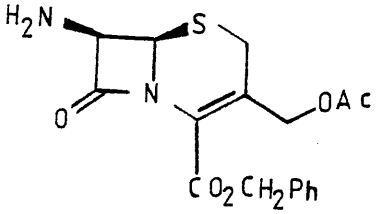

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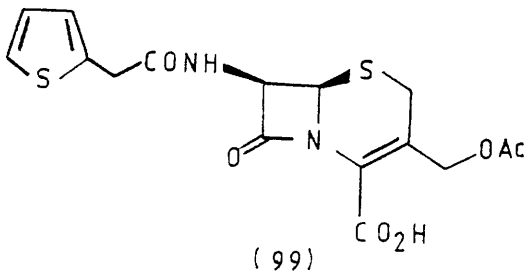
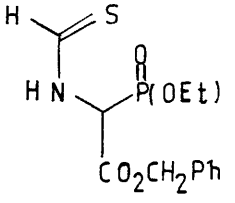


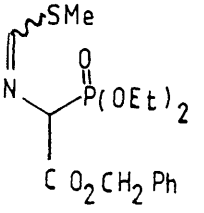
(99)

of this reaction, does not appear to be formed. The isolated β-lactam (88) was reduced to the corresponding amine and then acylated to give the racemic penicillin derivative (90). This synthesis does not produce an active antibiotic and also suffers from a very low yield of the cyclisation product (88).

The Merck group, on the other hand, achieved the synthesis of a biologically active cephalosporin[38] in far superior yield. The triazine (91), prepared from benzylamine and formaldehyde, was heated with diethylphosphite to give diethyl N-benzylaminomethylphosphonate, isolated as its hydrochloride salt (92). Hydrogenolysis and neutralisation furnished diethyl aminomethylphosphonate (93). The Schiffs base from amine (93) and benzaldehyde was acylated by sequential treatment with phenyl lithium and benzyl chloroformate. Deprotection then gave the key synthon (94), which was converted into the thioformamide (95) by treatment with ethyl thionoformate. Condensation with 1-chloro-3-acetoxy-2-propanone produced the thiazine (96). Cycloaddition using azidoacetyl chloride and triethylamine yielded the 7α-azidocephem (97). Epimerisation at C-7 was achieved by reduction to the amine, formation of the Schiffs base with p-nitrobenzaldehyde, deprotonation of the Schiffs base with phenyl lithium, and kinetically controlled quenching of the resulting anion, to give a 55:45 ratio[39] of the 7β:7α isomers. Liberation of the amine and separation of the cis-isomer by chromatography afforded the 7-aminocephem (98). Acylation with thienylacetyl chloride, followed by hydrogenolysis produced racemic cephalothin (99), which possessed half the activity of the naturally derived compound. The kinetically controlled epimerisation described above has been applied by the Merck group to a synthesis[39] of penicillin, in a synthesis which closely parallels that of Bose but which produces a biologically active species.



(95)



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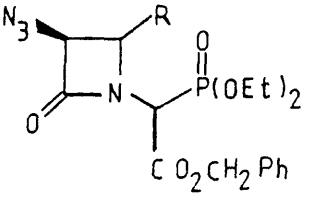
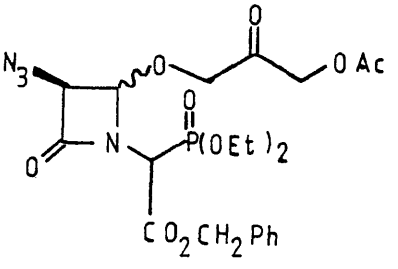


(101) R = ----SMe

(102) R = ~~Cl



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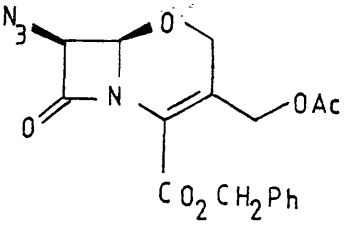
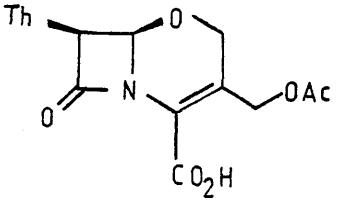
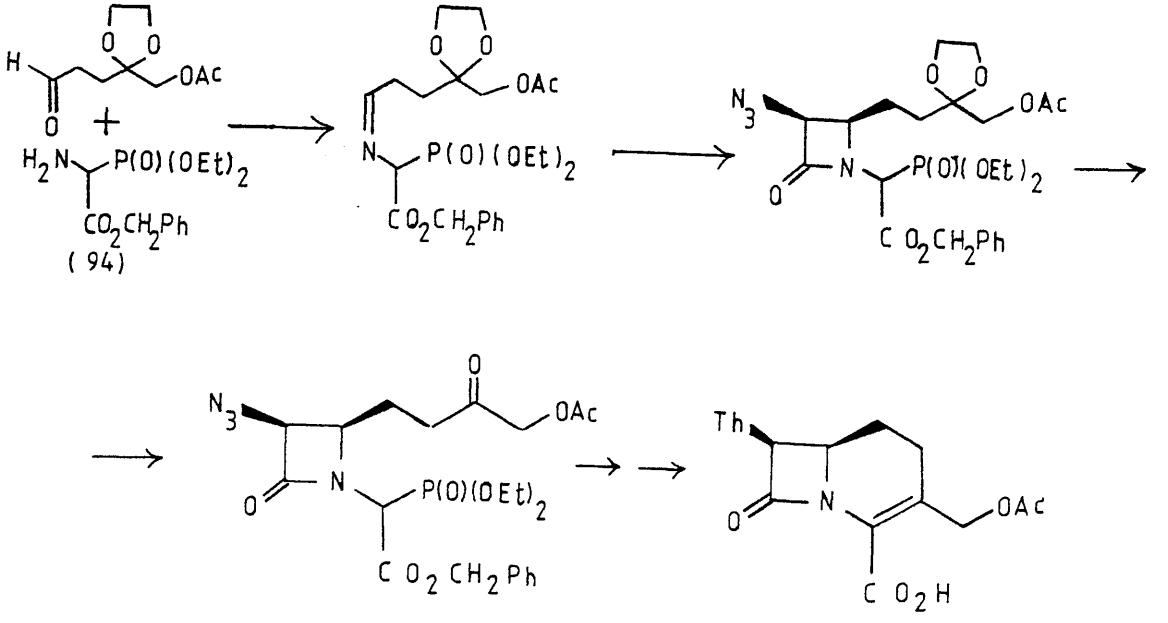


(104)



(105)

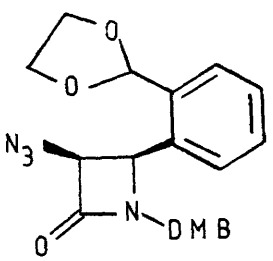


(94)

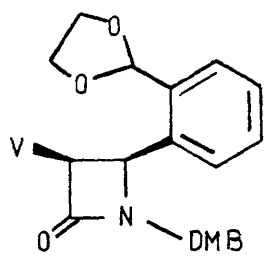
(106)

SCHEME 4.

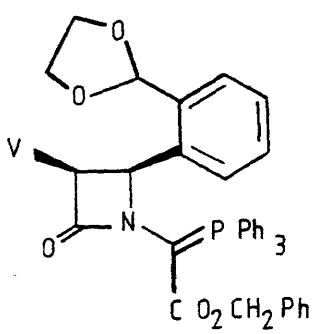
The Merck synthesis of cephalosporin is convergent and, as such, is ideally suited to the preparation of nuclearly modified analogues of cephalosporin. Before 1973 it was generally believed that the sulphur atom in cephalosporin was necessary for antimicrobial activity. To test this hypothesis, the Merck group synthesised analogues of cephalosporin in which the sulphur atom is replaced by an oxygen atom or a methylene group. (±)-1-Oxacephalothin was prepared[40] as follows. The thioformimidate (100), obtained by treating thioformamide (95) with methyl iodide and potassium carbonate, reacted with azidoacetyl chloride and triethylamine to give the trans-β-lactam (101). Non-stereospecific conversion to the chlorides (102) was achieved using chlorine gas. Silver (I) catalysed displacement with 1-hydroxy-3-acetoxypropanone gave lactam (103) as a cis-trans mixture. Emmons cyclisation then produced, after chromatography, the cis-oxacephem (104). Hydrogenolytic ester deprotection was accompanied by concomitant reduction of the azide to the amine. Acylation of the resulting amino acid with thienylacetyl chloride gave (±)-1-oxacephalothin (105). This possessed antibacterial activity comparable with racemic cephalothin; against some organisms the oxygen analogue was actually more potent than the parent compound. This demonstrates that sulphur is not essential for activity, but does not rule out the possibility that oxygen is replacing sulphur in binding to an electrophilic site on the enzyme with which these molecules interact. However, the synthetic route was adapted[41] to allow the preparation of (±)-1-carbacephalothin (106) (Scheme 4). This, too, was found to retain most of the activity of the parent compound, thereby proving that the presence of sulphur is not required for biological activity. Interestingly, in the first two examples the cycloaddition reaction gave the trans-oriented β-lactams (97) and (101) but in the carbacephalothin synthesis only the



(115)



(116)



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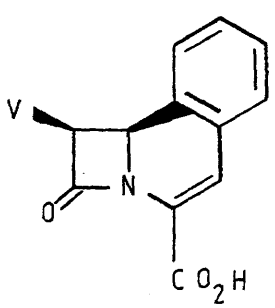

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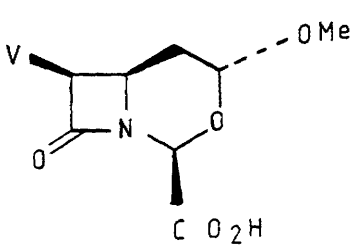
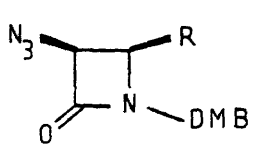


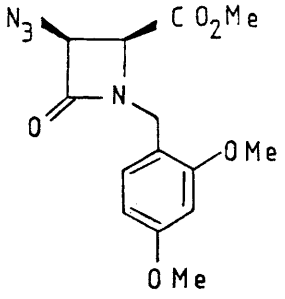
(127)



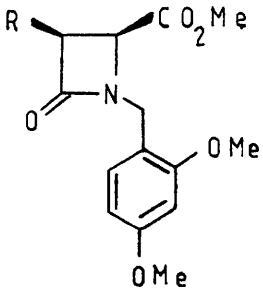
(119) R = CHO

(120) R = $CH(OH)CH_2NO_2$

(121) R = $CH_2CH_2NO_2$

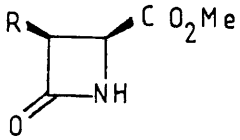


(112)

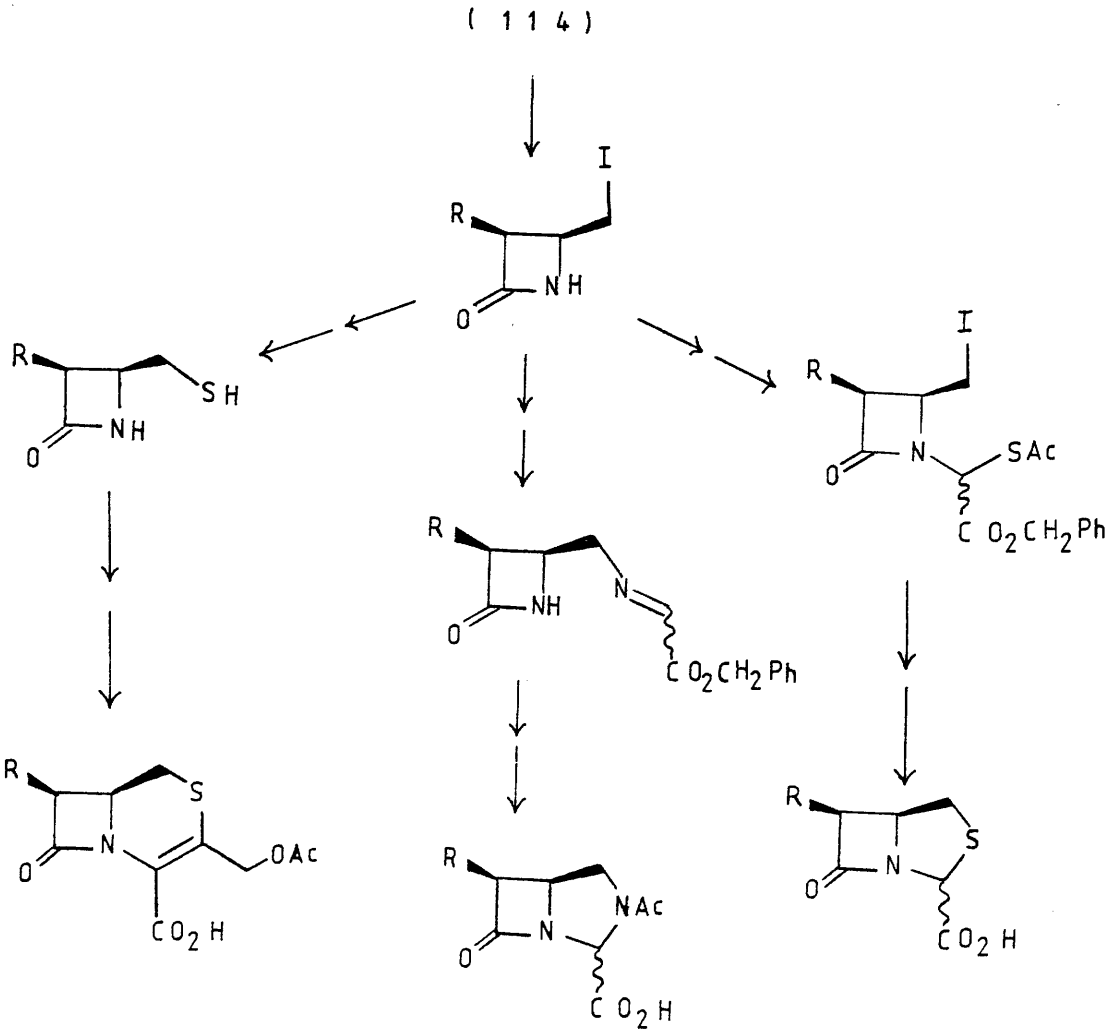


R = V or Th

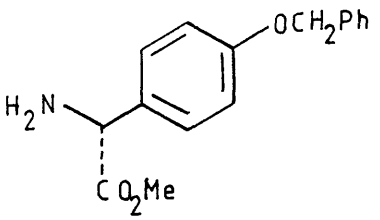
(113)



(114)



SCHEME 5.



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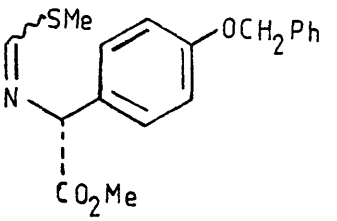
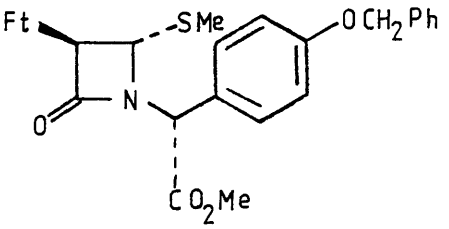
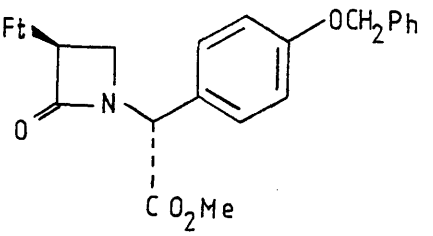


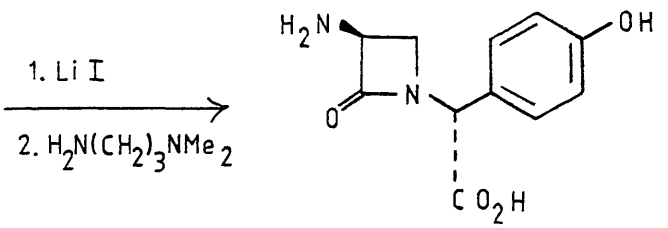
(1 0 8)



(1 0 9)



(1 1 0)



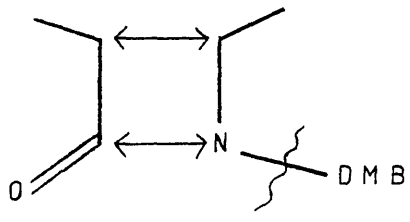
(1 1 1)

cis-substituted product was detected.

A very similar sequence of reactions was used for the first total synthesis of 3-ANA (111) reported[42] in 1976 from the Fujisawa laboratories. The protected (D)-p-hydroxyphenylglycine (107) was converted into the thioimidate (108). β-Lactam formation using phthalimidoacetyl chloride resulted in the formation of lactam (109), together with the other possible trans-isomer. Desulphurisation with Raney nickel followed by hydrogenolysis of the benzyl ether gave a diastereoisomeric mixture of reaction products, from which the desired isomer (110) could be obtained by chromatography. The synthesis of 3-ANA (111) was completed by halogenolytic fission of the methyl ester and liberation of the amino group.

The achievement of the Merck chemists in synthesising nuclearly modified cephalosporins which were potent antibiotics has stimulated many research groups to seek other nuclearly modified analogues with even greater antimicrobial activity. The cycloaddition of imines with ketenes has been used extensively in this search and one particularly noteworthy variation was developed in the laboratories of Smith Kline and French (SKF). This is discussed in detail in the following section.

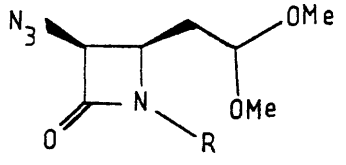
(f) Smith Kline and French Route (1977)



The innovation introduced[43] by chemists at SKF was the use of 2,4-dimethoxybenzylamine as the amine component for imine formation. The dimethoxybenzyl group could then be removed from the β-lactam thus produced to give a nitrogen-unsubstituted monocyclic azetidinone. This sequence is effectively equivalent to using ammonia in the formation of a monocyclic β-lactam. The β-lactam (112) was prepared by addition of the mixed anhydride of azidoacetic acid and TFA to the imine from 2,4-dimethoxybenzylamine and methyl glyoxylate; only the cis-isomer was obtained. Reduction of the azide followed by acylation gave the amide (113). The dimethoxybenzyl group was then oxidatively removed with buffered potassium persulphate[43] to give the free lactam (114). This species is a versatile intermediate for the synthesis[43-45] of nuclearly modified penicillin and cephalosporin analogues, some of which are shown in Scheme 5.

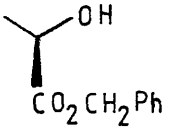
Removal of the dimethoxybenzyl group leaves a β-lactam capable of undergoing the Woodward glyoxylate condensation sequence. Such a strategy[46] can be seen in the synthesis of the benzo-fused carbocyclic β-lactam (118). Oxidative cleavage of the dimethoxybenzyl group of lactam (115), followed by reduction and acylation afforded the amide (116). The glyoxylate condensation sequence then furnished the phosphorane (117). Aldehyde deprotection was followed by spontaneous cyclisation. Hydrogenolysis to the free acid (118) gave a compound inactive against a range of organisms.

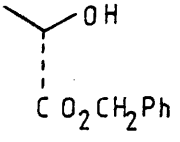
Recently the same group reported[47] the synthesis of a very interesting compound (127). Reduction of the ester (112) to the corresponding alchol with $NaBH_4$, followed by oxidation, gave the aldehyde (119). Condensation with nitromethane afforded nitro-alchol (120) which was converted into the nitroethylazetidinone (121) by dehydration and reduction of the resulting olefin (again using $NaBH_4$). The

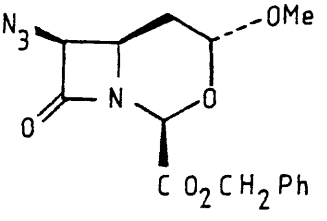


(122) R = DMB

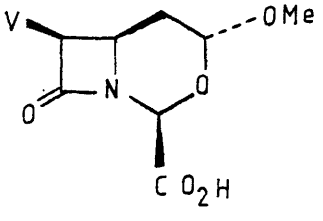
(123) R = H

(124) R = 

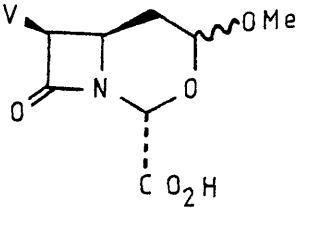
(125) R = 



(126)



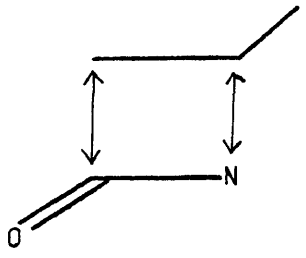
(127)



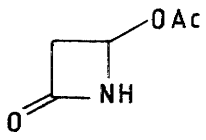
(128)

nitroethylazetidinone (121) was transformed by a modified Nef reaction into the acetal (122). Oxidative removal of the dimethoxybenzyl group and condensation with benzyl glyoxylate furnished an epimeric mixture of adducts, which could be separated chromatographically into epimers (124) and (125). On treatment with TsOH, epimer (124) cyclised to the single stereoisomer (126), the structure of which was confirmed by X-ray analysis. This synthesis was completed by hydrogenolysis of the benzyl ester with concomitant reduction of the azide group. Acylation then gave the cepham analogue (127). That this proved to be a potent antibiotic is surprising because (i) compound (127) lacks the enamine group considered necessary[48] for activity in a 4:6 fused β-lactam and (ii) the 4β-carboxyl group in (127) is of the opposite configuration to that of the 3α-carboxyl group found in penicillin. The epimeric compound (128) prepared from isomer (125) did not possess any significant antibacterial properties.

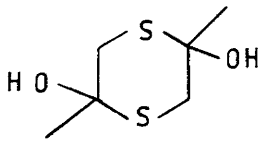
(g) The Hoechst Approach (1974)



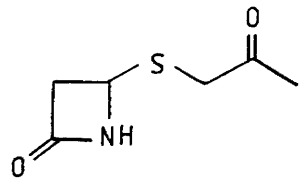
The discovery by Graf[49] that N-chlorosulphonyl isocyanate (CSI) reacts with olefins to give β-lactams, from which the chlorosulphonyl group can readily be removed, stimulated chemists at Hoechst in West Germany to prepare derivatives of the β-lactam antibiotics <u>via</u> this



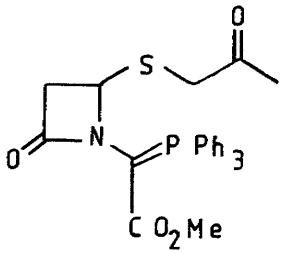
(1 2 9)



(1 3 0)



(1 3 1)



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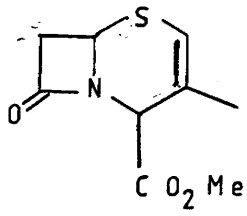

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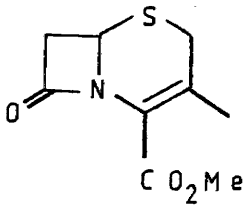

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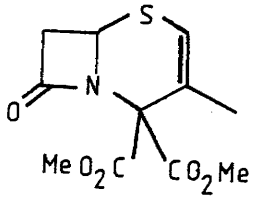

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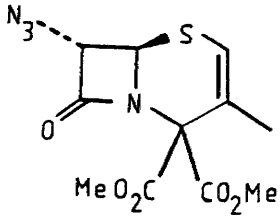

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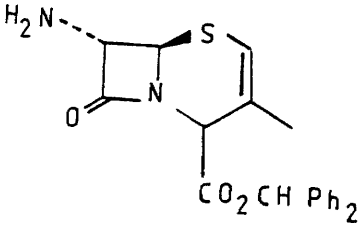
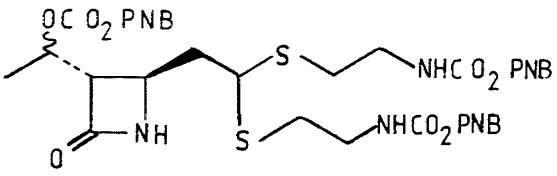


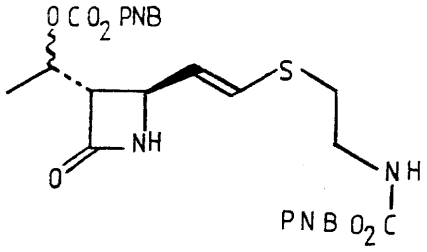
(1 3 7)

cycloaddition reaction. After much experimentation the following synthesis of a cephem was achieved[50]. The acetoxyazetidinone (129) was prepared by reaction of vinyl acetate with CSI, followed by hydrolysis of the chlorosulphonyl group. The dimeric compound (130) reacted with lactam (129) in the presence of base to give a product (131) corresponding to the displacement of acetate by mercaptoacetone. Lactam (131) was then converted into phosphorane (132) by Woodward's method. Pyrolysis of phosphorane (132) gave a mixture of the Δ^2-cephem (133) and the Δ^3-cephem (134), converted into pure isomer (134) on treatment with base. Attempts to functionalise the C-7 position of cephem (134) *via* deprotonation failed due to preferential deprotonation in the six membered ring. This latter process could be blocked by acylation of cephem (134) to give diester (135). Deprotonation then occurred at the C-7 position and reaction of this anion with TsN_3 gave the *trans*-azide (136). Reduction, hydrolysis and decarboxylation, and finally re-esterification gave the amine (137). The transformations necessary for the conversion of amine (137) into an active cephalosporin have been reported earlier. The *trans*-amine can be isomerised into its *cis*-isomer *via* deprotonation-kinetic reprotonation[38] of its Schiffs base. A deacetoxy- Δ^2-cephem has been converted into an acetoxy- Δ^3-cephem *via* bromination, displacement with acetate, and oxidation-reduction of the sulphur atom to isomerise the Δ^2-double bond[51].

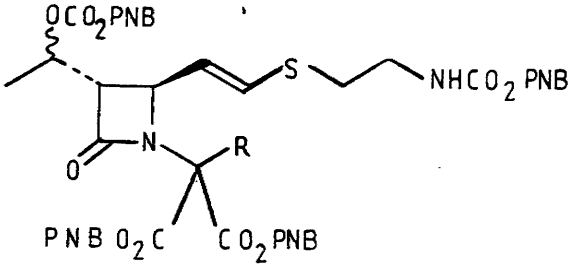
This synthesis was abandoned because the many steps involved rendered it commercially impractical. However, the above transformations demonstrate that this approach does lend itself to the preparation of bicyclic β-lactams. In particular, preparation of the acetoxy β-lactam (129) and nucleophilic displacement of the acetate group were significant as can be seen in the following synthesis[52] of clavulanic



(151)



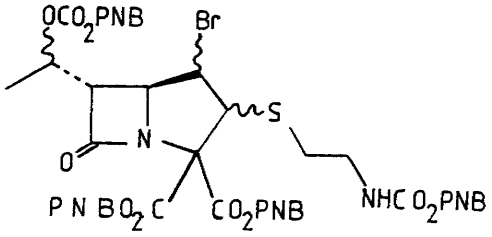
(152)



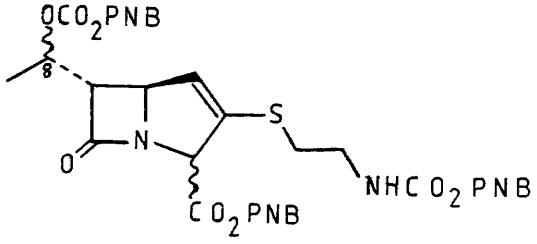
(153) R = OH

(154) R = Cl

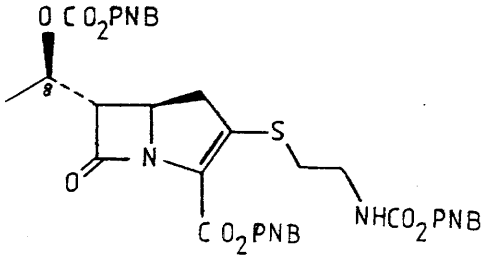
(155) R = H



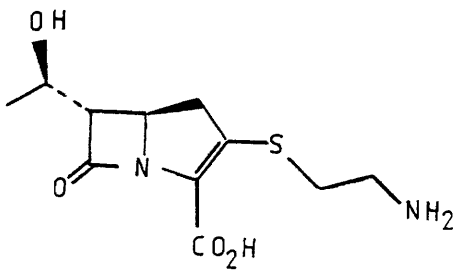
(156)



(157)



(158)



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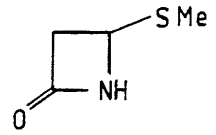

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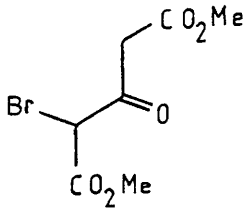

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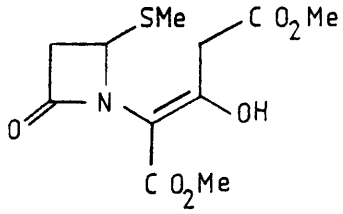

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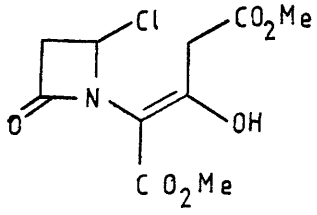

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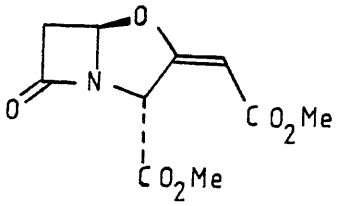

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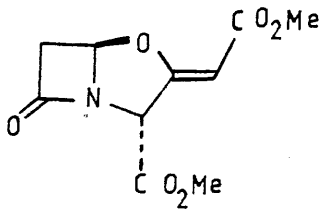

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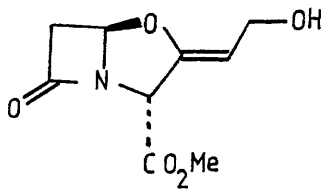

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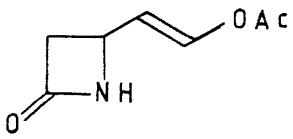

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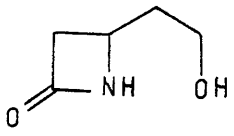

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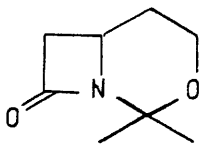

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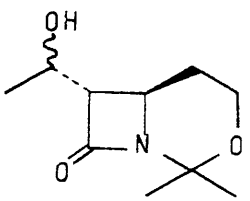

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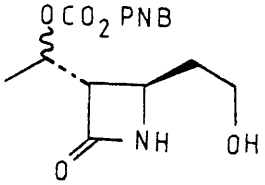
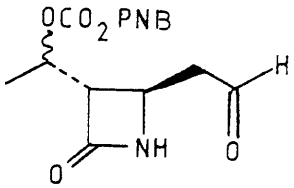


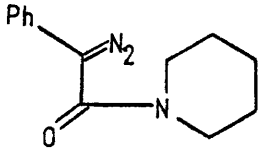
(1 4 9)



(1 5 0)

acid. The Beechams group used as their starting material the thiomethyl β-lactam (138), first prepared by the Hoechst team[53]. Alkylation with dimethyl 2-bromo-3-oxoglutarate (139) gave the β-ketodiester (140), which on treatment with a slight excess of chlorine afforded the chloro compound (141). Cyclisation to the bicyclic compound (142), isolated as a single racemic isomer, was achieved with anhydrous potassium carbonate in dry DMF. Photolysis of a benzene solution of (142) resulted in isomerisation about the double bond to an inseparable mixture of (142) and (143). Reduction of the mixture with Bu^i_2AlH gave a mixture of the corresponding allylic alchols, from which the desired methyl (±)-clavulanate (144) was isolated. This represents a formal total synthesis of (±)-clavulanic acid, methyl clavulanate being hydrolysable to clavulanic acid[54].

