NEW ROUTES TO SOME SUBSTITUTED PENICILLINS AND CEPHALOSPORINS

by

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

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To my parents, Rosie and Harvey,
my brother, Tim, and to Tash,
with many thanks.

Abstract

Literature methods for the synthesis of benzyl 6-oxo-penicillanate have been studied, it was apparent that some of these reports contained conflicting information with regard to reaction conditions and yields. The same methodology was also used to synthesise the cephalosporin analogue t.butyl 7-oxocephalosporanate. The reactions of these compounds with various trimethylsilyl amides and with the Wittig reagent 5'triphenylphosphoranylidenecyclo-hexanespiro-2'-(1',3'dioxolan)-4'-one have been investigated.

The potential for attaching penicillin and cephalosporin nuclei to polymer supports, and thereby utilising the well developed methodology of solid phase peptide synthesis in the field of the discovery of novel β-lactam compounds, has been investigated. Difficulties were encountered when attempting to remove the β-lactams from the resin, however both penicillin and cephalosporin compounds were recovered, in low yield, having had the 6β- (7β-) amino protecting group changed whilst attached to the resin.

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Abbreviations

Ac	Acetyl
6-APA	6-aminopenicillanic acid
7-ACA	7-aminocephalosporanic acid
Bnpeoc	2,2-bis(4'-nitrophenyl)ethoxycarbonyl
Boc	t.butyloxycarbonyl
Bz	benzyl
CHA	cyclohexylamine
DBN	1,5-diazabicyclo[4.3.0]non-3-ene
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCCI	1,3-dicyclohexylcarbodiimide
DCM	dichloromethane
DICI	1,3-diisopropylcarbodiimide
DMA	N,N-dimethylacetamide
DMF	N,N-dimethylformamide
DMSO	dimethyl sulphoxide
Dpp	diphenylphosphinyl
DppODpp	diphenylphosphinic anhydride
Fmoc	9-fluorenylmethoxycarbonyl
HOBt	N-hydroxybenzotriazole
hplc	high performance liquid chromatography
NMM	N-methylmorpholine
n.m.r (nmr)	nuclear magnetic resonance
ONSu	N-succinimidyl
SPPS	solid phase peptide synthesis
t.But	tertiary butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
tlc	thin layer chromatography
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl
MCpba	M.chloroperoxybenzoic acid

CHAPTER 1

INTRODUCTION

1.1 Historical development of β-Lactam Antibiotics

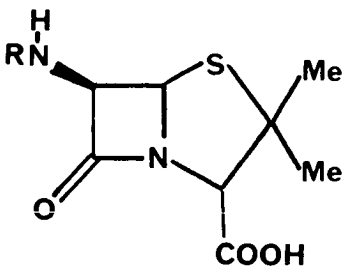
β-Lactam antibiotics have been marketed as antibacterial agents for over 40 years. Today they account for nearly half of all prescribed antibiotics and are used to treat many common infections.

The biological activity of β-lactams was first discovered by Fleming[1] in 1929; he noticed the partial lysis of colonies of staphylococci on a plate that had been contaminated by Penicillium notatum. This led others to the isolation and characterisation of the first clinically useful antibiotic, benzyl penicillin (penicillin G) (1) by 1945.

Problems were soon encountered; some bacteria were able to produce enzymes that could destroy the biological activity of the antibiotic before it had chance to act. These enzymes were initially called penicillinases, though later the more general term ' β-lactamase' became common when a similar problem was found to exist with cephalosporins. Much work has been carried out over the years attempting to synthesise β-lactamase resistant compounds.

The chemical and biological properties of penicillins were found to be dependent on the acylamino side chain at the 6β-position. New compounds were made by the addition of an alternative acyl side chain precursor to the

fermentation broth. This resulted in the discovery of the acid stable compound penicillin V (2) which has a phenoxymethyl group instead of the benzyl group of penicillin G. Penicillin V was found to be stable under the acidic conditions of the stomach, hence allowing oral administration.



(1) R = $C_6H_5CH_2CO$-

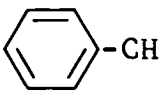
(2) R = $C_6H_5OCH_2CO$-

(3) R = H

(4) R = $C_6H_5CH(NH_2)CO$-

(5) R = $C_6H_5CH(CO_2H)CO$-

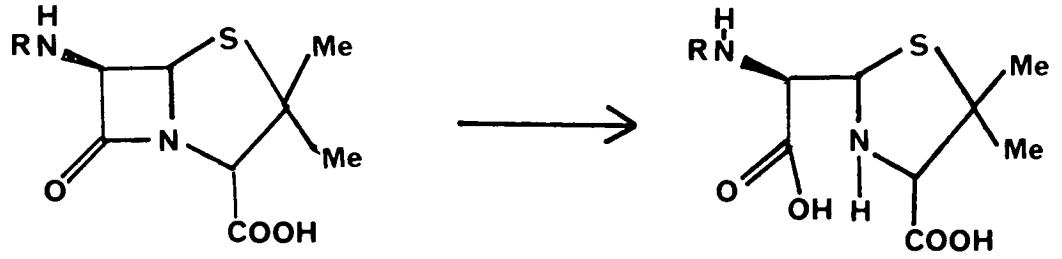
(6) R = $H_2NCH_2(CH_2)_3CO$-
CO_2H

(7) R = HO-  -$CH(NH_2)CO$-

The initial attempts to chemically modify the side chain were hindered because of a shortage of the required intermediate 6-aminopenicillanic acid (6-APA) (3). 6-APA was formed by the enzymatic removal of the side chain of penicillin G[2], but the high demand for the latter by clinicians left little for researchers. Workers at Beecham[3] then discovered that 6-APA could be isolated from a precursor starved fermentation of Penicillium chrysogenum. Once a ready supply of 6-APA was available, many new semisynthetic penicillins were made, with a wide