The first total synthesis[55] of thienamycin (6) also employed CSI cycloaddition with olefins as the initial step. β -Lactam (145) was formed by reaction of CSI with 1-acetoxybutadiene, followed by hydrolysis of the chlorosulphonyl group. Hydrogenation and ester exchange gave the hydroxyalkyl azetidinone (146) which was protected as the corresponding acetonide (147). Treatment with lithium diisopropylamide and acetaldehyde at -78°C then produced the trans-alkylated product (148), epimeric at the carbinol carbon. The alcohol was protected as its p-nitrobenzyl carbonate and the acetonide cleaved to give alcohol (149). This was oxidised to the aldehyde (150), which was converted into the dithioacetal (151). Sequential treatment with bromine and triethylamine caused elimination of one of the protected cysteamine groups, forming thioenol ether (152). Condensation with bis(p-nitrobenzyl) oxomalonate afforded hydroxy diester (153) which was converted into chloride (154). Reduction with tri-n-butyl phosphite to diester (155) followed by bromine-induced cyclisation gave the bicyclic compound (156).



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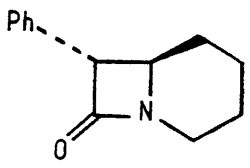

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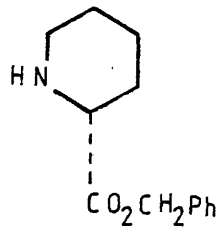

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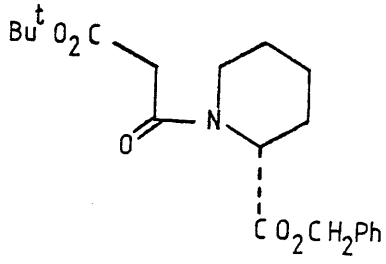

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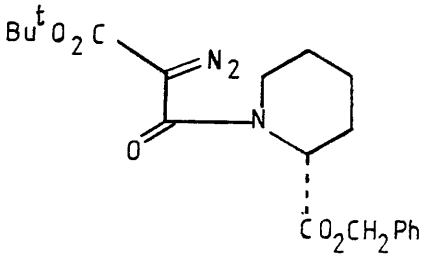

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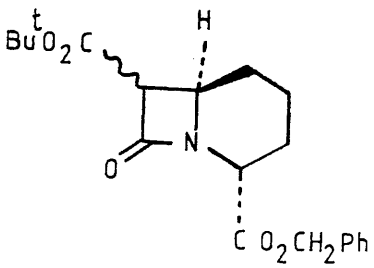
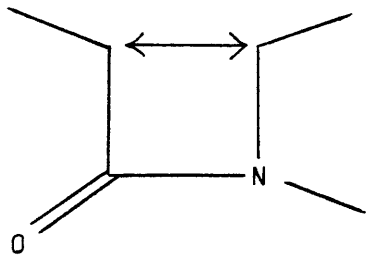


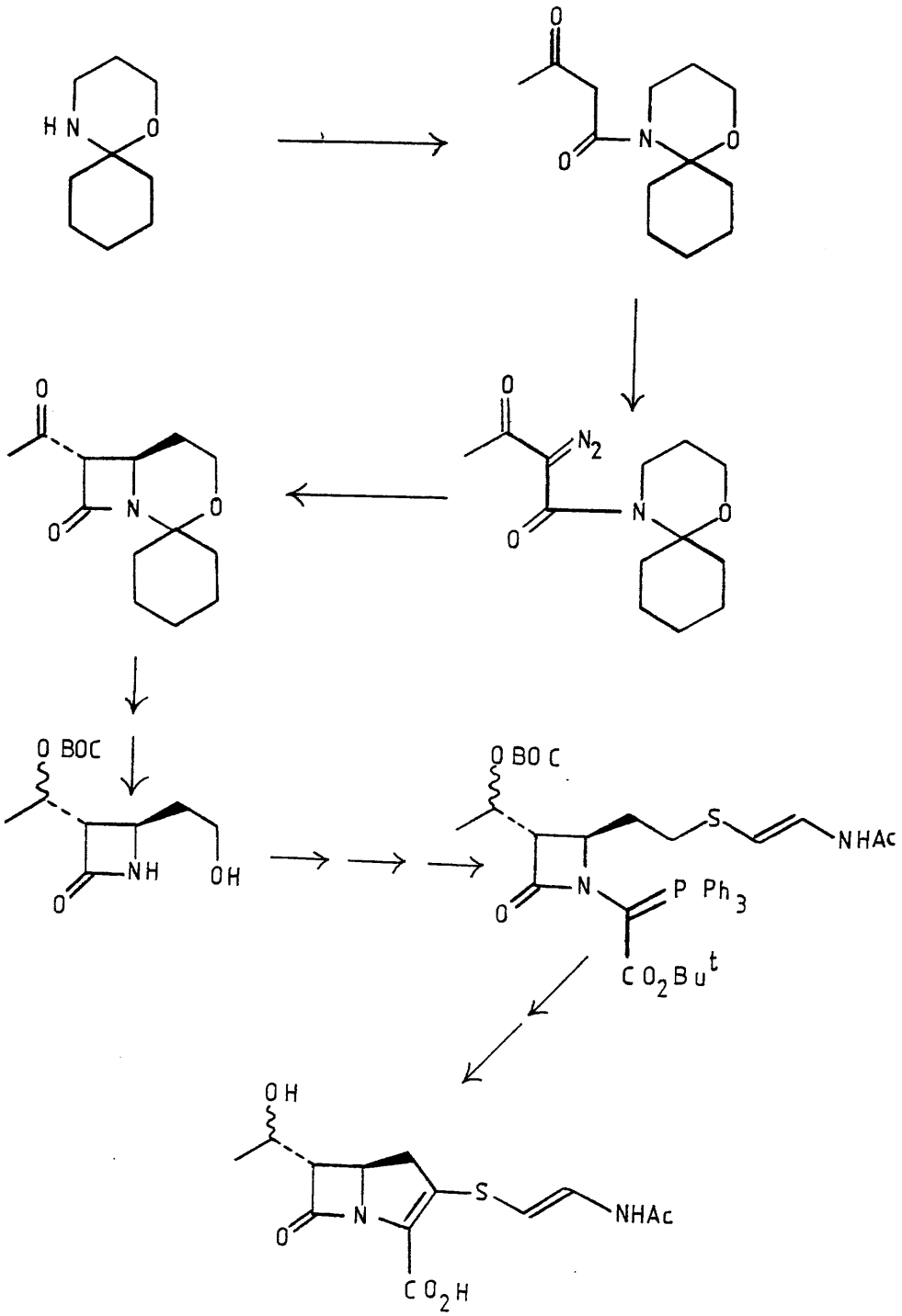
(164)

Dehydrobromination was followed by selective deprotection of one p-nitrobenzyl ester with one equivalent each of collidine and methyl iodide. The resulting malonate half-acid underwent decarboxylation on acidic work up, affording a diastereoisomeric mixture of monoesters (157) from which the desired (8R)-isomer could be obtained by chromatography. Base catalysed double bond isomerisation produced a separable mixture of alkene isomer (158) and starting material. Hydrogenolysis of the isolated protected thienamycin (158) gave (±)-thienamycin, possessing half the antibacterial activity of the naturally occurring antibiotic.

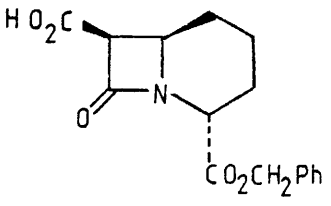
(h) The Lowe Synthesis (1971)



In 1965, Corey reported[56] formation of the β-lactam (160) upon photolysis of the diazoamide (159). Lowe, at Oxford, has extended this reaction to the synthesis[57] of analogues of the β-lactam antibiotics. (D)-Pipecolic acid benzyl ester (161) was condensed with the t-butyl half-ester of malonic acid using DCC and producing the amide (162). The requisite α-diazoamide (163) was generated by diazo-group transfer from TsN_3 in the presence of triethylamine. Photolysis of this diazo compound gave a mixture of the cis- and trans-β-lactams (164), with trans-stereochemistry in the six-membered ring. The cis-



SCHEME 7.



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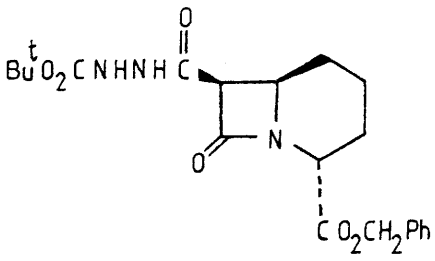

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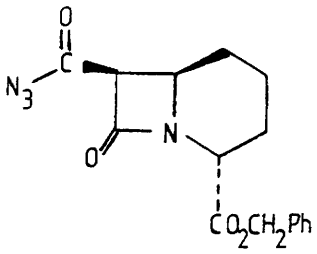

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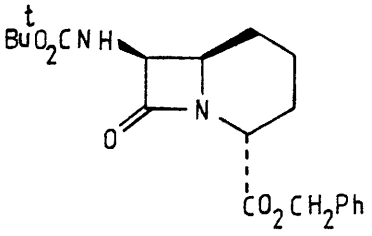

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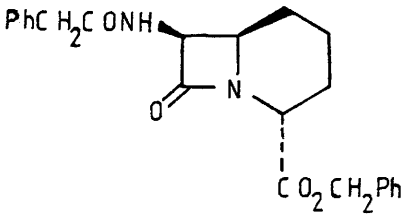

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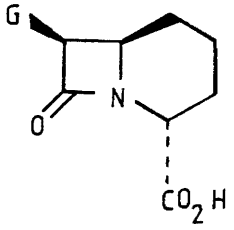
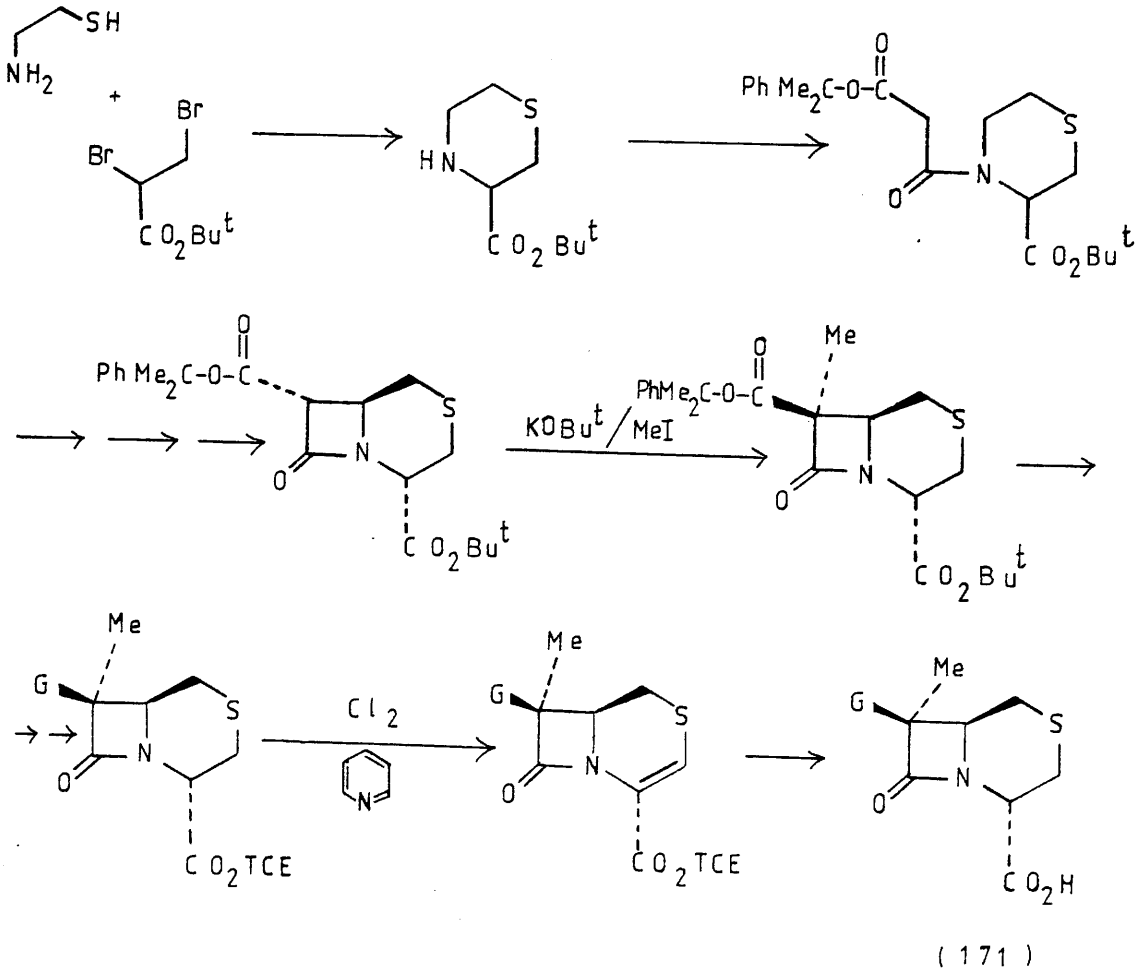


(170)



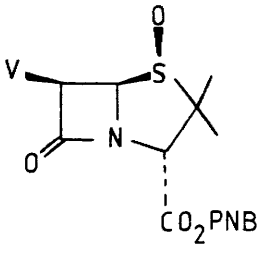
(171)

SCHEME 6.

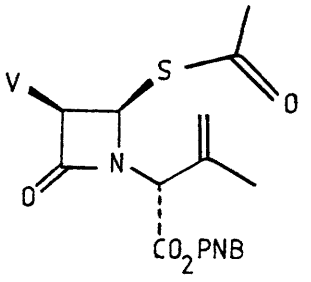
β-lactam was isolated chromatographically and the free acid (165) liberated. Curtius rearrangement of the corresponding acid azide (167) would then enable transformation of the C-7 carboxyl group into the required amino/amido group. However, attempts to convert the acid (165) into the acid azide (169) *via* the acid chloride or mixed anhydride caused epimerisation at C-7. The azide was therefore obtained by coupling of the acid with *t*-butyl carbazate to give the hydrazide (166) and thence the azide (167). The isocyanate resulting from Curtius rearrangement was not isolated, but was transformed into the t-butyl urethane (168) by the addition of *t*-butanol to the refluxing benzene solution. Deprotection of the urethane and acylation of the resulting amine with phenylacetyl chloride afforded amide (169). Catalytic hydrogenolysis then gave the inactive nuclearly modified analogue[58] (170).

This compound's lack of antimicrobial activity is not surprising in view of the absence of a Δ^3-double bond in the six-membered ring. The synthesis was therefore adapted[59], replacing the starting pipecolyl ester with a thiamorpholine (Scheme 6). The sulphur atom in the thiamorpholine enables the late introduction of unsaturation into the six-membered ring. In this synthesis it proved extremely difficult to obtain a product with the *cis*-orientation of the β-lactam substituents. To overcome this, a methyl group was introduced at C-7, alkylation occurring from the less hindered α-face of the molecule. The isocephem (171) ultimately obtained was also inactive. Since the isocephem obtained by the SKF group (Scheme 5) is a very potent antibiotic, it can be deduced that the inactivity of (171) is due to the presence of the 7-α-methyl substituent.

Very recently the Beechams research group[60] have used an α-diazo amide for the synthesis of an olivanic acid derivative (Scheme 7). The resulting product was racemic, possessing half the antimicrobial activity of the naturally occurring compound.



(1 7 2)



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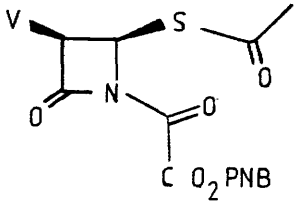

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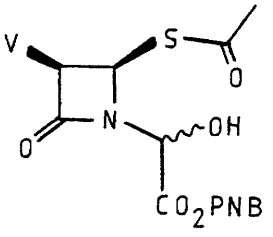

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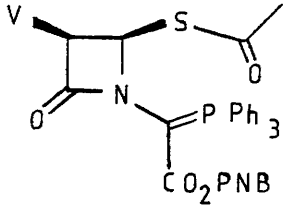
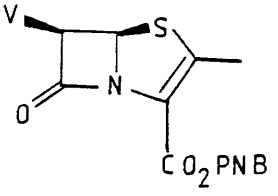


(1 7 6)



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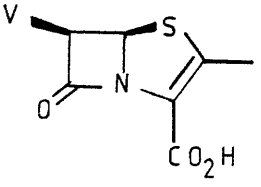


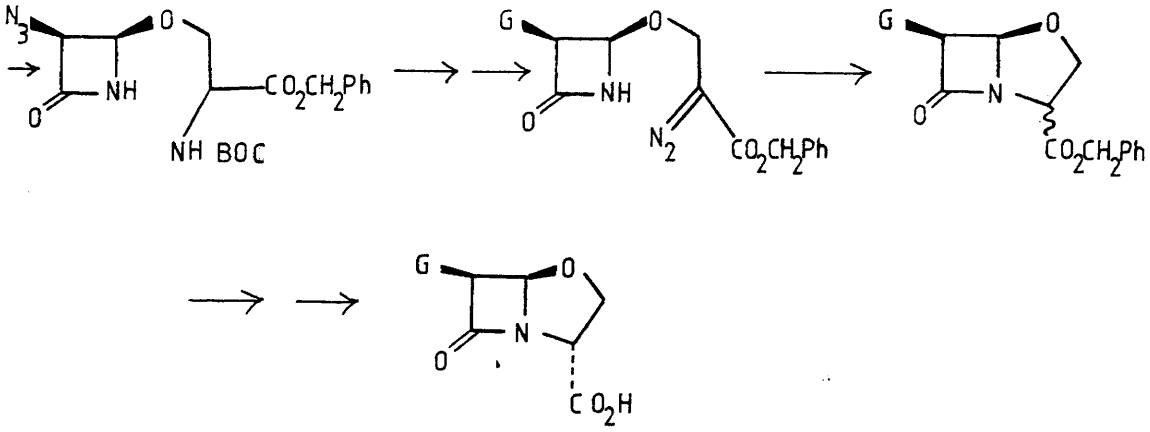
(1 1)

(i) Recent Developments

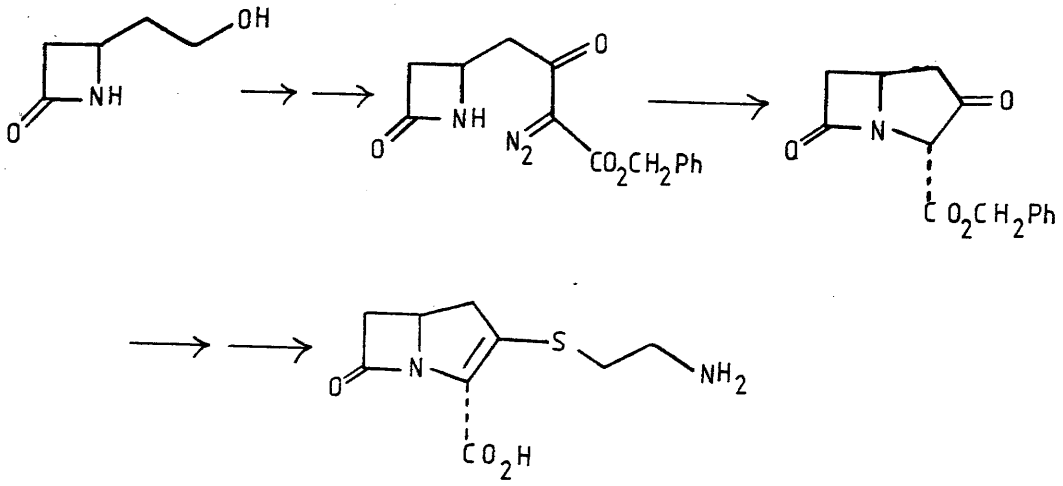
Woodward's Penem Synthesis

The β-lactam ring in penicillin is more reactive than simple monocyclic azetidinones towards nucleophilic cleavage. This can be explained in terms of the fused five-membered ring preventing the nitrogen substituents from adopting a planar conformation and thereby reducing the delocalisation of the nitrogen lone pair into the adjacent carbonyl π-orbital. In cephalosporin, the nitrogen substituents can adopt a planar conformation; however the β-lactam in cephalosporin is also chemically more reactive than a monocyclic β-lactam. The explanation for the lability of the amide bond in cephalosporin is that the nitrogen lone pair is delocalised into the π-system of the adjacent Δ^3-double bond. This reduces the delocalisation into the adjacent carbonyl π-system, rendering the carbonyl group more susceptible to nucleophilic attack. Woodward envisaged that a compound which incorporated both of these activating features would be very reactive towards nucleophiles and might therefore be an extremely potent antibiotic. In 1976 he reported the synthesis of such a compound, the penem (11), a β-lactam possessing a fused five-membered ring containing an enamine group. This was indeed a very labile system and several approaches to it met with failure. After extensive investigation the following high yield synthesis[61] of (11) was developed.

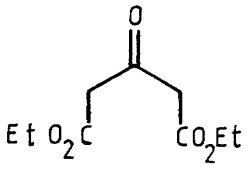
The penicillin sulphoxide (172) was heated in acetic anhydride and trimethyl phosphite to give the thioacetate (173). Double bond isomerisation followed by ozonolysis produced the oxalyl derivative (174). Diborane reduction afforded the hemiaminal (175), a species obtained in earlier investigations by methanolysis of (174) to the nitrogen



SCHEME 8.



SCHEME 9.



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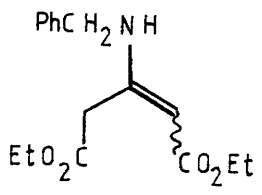

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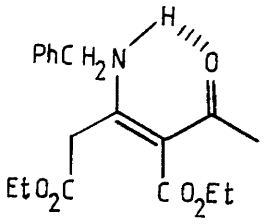

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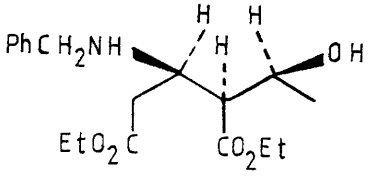


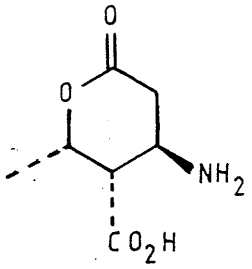
(181)

unsubstituted β-lactam followed by condensation with p-nitrobenzyl glyoxylate. Continuation of the sequence gave the phosphorane (176). The synthesis was completed by Wittig condensation to give ester (177) followed by hydrogenolysis. Penem (11) was an antimicrobial agent but its potency was reduced by its extreme lability. Nevertheless the synthesis of this important compound, by a very short sequence of reactions giving optically pure material, is an outstanding achievement in the field of β-lactam synthesis. It is also noteworthy that the synthesis was accomplished by well established transformations, most of which have been discussed in the previous sections.

Merck C-3 to N Cyclisation

In 1978 the Merck group published[62] a synthesis of (±)-1-oxabisnorpenicillin G (Scheme 8) involving insertion of a C-3 carbene into the N-H bond, the carbene being generated by $Rh(OAc)_2$ catalysed decomposition of the appropriate diazo compound. This novel cyclisation was then extended to the synthesis[63] of the 1-carbapen-2-em system found in thienamycin (Scheme 9). In this example the ring closure proceeded in near quantitative yield to give a single isomer. Very recently the process research team at Merck published[64] a total synthesis of thienamycin based on the above reactions.

Diethyl 1,3-acetonedicarboxylate (178) was condensed with benzylamine to give the enamine (179). Acylation with ketene gave, cleanly, the C-acylated product (180). This exhibited a high degree of rigidity due to the strong hydrogen bonding between the nitrogen proton and the carbonyl oxygen. Sodium cyanoborohydride reduction of enamine (180) proceeded stereoselectively, probably *via* a boron chelate, to the all *cis* reduction product (181). Acid catalysed ester hydrolysis was



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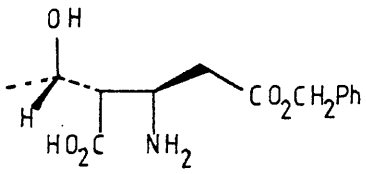

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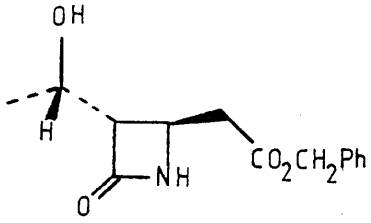

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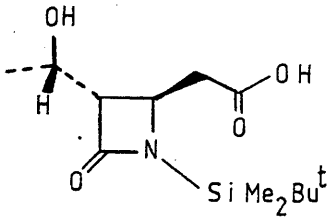

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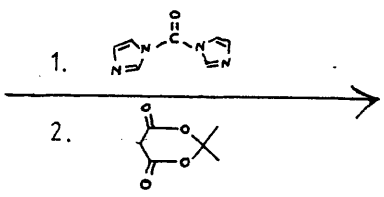

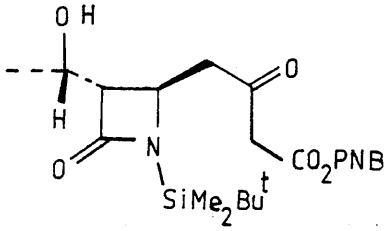

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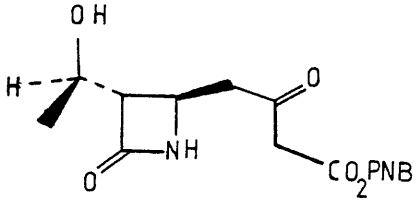

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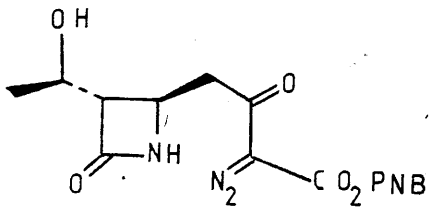

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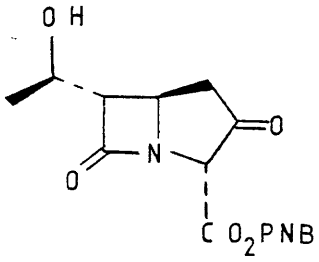

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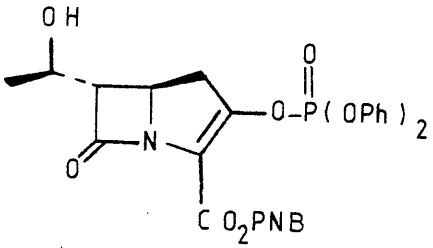

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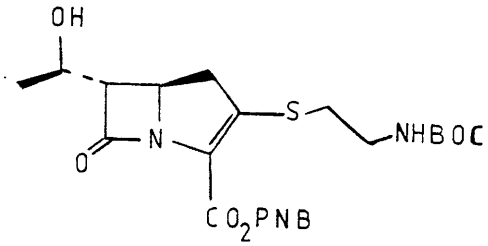

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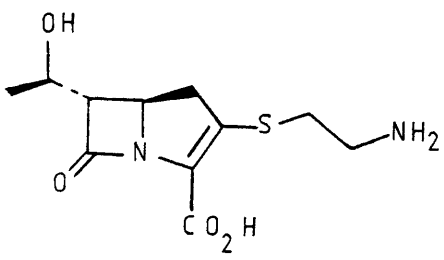
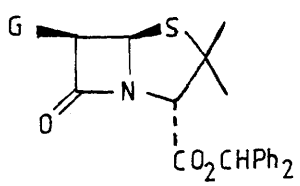


(6)

accompanied by lactonisation to give, after hydrogenolysis, the crystalline lactone (182). Lactonisation introduces a necessary discrimination between the two carboxyl groups; treatment with benzyl alcohol afforded an equilibrium mixture of lactone (182) and ester (183), from which lactone (182) could be removed and recycled. The amino acid (183) was cyclised to the β-lactam (184) with DCC. Protection of the amide nitrogen as its t-butyldimethylsilyl derivative and hydrogenolysis gave the carboxylic acid (185). Chain elongation to β-keto ester (186) was achieved by modification of a published[65] procedure. After removal of the protecting group from the β-lactam nitrogen, the configuration of the hydroxyethyl side chain was inverted by a "Mitsunobu[66] procedure" (Scheme 10). The inverted alcohol (187) was treated with TsN_3 and triethylamine to give the diazo compound (188) and thence the bicyclic β-ketoester (189). Transformation via the enol phosphate (190) into the protected thienamycin (191) and hydrogenolysis then gave (±) thienamycin (6). The overall yield of this synthesis is greater than 10% and, since it employs only simple reagents, the Merck group believes that it will form the basis of an industrial preparation of thienamycin.

Stereocontrolled Synthesis of 7α-Methoxy-1-oxacephalosporin

In 1979 a research group at the Shionogi Company in Japan reported that the 7α-methoxy-1-oxacephalosporin (203) possessed potent antibiotic activity against a wide range of Gram-negative organisms. These included β-lactamase producing resistant strains, pathogenic anaerobic bacteria and Pseudomonas species, which were not susceptible to other cephalosporins[67]. In 1980 the same group published[68] the following commercially feasible, stereocontrolled, synthesis of the antibiotic (203) from penicillin.



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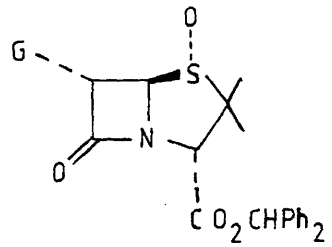

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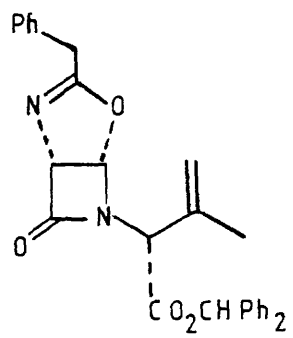

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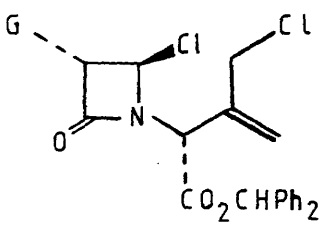

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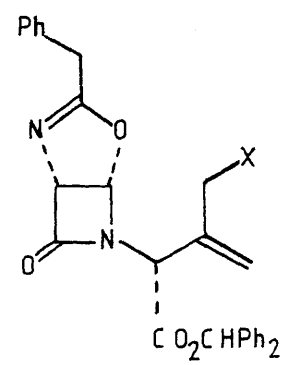
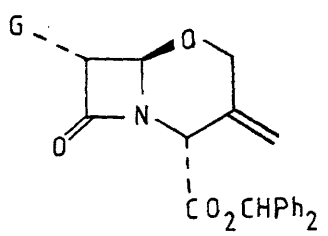


(196) X = Cl
(197) X = I
(198) X = ONO_2
(199) X = OH



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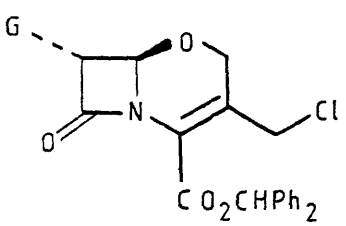

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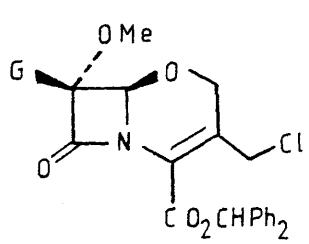
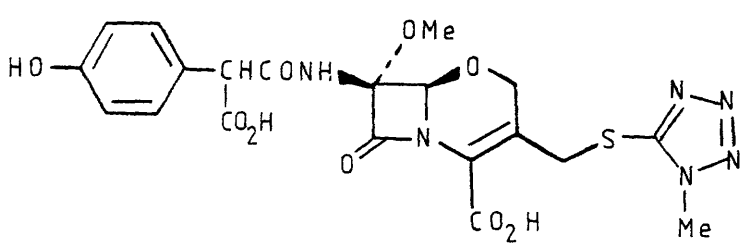
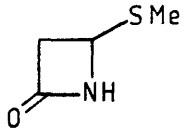


(202)



(203)

This synthesis is a development of the group's original preparation[69] in that all the carbon atoms of the penicillin starting material are retained in the product and that expensive reagents have been replaced. Penicillin G diphenylmethyl ester (192) was converted into the 6-epipenicillin sulphoxide (193) by base catalysed isomerisation, followed by oxidation of sulphur. The epi-oxazoline (194), obtained by heating sulphoxide (193) in the presence of triphenylphosphine, was functionalised at the allylic methyl group by reaction with chlorine to give the dichloride (195). In this reaction, the allylic chloride arises by an "ene" reaction of the alkene (194) with chlorine gas. Treatment of the dichloride with aqueous sodium bicarbonate furnished the epi-oxazoline (196). Direct conversion of chloride (196) into alcohol (199) failed, but the required alcohol was obtained by conversion of the chloride into the nitrate (198) *via* the iodide (197). Reduction of the nitrate with zinc and acetic acid then gave the desired alcohol (199), which underwent stereospecific BF_3-catalysed cyclisation to the exomethylene oxacepham (200). Addition of chlorine to the exomethylene double bond, followed by base-induced elimination of HCl gave the 7-epi-1-oxacephem (201). This was converted into the 7α-methoxy compound (202) by sequential treatment with *t*-butyl hypochlorite and lithium methoxide, a reaction originally developed[70] for the chemical preparation of cephamycins from cephalosporins. The synthesis was completed by chloride displacement with sodium 1-methyl-1-*H*-tetrazole-5-thiolate, phosphorus pentachloride mediated deacylation to the 7-amino compound, reacylation, and finally removal of the ester protecting group.