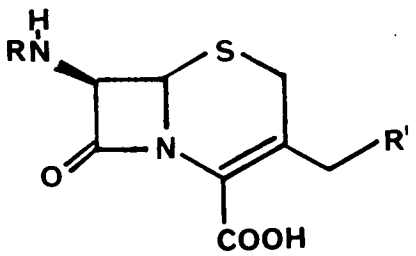
variety of acylamino side chains[4]. Two important compounds resulting from this work were ampicillin (4) and carbenicillin (5). Compared to penicillin G these were found to have improved activity against Gram-negative and penicillinase producing bacteria, but poorer activity against non lactamase producing staphylococcal strains.

Another major development was the discovery of the cephalosporins. In 1945 Brotzu noticed that culture filtrates from a mould, found near a sewage outlet in Sardinia, exhibited some activity against both Gram-negative and Gram-positive bacteria[5]. Isolation of the active ingredient was beyond his means and a sample of the mould was sent to Oxford. There it was identified as Cephalosporium acremonium and two antibiotics were isolated. The first was called cephalosporin N[6]; studies showed that this consisted of a penicillin nucleus with a D-α-aminoadipic side chain, and it was correspondingly renamed penicillin N (6). The second, cephalosporin C (8)[7], was discovered during work on penicillin N and was later isolated by the same researchers[8]. It did not show several of the characteristic properties of penicillins; penicilloic acid was not a product of its hydrolysis (Scheme 1.1), and it was resistant to attack by penicillinase. Even though it was not a very potent antibacterial agent, it was active against both Gram-negative and Gram-positive organisms.



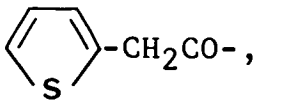
Scheme 1.1 Hydrolysis of penicillins to penicillinoic acid

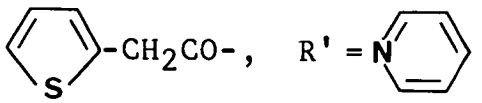
The structure of cephalosporin C was reported by Abraham in 1961[9]. It was found to be related to penicillin, with a dihydrothiazine ring fused to the β-lactam instead of a thiazolidine ring. This was confirmed by X-ray crystallography[10].



(8) R = $H_2NCH(CO_2H)(CH_2)_3CO-$, R' = OAc

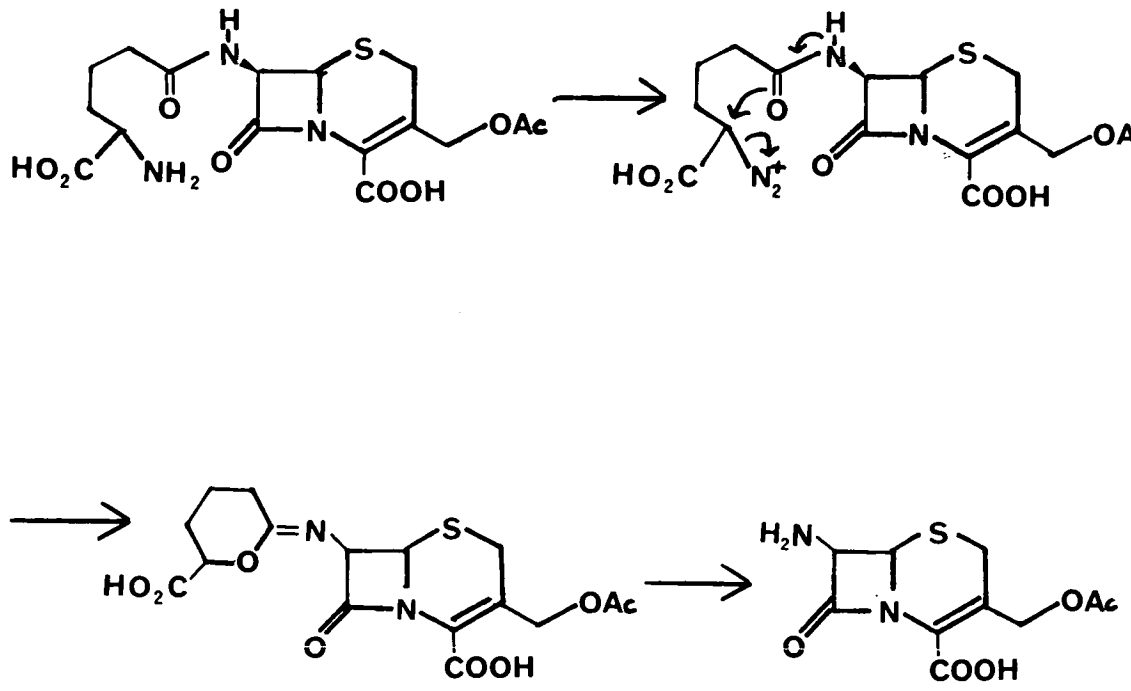
(9) R = H, R' = OAc

(10) R =  , R' = OAc

(11) R = 

Following on from work with the penicillins, attempts were made to modify the acylamino side chain at the 7β-position of cephalosporins. 7-Aminocephalosporanic acid (7-ACA) (9) could not be made using methodology already developed for the synthesis of 6-APA, and was eventually