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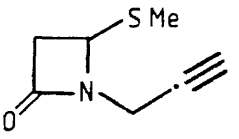

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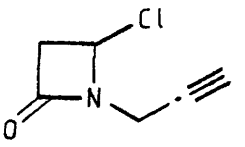

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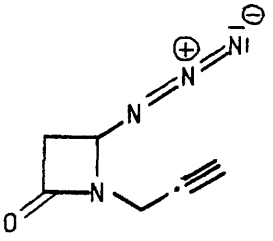

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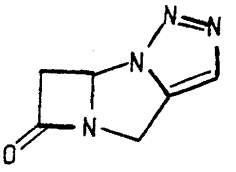

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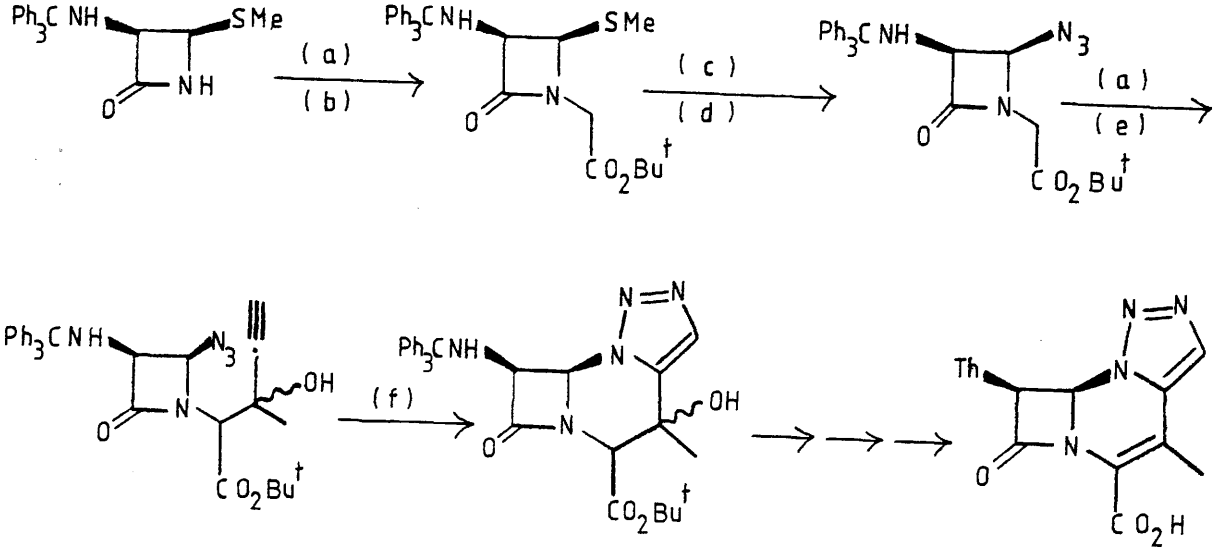
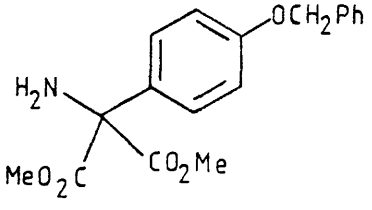


(a) $LiN(SiMe_3)_2$ (b) $BrCH_2CO_2Bu^t$

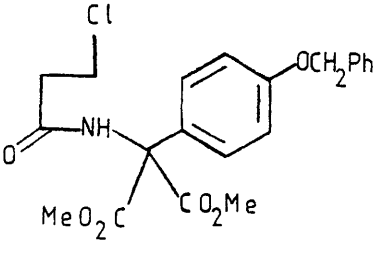
(c) Cl_2 (d) NaN_3 (e) $CH{\equiv}CCOCH_3$ (f) Δ

(208)

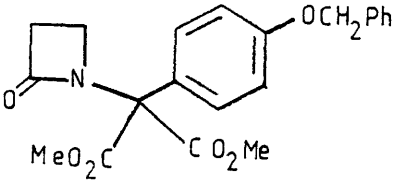
SCHEME 11.



(209)



(210)



(211)

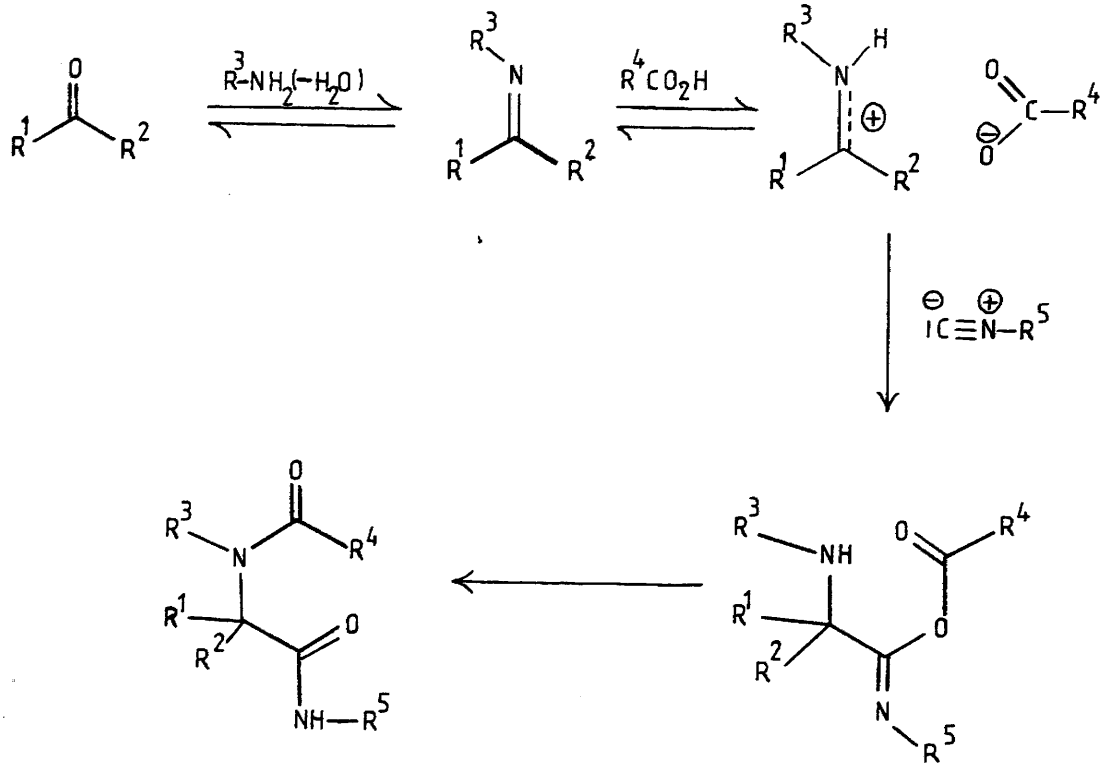
Fused β-Lactams by 1,3-Dipolar Cycloadditions

Very recently, a research group at Beechams has reported[71] the synthesis of biologically interesting compounds *via* intramolecular dipolar cycloaddition between an azido group and an alkyne. The synthesis of the tricyclic 1-azapenam analogue (207) employed the thiomethyl β-lactam (138) as starting material. Treatment with lithium hexamethyldisilazane and 3-bromoprop-1-yne gave the acetylenic β-lactam (204), which was converted into the chloride (205) by treatment with chlorine. Reaction with sodium azide afforded the tricyclic compound (207), presumably *via* the intermediate azide (206); this reaction was carried out at high dilution in order to minimise intermolecular interactions. The tricyclic β-lactam proved to be a β-lactamase inhibitor with a potency comparable to that of clavulanic acid. A similar sequence of reactions (Scheme 11) produced a highly active tricyclic 1-azacephalosporin analogue (208). The starting material here was a chiral β-lactam, obtained by degradation of a suitably protected penicillin.

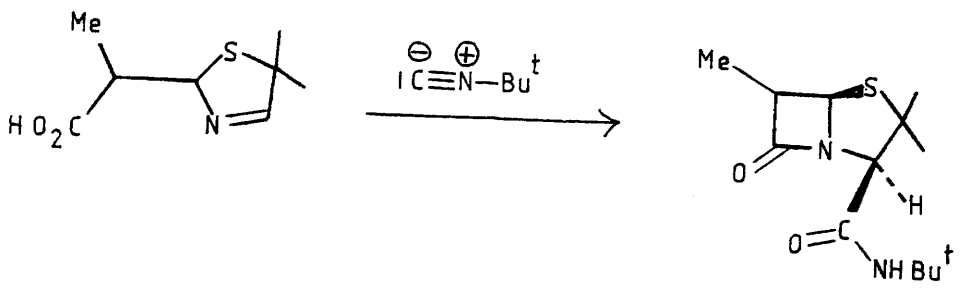
Novel Approaches to the Nocardicins

The nocardicin skeleton, being monocyclic, allows greater variation in synthetic approach than do the more usually encountered fused bicyclic β-lactam structures. Wasserman has been particularly active in the development of novel methods in this area, as the following two syntheses of nocardicin illustrate.

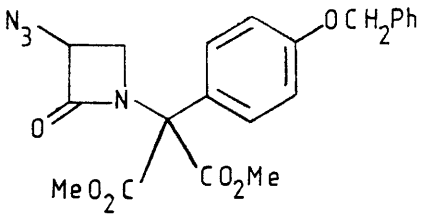
In one synthesis, described[72] in 1978, the β-lactam ring is created by acylation of the aminomalonate (209) with 3-chloropropionyl chloride to give the β-chloroamide (210), which cyclised to the β-lactam (211) on treatment with sodium hydride. This is a more



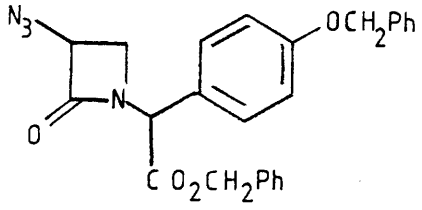
SCHEME 12.



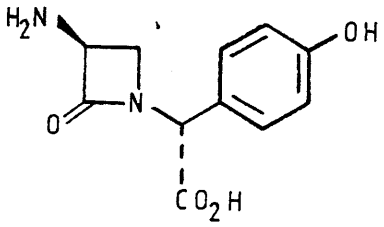
SCHEME 13.



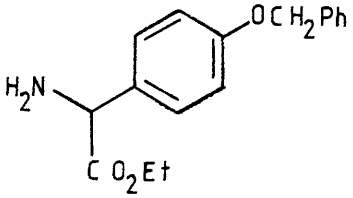
(212)



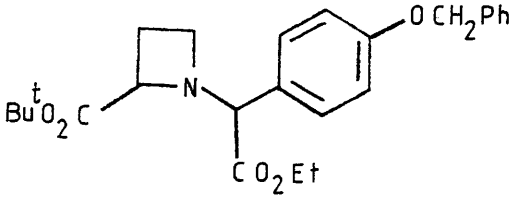
(213)



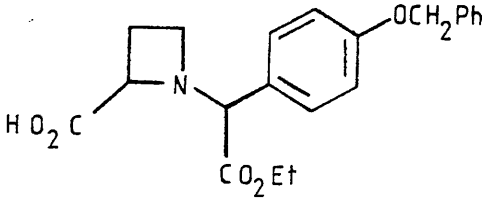
(111)



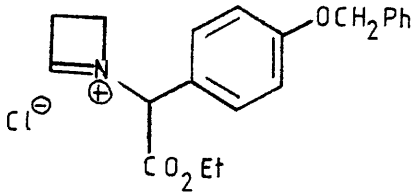
(214)



(215)



(216)



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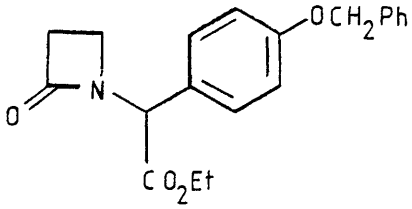

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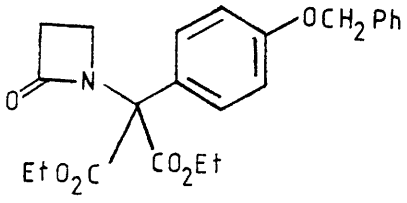


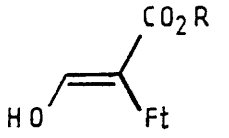
(219)

general example of the mode of β-lactam ring formation employed by Baldwin[25] in a stereocontrolled synthesis of penicillin. The 3-azido derivative (212) was obtained by sequential treatment of lactam (211) with lithium diisopropylamide and TsN_3. Saponification and decarboxylation gave the mono-acid, which was then protected as its benzyl ester (213). This ester was obtained as a mixture of diastereoisomers which could be separated chromatographically and the undesired isomer equilibrated with base and thus recycled. Hydrogenation of the desired isomer gave ($\pm$)-3-ANA (111).

A second synthesis proceeds[73] *via* an azetidine with potential for oxidation to an azetidin-2-one. The azetidine (215) was prepared from *t*-butyl 2,4-dibromobutyrate and the *p*-hydroxyphenylglycine derivative (214). Cleavage of the ester with TFA gave the acid (216), which was converted directly into the decarboxylated iminium salt (217), by sequential treatment with oxalyl chloride and perchloric acid. Oxidation of the salt (217) with *m*-chloroperbenzoic acid and pyridine gave the azetidinone (218). This was transformed into the malonate derivative (219) and thence to ($\pm$)-3-ANA.

Synthesis *via* the Ugi Reaction

The Ugi reaction[74] is a four-component condensation involving carbonyl, amino, carboxyl and isonitrile groups which results in the formation of an α-acylamino amide (Scheme 12). Creation of two amide moieties provides a strong driving force for the reaction. In 1962 Ugi applied[75] this condensation to the synthesis of a penicillin derivative (Scheme 13). This approach was not pursued further because it produced the undesired stereochemistry at C-3.



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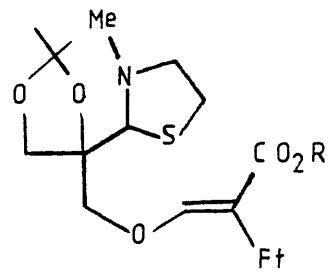
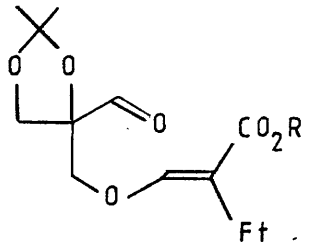
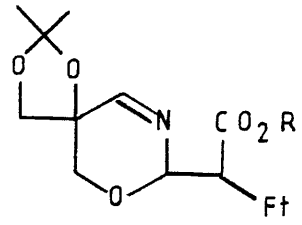


(224)

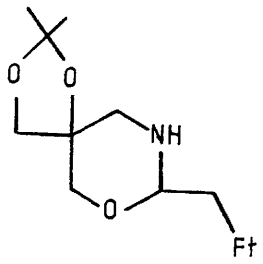


(225)

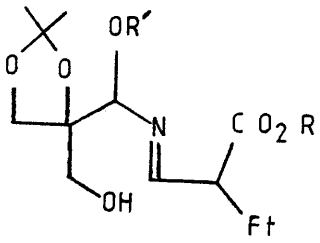


(226a) R = Me

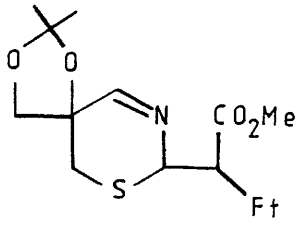
(226b) R = CH_2Ph



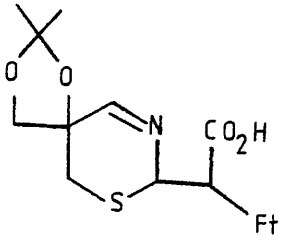
(227)



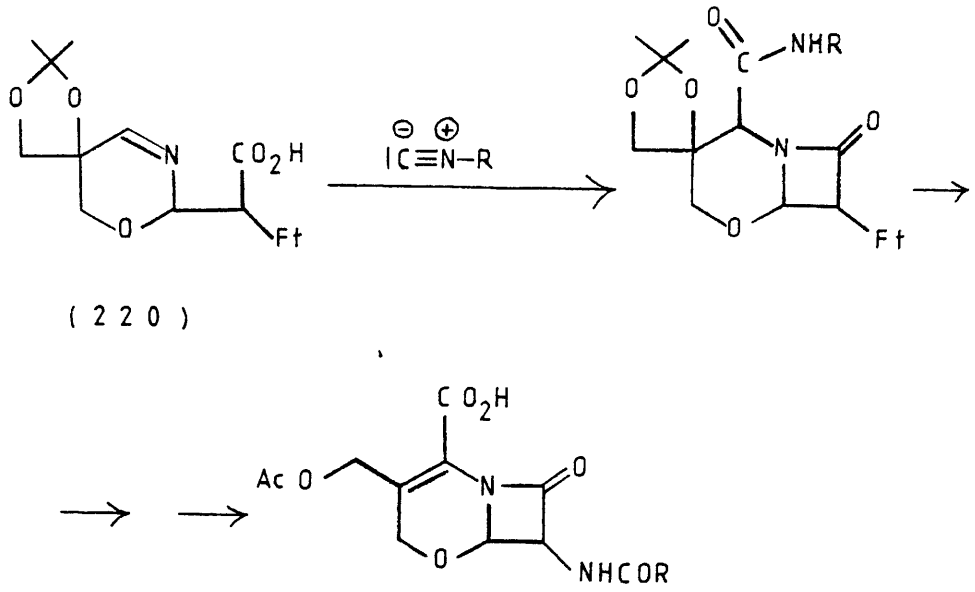
(228)



(229)

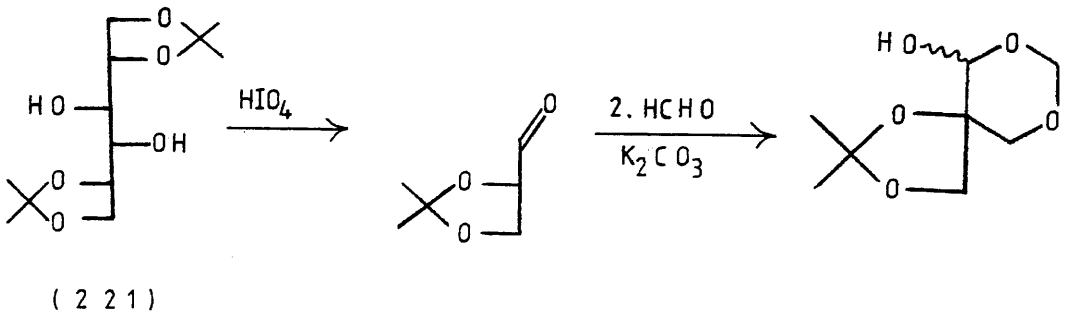


(230)

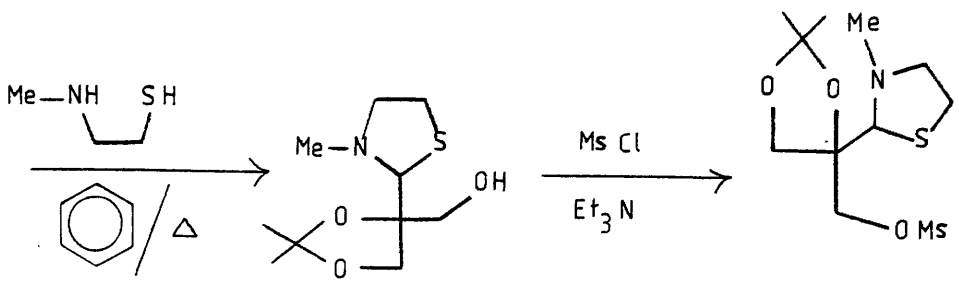


(2 2 0)

SCHEME 14.



(2 2 1)

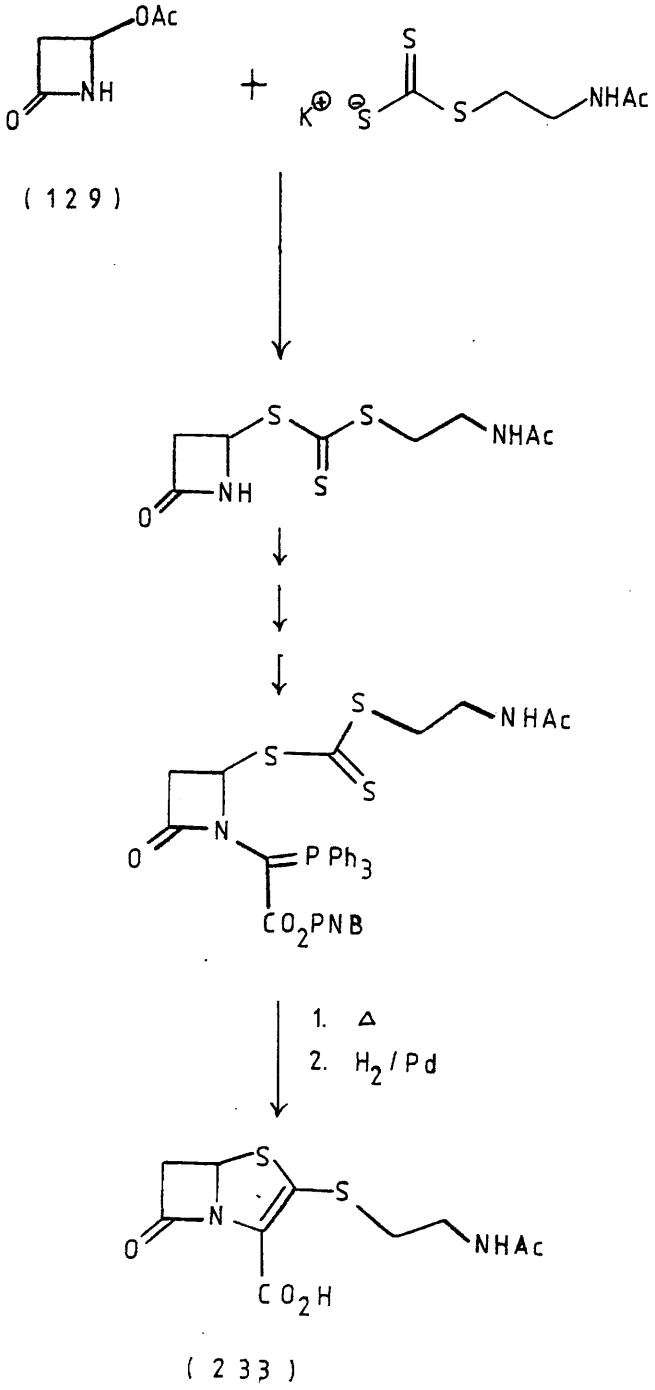


(2 2 2)

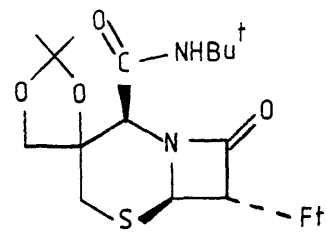
SCHEME 15.

In 1977 Just unsuccessfully attempted[76] to use the Ugi condensation for the synthesis of a 1-oxacephalosporin. The main features of the proposed synthesis are outlined in Scheme 14. The stereochemistry introduced at C-4 is irrelevant since it will be destroyed in the subsequent formation of the Δ^3-double bond. The thiazolidine mesylate (222), prepared[77] from mannitol diacetonide (221) (Scheme 15), was employed as starting material. Condensation with the 2-phthalimido-3-hydroxy-acrylate ester (223) afforded the enol ether (224). Mercury-catalysed hydrolysis of the thiazolidine to aldehyde (225), followed by reaction with ammonia furnished the monocyclic imine (226). Attempts to hydrolyse the methyl ester in (226a) caused decomposition of the ring system. To circumvent this problem the benzyl ester (226b) was prepared. This again did not give the desired acid but instead gave a product (227) of decarboxylation. An additional complication in these reactions was the reactivity of imine (226) towards nucleophiles, which resulted in formation of the ring-opened compounds (228). The nucleophile could be an alcohol or water, water being the solvent normally used in the Ugi condensation. Since it appeared that the acid (220) could not be prepared from the ester (226), the synthesis was abandoned.

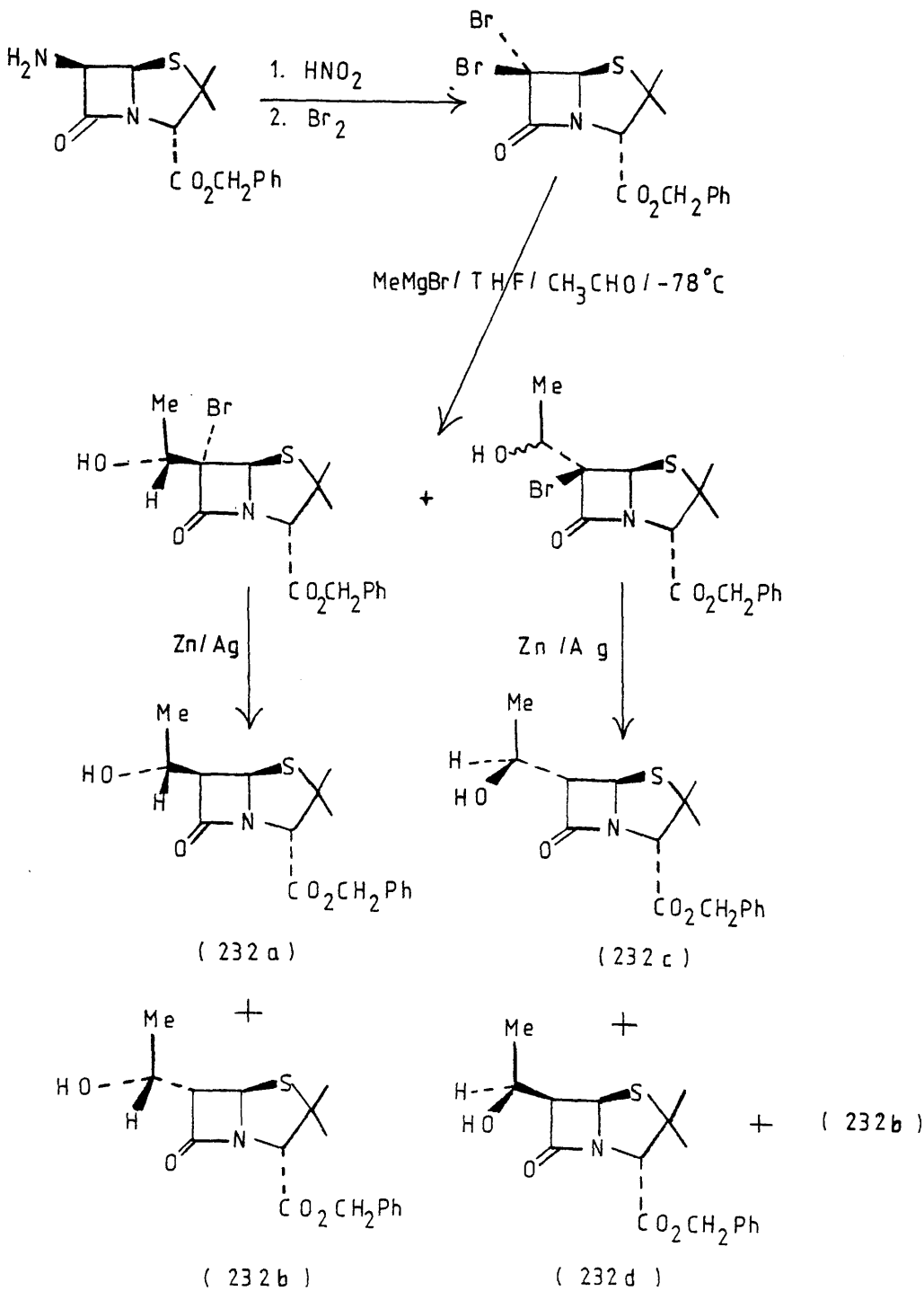
Ugi took up the challenge and in 1979 published[78] a successful application of a four-component condensation in Just's synthetic scheme. Concentrating attention on the natural cephalosporin system, rather than a 1-oxacephalosporin, the thiazoline (229) was prepared using the methods developed by Just. Ugi also commented on the instability of this compound but, with careful control of the reaction conditions, was able to cleave the methyl ester with lithium iodide and pyridine in DMF. The resulting acid (230) was too unstable for purification and was therefore condensed directly with t-butyl isonitrile to give the


OAc
NH
O
+
K⊕
⊖S
S
S
NHAc
(129)
S
S
NHAc
NH
O
S
S
S
NHAc
N
O
P Ph3
CO2PNB
1. Δ
2. H2 / Pd
S
S
NHAc
N
O
CO2H
(233)

SCHEME 17.



(231)



SCHEME 16.

cepham (231). Here the usual solvent, water, could not be employed but a CH_2Cl_2-Et_2O mixture proved to be effective. The stereochemistry at C-4 was β, as expected, and the stereochemistry across the β-lactam ring was trans. The cepham (231) contains sufficient functionality for transformation into a biologically active cephalosporin. Hopefully, one of the two research groups will complete the synthesis. However, the difficulties inherent in this approach may render it of more esoteric interest than of practical importance.

(j) Epilogue

Although work continues on the development of new approaches to the synthesis of β-lactam ring systems, the major synthetic effort lies in the preparation of analogues of the known β-lactam antibiotics using the above routes. Recently, considerable effort has also been expended on interchanging various structural features of the different classes of β-lactam antibiotics, for example, replacement of the 6-amido side chain in penicillin by the hydroxyethyl side chain of the olivanic acids. The synthetic route employed[79] in this transformation is outlined in Scheme 16. The isomers (232a)-(232d) were separated by chromatography and the free acids obtained by hydrogenolysis. It was found that the trans-isomers were of equivalent activity but were less active than the cis-isomers which also possessed equal activity. All of the isomers were, however, considerably less active than penicillin. Another example of the hybridisation of different classes of β-lactam antibiotics is the penem (233), prepared[80] by a standard sequence of reactions (Scheme 17). This compound, being unsubstituted at C-6, is reminiscent of clavulanic acid, and it carries a cysteamine side chain at C-2 like the olivanic acids. The penem nucleus itself

is a hybrid of penicillin and cephalosporin, hence structure (233) represents a hybrid of four classes of β-lactam antibiotics. In this case the novel substitution pattern resulted in improved antibiotic properties.

This is but a short survey of the more important syntheses of β-lactam antibiotics. For an extensive account of synthesis in this field the reader is directed to an excellent comprehensive review[81] which appeared during the preparation of this account.

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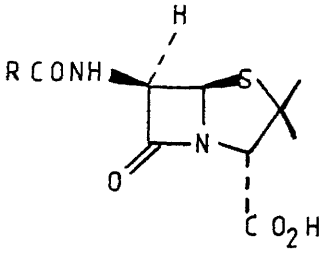

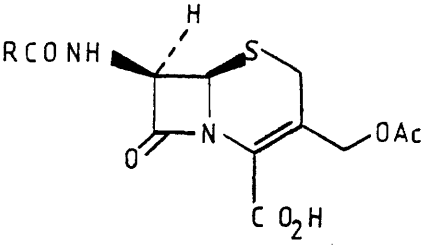

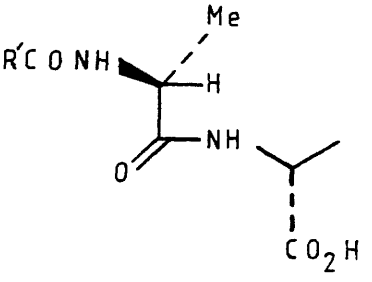

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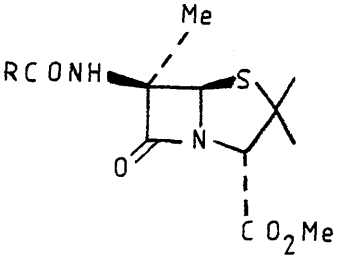

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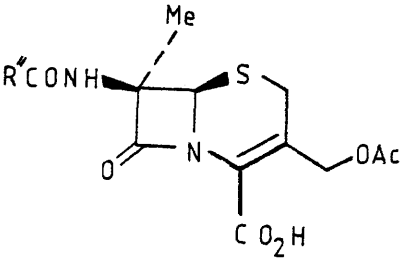
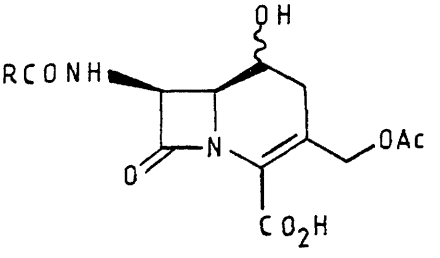


(2b)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RCONH
N
OAc
O
CO_2H

(1)

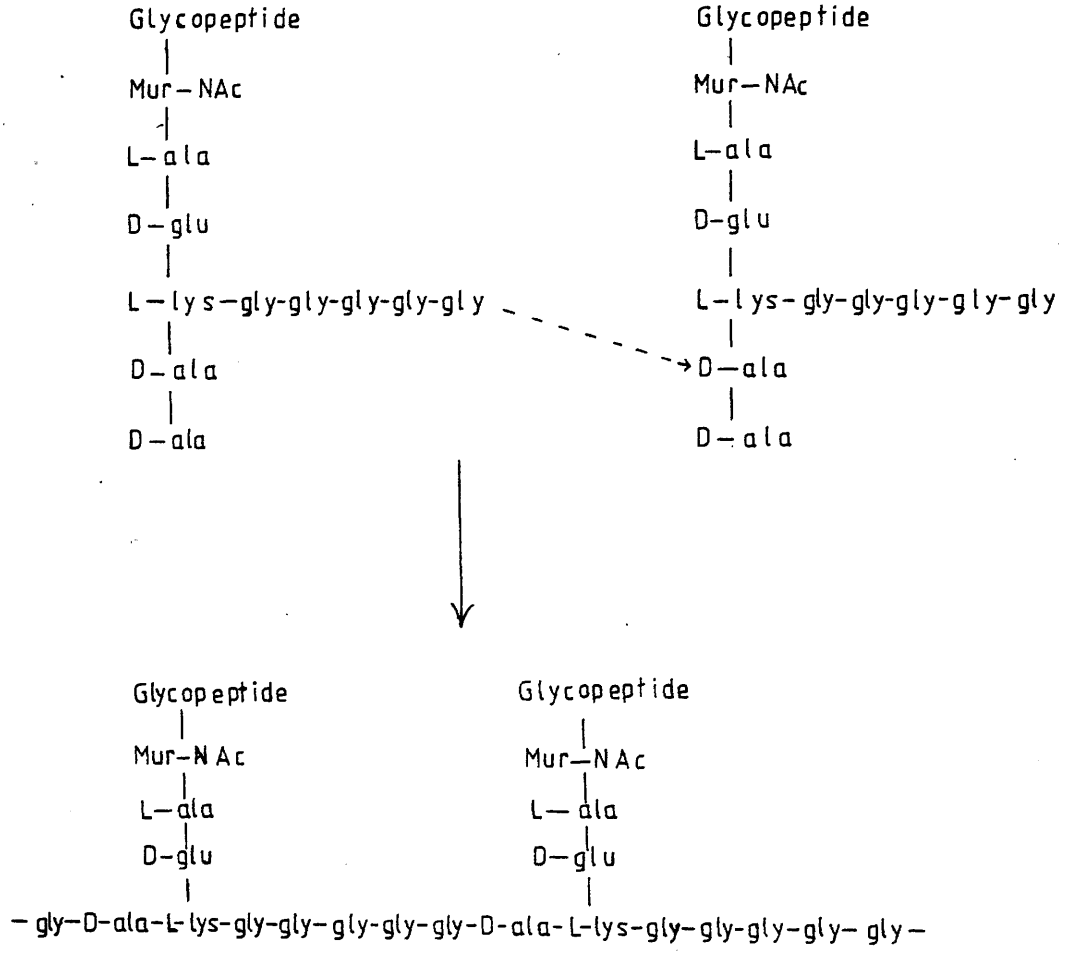

Glycopeptide
Mur–NAc
L–ala
D–glu
L–lys–gly–gly–gly–gly–gly
D–ala
D–ala
Glycopeptide
Mur–NAc
L–ala
D–glu
L–lys–gly–gly–gly–gly–gly
D–ala
D–ala
Glycopeptide
Mur–NAc
L–ala
D–glu
Glycopeptide
Mur–NAc
L–ala
D–glu
– gly–D–ala–L–lys–gly–gly–gly–gly–gly–D–ala–L–lys–gly–gly–gly–gly– gly –

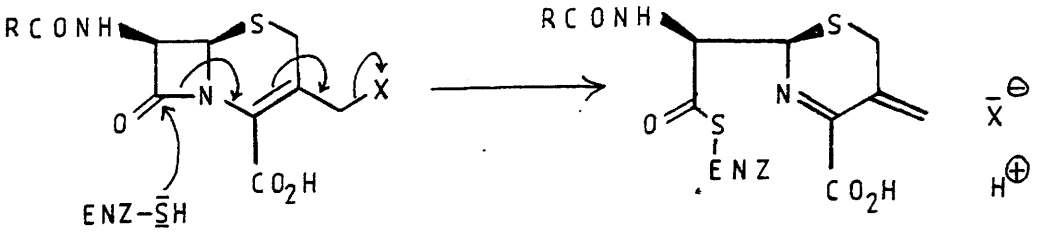
FIGURE 1.

Results and Discussion

The research described herein has been directed towards synthesis of the nuclearly modified cephalosporin (1). The motivations for initiating such an investigation were manifold. The target compound may be superior or complementary to the parent antibiotic. Additionally, by observing changes in biological activity associated with variation in the molecular framework of a series of analogues, structure-activity relationships may be determined. The last, and by no means least, justification is that such an endeavour is chemically challenging; as such it should result in increased knowledge of existing synthetic processes, and could lead to new methodology.

It is well established that the β-lactam antibiotics interfere with the synthesis of mucopeptide, the rigid structural component of bacterial cell walls. The antibiotic affects the final stage of mucopeptide synthesis, which is a crosslinking of peptidoglycan strands (Figure 1). In 1965 Tipper and Strominger proposed[1] that the transpeptidase enzyme responsible for this crosslinking falsely recognises the β-lactam antibiotic as the terminal (D)-alanyl-(D)-alanine residue of a peptidoglycan strand. In doing so, it becomes irreversibly acylated, and thus inactivated. The similarities between the three-dimensional structures of penicillin, cephalosporin and (D)-alanyl-(D)-alanine are represented in Figure 2. With inactivation of transpeptidase, and as it grows, the bacterium cell wall structure becomes increasingly disordered until it can no longer resist the high osmotic pressure of its contents; the cell then bursts.

Structure-activity relationships, in contrast, are but poorly understood. A striking example of this is presented by the cases of the 6α-methyl penam (2 a) and the 7α-methyl cephem (2b). Tipper



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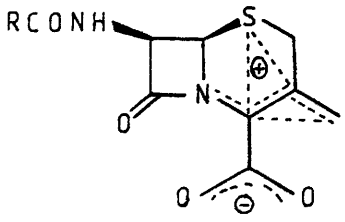

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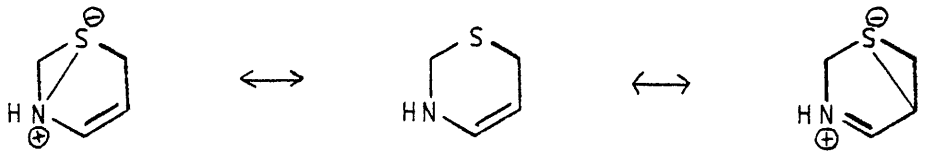
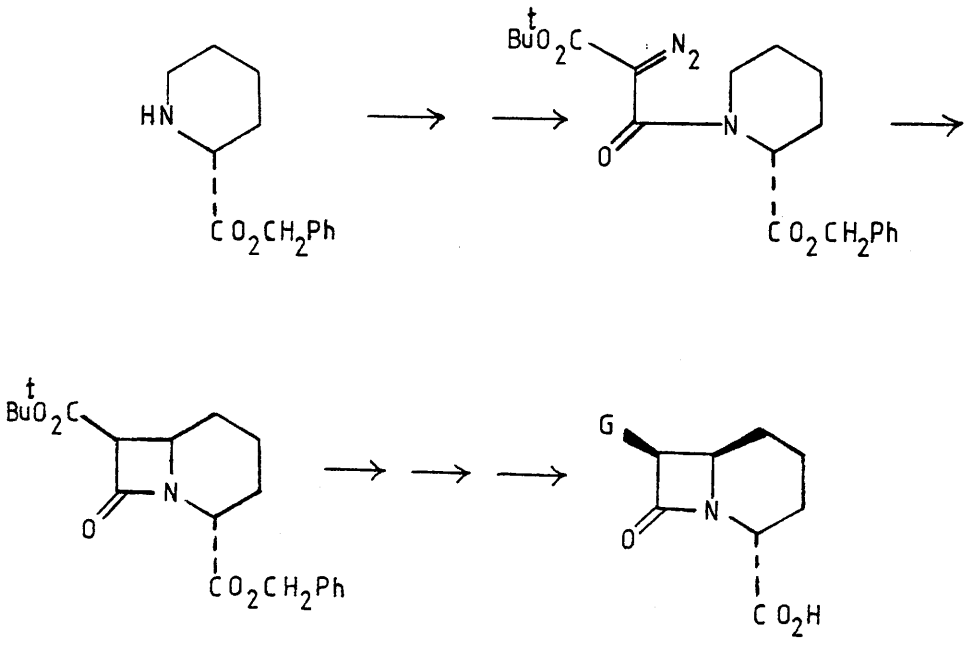


FIGURE 3.

and Strominger suggested[2] that the spatial similarity between penicillin, cephalosporin and (D)-alanyl-(D)-alanine (Figure 2) could be increased by introduction of methyl groups at the 6α- and 7α- positions, respectively, and therefore produce more powerful antibiotics. In 1971, Bohem reported[3] the synthesis of penam (2a) and cephem (2b); surprisingly, they were much less active than the naturally occurring parent compounds.

However, there is some correlation between antibiotic potency and chemical reactivity of the β-lactam ring towards nucleophiles. This is especially evident for a particular series of cephalosporins[4] and must be related to the ability of the antibiotic to acylate the transpeptidase (Scheme 1). The ability of the 3'-substituent, X, to influence the reactivity of the β-lactam ring depends upon the following electronic effects. The degree of σ- and, more importantly, π-electron withdrawal will influence the electrophilicity of the β-lactam carbonyl carbon atom and also the stability of the transition state structure involved in opening of the β-lactam ring. Additionally, the tendency of the substituent to leave with a bonding pair of electrons will affect the ease of ring opening.

The target compound (1) of this research involves modification of position-1 of cephalosporin. Relatively little is understood about structure-activity relationships associated with this position. The role played by sulphur in the chemistry and biology of cephalosporin has been the subject of considerable speculation for several years. The delocalised structure (3) has been proposed[5] as an intermediate in the solvolysis of the 3'-acetoxy group. The ultraviolet spectrum of cephalosporin exhibits an absorption at ca. 260nm, which is of abnormally long wavelength for a simple enamine system. It has been suggested[6] that the sulphur d-orbitals contribute to the chromophore (Figure 3), to produce a bathochromic shift. In 1974, Topp and Christensen



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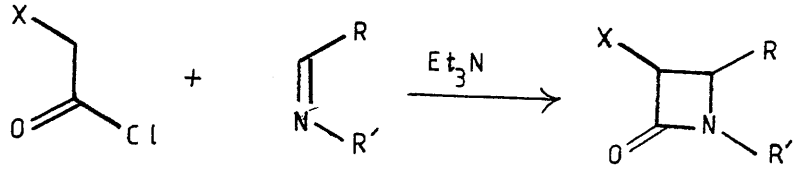

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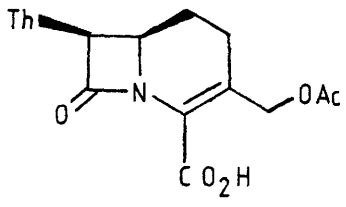

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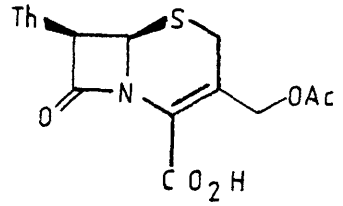

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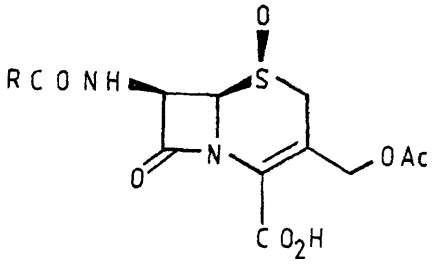

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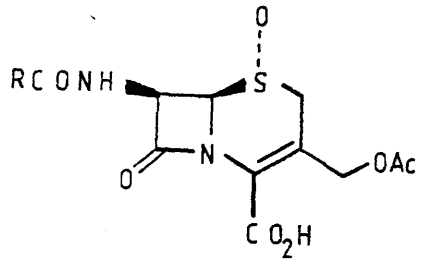

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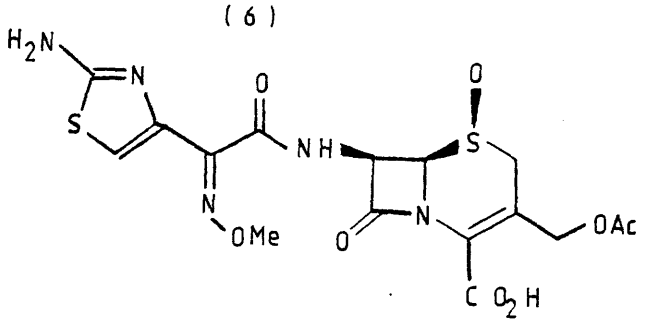

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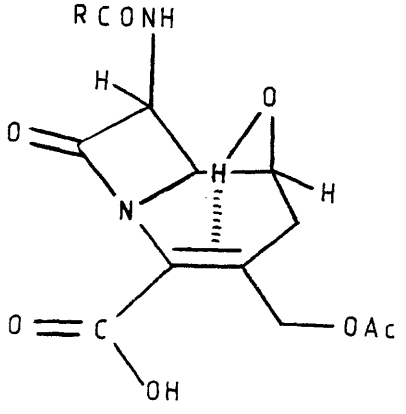
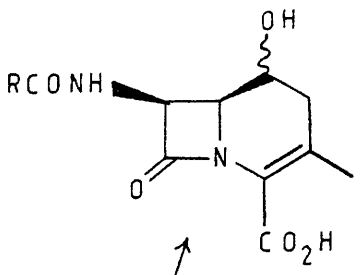


FIGURE 4.

showed[7b] that the presence of the sulphur atom was not mandatory for biological activity, the 1-carbacephalothin (4) exhibiting activity comparable with natural cephalothin (5) (interestingly, the predicted hypsochromic shift in the ultraviolet spectrum of analogue (4) was indeed observed). Another important modification of position-1 of cephalosporin is oxidation of the thio-ether to the two sulphoxides (6) and (7). In general[8], the (S)-sulphoxide (6) is inactive; in contrast, the (R)-sulphoxide (7) retains biological activity, and in some cases may be even more potent than the parent thio-ether. A recently reported[9] exception to this generalisation is that the (S)-sulphoxide (8) is more active than the corresponding (R)-sulphoxide.

An appraisal of the above information made 1-hydroxy-1-carbacephalosporin (1) an interesting synthetic target. The hydroxyl group is ideally situated in at least one of the two possible carbinol epimers for intramolecular hydrogen bonding[10] with the Δ^3-double bond (Figure 4). This could activate the β-lactam ring by π-electron withdrawal, and so produce a more potent antibiotic. Two approaches to the synthesis of 1-hydroxy-1-carbacephalosporin (1) were investigated. The first was based on the Lowe methodology,[11] which involves annelation of a β-lactam ring on to a preformed cyclic system via a carbene insertion reaction (Scheme 2). The second route was modelled on the Merck[7b] and SKF syntheses, cycloaddition of imines with ketene equivalents being employed for β-lactam ring formation (Scheme 3). The Lowe, Merck, and SKF synthetic strategies, and their use in the preparation of nuclearly modified β-lactam ring systems are discussed in detail in the Introduction. The results of their application to the synthesis of 1-hydroxy-1-carbacephalosporin (1) are revealed below.



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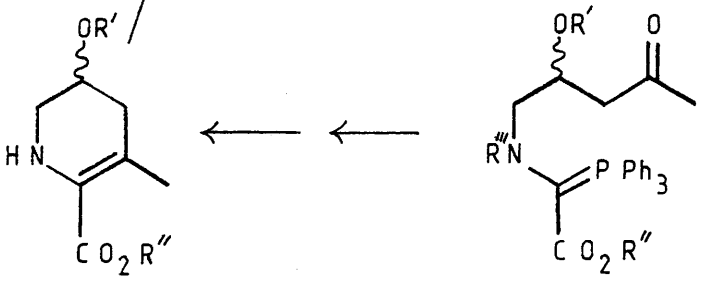

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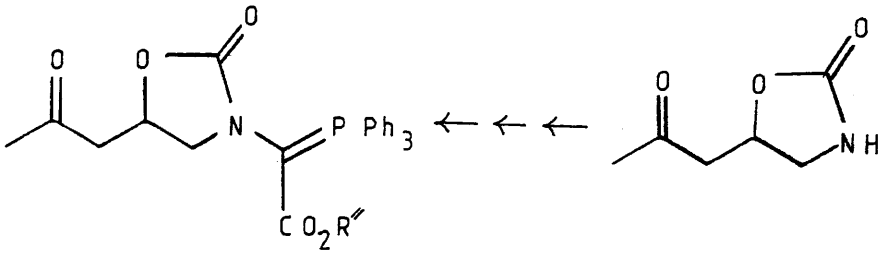

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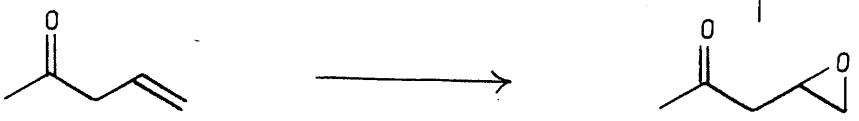

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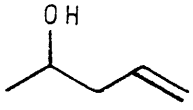
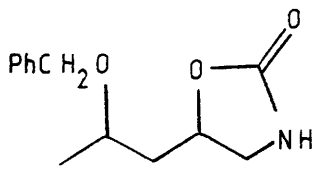


(16)

1. Oxazolidone Approach

The less highly functionalised 3'-deacetoxy-1-hydroxy-1-carbacephalosporin (9) was chosen as the initial synthetic target for several reasons. Although their activity is somewhat reduced, 3'-deacetoxycephalosporins are nevertheless potent antibiotics. Additionally, a reduction in functionality would facilitate the synthesis both by reducing the number of necessary transformations, and by involving more tractable intermediates. Finally, if antimicrobial activity is observed in the deacetoxy compound (9), then the acetoxy compound (1) could be prepared either by allylic functionalisation or by modification of the synthesis at an earlier stage. Preparation of the target compound (9) by Lowe's methodology (Scheme 2) requires a suitably protected tetrahydropyridine (10). This compound could be obtained in turn by intramolecular Wittig condensation of the keto-phosphorane (11). In this intermediate the amine and hydroxyl groups could be protected simultaneously by their incorporation into an oxazolidone ring system (12). When required, deprotection could be acheived by either acidic[13a] or basic[13b] hydrolysis, or by hydride[14] reduction. It should be possible to prepare the phosphorane (12) from the oxazolidone (13) by the glyoxylate condensation procedure developed[15] by Woodward.

Oxazolidones can be prepared[16] by the reaction of epoxides with carbamates. A small amount of nucleophilic catalyst is necessary and a possible mechanism involves initial opening of the epoxide by the catalyst. To use this method to obtain the oxazolidone (13), one would require the epoxide (14) which, in turn, could be prepared by peracid oxidation of the $\beta\gamma$-unsaturated ketone (15). To avoid double bond conjugation, the alcohol (16) was employed as the starting material for the synthesis.



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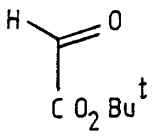

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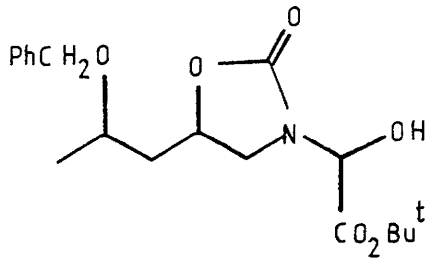

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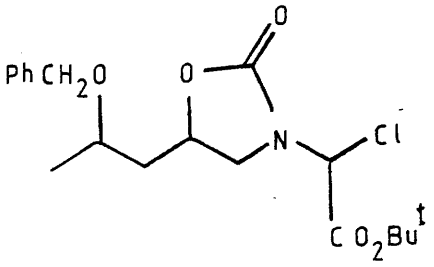

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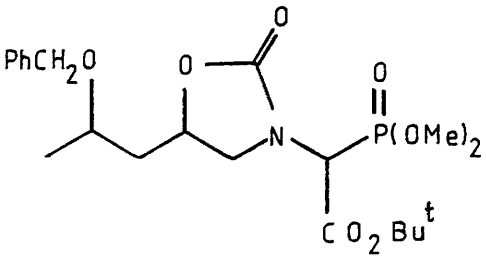

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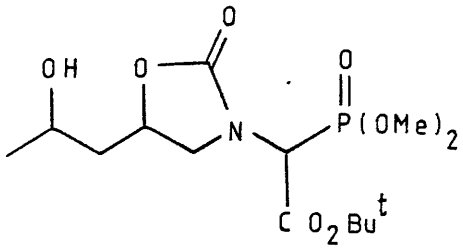

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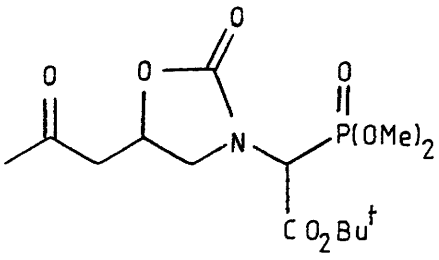

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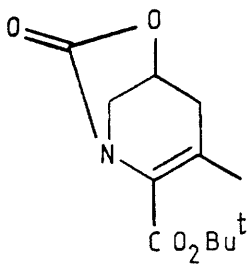

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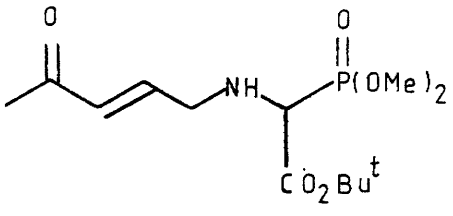

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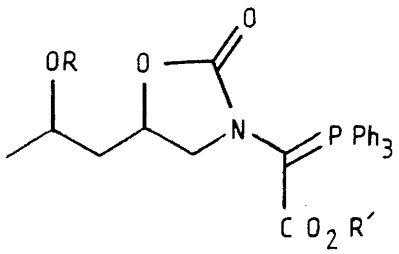

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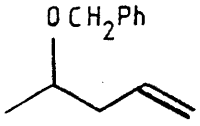

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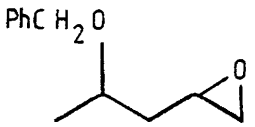

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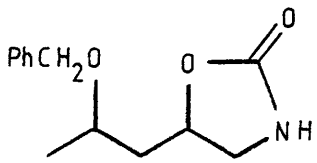

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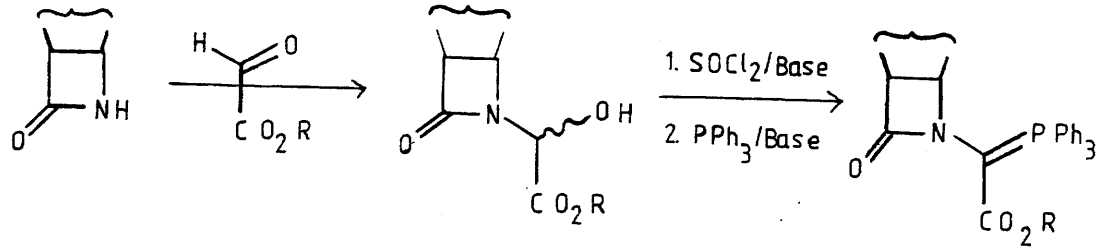

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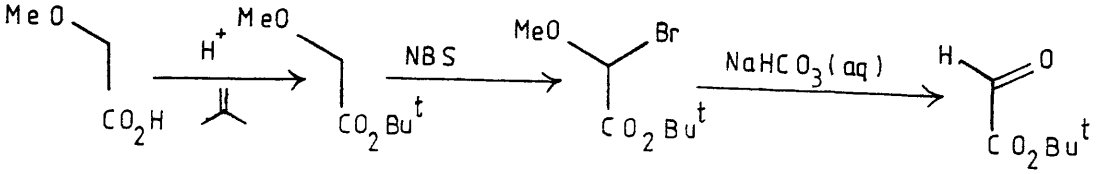
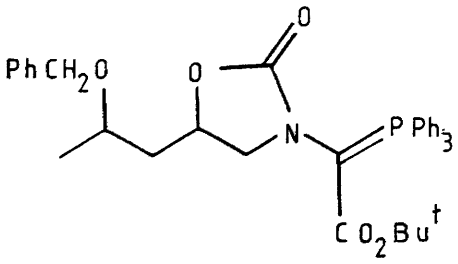


SCHEME 5.

The alcohol (16) was obtained by a published procedure involving Grignard reaction of allyl magnesium bromide with acetaldehyde. The nucleophilic hydroxyl group might be an unwelcome participant in the proposed oxazolidone ring formation and was therefore masked. The choice of masking group was constrained by the need for its subsequent removal from a target molecule (17) containing an oxazolidone ring system and an as yet unspecified carboxylic acid protecting group. The hydroxyl group was therefore masked as the hydrogenolytically labile benzyl ether (18). Oxidation with permaleic acid, a reagent reported[18] to be superior for the preparation of epoxides from terminal olefins, afforded the epoxide (19). Treatment with ethyl carbamate and a catalytic amount of triethylamine then produced the oxazolidone (20). This brought the synthesis to the stage where Woodward's procedure (Scheme 4) could be applied. The t-butyl, benzyl, p-nitrobenzyl, or trichloroethyl glyoxylate esters are normally used in this reaction sequence because they can be removed selectively from the sensitive cephalosporin nucleus. Since the oxazolidone (20) contains a hydroxyl group masked as its benzyl ether, only the t-butyl and trichloroethyl gloxylates are applicable; the former was chosen because of its relatively simple preparation[19] (Scheme 5). The oxazolidone (20) was condensed with t-butyl glyoxylate (21) to give the hemiaminal (22). Model studies[20] had indicated that the required oxazolidone-phosphorane could not readily be prepared but that the corresponding phosphonate (24) was more amenable to synthesis. Accordingly, the phosphonate was prepared by treatment of the hemiaminal (22) with thionyl chloride to give the chloride (23) which, without purification, was heated with trimethyl phosphite. Hydrogenolysis of the benzyl ether afforded the deprotected alcohol (25). Conversion into the desired bicyclic oxazolidone (27) requires oxidation to the ketone (26) and intramolecular Emmons condensation. The presence of the acid labile t-butyl ester and



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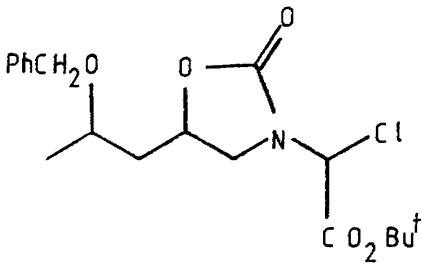

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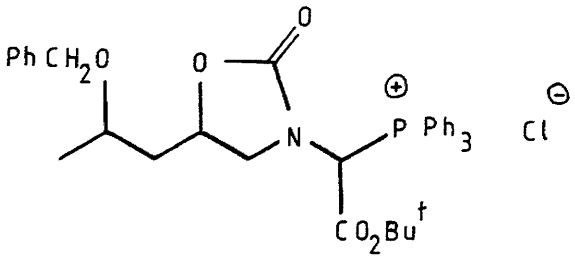

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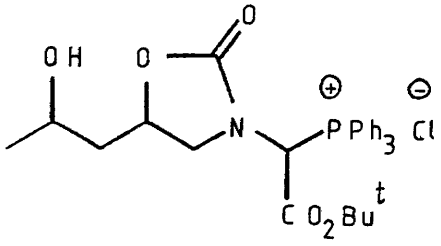
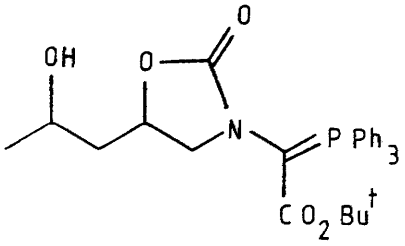
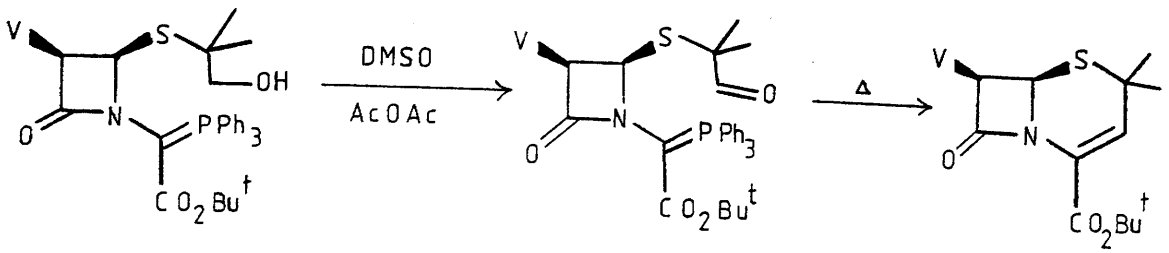


(3 1)

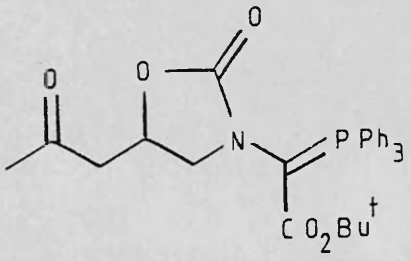


(3 2)

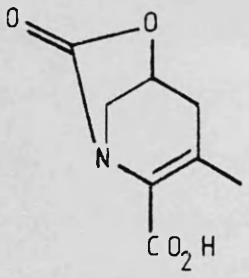


SCHEME 6.

the oxazolidone system necessitated mild oxidation conditions. Attempted oxidation with Collins[21] reagent gave, after chromatography, a low yield of a mixture of methyl ketones as indicated by ^{1}H-nmr. Oxidation with silver carbonate supported on Celite[22] offered no improvement. The desired transformation was ultimately achieved using a low temperature, two-phase Jones oxidation. The spectral characteristics of the crude reaction product were consistent with formation of the ketone (26). However, attempted purification by even short-contact chromatography yielded enone (28), an apparently facile β-elimination having cleaved the oxazolidone ring. The ketone (26) was therefore used without purification in an attempted cyclisation reaction. This unfortunately resulted only in decomposition of the keto-phosphonate, none of the desired bicyclic tetrahydropyridine (27) being detected. In hindsight, such a result is not surprising in view of the demonstrable lability of the ketone (26). To be successful, any transformation involving such a ketone would need to employ essentially neutral reaction conditions. The corresponding Wittig condensation was therefore investigated. As anticipated, synthesis of the phosphorane (29) proved to be very difficult. After considerable variation of reaction conditions, the desired compound was prepared in satisfactory yield from the chloride (23) by heating under reflux in toluene with triphenylphosphine and lutidine. Unexpectedly, the benzyl ether could not be cleaved from the phosphorane (29) by hydrogenolysis. This problem was circumvented by performing the hydrogenolysis on the intermediate phosphonium salt (30). The resulting alcohol (31) was converted into the hydroxy-phosphorane (32) by treatment with sodium hydroxide. In view of the established lability of the keto-oxazolidone system it was decided to attempt oxidation and cyclisation without isolation of intermediates. Such a process has been described[15] for a similar system (Scheme 6). The hydroxy-



(3 3)



(3 4)