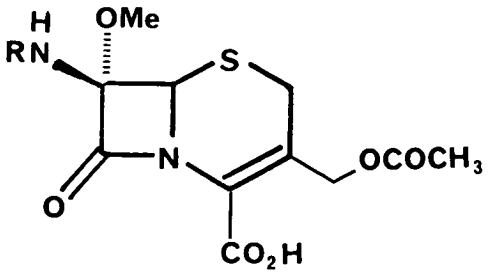
obtained by chemically removing the side chain of cephalosporin C using nitrosyl chloride[11] (scheme 1.2).



Scheme 1.2 The synthesis of 7-ACA

This led to other 7-acylamino side chains being investigated[12] and resulted in the first commercially available cephalosporins: cephalothin (10) and cephaloridine (11). They were found to be active against penicillinase producing bacteria and could be used to treat patients who were allergic to penicillins. They are also more acid stable and have a broader spectrum of activity than penicillins.

Further developments in the field of β-lactam antibiotics resulted from the discovery of the naturally occuring 7α-methoxy cephalosporins (cephamycins) (12)[13]. It was initially thought that the addition of a 6α-methyl group to penicillins would increase the similarity to N-acyl-D-ala-D-ala (see section 1.3.1), but compounds of this type were found to be inactive[14]. However, it was discovered that cephamycins were highly active and resistant to β-lactamase. The 6α-methoxy penicillin analogues (13) were synthesised and temocillin (14), the first penicillin with a broad spectrum of activity coupled with a high level of β-lactamase resistance, was introduced by Beecham[15].



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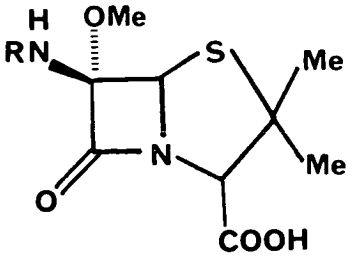

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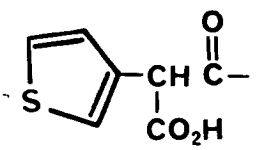
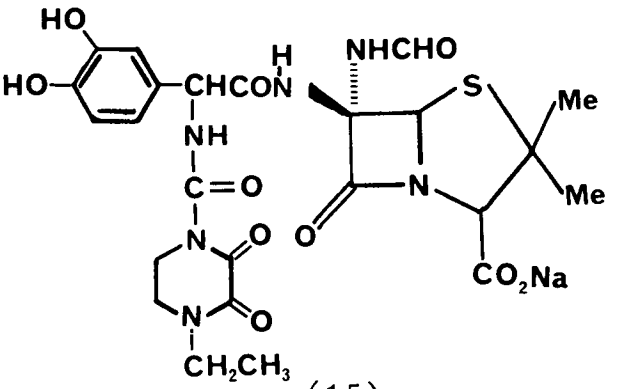
(14) R = 

Workers at Beecham later synthesised approximately 60 different 6α-(7α-)substituted penicillins and cephalosporins in a search for improved antibacterial activity and β-lactamase stability[90]. This work resulted in the chance discovery of the 6α-(7α-) formamido analogues[16]. The 6α-formamido penicillin (15) was found to be more active than the 6α-methoxy compounds whilst retaining the stability to β-lactamase.



(15)

Annual reviews covering research in the field of β-lactam antibiotics are published by the Royal Society of Chemistry in a series of specialist periodical reports entitled "Amino Acids and Peptides".

1.2 Some biological aspects of β-lactam antibiotics

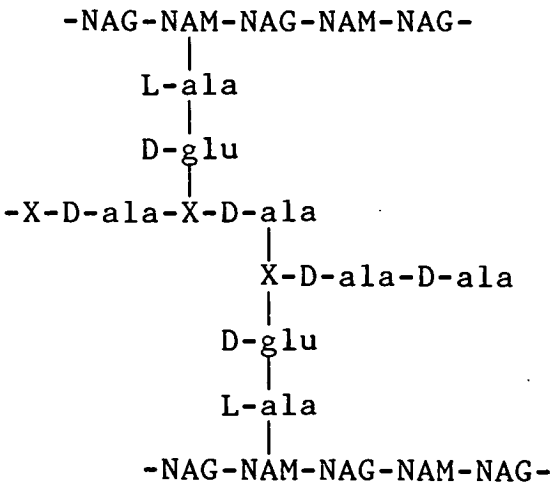
1.2.1 Mode of action

Unlike eukaryotic cells, both Gram-positive and Gram-negative bacterial cells have a complex cell wall surrounding the cytoplasm. As well as maintaining the shape of the bacterium, the cell wall also acts as an osmotic barrier allowing the retention of nutrients and the exclusion of other unwanted compounds. The rigid structure of the cell wall in both types of bacteria is due to the crosslinking of linear polysaccharide chains by short peptide chains.

It is generally accepted that β-Lactam antibiotics interfere with bacterial cell wall synthesis, hence the reason for the low levels of toxicity towards mammalian cells. However this has been subject to some debate, and at least one report indicating that this may not be the case has been published[17].

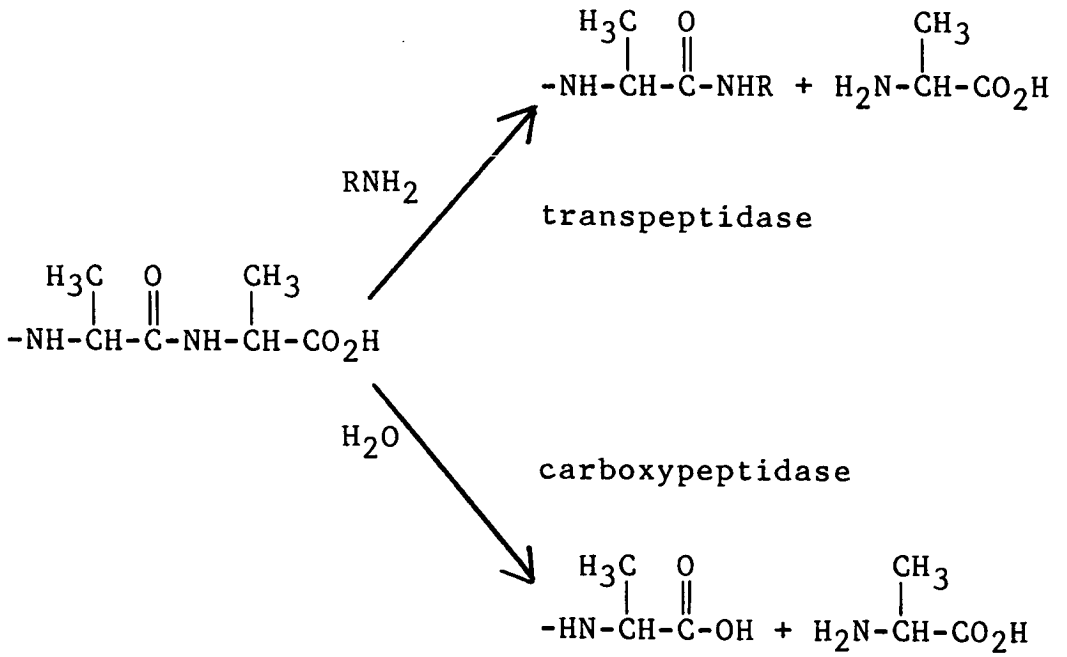
β-lactams only act against growing bacterial cells. Within a short time of adding penicillin to a bacterial culture, bulges begin to form in the bacteria. These increase in size until the bacterium bursts open. During this process the penicillin becomes covalently bonded to proteins, called penicillin binding proteins (PBP's)[18]. These proteins are located on an inner membrane and a series of barriers may have to be overcome before they can be reached by the antibiotic.

The cell wall is made up of peptidoglycan which consists of polysaccharide or 'glycan' chains that are cross-linked by branched peptide chains. The length and nature of the crosslinks vary with bacteria, but the structure of the glycan is universal, consisting of alternating N-acetyl-D-glucosamine (NAG) and N-acetylmuramic acid (NAM). During the cell wall biosynthesis all peptidoglycans have a pentapeptide which commonly has the sequence: L-ala-D-glu-γ-L-X-D-ala-D-ala, in which X is usually an amino acid with a free amino group such as L-lysine. Crosslinking occurs by a transpeptidation reaction, this involves displacing the terminal D-alanine residue with the free amine group (X) of an adjacent pentapeptide (Scheme 1.3). This reaction is highly penicillin sensitive. The extent of crosslinking can vary between 25% and 80% depending on the bacterial species.



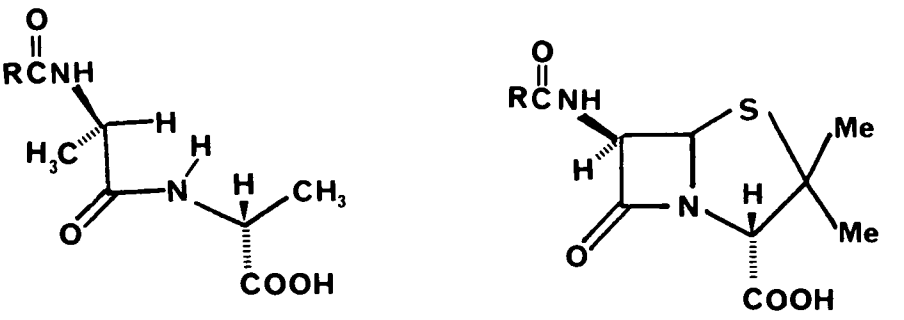
Scheme 1.3 General structure of peptidoglycans

The D-ala-D-ala residues of the uncrosslinked sections are removed by carboxypeptidases. The transpeptidase and carboxypeptidase reactions are similar in that both involve a reaction of the carbonyl of the penultimate D-alanine with a nucleophile. When the nucleophile is water then hydrolysis results, whereas if it is the amino group of another peptide, a peptide bond will be formed (Scheme 1.4).



Scheme 1.4

A historical model describing the selectivity and action of β-lactam antibiotics was proposed by Tipper and Strominger over 25 years ago[19]. They suggested that penicillin may be a structural analogue of D-alanine-D-alanine. It should however be noted that the configuration at C-6 in penicillins is opposite to what would be required for a complete structural analogy (Scheme 1.5).