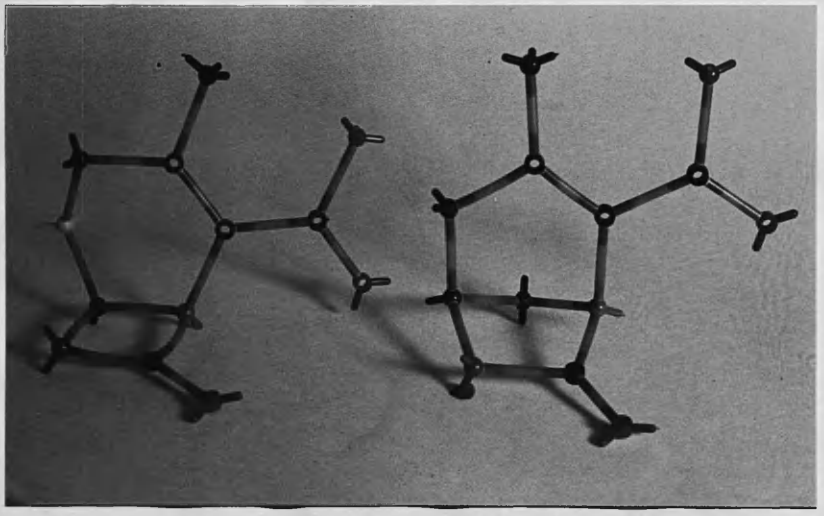


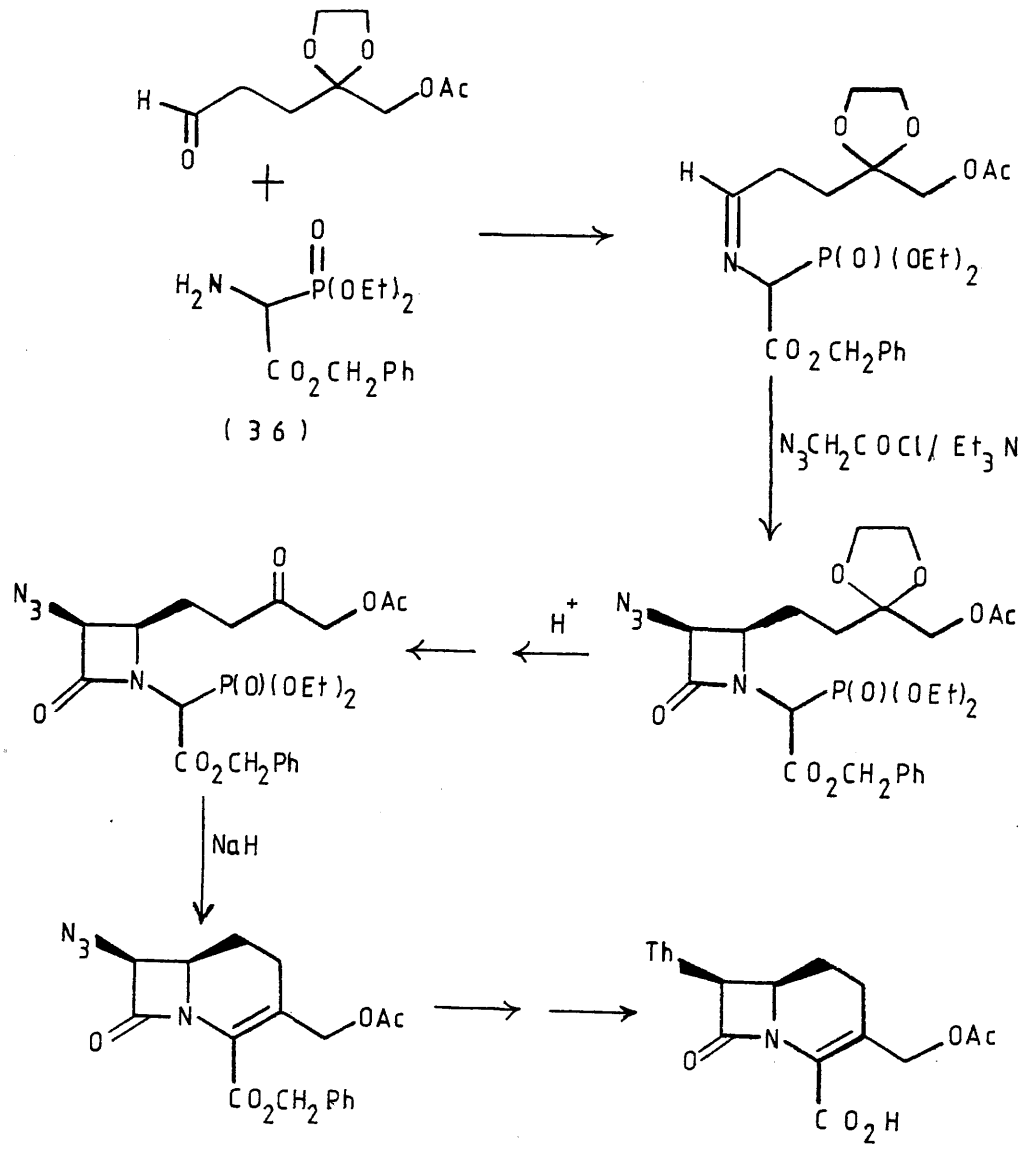
PLATE 1.

(1)

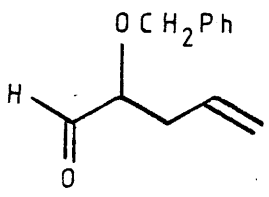
phosphorane (32) was therefore treated with DMSO and acetic anhydride. Tlc Monitoring indicated that, by analogy with the oxidation of the hydroxy-phosphonate, oxidation had occurred and the mixture was therefore heated in an attempt to bring about cyclisation. No products of cyclisation were detected from this reaction, tlc analysis indicating extensive decomposition. It may be that the small amount of acetic acid present is catalysing decomposition of the desired keto-phosphorane (33). Some effort should therefore be made to isolate this compound before subjecting it to the elevated temperatures required for Wittig reaction. Further investigation of this sequence was postponed because of developments in the alternative approach, which eventually led to the preparation of 1-substituted-1-carbacephem compounds. Nevertheless, this does not mean that the oxazolidone route should be abandoned. The bicyclic tetrahydropyridine carboxylic acid (34) has a three-dimensional structure very similar to that of cephalosporin, especially in the important α-acylamino-$\alpha\beta$-unsaturated acid region (Plate 1). This bicyclic compound may therefore possess interesting biological properties and has accordingly become a synthetic target in its own right.

Imine-Ketene Cycloaddition Approach

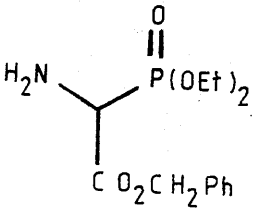
The second synthetic investigation of 1-hydroxy-1-carbacephalosporin (1) was constructed around creation of the β-lactam ring by reaction of an imine with a ketene or its equivalent. The cycloaddition reaction of imines with ketenes was initially discovered[23] in 1907 by Staudinger. In more recent times, it has been studied extensively[24-26] by Bose and his collaborators. Although the mechanism of this reaction is complex and incompletely understood, some empirical generalisations can be made.



SCHEME 9.



(35)



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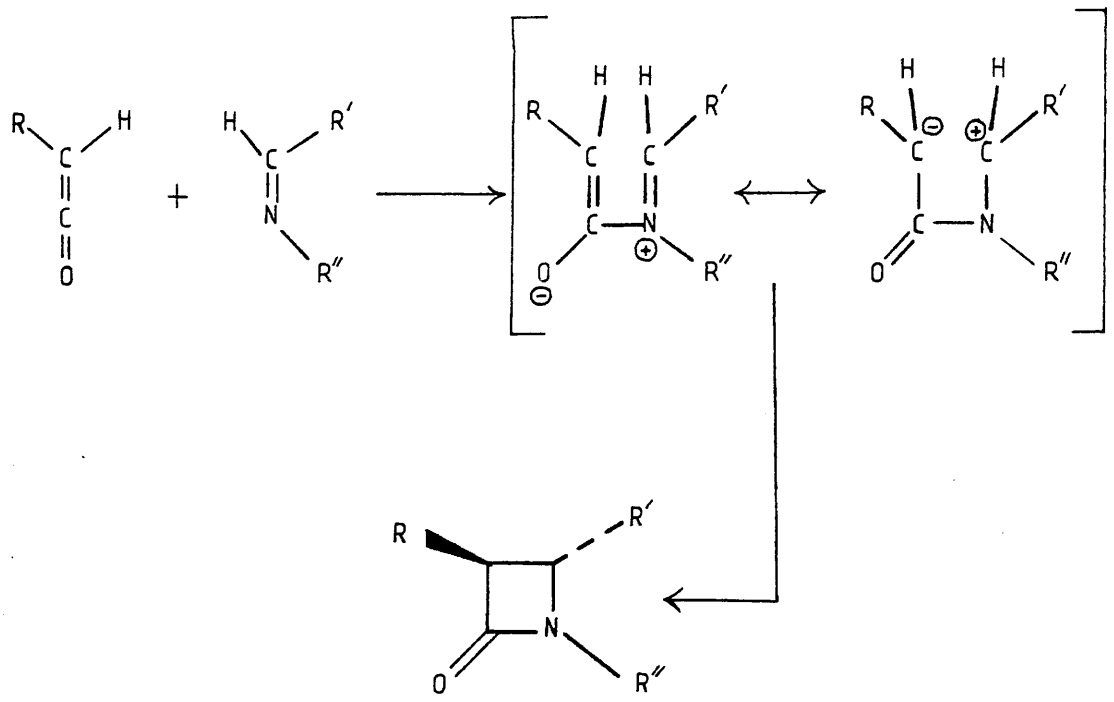
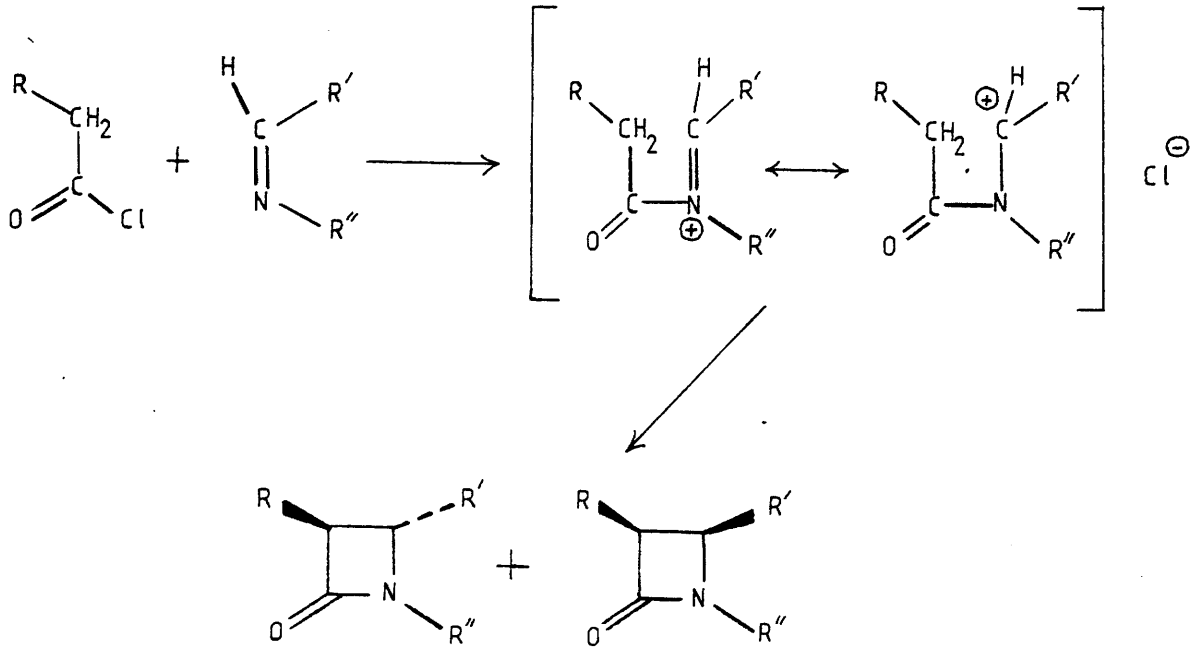


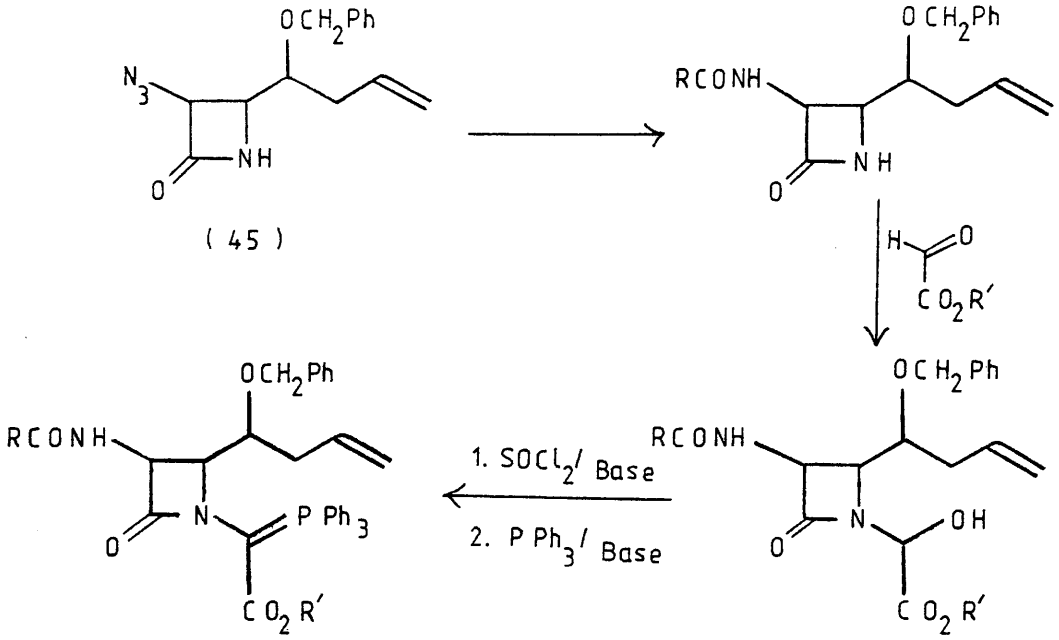
SCHEME 7.



SCHEME 8.

The reaction of an imine with a preformed ketene[27,28] involves a zwitterionic intermediate (Scheme 7); such a pathway usually leads to a trans-3,4-disubstituted β-lactam. On the other hand, reaction with an activated carboxylic acid derivative probably proceeds via an acyl iminium salt (Scheme 8); triethylamine will then remove an α-proton, and the resulting cyclisation will lead to a mixture of cis- and trans-3,4-disubstituted β-lactams. The ratio of cis to trans isomers obtained depends upon several factors, including the mode of addition[24] of reagents; addition of acid chloride to a mixture of imine and triethylamine favours cis-disubstitution whereas addition of triethylamine to a solution of imine and acid chloride favours trans-disubstitution. In those cases in which the acid chloride carries an α-substituent with a lone pair of electrons, mixtures of cis- and trans-β-lactams are obtained, otherwise[26] the trans-isomers are normally observed. The nature of the imine substituent R' is also important; when R' is a thioalkyl group trans-disubstituted products predominate[29], when it is an alkyl chain cis-disubstitution is the major isomer[7b], and when it is an aromatic group cis-trans mixtures[24] of isomers are obtained. The outcome of this reaction is further complicated in that the two general pathways outlined in Schemes 7 and 8 are not necessarily exclusive, mixed pathways being highly probable in some cases.

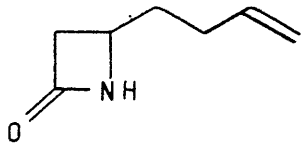
The Merck group have employed cycloaddition of an imine and a ketene equivalent in a synthesis[7b] of 1-carbacephalothin (Scheme 9). The results of a previous attempt[20] to adapt this synthesis to the preparation of 1-hydroxy-1-carbacephem structures strongly influenced the direction of the present work; these are therefore summarised below. The aldehyde (35), in which the terminal double bond permits introduction of the requisite functionality at a later stage, was chosen as one of the key building blocks. Condensation with the amine (36) then gave



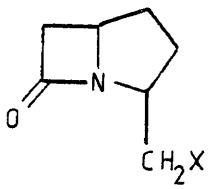
(45)

(46)

SCHEME 14.

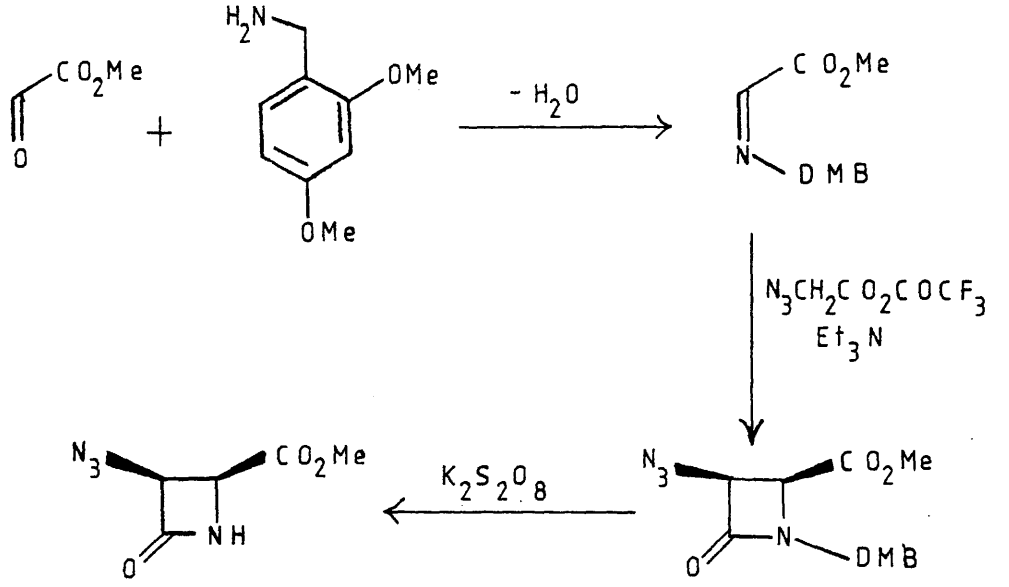


(47)

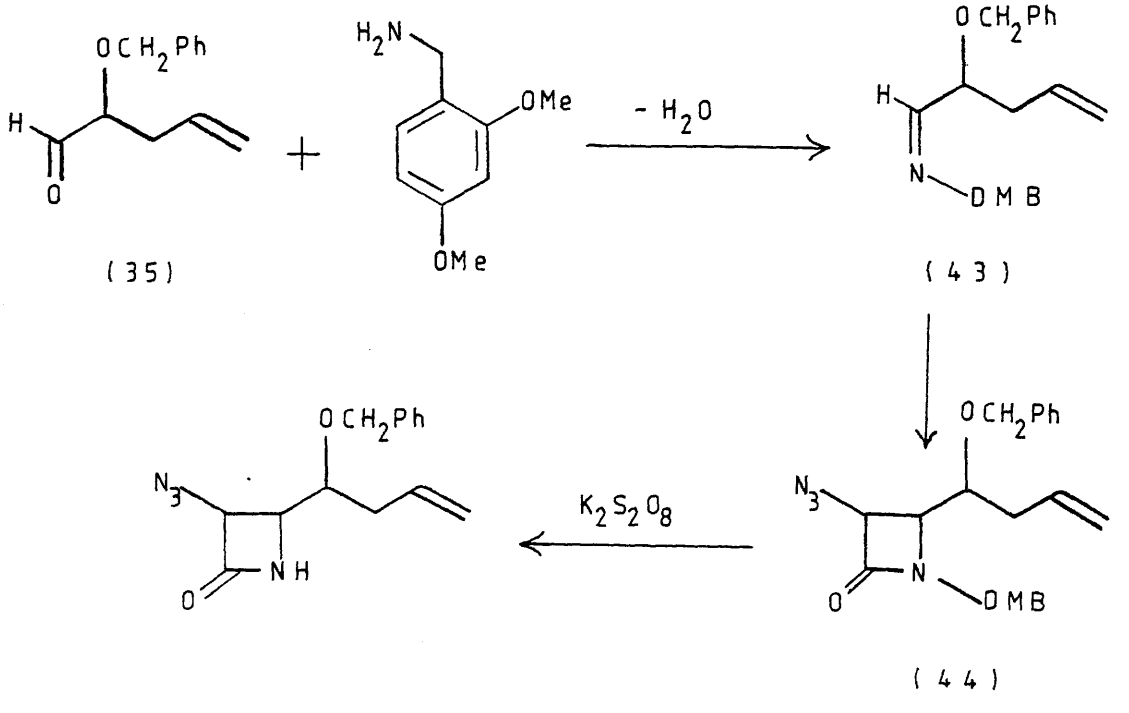


(48 a) X = I

(48b) X = HgOAc



SCHEME 12.

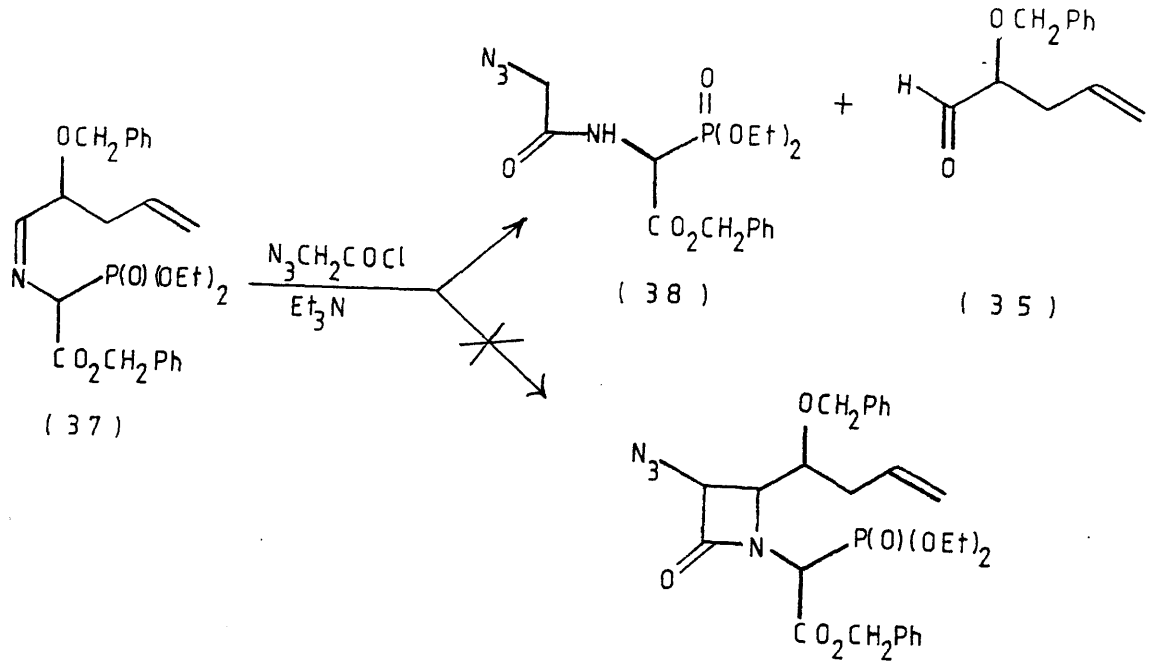


(35)

(43)

(44)

SCHEME 13.



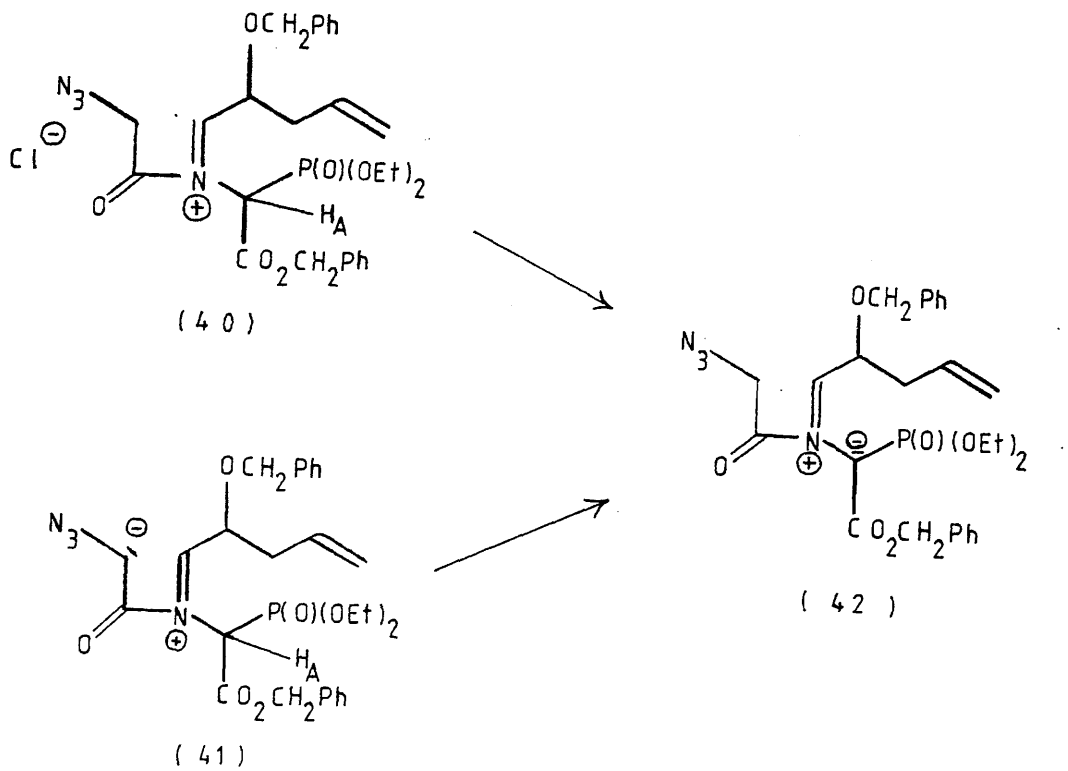
(37)

(38)

(35)

(39)

SCHEME 10.



(40)

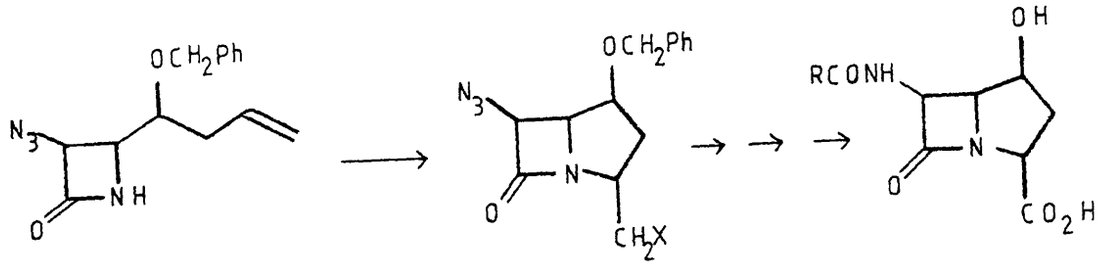
(41)

(42)

SCHEME 11.

the imine (37). This imine was treated with azidoacetyl chloride and triethylamine under a considerable range of reaction conditions, but the desired β-lactam (39) was never detected in these reactions, the sole products being the acylated amine (38) and the starting aldehyde (35) (Scheme 10). To account for these results, it was proposed that the initially formed adducts (40) and/or (41) could undergo either base abstraction or proton transfer of the highly acidic proton H_A to form in both cases the ylid (42) (Scheme 11). This ylid will lie in a thermodynamic well on the reaction coordinate, and the driving force for cyclisation will then be lost. On isolation, protonation of the ylid (42) will reform the reactive iminium ion (40) which will be hydrolysed immediately to the observed products, the aldehyde (35) and the amide (38). This particular investigation was therefore abandoned.

Recently, chemists at SKF have developed[11] a method for the preparation of β-lactams <u>via</u> imine + ketene cycloaddition in which the amine component of the imine can be regarded as equivalent to ammonia itself (Scheme 12). If the above rationalisation is correct, the SKF methodology should offer a solution to the problem of β-lactam formation. The imine (43) which would be formed by condensation of the aldehyde (35) with 2,4-dimethoxybenzylamine does not possess such a highly acidic proton as before, and the formation of the β-lactam (44) should therefore be relatively uncomplicated. Continuation of the SKF route would furnish the deprotected β-lactam (45) (Scheme 13). After reduction of the azide and acylation, application of Woodward's procedure[15] (Scheme 14) would give a compound (46) functionally equivalent to the β-lactam (39) which could not be obtained directly. Recently, Durst has reported[30] that 4-(3'-butenyl)azetidin-2-one (47) undergoes a novel cyclisation to give carbapenam structures (48) when treated with electrophiles such as iodine or mercuric acetate. This makes the β-lactam



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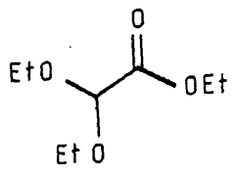
SCHEME 15.



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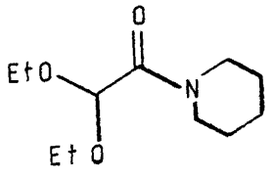

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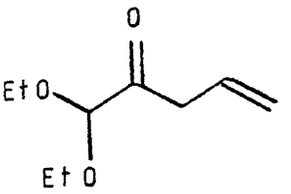

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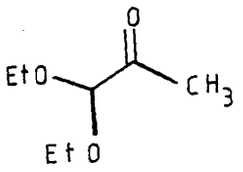

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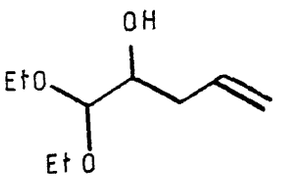

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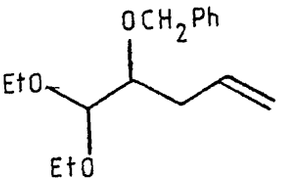

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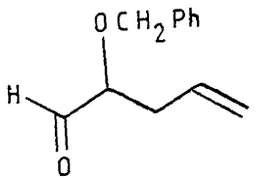

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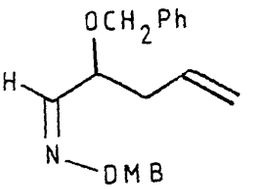

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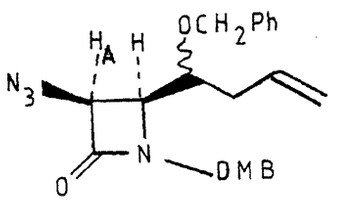

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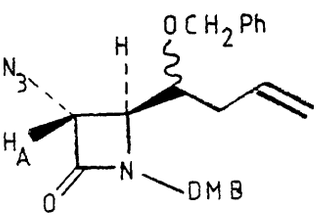
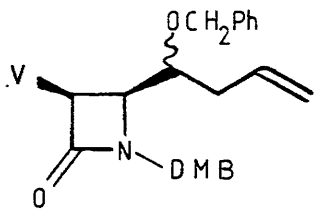


(56)

(45) additionally attractive since it could be converted by such a procedure into 1-hydroxy-1-carbapenam analogues (Scheme 15).

To return to the investigation of β-lactam formation, the aldehyde (35) was prepared as follows. The glyoxylate acetal (49) was converted into the piperidide (50), which was subjected to a Grignard reaction with allyl magnesium chloride to give the $\beta\gamma$-unsaturated ketone (51). This is a modification of a preparation[31] of the methyl ketone (52). The unsaturated ketone (51) proved to be extremely labile with regard to double bond conjugation and was therefore reduced directly at low temperature to the alcohol (53). Protection of the alcohol as its benzyl ether (54) and acid-catalysed hydrolysis of the acetal then gave the required aldehyde (35). The imine (43) was obtained by condensation of the aldehyde with 2,4-dimethoxybenzylamine. Treatment of a mixture of this imine and triethylamine in THF at $-78^{\circ}C$ with azidoacetyl chloride resulted most gratifyingly in efficient β-lactam formation. Chromatographic separation furnished pure <u>cis</u>-β-lactam (55), and a mixture consisting of equal amounts of <u>cis</u>-β-lactam (55) and <u>trans</u>-β-lactam (56), in a ratio of 2:1 respectively. The stereochemical assignment was based on the observed ^{1}H-nmr coupling constants[32] of a one proton doublet at δ 4.5ppm (H_A); these were J=5Hz in the <u>cis</u>-isomer (55) and J=2Hz in the <u>trans</u>-isomer (56).