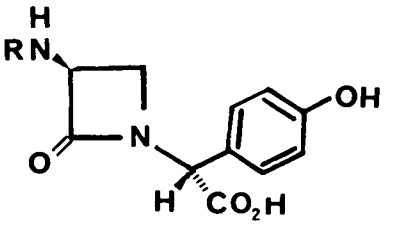


(a) (b)

Scheme 1.5 Comparison of the configuration of N-acylated D-alanine-D-alanine (a) with a 3S,5R,6R-penicillin (b).

It was suggested that transpeptidation was a two step process involving an acyl-enzyme intermediate, formed by a serine hydroxyl on the enzyme displacing the terminal D-alanine. Because of the reactivity of the β-lactam ring, it was thought that penicillin was capable of irreversibly acylating bacterial transpeptidases thereby preventing the formation of crosslinks. This would lead to a weakening of the peptidoglycan and the eventual death of the cell.

This theory provided a simple model which helped to explain the observations, but when one considers the activity of compounds such as the norcardicins (16) or the monobactams (17), its limitations become apparent.



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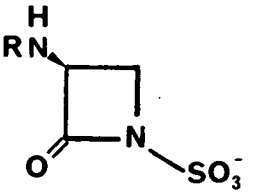
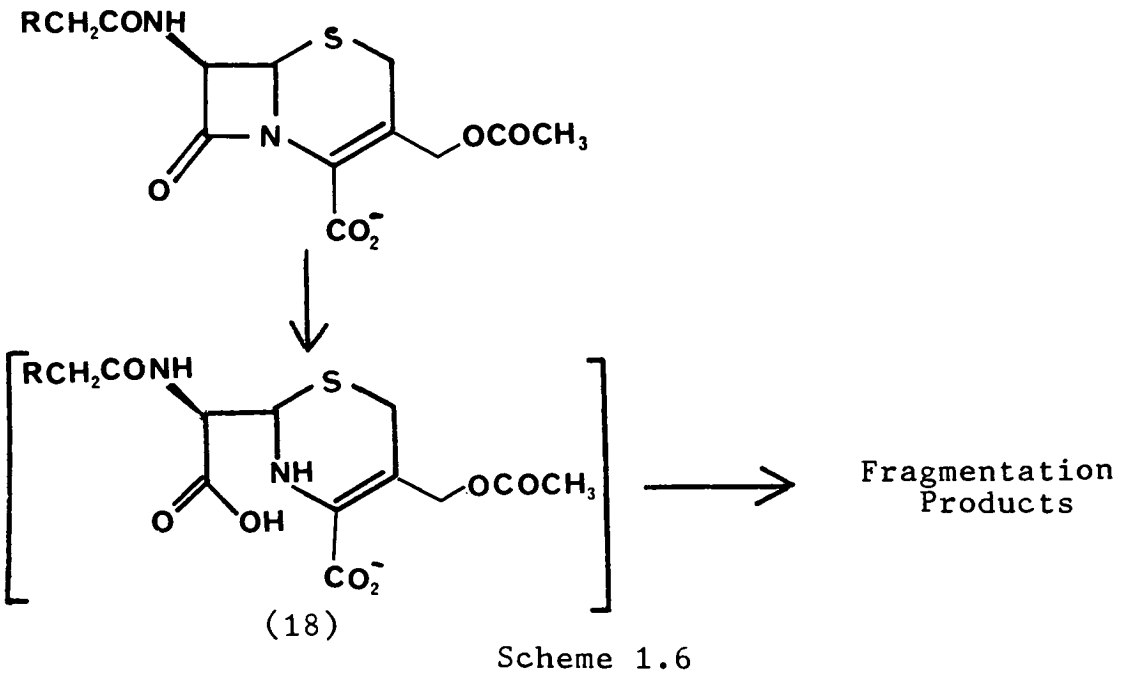


(17)

1.2.2 Action of β-lactamases on penicillins and cephalosporins.

The biological activity and microbial resistance of penicillins and cephalosporins are linked to the reactivity of the β-lactam ring which is strained and susceptible to various cleavage reactions. The ring opening reactions of penicillins and cephalosporins are totally different. The hydrolysis of penicillins has been illustrated in scheme 1.1; this reaction will occur in the presence of dilute alkali, penicillinase or amino groups. The resulting penicilloates can be isolated and are well characterised. Cephalosporins however are more complicated because the corresponding cephalosporoate (18) is unstable (scheme 1.6). Newton et al[20] reported extensive fragmentation and the production of free acetate when

cephalosporin C was subjected to aqueous cleavage conditions.