The lability of these monocyclic β-lactams encouraged the thought that reduction of the azide followed by acylation might confer some stability and thus improve the chance of success in the oxidative removal of the dimethoxybenzyl group. Azides are usually reduced to amines by catalytic hydrogenation, but this could additionally cleave the benzyl ether protecting group, and would reduce the terminal double bond. However, 3-azidoazetidin-2-ones have been reduced[33] by treatment with a combination of hydrogen sulphide and triethylamine; this method



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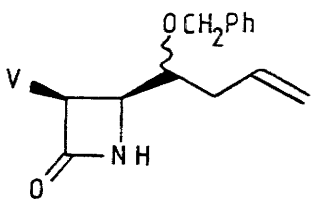

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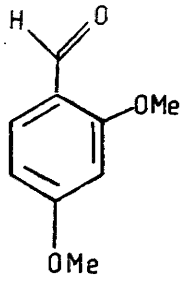

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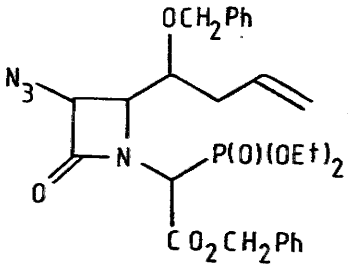

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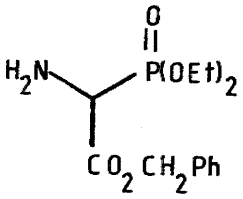

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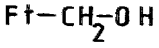

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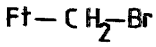
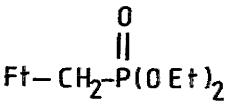


(61)



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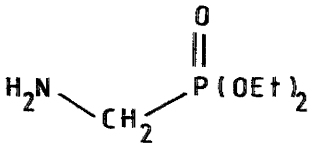
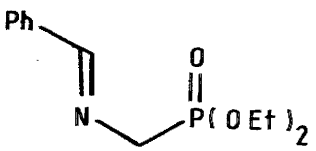


(63)

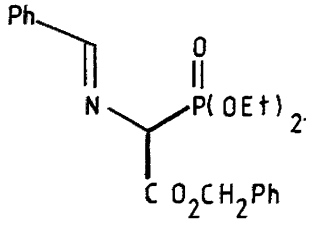


(64)

proved successful for the reduction of the cis-fused azido-β-lactam (55). The resulting amine was treated, without isolation, with phenoxyacetyl chloride to give the stable crystalline acetamido-β-lactam (57); additional amounts of this compound could be obtained by reduction and acylation of the cis-trans mixture, followed by fractional crystallisation.

When the protected β-lactam (57) was subjected to the conditions reported[11] for the removal of the dimethoxybenzyl group, the deprotected compound (58) was not obtained. The only discrete product isolated was 2,4-dimethoxybenzaldehyde (59), indicating that deprotection had certainly occurred. Tlc Analysis of the crude reaction products showed the presence of dimethoxybenzaldehyde, together with highly polar material, suggestive of considerable decomposition having taken place. Extensive variation of the reaction conditions, including the addition of silver nitrate[34], did not alter the outcome. Benzylic[35] or allylic oxidation of the other active methylene positions in lactam (57) may well be complicating the deprotection, which, to date, has still not been achieved. Although this apparent dead end was met with disappointment, the success of the β-lactam formation itself prompted a reinvestigation of the synthesis of lactam (39), employing the same reaction conditions.

The required amine component (36) was therefore prepared in the following manner. Condensation of phthalimide and formaldehyde gave the hemiaminal (60), which was converted into the bromide (61). An Arbusov reaction with triethyl phosphite then gave the phthalimidophosphonate (62), which was subjected to hydrazinolysis. The resulting aminomethylphosphonate[36] (63) was both protected and activated as its Schiffs base (64). C-Acylation with benzyl chloroformate using lithium isopropylcyclohexylamide as base afforded the glycine derivative



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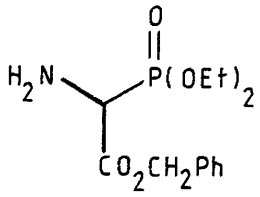
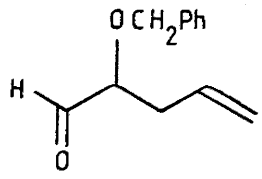


(36)



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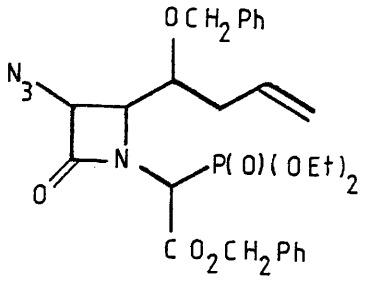
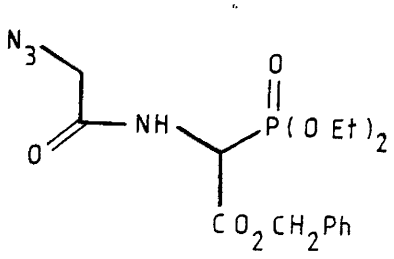
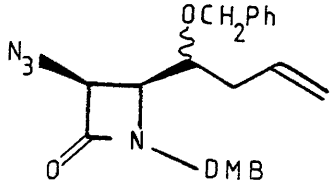


(39)



(38)



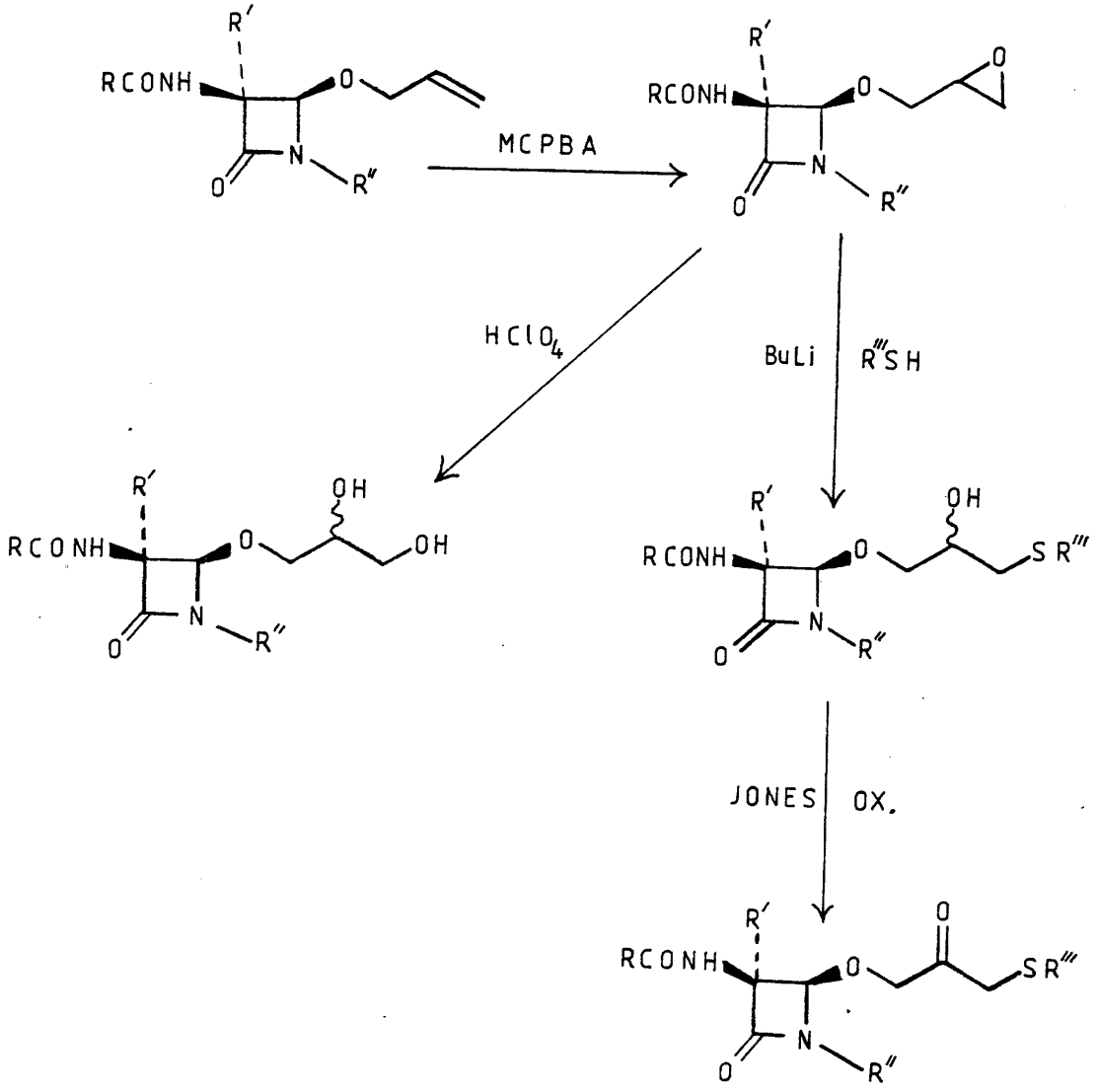
(55)

(65). Deprotection then gave the required amine component (36). The sequence from amine (63) to the acylated species (36) is a modification of the reported[37] procedure.

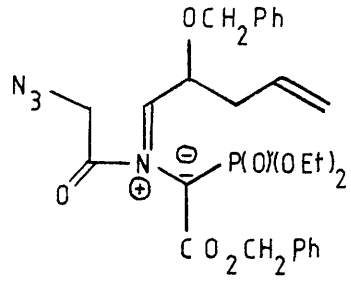
When the amine (36) was condensed with the aldehyde (35) and the resulting imine treated with azidoacetyl chloride under the previously successful cycloaddition conditions, a low yield (5%) of the desired β-lactam (39) was obtained. The ^{1}H-nmr spectrum of this compound was broad and unresolved, indicating that a gross mixture of stereoisomers was present, although this was not apparent from its behaviour on tlc analysis. Initially it was believed that this product was a mixture of cis- and trans-3,4-disubstituted β-lactams; the results of subsequent transformations on a similar series of compounds will indicate that this assumption is probably incorrect.

On subsequent repetitions of the cycloaddition reaction the yield of lactam varied between 0 and 5%, despite strenuous efforts to maintain constant reaction conditions. In an attempt to obtain the β-lactam (39) on a preparative scale, the reaction conditions were subjected to extensive investigation. The order of addition of the reactants was varied, ranging from modes thought to favour pre-formation of a ketene (addition of the imine to a solution of azidoacetyl chloride and triethlamine) to modes where the iminium salt is pre-formed (addition of triethylamine to a mixture of the imine and azidoacetyl chloride). No improvement in yield was attained; moreover, for any particular order of addition, the yield observed was not reproducible. It would therefore appear that the desired β-lactam (39) is occurring only as a side-product in this reaction, the main product being the amide (38).

Since the formation of the N-dimethoxybenzyl substituted β-lactam (55) is a facile process, whereas the formation of the N-phosphonatoacetate substituted β-lactam (39) is extremely intractable, it is



SCHEME 17.



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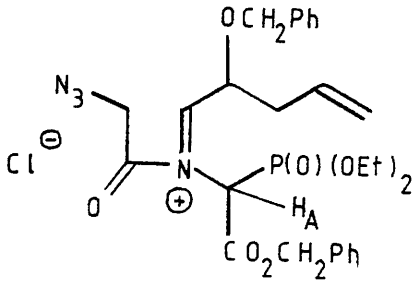
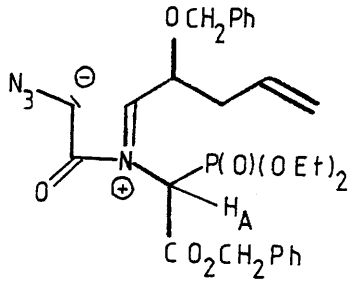
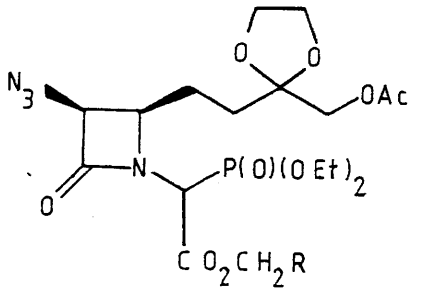


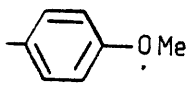
(4 1)

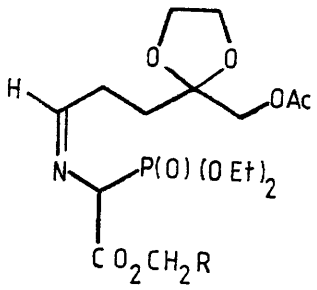


(4 0)

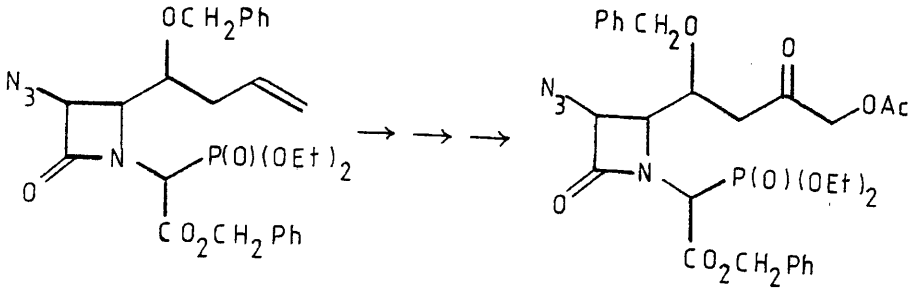


(6 6 a) R = Ph

(6 6 b) R = 



(6 7)

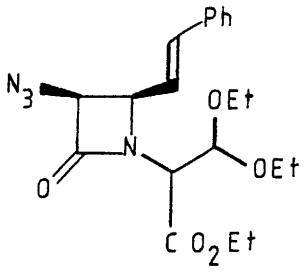


(3 9)

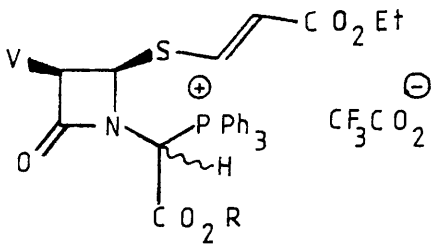
SCHEME 16.

apparent that the explanation proposed for the difficulties previously encountered is correct. This encouraged speculation as to how the formation of the thermodynamically favoured intermediate (42) might be precluded. If this intermediate arises *via* intermolecular proton transfer in the initial adduct (41) the rate of such a process could be reduced by decreasing the concentration of reactants. Also, an increase in the reaction temperature could increase the amount of kinetic product (β-lactam) formed from either adducts (40) or (41). However, before these speculations were tested experimentally a literature search revealed a publication[38] by the Merck group, in which they reported formation of a β-lactam (66b) in which the reaction conditions had been altered drastically from those previously reported[7b] for formation of the closely-related lactam (66a) outlined earlier in Scheme 9. The original conditions involved addition of an ethereal solution of the imine (67) to a solution of azidoacetyl chloride and triethylamine in ether at $-78^{\circ}C$, whereas the conditions reported three years later involved slow addition of a cyclohexane solution of azidoacetyl chloride to a dilute solution of imine (67) and triethylamine in benzene-cyclohexane, at $25^{\circ}C$. In the former reaction, the yield of β-lactam was 30% and in the latter 54%. Interestingly the new conditions involve a reduction in reactant concentration and an increase in reaction temperature. When these conditions were applied to the case in hand, the desired lactam (39) was obtained in the very respectable yield of 42%.

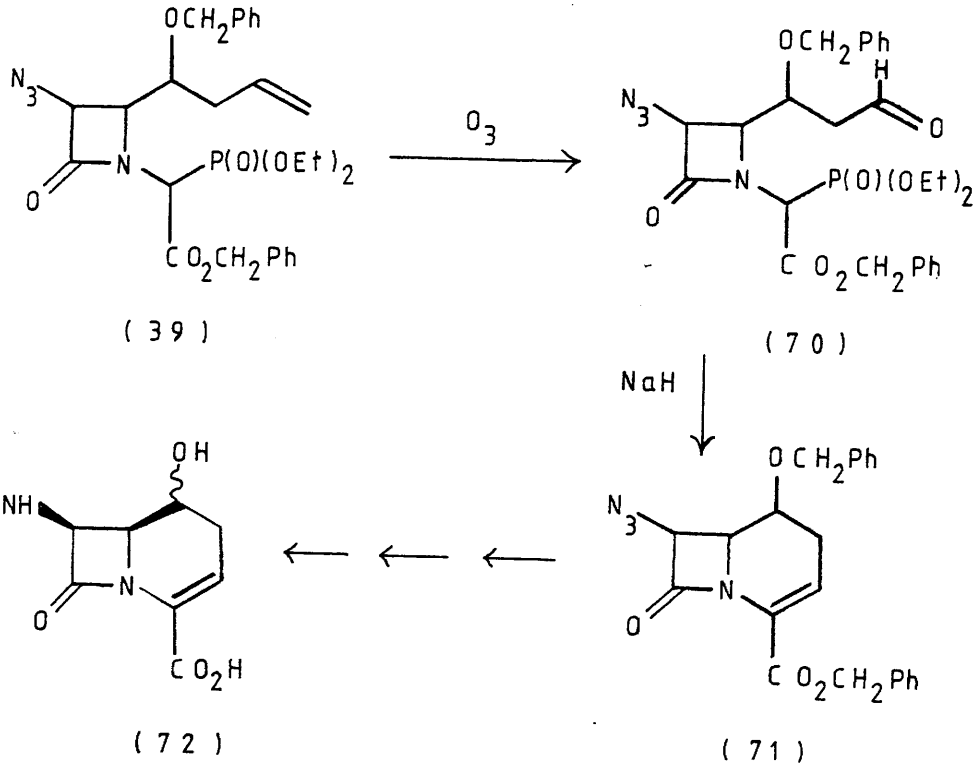
With the problem of β-lactam formation overcome, consideration was then given to functionalisation of the terminal double bond, which requires overall oxidation to an α-acetoxy ketone (Scheme 16). Similar transformations[39,40] have been reported (Scheme 17), but these are multistep processes usually involving both acidic and basic reagents. It was considered inappropriate to subject a compound such as lactam



(68)

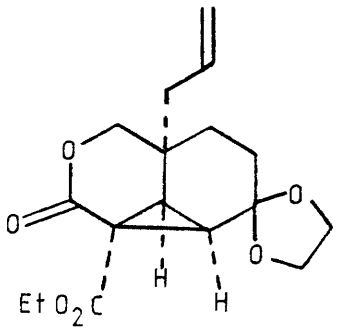


(69)



(39) (70) (71) (72)

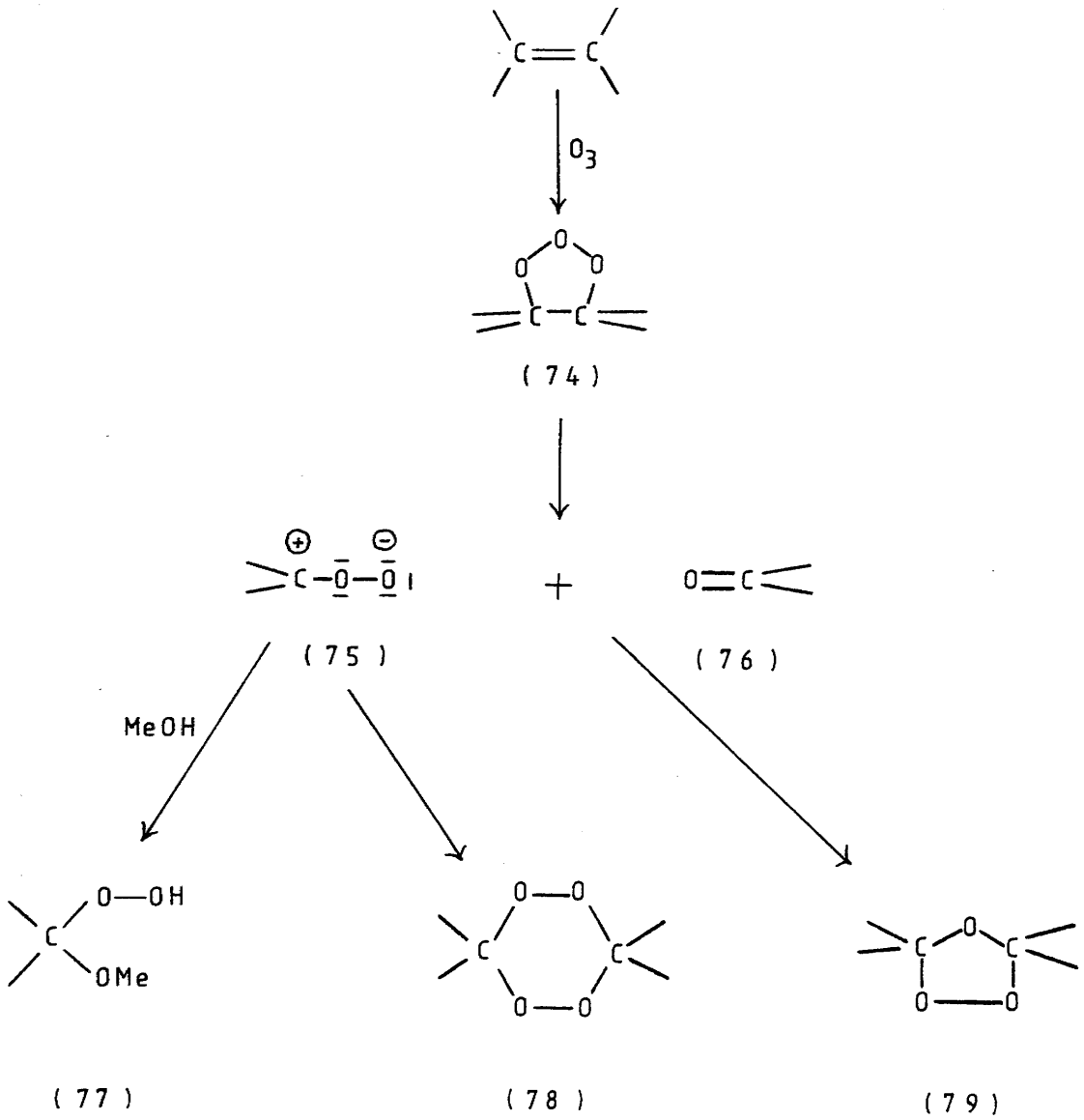
SCHEME 18.



(73)

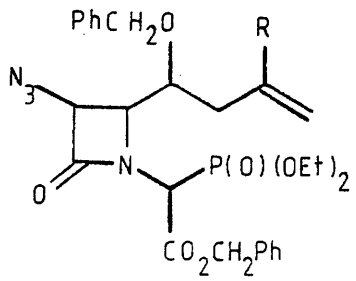
(39), containing as it does a reactive azido group and a potentially acidic hydrogen atom, to such a protocol; accordingly an alternative oxidation procedure was sought. Ozonolysis offers the most direct oxidation of an olefin to a carbonyl compound, and furthermore has been performed on an azetidinone (68) with[33] an azido substituent and also on β-lactam structures (69) containing[41] a phosphonium-activated proton. It was therefore decided to subject β-lactam (39) to ozonolysis, in the expectation that the aldehyde (70) could thus be readily prepared. This modification to the synthetic strategy was thought desirable because it would very quickly give a compound which could be used to investigate the proposed intramolecular Emmons condensation to give, in this case, cephem (71). There was some anxiety that this base-induced reaction might be complicated by β-elimination of benzyl alcohol in a process similar to that observed in the attempted Emmons condensation in the earlier oxazolidone route. The preparation of the cephem (71) need not be considered only as an academic exercise since completion of the planned synthesis (Scheme 18) would give the 3-desmethyl analogue (72); similarly unsubstituted cephems are known[42] to possess good antibacterial activity.

Ozonolysis of lactam (39) followed by reductive treatment with dimethyl sulphide[43] gave a compound which on tlc analysis was shown not to be starting material but whose nmr and ir spectra did not reveal the presence of an aldehyde function. It was therefore presumed that ozonolysis had produced a relatively stable ozonide. In a sesquiterpene synthesis, Isobe has reported[44] that ozonolysis of the terminally olefinic compound (73) gave an ozonide which could not be reduced by dimethyl sulphide, but which was reduced to the corresponding aldehyde on treatment with triethylamine. Unfortunately, triethylamine did not reduce the ozonide obtained from lactam (39). In view of the



(74) (75) (76) (77) (78) (79)

SCHEME 19.



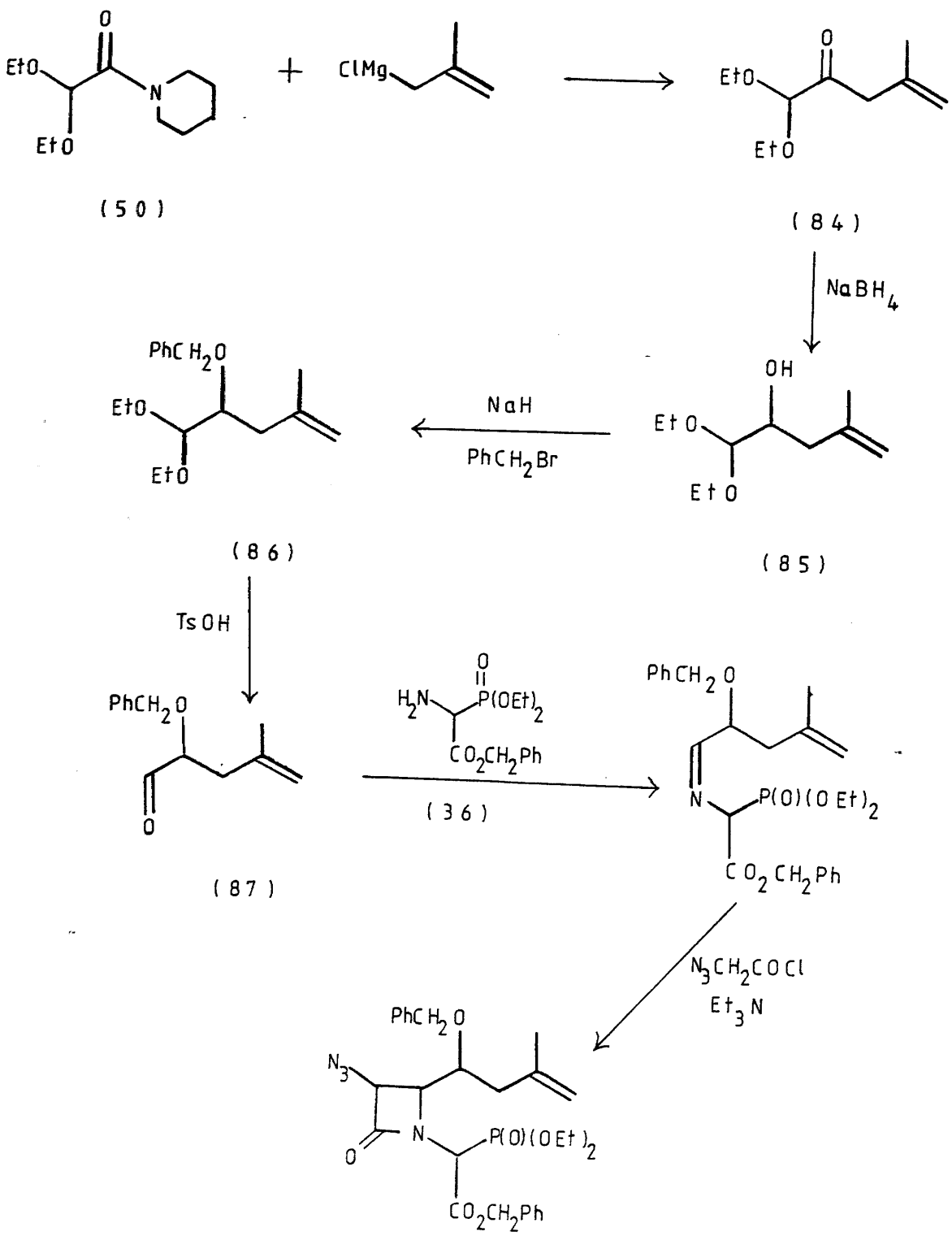
(80) R = CH_3

(39) R = H

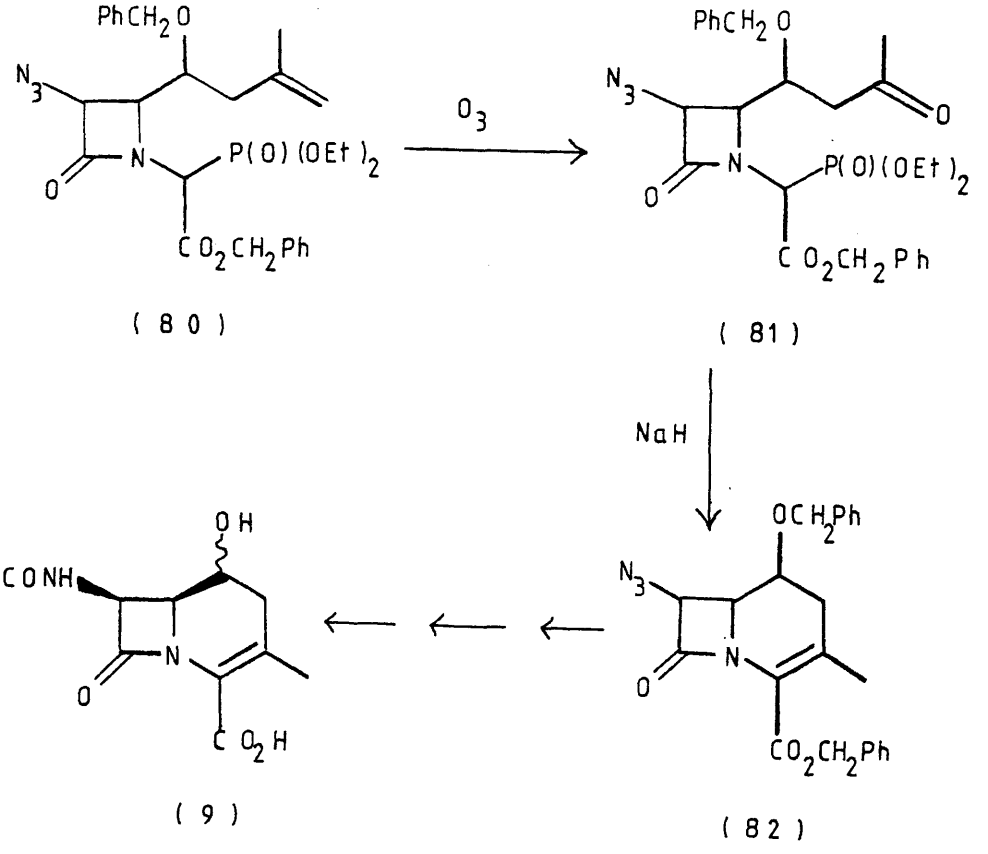
failure of these mild and selective reducing agents, more powerful reagents were employed in the hope that the ozonide would be more labile towards reduction than either the azido or benzyl groups. Ozonolysis followed by treatment with triphenylphosphine[45] or catalytic hydrogenation[46] did not produce aldeydic compounds but instead gave very polar products indicating that reduction of the other functional groups had indeed occurred.

An appraisal of the mechanism of ozonolysis was necessary to help devise a method of avoiding the formation of such a stable ozonide. Criegee[47] has studied the ozonolysis reaction extensively and has arrived at the following conclusions. Ozone reacts with the olefin to form an unstable molozonide (74), which fragments to give a zwitterion (75) and a carbonyl component (76) (Scheme 19). The carbonyl component is usually derived from the less substituted olefinic carbon and the ultimate fate of the zwitterion depends on three factors; its structure, its environment, and the structure of the carbonyl component. Protic solvents such as methanol usually react with the zwitterion to give hydroperoxides (77) in high yields. In inert solvents the zwitterion may dimerise to give diperoxides (78), or polymerise, or react with the carbonyl component (76) to give the classical ozonide (79). This last process is particularly dependent upon the structure of the carbonyl component and, in general, only occurs where the carbonyl compound is an aldehyde.

The stability of the ozonide obtained from lactam (39) indicated that a structure of type (79) was being formed by reaction of the zwitterion with the carbonyl component, formaldehyde. It was speculated that this process might be obviated by increasing the substitution pattern of the olefin. The simplest and most appropriate increase in substitution is seen in the methyl homologue (80) of lactam (39); this


EtO
O
N
EtO
ClMg
(50)
(84)
NaBH4
OH
(85)
NaH
PhCH2Br
PhCH2O
(86)
TsOH
(87)
H2N
P(OEt)2
CO2CH2Ph
(36)
P(O)(OEt)2
N3CH2COCl
Et3N
N3
(80)

SCHEME 21.



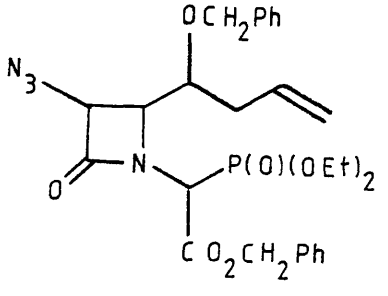
(80)

(81)

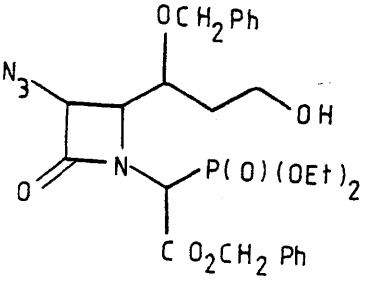
(82)

(9)

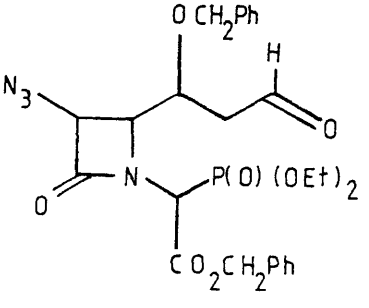
SCHEME 20.



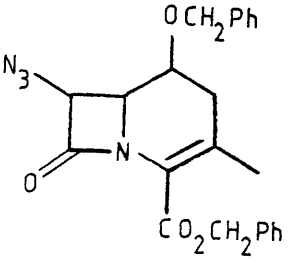
(39)



(83)



(70)



(71)

would give the 3-methyl cephem analogue (9) on successful completion of the proposed synthetic strategy (Scheme 20). This compound more closely resembles the natural cephalosporin skeleton and, indeed, has been discussed previously as a synthetic target.

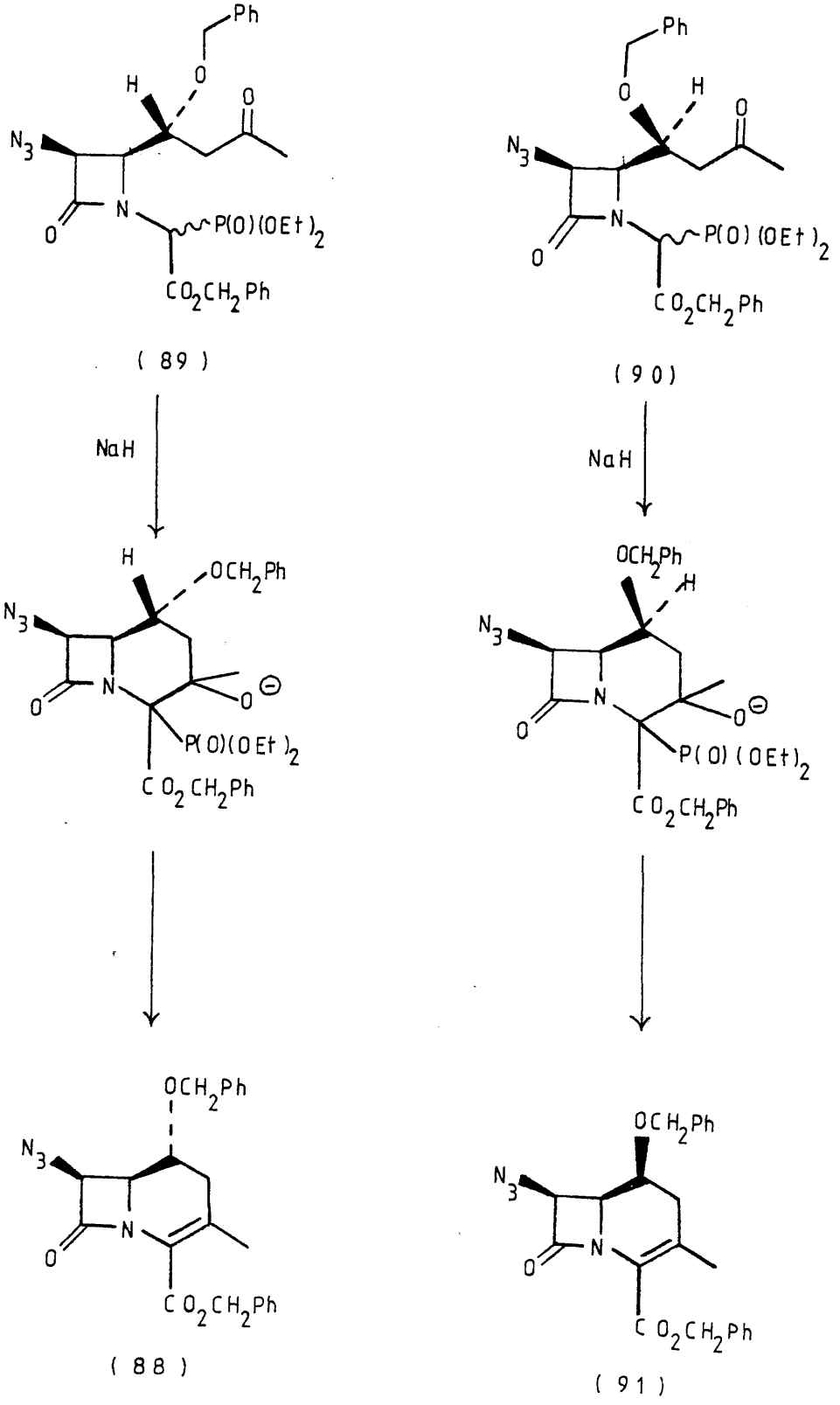
Simultaneously with the preparation of lactam (80), the remaining supply of desmethyl lactam (39) was subjected to ozonolysis, followed by treatment[33] with $NaBH_4$ in the hope that the ozonide would be reduced to the alcohol (83). Unexpectedly, the initial reduction product, the phosphonato-aldehyde (70), underwent intramolecular condensation in the basic medium at a rate greater than that of further reduction. The product isolated from this reaction was surprisingly the cephem (71). There was insufficient material to allow the determination of the stereochemistry and this result, though interesting, was not investigated further because of developments in the homologous series.

The methyl analogue (80) of β-lactam (39) was prepared *via* the intermediates (84) to (87) by substituting methallyl chloride in place of allyl chloride and performing the synthesis as before (Scheme 21). The compound (80) so obtained exhibited similar physical characteristics to its desmethyl parent (one spot on tlc and a largely unresolved ^{1}H-nmr spectrum) and again it was assumed that a *cis-trans* mixture of β-lactams had been obtained. Ozonolysis, followed by treatment with dimethyl sulphide now gave the ketone (81) smoothly and in virtually quantitative yield. Analytical tlc examination of this ketone showed two overlapping components which were assumed to be the *cis-* and *trans-* disubstituted isomers. Some effort was expended in obtaining pure samples of each; separation was achieved on a small scale by preparative tlc employing multiple elution. The ^{1}H-nmr spectra of both components were very complicated, with doubling of signals indicating that each was a mixture of diastereoisomers, and no relative stereochemical assignments could

be made at this stage. In view of this, and in the expectation that separation would become easier after cyclisation (if only because this destroys one chiral centre), no attempt was made to obtain the individual components of the mixture of ketones on a preparative scale.

When the mixture was treated with sodium hydride in DME and heated at 50°C, cyclisation of <u>one</u> of the diastereoisomeric components was observed to occur. After chromatographic separation, this gave a crystalline carbacephem compound (88) and a diastereoisomeric mixture of ketones whose ^{1}H-nmr spectrum was superimposable with that of the more polar of the two separated ketones. The melting point of the cyclised product (88) was extremely sharp, suggesting that it was not a diastereoisomeric mixture but was a single compound. Its relative stereochemistry was assigned on the basis of ^{1}H-nmr coupling constants (see ^{1}H-nmr opposite), which were determined using scale expansion and decoupling techniques. The coupling constant of J_{ab} = 4.5Hz for the β-lactam 6,7-protons is characteristic of <u>cis</u>-6,7-disubstitution. With this established, it then became possible to ascertain the relative stereochemistry at C-1 by examining the vicinal coupling constants associated with H_c.

The coupling constant of J_{bc} = 8.5Hz is very large, indicating a <u>trans</u> orientation of H_c and H_b in which the dihedral angle approaches 180°. This relative configuration of H_c is further evidenced by its coupling with the methylene protons H_d and H_e; here there is one large coupling constant J_{cd} = 8.5Hz and a slightly smaller one J_{ce} = 6Hz. In the alternative configuration for the substituent at C-1 these coupling constants would be approximately equal and small. The appearance of the benzyl ether benzylic protons as a sharp two-proton singlet lends further support to the α-configurational assignment for this substituent, such equivalence indicating that it is rotating freely.

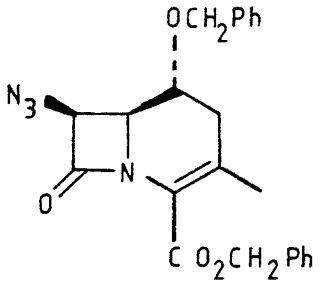


SCHEME 22.

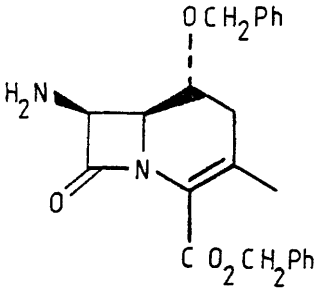
In the alternative β-configuration, it will be seen (vide infra) that free rotation is restricted by intramolecular interaction between the benzyloxy and the azido groups.

The assignment of the benzyloxy and azido substituents to opposite faces of a carbacephem in which the 6,7-disubstitution is cis can account for the isomer selectivity observed in the cyclisation reaction. Contrary to what had been assumed previously, β-lactam ring formation must have resulted in exclusive cis-3,4-disubstituted products, and the individual diastereoisomeric ketone components must therefore have been the benzyloxy epimers (89) and (90). As Emmons cyclisation takes place (Scheme 22), considerable conformational rigidity will be created at an early stage in the reaction coordinate. In isomer (90), this will introduce a comparatively unfavourable steric interaction between the azido and the benzyloxy substituents, resulting in a higher activation energy requirement for production of carbacephem (91). There will be no such unfavourable interactions in the pathway leading from isomer (89) to carbacephem (88), and the observed selective production of this bicyclic epimer under mild conditions is therefore unsurprising.

Such an interpretation required cyclisation of the recovered unreacted keto-phosphonate isomer (90) for verification. This was achieved by similar treatment with NaH in DME, but at an elevated reaction temperature, to give carbacephem (91). The ^{1}H-nmr of this compound (see opposite) again includes a characteristic cis-coupling constant of J_{ab} = 5Hz for the β-lactam protons. As expected, all the vicinal coupling constants associated with H_c are small, as anticipated if the benzyloxy and azido substituents occupy the same face of the molecule. This is further evident from the non-equivalence of the benzylic protons of the benzyl ether, which appear as an AB quartet, indicating that



(88)



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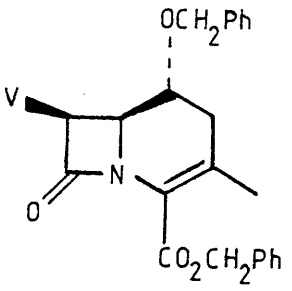

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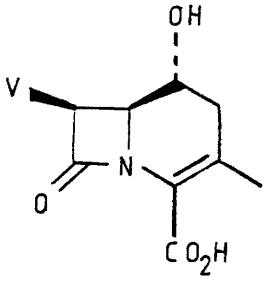

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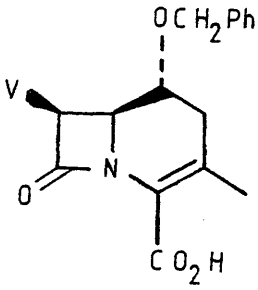

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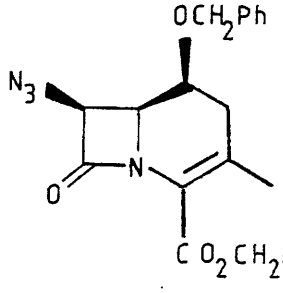

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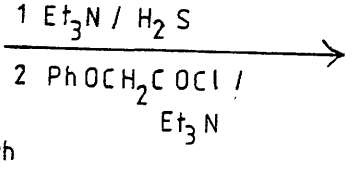

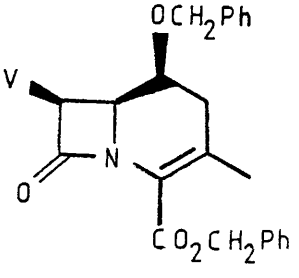

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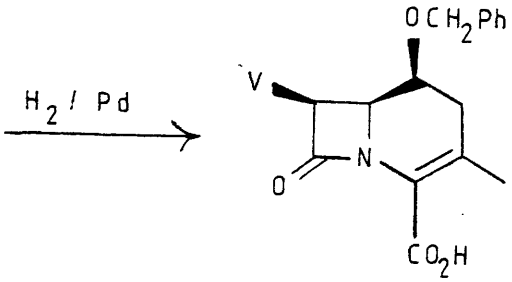

(97)

SCHEME 23.

there is restricted rotation at this position. Additionally, the aromatic protons of this moiety now occur as a broadened multiplet indicative of some rotational immobility. It is therefore evident that β-lactam ring formation resulted in cis-3,4-disubstitution exclusively; by analogy, this was also the case in formation of the desmethyl β-lactam (39).

Reduction of the 7β-azido-1α-benzyloxycarbacephem (88) with hydrogen sulphide/triethylamine to the corresponding amino compound (92) followed, without purification, by acylation gave the 7β-phenoxyacetamidocarbacephem (93). Disappointingly, hydrogenolysis did not produce the target 1-hydroxy-1-carbacephem (94), but instead gave the mono-deprotected compound (95). Reduction of the Δ^3-double bond (as evidenced by the gradual appearance in the ^{1}H-nmr spectrum of a doublet at δ 1.2 ppm) was occurring before there was any indication of benzyl ether cleavage. The addition of TFA to the hydrogenolysis mixture did not alter the course of the reaction. The epimeric cephalosporanic acid analogue (97) was similarly obtained from the azido compound (91), via the protected carbacephem (96) (Scheme 23).

Trimethylsilyl iodide has been reported[48] to be a mild and selective reagent for the removal of benzyl esters and ethers from labile peptide compounds. However, treatment of the protected carbacephem (93) with this reagent led to extensive decomposition of the starting material.

The nuclearly modified cephalosporin analogues (95) and (97) were tested for antibiotic activity against a strain of S. aureus. The 1α-isomer (95) produced a 1mm zone of total inhibition at a concentration of 1mg per ml using the agar well method for determining susceptibility; the 1β-isomer was inactive at this concentration.

This project has been successful in the synthesis of oxygenated 1-carbacephalosporin analogues. Only removal of a protecting group impedes attainment of the target 1-hydroxy-1-carbacephalosporins. Deprotection, though occasionally troublesome, is rarely an insurmountable problem in a molecule of this size. The wealth of protecting groups available and the variety of methods employed for their removal usually enable modification of a synthesis to overcome such difficulties. Electrochemical oxidation[49] is one particularly promising method of benzyl ester and ether cleavage which has yet to be explored. Time has not permitted such an investigation which now becomes the responsibility of Mr. A. McLeod who is continuing this research.

Experimental

Experimental - Contents

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General Experimental and Abbreviations

Melting points are uncorrected and were determined on a Kofler hot stage apparatus. Microanalyses were obtained by Mrs. Harkness and her staff. Mass spectra were recorded by Mr. A. Ritchie on A.E.I. - G.E.C./MS 12 and A.E.I. - G.E.C./MS 902 mass spectrometers. Routine ir spectra were recorded on a Perkin Elmer 197 grating spectrophotometer and were liquid film. Solution and KBr disc ir spectra were obtained using a Perkin Elmer 580 grating spectrophotometer. Routine nmr were recorded on a Varian T 60 and high resolution nmr spectra were recorded on a Perkin Elmer R32 spectrometer, with tetramethyl silane as internal standard in both cases.

Kieselgel G (Merck) was used for preparative tlc and, unless otherwise stated, Silica TSC (Woelm, Grade III) was used for column chromatography. Analytical tlc plates were stained with ceric sulphate followed by heating to approximately 150°C.

All dilute mineral acids were 6N aqueous unless otherwise stated. Light petroleum refers to the fraction boiling in the range 40-60°C. All organic solutions were dried, unless otherwise stated, over anhydrous magnesium sulphate.

Tetrahydrofuran and dimethoxyethane were heated under reflux with lithium aluminium hydride and distilled prior to use. Ether, benzene and toluene were dried over sodium wire. Methylene chloride was dried by percolation through a column of alumina (Woelm, Grade I basic).

The following abbreviations and symbols have been used throughout this section.

tlc thin layer chromatography

ir infrared

nmr	nuclear magnetic resonance
uv	ultraviolet
s	singlet
d	doublet
t	triplet
q	quartet
m	multiplet
b	broad
M^+	molecular ion

Oxazolidone Approach

4-Hydroxypent-1-ene (16)

This was prepared by the published procedure[17], and was obtained as a colourless oil bp 119°C at 760 mmHg (lit. bp 115-116°C at 760 mmHg).

δ ($CDCl_3$) 1.1 (3 H, d, J 5 Hz, CH_3) 2.21 (2 H, d t, J 7 Hz and J 2 Hz, $-CH_2-$), 3.2 (1 H, bs, exchanges with D_2O, OH), 3.4-4.0 (1 H, m, $\underline{CH}OH$), 4.9-5.3 (2 H, m, $CH=\underline{CH_2}$), and 5.5-6.2 (1 H, m, $\underline{CH}=CH_2$).

4-Benzyloxypent-1-ene (18)

To a stirred suspension of sodium hydride (9.0 g, 100%, 0.38 mol) in THF (150 ml) was added 4-hydroxypent-1-ene (16) (30 g, 0.37 mol) dropwise over 30 min with stirring in an atmosphere of nitrogen. The mixture was heated under reflux for 2 h then cooled to 0°C, when a solution of benzyl bromide (63.6 g, 0.37 mol) in THF (30 ml) was added dropwise with stirring over 30 min. The mixture was heated under reflux for a further 2 h, then allowed to stand for 18 h at room temperature. Ethanol was added dropwise to quench excess sodium hydride and the reaction mixture poured on to water (250 ml). The aqueous solution was extracted with ether (2 x 500 ml) and the combined organic extracts washed with brine, dried and concentrated in vacuo. Distillation of the residue afforded the benzyl ether (18) as a colourless oil (53.6 g, 87%) bp 94°C at 2 mmHg.

ν max 3065, 3030, 1640, 1450, 1170, 1140, 1125, and 1090 cm^{-1}.

δ ($CDCl_3$) 1.22 (3 H, d, J 6 Hz, CH_3), 2.35 (2 H, m, $-CH_2-$), 3.61 (1 H, q, J 6 Hz, CHO), 4.6 (2 H, s, $\underline{CH_2}Ph$), 4.9-

5.3 (2 H, m, CH=C$\underline{H}_2$), 5.6-6.2 (1 H, m, C$\underline{H}$=C$\underline{H}_2$), and 7.3 (5 H, s, Ph)

(Found: C, 81.9; H, 9.3. $C_{12}H_{16}O$ requires C, 81.7; H, 9.2%).

1,2-Epoxy-4-benzyloxypentane (19)

To a solution of maleic anhydride (7.46 g, 76 mmol, freshly distilled) in methylene chloride (30 ml) at 0°C was added hydrogen peroxide (2.3 g, 90%, 66 mmol) in one portion with stirring. The solution was stirred at this temperature for a further 2 h, and a solution of 4-benzyloxypent-1-ene (18) (7 g, 40 mmol) in methylene chloride (7 ml) was added dropwise at 0°C. The mixture was stirred at this temperature for 14 h, filtered, and the filtrate washed with saturated aqueous $NaHCO_3$ solution, brine, and dried. The solvent was removed under reduced pressure and the residue distilled to furnish a mixture of the diastereoisomeric epoxides (19) as a colourless oil (6.3 g, 82%), bp 100°C at 1 mmHg.

νmax 1460, 1380, 1350, 1130, 1100, 1070, and 1030 cm^{-1}.

δ($CDCl_3$) 1.26 (3 H, d, J 6 Hz, CH_3), 1.28 (3 H, d, J 6 Hz, CH_3), 1.47-3.7 (12 H, m, 2 x C$\underline{H}_2$-C$\underline{H}$C$\underline{H}_2$C$\underline{H}$), 4.53 (4 H, bs, 2 x C$\underline{H}_2$Ph), and 7.35 (10 H, s, 2 x Ph).

(Found: C, 74.9; H, 8.6. $C_{12}H_{16}O_2$ requires C, 74.9; H, 8.4%).

Preparation of the Oxazolidone (20)

A mixture of the epoxide (19) (22.5 g, 0.12 mol), ethyl carbamate (15 g, 0.17 mol) and triethylamine (2.25 g, 0.022 mol) was heated for 4 h at 110°C in a tube sealed with a Teflon valve. Column chromatography (eluting solvent ethyl acetate - light petroleum) of the crude

reaction product on alumina (Woelm, Grade III acidic) afforded the oxazolidone (20) as a pale yellow oil (15 g, 55%) which gave white needles mp 46-49°C on crystallisation from ether.

ν_{max} 3380, 1750, 1490, 1450, 1375, 1230, 1070, and 1030 cm^{-1}.

$\delta(CDCl_3)$ 1.22 (3 H, d, J 7 Hz, CH_3), 1.24 (3 H, d, J 7 Hz, CH_3), 1.6-2.1 (4 H, m, 2 x $-CH_2-$), 3.2-4.0 (8 H, m, 2 x OCH CH_2N and 2 x $CHOCH_2Ph$), 4.48 (2 H, s, CH_2Ph), 4.52 (2 H, s, CH_2Ph), 6.35 (2 H, bs, 2 x NH), and 7.35 (10 H, s, 2 x Ph).

(Found: C, 66.1; H, 7.4; N, 5.75. $C_{13}H_{17}O_3N$ requires C, 66.4; H, 7.3; N, 5.95%).

t-Butyl Glyoxylate (21)

This was prepared by the literature method[19] and was obtained as a colourless oil, bp 76°C at 30 mmHg (lit. bp 59-61°C at 20 mmHg), as a 1:2 mixture of the aldehyde (21) and its hydrate.

Preparation of the Hemiaminal (22)

A solution of the oxazolidone (20) (7 g, 29.8 mmol), t-butyl glyoxylate (3.85 g, 29.6 mmol) and triethylamine (5 drops), in toluene (50ml) was heated under reflux for 18 h. The mixture was concentrated in vacuo and the crude reaction product purified by column chromatography (eluting solvent ethyl acetate - light petroleum) to give the hemiaminal (22) as a pale yellow oil (7.1 g, 66%).

ν_{max} 3320, 1750, 1740, 1370, 1230, 1150 and 1060 cm^{-1}.

$\delta(CDCl_3)$ 1.2 (3 H, m, CH_3), 1.5 (9 H, s, Bu^t), 1.9 (2 H, m,

$-CH_2-$), 2.8-3.8 (4 H, m, $OC\underline{H}C\underline{H}_2N$ and $C\underline{H}OCH_2Ph$), 4.5 (2 H, m, $C\underline{H}_2Ph$), 5.5 (1 H, s, OH), and 7.35 (5 H, s,Ph) (Found: m/e 321. $C_{15}H_{19}NO_6$ $(M-C_4H_8)$ requires M, 321).

Preparation of the Phosphonate (24)

To a solution of the hemiaminal (22) (2.2 g, 6 mmol) and lutidine (0.77 ml, 6.6 mmol) in THF (40 ml) in an atmosphere of argon was added thionyl chloride (0.48 ml, 6.6 mmol) dropwise with stirring at -10°C. The solution was stirred for 1 h at room temperature and then filtered. To the filtrate was added trimethyl phosphite (1.6 g, 12.3 mmol) and the mixture heated under reflux for 16 h. Volatiles were removed in vacuo. Column chromatography (eluting solvent ethyl acetate - light petroleum) of the residue on alumina (Woelm, Grade III, neutral) afforded the phosphonate (24) as a pale yellow oil (1.5 g, 55%).

νmax ($CHCl_3$) 1760 (shoulder), 1730, 1370, 1230, 1150, and 1060 cm^{-1}.

δ($CDCl_3$) 1.62 (9 H, s, Bu^t), 3.85 (6 H, 2 x d, J_{H-P} 11 Hz, 2 x OMe), 5.0 (1 H, d, J_{H-P} 24 Hz, $C\underline{H}PO$), and 7.35 (5 H, s, Ph).

(Found: m/e, 401.12380. $C_{17}H_{24}O_8PN$ $(M-C_4H_8)$ requires 401.12392).

Preparation of the Alcohol (25)

To a solution of the benzyl ether (24) (300 mg, 0.66 mmol) in ethyl acetate (30 ml) was added 10% Pd/C (300 mg) and the mixture stirred for 5 days under 1 atmosphere of hydrogen. The solution was filtered and volatiles removed in vacuo to give the alcohol (25) as a colourless oil (215 mg, 90%).

νmax ($CHCl_3$) 3470, 1760 (shoulder), 1735, 1370, 1250, 1150, and 1040 cm^{-1}.

δ($CDCl_3$) 1.23 (3 H, d, $C\underline{H}_3CHOH$), 1.54 (9 H, s, Bu^t), 2.5 (1 H, bs, exchanges with D_2O, OH), 3.85 (6 H, 2 x d, J_{H-P} 11 Hz, 2 x OCH_3), and 5.0 (1 H, 2 x d, J_{H-P} 26 Hz, $C\underline{H}PO$).

(Found: M^+, 367.13968. $C_{14}H_{25}NO_8P$ requires M, 367.13957).

Oxidation of the Alcohol (25)

To a solution of the alcohol (25) (300 mg, 0.82 mmol) in benzene (3ml) at 10°C was added Jones' reagent (2N, 1.65 ml) and the mixture was stirred at 10°C for 45 min. The solution was diluted with benzene, washed with brine, dried and concentrated in vacuo to give the ketone (26) as a pale yellow oil (176 mg, 59%).

νmax ($CHCl_3$) 1740 (b), 1720, 1370, 1250, 1150, 1060, and 1040 cm^{-1}.

δ($CDCl_3$) 1.45 (9 H, s, Bu^t), 2.15 (3 H, s, CH_3CO), 3.8 (6 H, 2 x d, J_{H-P} 11 Hz, 2 x OMe), and 4.9 (1 H, 2 x d, J_{H-P} 26 Hz, $C\underline{H}PO$).

Attempted purification of this compound by preparative tlc (developing solvent ethyl acetate) resulted in β-elimination and loss of carbon dioxide to give the $\alpha\beta$-unsaturated ketone (28).

νmax ($CHCl_3$) 1730, 1680, 1630, 1450, 1370, 1250, 1150, and 1030 cm^{-1}.

δ($CDCl_3$) 1.45 (9 H, s, Bu^t), 1.85 (1 H, bs, NH) 2.2 (3 H, s, CH_3CO), 3.85 (6 H, 2 x d, J_{H-P} 11 Hz, 2 x OCH_3), and 6.2-7.0 (2 H, m, CH=CH)

(Found: M^+, 321. $C_{13}H_{24}O_6N$ requires M, 321).

Attempted Intramolecular Emmons Condensation of Keto-Phosphonate (26)

A solution of the keto-phosphonate (26) (195 mg, 0.5 mmol) in THF (20 ml) was added, in one portion, to a slurry of sodium hydride (14 mg, 0.55 mmol) in THF (20 ml) at -40°C. The mixture was stirred and allowed to warm up to room temperature. It was then heated under reflux, with tlc monitoring, until all the starting material was consumed (16 h). The mixture was poured onto water and extracted with ether. The combined ethereal extracts were dried, and concentrated *in vacuo* to give a mixture of polar products, none of which could be identified.

Preparation of the Phosphorane (29)

To a solution of the hemiaminal (22) (1 g, 2.7 mmol) and lutidine (0.35 ml, 3 mmol) in THF (20 ml), under an atmosphere of argon, was added thionyl chloride (0.22 ml, 3 mmol) dropwise with stirring at -10°C. The mixture was stirred for 2 h at room temperature, filtered, the filtrate concentrated *in vacuo*, and redissolved in toluene (20 ml). Triphenylphosphine (0.72 g, 2.7 mmol) was added and the mixture heated under reflux, in an atmosphere of argon, for 40 h. The reaction mixture was evaporated *in vacuo*, and the residue partitioned between water and ether. The separated aqueous layer was basified with dilute sodium hydroxide solution and extracted with ethyl acetate. The combined ethyl acetate extracts were dried, and concentrated *in vacuo*. Column chromatography (eluting solvent ethyl acetate - light petroleum) afforded the phosphorane (29) as a white foam (0.85 g, 51%).

ν_{max} ($CHCl_3$) 1745 (shoulder), 1640, 1440, 1240, 1170, and 1110 cm^{-1}.

δ ($CDCl_3$) 0.8 (9 H, s, Bu^t), 7.35 (5 H, s, $OCH_2\underline{Ph}$), and 7.5-7.7 (15 H, bm, PPh_3)

(Found: M^+, 609.26404. $C_{37}H_{40}O_5NP$ requires M, 609.26438).

Attempts to remove the benzyl ether from this compound by hydrogenolysis were unsuccessful.

Preparation of the Hydroxy-Phosphorane (32)

To a solution of the hemiaminal (22) (1 g, 2.7 mmol) and lutidine (0.35 ml, 3 mmol) in THF (20 ml) under an atmosphere of argon, was added thionyl chloride (0.22 ml, 3 mmol) dropwise with stirring at -10°C. The mixture was stirred for 2 h at room temperature, filtered, concentrated *in vacuo* and redissolved in toluene (20 ml). Triphenylphosphine (0.72 g, 2.7 mmol) was added and the mixture heated under reflux, in an atmosphere of argon, for 40 h. The reaction mixture was evaporated *in vacuo* and the residue triturated with ether to give the crude phosphonium chloride (30) as a white foam.

δ ($CDCl_3$) 1.85 (9 H, s, Bu^t), 7.35 (5 H, s, $OCH_2\underline{Ph}$), and 7.4-8.2 (15 H, bm, PPh_3).

This was dissolved in ethanol (25 ml) and 10% Pd/C (600 mg) catalyst added to the solution. The mixture was stirred in an atmosphere of hydrogen (50 psi) for 5 days, filtered, and volatiles removed *in vacuo*. The residue was dissolved in water and extracted with ether. The separated aqueous layer was basified with dilute sodium hydroxide solution and extracted with ethyl acetate. The combined ethyl acetate extracts were dried and concentrated *in vacuo*. Column chromatography

(eluting solvent ethyl acetate - light petroleum) of the residue afforded the hydroxy-phosphorane (32) as a white foam [0.24 g, 16% from hemiaminal (22)]

νmax ($CHCl_3$) 3610, 3400 (b), 1740 (shoulder), 1630, 1440, 1240, 1170, and1110 cm^{-1}.

δ($CDCl_3$) 0.9 (9 H, s, Bu^t), and 7.5-7.9 (15 H, m, PPh_3)

(Found: M^+, 519.21784. $C_{30}H_{34}O_5NP$ requires M, 519.21744).

Attempted Oxidation-Intramolecular Wittig Condensation of Hydroxy-Phosphorane (32)

The hydroxy-phosphorane (32) (100 mg, 0.2 mmol), was dissolved in a mixture of DMSO (2 ml) and acetic anhydride (2 ml). The solution was stirred, under an atmosphere of argon, for 16 h when tlc monitoring indicated complete oxidation. The mixture was heated at 50°C for 2 h, concentrated in vacuo, the residue dissolved in ethyl acetate, washed with water, dried and evaporated in vacuo. Preparative tlc (developing solvent ethyl acetate - light petroleum) afforded only products of decomposition.

Imine-Ketene Cycloaddition Approach

N-(Diethoxyacetyl) piperidine (50)

This was prepared by the literature method[31], and was obtained as a colourless oil, bp 120°C at 1 mmHg (lit. bp 87-90°C at 0.12-0.15 mmHg).