(18)

Scheme 1.6

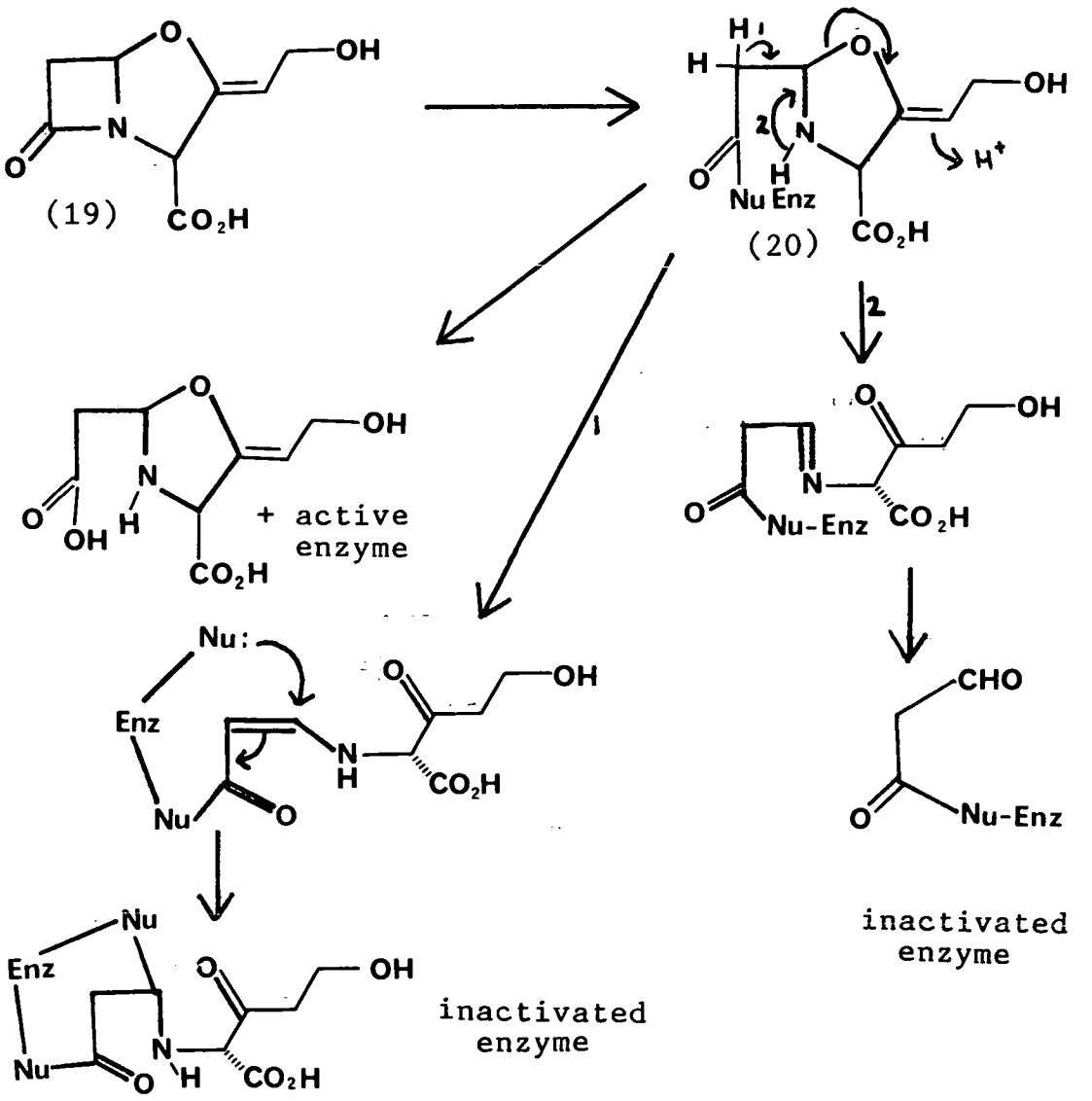
Hamilton-Millar and co workers[21] carried out a detailed study on the aminolysis and enzymatic hydrolysis of cephalosporins which led to some understanding of the chemistry involved.

Excellent reviews are available regarding the types of β-lactamases and their modes of action[22].

1.2.3 β-lactamase inhibitors.

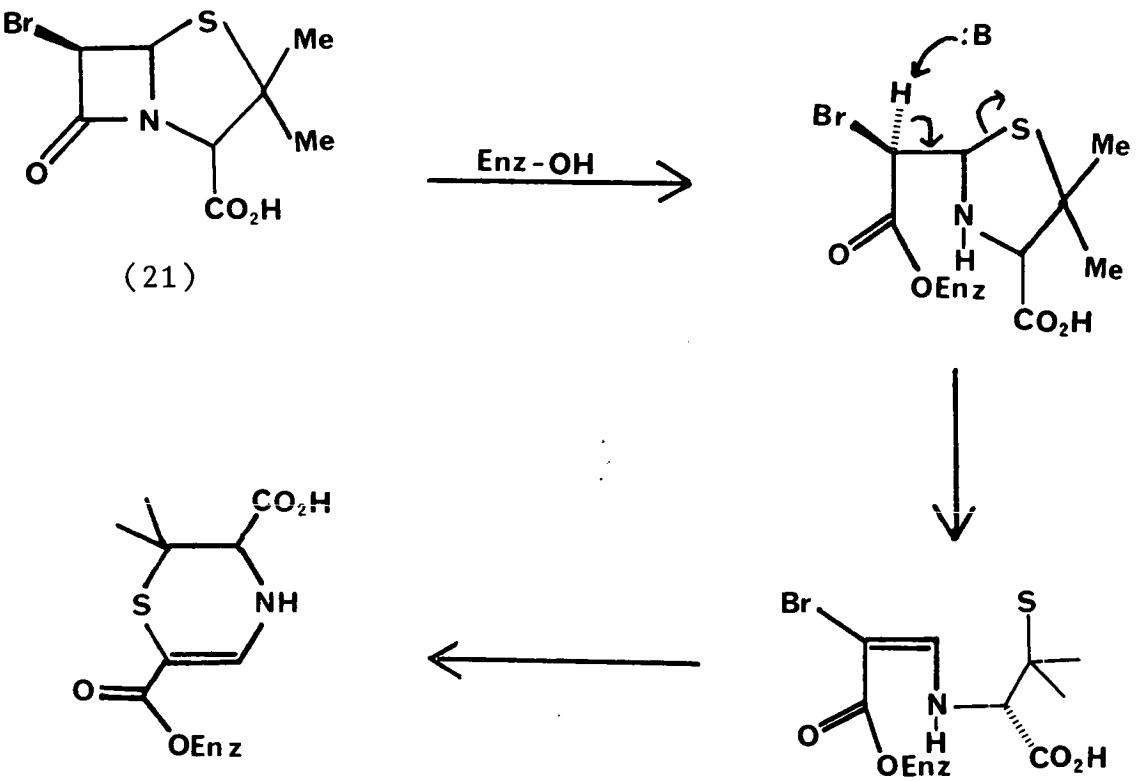
A β-lactamase inhibitor is a β-lactam compound that will irreversibly react with β-lactamases thereby preventing these enzymes from attacking the antibiotic. β-Lactamase inhibitors will often have only poor antibiotic activity, and are used in conjunction with more active compounds.