$\delta(CDCl_3)$ 1.12 (6 H, t, J 6 Hz, 2 x $OCH_2\underline{CH_3}$), 1.55 (6 H, m, $\{CH_2\}_3$), 3.60 (8 H, m, 2 x $O\underline{CH_2}CH_3$ and CH_2NCH_2), and 4.95 (1 H, s, CH)

1,1-Diethoxypent-4-en-2-one (51)

This was prepared by an extension of a published[31] procedure. To a stirred solution of allyl magnesium chloride[50] [from magnesium (24.3 g, 1 mol) and allyl chloride (27.4 g, 0.36 mol)] in ether (400 ml) was added the piperidine (50) (31 g, 0.14 mol), dropwise, with stirring, over 30 min. at 0°C in an atmosphere of nitrogen. The solution was heated under reflux, with stirring, under nitrogen, for 16 h. The reaction mixture was cooled to -15°C and poured on to saturated ammonium chloride solution (1 l) at -15°C. The aqueous solution was extracted with ether (3 x 500 ml), the ethereal extracts dried over anhydrous sodium sulphate, and the solvent removed under reduced pressure to give the crude ketone (51).

$(CDCl_3)$ 1.23 (6 H, t, J 7 Hz, 2 x $OCH_2\underline{CH_3}$), 3.5 (6 H, m, 2 x $O\underline{CH_2}CH_3$ and $COCH_2$), 4.6 (1 H, s, CH), 5.1 (2 H, m, $C{=}CH_2$), and 5.9 (1 H, m, $\underline{H}C{=}CH_2$).

Due to its lability towards conjugation, (51) was used immediately in the following reaction, without purification.

1,1-Diethoxy-2-hydroxypent-4-ene (53)

The crude ketone (51), from above, was dissolved in ethanol (100 ml, 95%) and added rapidly to a stirred solution of sodium borohydride (60 g, 1.5 mol) in water (225 ml) and ethanol (50 ml, 95%) at 0°C. The solution was allowed to warm up to room temperature, with stirring, over a period of 3 h. The reaction mixture was poured on to water (400 ml) and extracted with ether (3 x 400 ml). The combined ethereal extracts were dried and concentrated under reduced pressure. Distillation afforded the alcohol (53) as a colourless liquid [18 g, 72% based on piperidine (50)] bp 80°C at 1.5 mmHg.

ν_{max} 3450 (broad), 1640, 1440, 1370, 1280, and 1110 cm^{-1}.

$\delta(CDCl_3)$ 1.2 (6 H, t, J 7 Hz, 2 x $OCH_2\underline{CH_3}$), 2.3 (1 H, bs, exchanges with D_2O, OH), 3.6 (7 H, m, 2 x $O\underline{CH_2}CH_3$ and $\underline{CH_2}\underline{CH}OH$), 4.3 (1 H, d, J 2 Hz, $\underline{CH}(OEt)_2$), 5.1 (2 H, m, $C=CH_2$), and 5.8 (1 H, m, $\underline{CH}=CH_2$).

(Found: C, 62.2, H, 10.2. $C_9H_{18}O_3$ requires C, 62.05, H, 10.4%).

1,1-Diethoxy-2-benzyloxypent-4-ene (54)

To a slurry of sodium hydride (1.82 g, 100%, 80 mmol) in THF (50 ml) under an atmosphere of argon was added alcohol (53) (12 g, 70 mmol) dropwise with stirring over 30 min. at room temperature. The reaction mixture was stirred and heated under reflux for 2 h., then cooled to 0°C when benzyl bromide (12.4 g, 75 mmol) was added dropwise, with stirring, over 30 min. The reaction mixture was heated under reflux for 2 h., cooled, and stirred for 16 h. Ethanol was added dropwise to quench excess sodium hydride, and the solution poured on to

water (100 ml). The aqueous solution was extracted with ether (3 x 150 ml), and the combined ethereal extracts washed with brine, dried, and concentrated *in vacuo*. Distillation of the residue afforded the benzyl ether (54) as a colourless liquid (13.5 g, 74%), bp 94°C at 0.15 mmHg.

ν_{max} 1650, 1440, 1360, 1060, 905, 725 and 690 cm^{-1}.

$\delta(CDCl_3)$ 1.23 (6 H, t, J 8 Hz, 2 x $OCH_2\underline{CH_3}$), 2.4 (2 H, m, $\underline{CH_2}CH=CH_2$), 3.6 (5 H, m, 2 x $O\underline{CH_2}CH_3$ and $\underline{CH}OCH_2Ph$), 4.4 (1 H, d, J 6 Hz, $\underline{CH}(OEt)_2$), 4.7 (2 H, s, $\underline{CH_2}Ph$), 4.9-5.3 (2 H, m, $C=CH_2$), 5.6-6.2 (1 H, m, $\underline{CH}=CH_2$), and 7.3 (5 H, m, Ph).

(Found: C,72.75, H, 9.05. $C_{16}H_{24}O_3$ requires C, 72.70, H, 9.15%).

2-Benzyloxypent-4-enal (35)

A solution of 1,1-diethoxy-2-benzyloxypent-4-ene (54) (4 g, 15 mmol) and *p*-toluenesulphonic acid monohydrate (400 mg, 2 mmol) in acetone (AnalaR, 40 ml) and water (4 ml) was heated under reflux for 3.5 h. The solution was cooled and concentrated *in vacuo*, salt was added to the aqueous residue which was then extracted with ether (3 x 40 ml). The combined ethereal extracts were washed successively with saturated sodium bicarbonate and brine, dried, and the solvent removed under reduced pressure. Distillation of the residue afforded the aldehyde (35) as a fragrant colourless liquid (2.1 g, 73%) bp 80°C at 0.25 mmHg.

ν_{max} 2705, 1740, 1650, 1455, 1060, 1045, 915, 740, and 700 cm^{-1}.

$\delta(CDCl_3)$ 2.5 (2 H, m, $\underline{CH_2}CH=C$), 3.84 (1 H, dt, J 6 Hz and J 2 Hz, $\underline{CH}OCH_2Ph$), 4.64 (2 H, s, $\underline{CH_2}Ph$), 5.1 (2 H,

m, $CH=C\underline{H}_2$), 5.9 (1 H, m, $C\underline{H}=CH_2$), and 7.4 (5 H, s, Ph). (Found: m/e, 161. $C_{11}H_{13}O$ (M-CHO) requires 161).

Azidoacetic Acid

This was prepared by the literature[51] method, and was obtained as a colourless oil which, due to its instability, was used without further purification.

νmax 3000 (broad), 2100, 1725, 1420, 1380, and 1215 cm^{-1}.

Azidoacetyl Chloride

This was prepared by the published[52] procedure and was obtained as a mobile colourless oil, bp 62-63°C at 25 mmHg (lit. bp 55-60°at 18 mmHg).

νmax 2100, 1800, 1740, 1410, 1270, 1200, 905, and 760 cm^{-1}.

Preparation of the β-Lactams (55) and (56)

A solution of 2-benzyloxypent-4-enal (35) (2.5 g, 13 mmol) and 2,4-dimethoxybenzylamine (2.2 g, 13 mmol) in ether (60 ml) was stirred in the presence of anhydrous magnesium sulphate for 2 h at room temperature. The mixture was filtered, concentrated in vacuo, and then dissolved in THF (60 ml) under an atmosphere of argon. Triethylamine (1.98 g, 19.6 mmol) was added and the solution was cooled to -78°C. Azidoacetyl chloride (2.33 g, 19.5 mmol) was added dropwise to the stirred solution. The cooling bath was removed and the mixture allowed to come to room temperature, stirred for 10 h, filtered, and concentrated in vacuo. Column chromatography (eluting solvent ethyl acetate-

light petroleum) of the crude reaction product afforded pure cis-β-lactam (55) as a colourless oil (2.84 g, 51%)

νmax 2100, 1760, 1610, 1590 and 1505 cm^{-1}.

$\delta(CDCl_3)$ 2.35 (2 H, b m, $C\underline{H}_2CH=CH_2$), 3.55 (1 H, m, $C\underline{H}NCO$), 3.64 (3 H, s, CH_3O), 3.72 (3 H, s, CH_3O), 4.07 and 4.64 (2 H, ABq, J 14.5 Hz, $C\underline{H}_2$ Ar), 4.35 and 4.64 (2 H, ABq, J 11 Hz, $C\underline{H}_2$ Ar), 4.1-4.4 (1 H, b m, $C\underline{H}OCH_2Ph$), 4.50 (1 H, d, J 5 Hz, $C\underline{H}N_3$), 5.05 (2 H, b m, $CH=C\underline{H}_2$), 5.75 (1 H, b m, $C\underline{H}=CH_2$), 6.30 and 6.86 (3 H, m, Ar), 7.25 (5 H, s, Ph)

(Found: M^+, 422. $C_{23}H_{26}O_4N_4$ requires M, 422). and a mixture of cis-β-lactam (55) and trans-β-lactam (56) as a cloudy oil (1.05 g, 19%)

$\delta(CDCl_3)$ 4.5 (1 H, d, J 5 Hz, $C\underline{H}N_3$ cis and 4.5 (1 H, d, J 2 Hz, $C\underline{H}N_3$ trans).

Preparation of the Phenoxyacetamido β-Lactam (57)

A stream of hydrogen sulphide gas was bubbled through a solution of azido-β- lactam (55) (475 mg, 1.13 mmol) and triethylamine (570 mg, 5.65 mmol) in methylene chloride (50 ml) at 0°C for 30 min. The solution was stirred for 1 h at room temperature, concentrated in vacuo, and the crude amine redissolved in methylene chloride (10 ml). Triethylamine (193 mg, 1.91 mmol) was introduced into the solution and phenoxyacetyl chloride (232 mg, 1.36 mmol) added dropwise with stirring at 0°C. The mixture was stirred for 1 h at room temperature, washed with water, dried, and concentrated in vacuo. Column chromatography (eluting solvent ethyl acetate - light petroleum) afforded phenoxyacetamido-β-lactam (57) as a gum [180 mg, 30% from azido-β-lactam (55)] which

crystallised from benzene - light petroleum, or ether, to give fine white crystals, mp 130-135°C

ν_{max} 3410, 1750, 1690, 1620, 1600, and 1590 cm^{-1}.

$\delta(CDCl_3)$ 2.3 (2 H, ABq, J 6 Hz, $C\underline{H}_2CH=CH_2$), 3.78 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 4.47 (2 H, s, $C\underline{H}_2OPh$), 7.29 (5 H, s, Ph), 3.3-5.8 (10 H, m, $C\underline{H}C\underline{H}(NC\underline{H}_2)C\underline{H}(OC\underline{H}_2Ph)CH_2C\underline{H}=C\underline{H}_2$), 6.3-7.0 (9 H, m, Ar and $N\underline{H}$)

(Found: M^+, 530.24158. $C_{31}H_{34}O_6N_2$ requires M, 530.24167).

Attempted N-Deprotection of β-Lactam (57)

The β-lactam (57) (250 mg, 0.5 mmol) was dissolved in 40% aqueous acetonitrile (30 ml) and the solution flushed with argon. Potassium persulphate ($K_2S_2O_8$) (510 mg, 2 mmol) and disodium hydrogen orthophosphate (Na_2HPO_4) (134 mg, 1 mmol) were added and the mixture was heated under reflux, in an atmosphere of argon, for 45 min. Volatiles were removed under reduced pressure, and the residue extracted with ethyl acetate. The combined ethyl acetate extracts were dried and concentrated in vacuo to give a dark viscous oil (210 mg). Preparative tlc (eluting solvent ethyl acetate - light petroleum) gave 2,4-dimethoxybenzaldehyde (59), mp 70-71°C (lit. mp 71°C), and a mixture of polar compounds which were not β-lactams, as evidenced by their lack of absorption in the region 1800-1700 cm^{-1} in the infra-red spectrum. Variations of the reaction temperature and time, and the addition of silver nitrate did not alter the outcome of this reaction. Attempted deprotection using TFA also failed.

Diethyl Aminomethylphosphonate (63)

This was prepared by the method of Regitz[36], and was obtained as a yellow oil which, due to its extreme lability, was used immediately without purification.

$\delta(CDCl_3)$ 1.36 (6 H, t, J 7 Hz, 2 x $OCH_2\underline{CH_3}$), 2.3 (2 H, bs, exchanges with D_2O, NH_2), 3.0 (2 H, d, J_{H-P} 11 Hz, $\underline{CH_2}NH_2$), and 4.3 (4 H, d q, J_{H-H} 7 Hz, J_{H-P} 8 Hz, 2 x $O\underline{CH_2}CH_3$).

Benzaldehyde Imine of Diethyl Aminomethylphosphonate (64)

A solution of benzaldehyde (14.5 g, 0.14 mol) in toluene (200 ml) was added to a solution of diethyl aminomethylphosphonate (64) (23 g, 0.14 mol) in toluene (200 ml) with stirring at 0°C. The reaction mixture was stirred for 1 h at room temperature and concentrated in vacuo with final azeotroping with toluene to remove the water produced in the reaction. Distillation gave the imine (64) as a pale yellow oil (20 g, 57%), bp 160°C at 1 mmHg.

$\delta(CDCl_3)$ 1.35 (6 H, t, J 7 Hz, 2 x $OCH_2\underline{CH_3}$), 3.9–4.4 (6 H, m, 2 x $O\underline{CH_2}CH_3$ and $\underline{CH_2}P$), and 7.2–8.4 (6 H, m, Ph and N=C$\underline{H}$).

Preparation of the Glycine Derivative (65)

This was prepared by a modification of the published procedure[37]. To a solution of isopropylcyclohexylamine (12.7 g, 90 mmol) in THF (150 ml) at room temperature, in an atmosphere of argon, was added

n-butyl lithium (48 ml, 1.6M in hexane, 90 mmol). The solution was cooled to -78°C and a solution of the phosphonato-imine (64) (8 g, 30 mmol) in THF (10 ml) was added dropwise with stirring. The mixture was stirred for 40 min, then a solution of benzyl chloroformate (13.0 g, 90 mmol) in THF (10 ml) was added dropwise. The reaction mixture was stirred for 2 h at -78°C, then allowed to warm slowly to room temperature and poured on to saturated aqueous ammonium chloride solution (100 ml). The aqueous solution was extracted with ether, and the combined ethereal extracts washed with brine, dried, and concentrated *in vacuo*. Column chromatography (eluting solvent ethyl acetate - light petroleum) of the residue on alumina (Woelm, Grade V basic) afforded the protected glycine derivative (65) as a yellow oil (5 g, 50%).

ν_{max} 1730, 1700, 1635, 1440, 1370, 1250, 1140, and 1020 cm^{-1}.

$\delta(CDCl_3)$ 1.2-1.7 (6 H, m, 2 x $OCH_2\underline{CH_3}$), 4.0-4.6 (4 H, m, 2 x OCH_2CH_3), 4.75 (1 H, d, J_{H-P} 20 Hz, $CHPO$), 5.3 (2 H, s, CH_2Ph), 7.2-8.0 (10 H, m, 2 x Ph), and 8.4 (1 H, d, J 5 Hz, CH=N).

Benzyl(2-Amino-2-diethylphosphonato)acetate (36)

To a solution of the above protected glycine derivative (65) (1.3 g, 3.34 mmol) in ether (60 ml) was added p-toluene sulphonic acid monohydrate (1.3 g, 6.8 mmol) and the mixture stirred for 3 h at room temperature, after which time a brown gum had separated. The supernatant solution was decanted and the gum triturated with ether and dissolved in saturated aqueous dipotassium hydrogen orthophosphate (K_2HPO_4) solution 40 ml) and extracted with ether. The combined ethereal extracts were dried and concentrated *in vacuo* below 25°C. Column chromatography

(eluting solvent ethyl acetate - light petroleum) afforded the amine (36) as a labile colourless oil (450 mg, 45%)

νmax 3600-3250, 1730, 1440, 1370, and 1240 cm^{-1}.

δ ($CDCl_3$) 1.35 (6 H, t, J 7 Hz, 2 x $OCH_2\underline{CH_3}$), 2.1 (2 H, bs, exchanges with D_2O, NH_2), 3.7-4.3 (5 H, m, 2 x OCH_2-CH_3 and $\underline{CH}PO$), 5.2 (2 H, s, OCH_2Ph), and 7.35 (5 H, s, Ph).

Preparation of the β-Lactam (39)

This was prepared by an extension of a published[38] procedure. A solution of amine (36) (450 mg, 1.5 mmol) and aldehyde (35) (285 mg, 1.5 mmol) in methylene chloride (40 ml) was stirred at room temperature for 2 h. Anhydrous magnesium sulphate (600 mg) was added and the mixture stirred for a further 1 h, filtered, concentrated in vacuo, and the residue azeotroped with benzene. The crude imine (37) was dissolved in benzene (15 ml) and cyclohexane (15 ml) and triethylamine (0.42 ml, 3 mmol) was introduced under an atmosphere of argon. A solution of azidoacetyl chloride (0.26 ml, 3 mmol) in cyclohexane (30 ml) was added dropwise with stirring, at room temperature, over a period of 1 h. The reaction mixture was diluted with benzene and washed successively with pH 3 phosphate buffer, water, pH 8 phosphate buffer and brine; it was then dried, and concentrated in vacuo. Column chromatography (eluting solvent ethyl acetate - light petroleum) of the residue afforded pure β-lactam (39) as a mixture of stereoisomers.

νmax 2100, 1775, 1740, and 1675 cm^{-1}.

δ ($CDCl_3$) broad and largely unresolved, peaks at 1.0-1.4 (6

H, bm, 2 x $OCH_2\underline{CH}_3$), 3.8-5.3 (12 H, bm), and 7.3 (10 H, s, 2 x Ph).

(Found: M^+, 556.20801. $C_{27}H_{33}N_4O_7P$ requires M, 556.20865).

Ozonolysis of β-Lactam (39) - Preparation of Desmethyl-carbacephem (71)

A stream of ozone and oxygen was bubbled through a solution of β-lactam (39) (100 mg, 0.18 mmol) in methanol (AnalaR, 5 ml) at -78°C until a faint blue colour persisted. A stream of nitrogen gas was then bubbled through the solution for 30 min to purge excess ozone. The dry-ice bath was removed and the solution allowed to warm up with stirring. When the reaction mixture reached -40°C sodium borohydride (6 mg, 0.16 mmol) was added and the solution then allowed to come to room temperature over 30 min with stirring. Dilute aqueous hydrochloric acid (10%, 3 drops) was added, and the solution concentrated in vacuo. The residue was dissolved in brine which was then extracted with ethyl acetate. The combined ethyl acetate extracts were dried, and concentrated under reduced pressure. Preparative tlc (eluting solvent ethyl acetate - light petroleum) of the residue afforded desmethyl-carbacephem (71) in low yield as a colourless oil, which became a waxy solid after storing at -20°C for several weeks.

ν_{max} 2100, 1780, 1715, and 1630 cm^{-1}.

(Found: m/e, 376.14237. $C_{22}H_{20}N_2O_4$ $(M-N_2)$ requires 376.1423).

Attempts to prepare the aldehyde (70) by reducing the ozonide, obtained in this reaction, with dimethyl sulphide, triethylamine, triphenylphosphine, or catalytic hydrogenation were unsuccessful.

2-Benzyloxy-4-methylpent-4-enal (87)

1,1-Diethoxy-2-benzyloxy-4-methylpent-4-ene (86) [4 g, 14.4 mmol, prepared[53] by substituting methallyl chloride for allyl chloride in a synthesis otherwise identical to that of the desmethyl analogue (54)] was deprotected as described above for liberation of the desmethyl aldehyde (35), to give the aldehyde (87) as a fragrant colourless oil (2 g, 68%) bp 84°C at 0.6 mmHg

ν max ($CHCl_3$) 1730, 1650, 1500, 1460, 1380, and 1110 cm^{-1}.

δ ($CDCl_3$) 1.75 (3 H, s, CH_3), 2.45 (2 H, d, J 7 Hz, $C\underline{H}_2C{=}C$), 3.95 (1 H, 6 lines, t, J 7 Hz, further coupled d, J 2 Hz, $C\underline{H}OCH_2Ph$), 4.7 (2 H, s, $C\underline{H}_2Ph$), 4.9 (2 H, bs, $C{=}CH_2$), 7.4 (5 H, s, Ph), and 9.7 (1 H, d, J 2 Hz, CH=O)

(Found: M^+, 204.11459. $C_{13}H_{16}O_2$ requires M, 204.115023).

Preparation of β-Lactam (80)

This was prepared from aldehyde (87) (2.23 g, 10.9 mmol), amine (36) (3.4 g, 11.3 mmol), and azidoacetyl chloride (2 ml, 23 mmol) by the method previously described for the synthesis of the desmethyl β-lactam (39), and was obtained as a pale yellow oil (4 g, 64%) which was a mixture of diastereoisomers.

ν max 2100, 1780, 1745, and 1650 cm^{-1}

δ ($CDCl_3$) broad and largely unresolved, peaks at 1.0-1.4 (6 H, bm, 2 x $OCH_2C\underline{H}_3$), 1.6-1.9 (3 H, bm, $CH_3C{=}C$), 3.8-5.3 (12 H, bm), and 7.3 (10 H, bs, 2 x Ph).

(Found: m/e 542.21839. $C_{28}H_{35}N_2O_7P$ ($M{-}N_2$) requires 542.21815).

Preparation of Keto-β-Lactam (81)

A stream of ozone and oxygen was bubbled through a solution of β-lactam (80) (3 g, 5.3 mmol) in methanol (AnalaR, 100 ml) at -78°C until a faint blue colour persisted. A stream of nitrogen gas was then bubbled through the solution for 1 h to purge excess ozone. Dimethyl sulphide (20 ml, 270 mmol) was added and the dry-ice bath removed. The solution was allowed to warm up to room temperature with stirring for 4 h. Volatiles were removed under reduced pressure, the residue was dissolved in ethyl acetate, washed with brine, dried, and concentrated in vacuo. Column chromatography (eluting solvent ethyl acetate - light petroleum) of the residue afforded the keto-β-lactam (81) as a colourless oil (2.4 g, 80%) which was a mixture of diastereoisomers as evidenced by a broad and unresolved nmr spectrum.

νmax 2100, 1775, 1745, and 1720 cm^{-1}.

(Found: m/e, 544.1973. $C_{27}H_{33}N_2O_8P$ ($M-N_2$) requires 544.19742).

This diastereoisomeric mixture of ketones was separable by preparative tlc (multiple elution with ethyl acetate - light petroleum) into two diastereoisomeric components.

less polar component (89)

δ ($CDCl_3$) 1.25 (6 H, t, J 8 Hz, 2 x $OCH_2\underline{CH_3}$), 1.26 (6 H, t, J 8 Hz, 2 x $OCH_2\underline{CH_3}$), 2.0 (3 H, s, CH_3CO), 2.22 (3 H, s, CH_3CO), 7.29 (10 H, s, 2 x Ph) and 7.35 (10 H, s, 2 x Ph).

more polar component (90)

δ ($CDCl_3$) 1.1-1.3 (12 H, m, 4 x $OCH_2\underline{CH_3}$), 2.11 (3 H, s, CH_3CO), 2.15 (3 H, s, CH_3CO), and 7.2-7.4 (20 H, m, 4 x Ph).

Preparation of Carbacephem (88)

To a stirred suspension of sodium hydride (10 mg, 0.42 mmol) in DME (3 ml) at -40°C under an atmosphere of argon was added a solution of keto-phosphonate (81) (250 mg, 0.44 mmol) in DME (2 ml). The cooling bath was removed and the solution allowed to come to room temperature, with stirring. The mixture was then heated to 50°C for 1 h, cooled to 0°C, and poured on to brine. The aqueous solution was extracted with ethyl acetate and the combined ethyl acetate extracts were dried and concentrated in vacuo. Preparative tlc (eluting solvent ethyl acetate - light petroleum) afforded unreacted keto-phosphonate (80 mg, 32%) identical in all respects with the more polar ketone component (90) obtained previously, and carbacephem (88) as a colourless oil (80 mg, 44%) which on crystallisation from ethyl acetate - light petroleum gave powdery white crystals mp 93.5 - 94°C.

ν_{max} ($CHCl_3$) 2110, 1775, 1720, and 1645 cm^{-1}.

δ ($CDCl_3$) 2.0 (3 H, s, $CH_3C{=}C$), 2.15 (1 H, four lines, d, J_{de} 18.5 Hz, further coupled, d, J_{cd} 8.5 Hz, H_d), 2.65 (1 H, four lines, d, J_{de} 18.5 Hz, further coupled, d, J_{ce} 6 Hz, H_e), 3.63 (1 H, four lines, d, J_{bc} 8.5 Hz, further coupled, d, J_{ab} 4.5 Hz, H_b), 3.9 (1 H, six lines, d, J_{bc} 8.5 Hz, further coupled, d, J_{cd} 8.5 Hz, further coupled, d, J_{ce} 6 Hz, H_c), 4.63 (2 H, s, $CHOC\underline{H}_2Ph$), 4.9 (1 H, d, J_{ab} 4.5 Hz, H_a), 5.21 (2 H, s, $CO_2C\underline{H}_2Ph$), and 7.3 (10 H, s, 2 x Ph)

λ_{max} (CH_3OH) 265 nm.(ε, 9550.)

(Found: C, 66.23; H, 5.07; N, 13.2. $C_{23}H_{22}N_4O_4$ requires C, 66.03; H, 5.26; and N, 13.4%).

Preparation of Carbacephem (91)

This was prepared from the unreacted keto-phosphonate (90) (334 mg, 0.58 mmol) by the method described above for the production of the diastereoisomeric compound (88), with the exception that the mixture was heated under reflux instead of at 50°C. The resulting carbacephem (91) was obtained (110 mg, 45%) as a white crystalline solid mp 104-106°C.

νmax ($CHCl_3$) 2110, 1775, 1720, and 1645 cm^{-1}.

δ($CDCl_3$) 1.92 (3 H, s, $CH_3C{=}C$), 2.3 (2 H, eight lines, AB_q, J 18.5 Hz, further coupled, d, J 2.5 Hz, $CH_2C{=}C$), 3.6 (1 H, four lines, d, J_{ab} 5 Hz, further coupled, d, J_{bc} 1.5 Hz, H_b), 4.07 (1 H, four lines, d, J_{ce} 2.5 Hz, further coupled, d, J_{cd} 2.5 Hz, further coupled, d, J_{bc} 1.5 Hz, H_c), 4.65 (1 H, d, J_{ab} 5 Hz, H_a), 4.7 (2 H, AB_q, J 12 Hz, $CHOC\underline{H}_2Ph$), 5.27 (2 H, s, $CO_2C\underline{H}_2Ph$), and 7.2-7.5 (10 H, m, 2 x Ph).

(Found: C, 66.32; H, 5.50; N,13.33. $C_{23}H_{22}N_4O_4$ requires C, 66.03; H, 5.26; and N, 13.40%).

Preparation of the Phenoxyacetamido-carbacephem (93)

A stream of hydrogen sulphide gas was bubbled through a solution of azido-carbacephem (88) (100 mg, 0.24 mmol) and triethylamine (121 mg, 1.2 mmol) in methylene chloride (10 ml) at 0°C for 30 min. The cooling bath was removed and the solution stirred for 1 h at room temperature then evaporated to dryness *in vacuo*. The crude amine (92) was redissolved in methylene chloride (5 ml) and triethylamine (48 mg, 0.48 mmol) introduced in to the solution. Phenoxyacetyl chloride (61 mg, 0.36 mmol)

was added dropwise with stirring at 0°C. The cooling bath was removed and the reaction mixture stirred for 1 h at room temperature. The solution was diluted with methylene chloride (15 ml) and washed successively with dilute hydrochloric acid (10%), water, and brine, then dried and concentrated in vacuo. Preparative tlc (eluting solvent ethyl acetate - light petroleum) of the residue afforded phenoxyacetamidocarbacephem (93) as an oil which crystallised from ethyl acetate - light petroleum to give white crystals (94 mg, 75%) mp 153-154°C.

ν_{max} ($CHCl_3$) 3400, 1770, 1720, 1690, 1640, 1600, and 1590 cm^{-1}.

δ ($CDCl_3$) 3.7-4.0 (2 H, bm, $C\underline{H}OCH_2Ph$ and $CH\underline{N}$), 4.5 (2 H, AB_q, J 11 Hz, $OC\underline{H}_2CO$), 5.6 (1 H, four lines, d, J 4.5 Hz, further coupled, d, J 9 Hz, $NHC\underline{H}CO$), 6.8-7.5 (15 H, m, 3 x Ph), 7.93 (1 H, d, J 9 Hz, NH), and other signals as for carbacephem (88).

λ_{max} (CH_3OH) 265 nm.(ε, 14530.)

(Found: C, 70.74; H, 5.9; N, 5.25. $C_{31}H_{30}O_6N_2$ requires C, 70.72; H, 5.7; and N, 5.32%).

Preparation of the Carbacephalosporanic Acid (95)

To a solution of the protected carbacephem (93) (100 mg, 0.19 mmol) in ethyl acetate (30 ml) was added 10% Pd/C catalyst (50 mg). The mixture was agitated under an atmosphere of hydrogen (45 psi) for 1 h, filtered, and the filtrate partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The separated aqueous layer was acidified with dilute hydrochloric acid and extracted with ethyl acetate. The combined ethyl acetate extracts were dried and concentrated in vacuo to give the crude carbacephalosporanic acid (95) as an oil which crystallised from acetone - light petroleum to give white crystals (43 mg, 52%) mp

206-208°C.

νmax (KBr disc) 3300, 1775, 1720, 1690, 1675, 1640, 1600, and 1590 cm^{-1}.

δ (d_6-DMSO) 3.7 (1 H, four lines, d, J 4.5 Hz, further coupled, d, J 8.5 Hz, CHN), 4.2 (1 H, bm, CHO-CH_2Ph), 4.5 (2 H, AB_q, J 11 Hz, OCH_2CO), 4.52 (2 H, s, $CHOCH_2Ph$), 5.5 (1 H, four lines d, J 4.5 Hz, further coupled, d, J 9 Hz, NH CHCO), 7.25 (5 H, s, $CHOCH_2Ph$), 6.9-7.4 (5 H, m, PhO), 9.03 (1 H, d, J 9 Hz, NH) and other signals as for carbacephem (88).

λmax (CH_3OH) 263 nm.(ε, 9870.)

(Found: C, 66.32; H, 5.76; N, 6.25. $C_{24}H_{24}O_6N_2$ requires C, 66.06; H, 5.50; and N, 6.42%).

The addition of TFA to the reaction mixture did not alter the course of the reaction. In another attempt to remove both benzyl ester and ether simultaneously, using trimethylsilyl iodide in $CDCl_3$, only decomposition of starting material was observed.

Preparation of Phenoxyacetamido-carbacephem (96)

The azido-carbacephem (91) (100 mg, 0.19 mmol) was reduced and acylated as described above, for the preparation of the diastereoisomeric amide (93), to give the phenoxyacetamide (96) as a white crystalline solid (50 mg, 40%) mp 137-138°C.

νmax (KBr disc) 3420, 1765, 1720, 1690, 1635, 1600, and 1590 cm^{-1}.

δ($CDCl_3$) 4.47 (2 H, s, $CHOCH_2Ph$), 4.50 (2 H, s, OCH_2CO), 5.82 (1 H, four lines, d, J 10 Hz, further

coupled, d, J 5 Hz, NHC<u>H</u>CO), 6.4-7.5 (15 H, m, 3 x Ph), 7.7 (1 H, d, J 10 Hz, NH), and other signals as for carbacephem (91).

λmax (CH_3OH) 267 nm.(ε, 8730.)

(Found: C, 70.5; H, 5.68; N, 5.47. $C_{31}H_{30}O_6N_2$ requires C, 70.72; H, 5.7; and N, 5.32%).

<u>Preparation of Carbacephalosporanic Acid (97)</u>

The protected carbacephem (96) (80 mg, 0.15 mmol) was deprotected as described above for the diastereoisomeric compound (95), to give the <u>carbacephalosporanic acid</u> (97) as a white crystalline solid (9 mg, 14%) mp 182-185°C.

δ (d_6-DMSO) 3.95 (1 H, d, J 5 Hz, CHN), 4.6 (2 H, s, OCH_2CO), 5.64 (1 H, four lines, d, J 5 Hz, further coupled, d, J 10 Hz, NH C<u>H</u>CO), 7.3 (5 H, s, $CHOCH_2$<u>Ph</u>), 6.5-7.4 (5 H, m, <u>Ph</u>O), 7.67 (1 H, d, J 10 Hz, NH), 12.9 (1 H, bs, CO_2H), and other signals as for carbacephem (91).

(Found: m/e, 392.17381. $C_{23}H_{24}N_2O_4$ (M-CO_2) requires 392.173596).

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