Clavulanic acid (19)[23], was the first β-lactamase inhibitor discovered by workers at Beecham, and has been marketed as a drug called augmentin, in which it is combined with amoxycillin (7). When (19) is used in excess, the enzyme becomes irreversibly acylated. The reaction initially involves the formation of an acyl-enzyme intermediate (20), after which it can follow one of three competing pathways (Scheme 1.7), two of which inactivate the enzyme.



Scheme 1.7

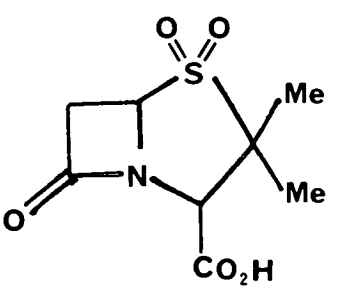
Other common β-lactamase inhibitors include 6β-bromo penicillanic acid (21) and penicillanic acid sulphone (23). The former is believed to produce a thiazolidinone-enzyme adduct (22) (scheme 1.8), whereas the sulphone is believed to react in a similar manner to clavulanic acid.



(21)

(22)

Scheme 1.8

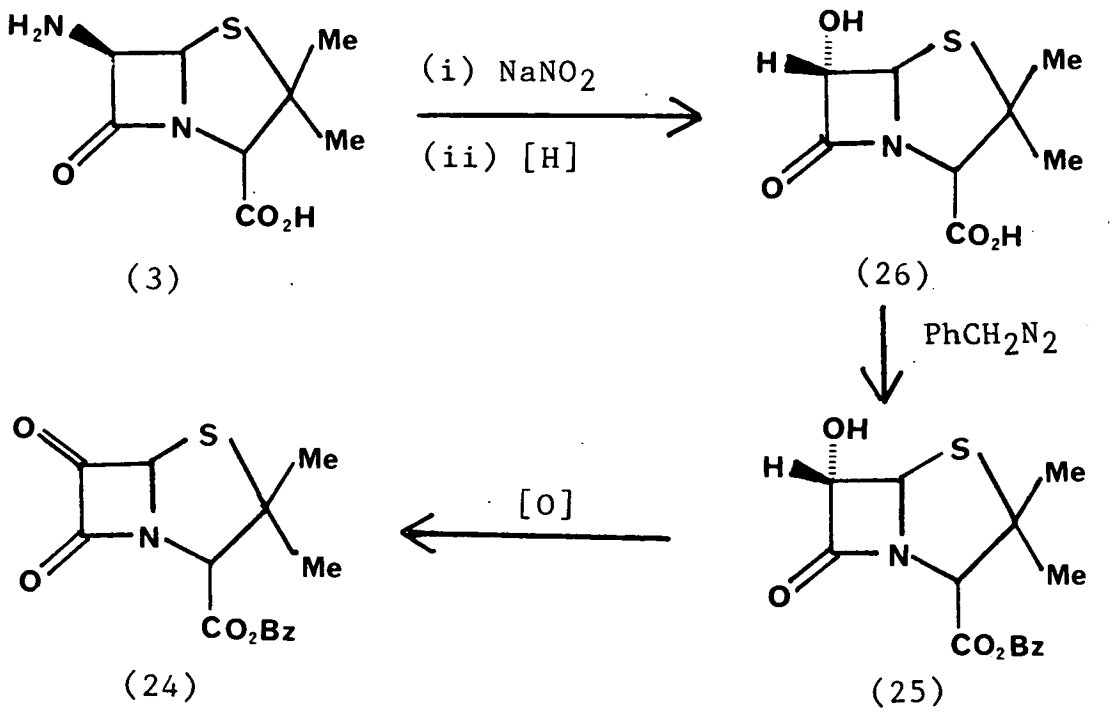


(23)

1.3 The synthesis and selected chemistry of 6-oxo-penicillins and 7-oxocephalosporins

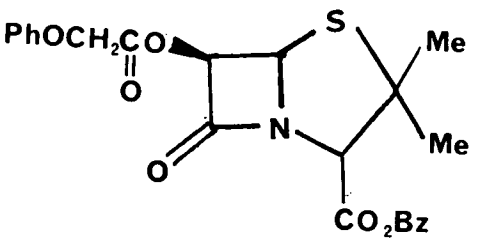
Compounds with a carbonyl group at the 6(7) position of a penicillin or cephalosporin nucleus were first synthesised by Y. S. Lo and J. C. Sheehan in 1972[24]. The potential for the synthesis of novel compounds from these intermediates was soon realised.

The first compound of this type synthesised by these researchers was benzyl 6-oxopenicillanate (24), which was obtained by the oxidation of benzyl 6α-hydroxy-penicillanate (25). The oxidation of (25) was carried using diisopropyl carbodiimide in the presence of dimethyl sulphoxide, oxidation methodology that is commonly referred to as the Moffat oxidation[25]. Benzyl 6 α -hydroxypenicillanate (25) was prepared by the method of Hauser and Sigg[26]. This involved the reaction of 6-APA (3) with sodium nitrite to produce 6α-hydroxypenicillanic acid (26), from which the benzyl ester (25) was obtained following treatment with phenyldiazomethane (Scheme 1.9). Difficulty was experienced in removing the byproduct resulting from the diisopropylcarbodiimide from the oxidation product, and the reaction was unsuccessful when water soluble carbodiimides were used in an attempt to overcome this problem. The final product was eventually isolated as a crystalline cyanohydrin following treatment with HCN.



Scheme 1.9 Synthesis of benzyl 6-oxopenicillanate

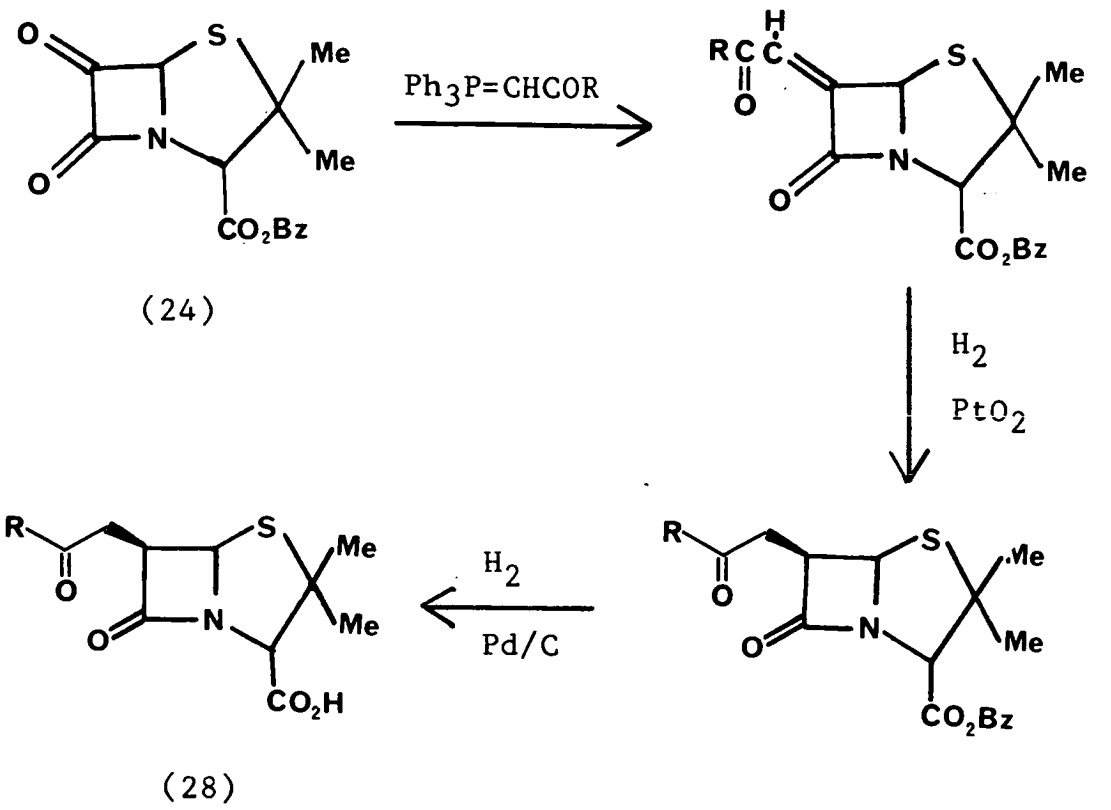
Sheehan and Lo then reduced (24) using potassium borohydride. This resulted in only the β-hydroxy isomer which was acylated with phenoxyacetylchloride to benzyl 6β -(phenoxyacetoxy)penicillanate (27).



(27)

No indication of yields were given in the initial report of this work, however in a later publication a yield of 22% was quoted for the conversion of 6-APA to benzyl 6-oxopenicillanate (24)[27].

Due to the absence of amide stabilisation, the carbonyl group at the 6 position of (24) was significantly more reactive than the adjacent β-lactam carbonyl group, and Wittig reactions were found to be highly regioselective towards it. Using this reaction, 6β-phenoxyacetylmethyl penicillanic acid (28), a carbon analogue of penicillin V, was synthesised[28] (Scheme 1.10).



(24)

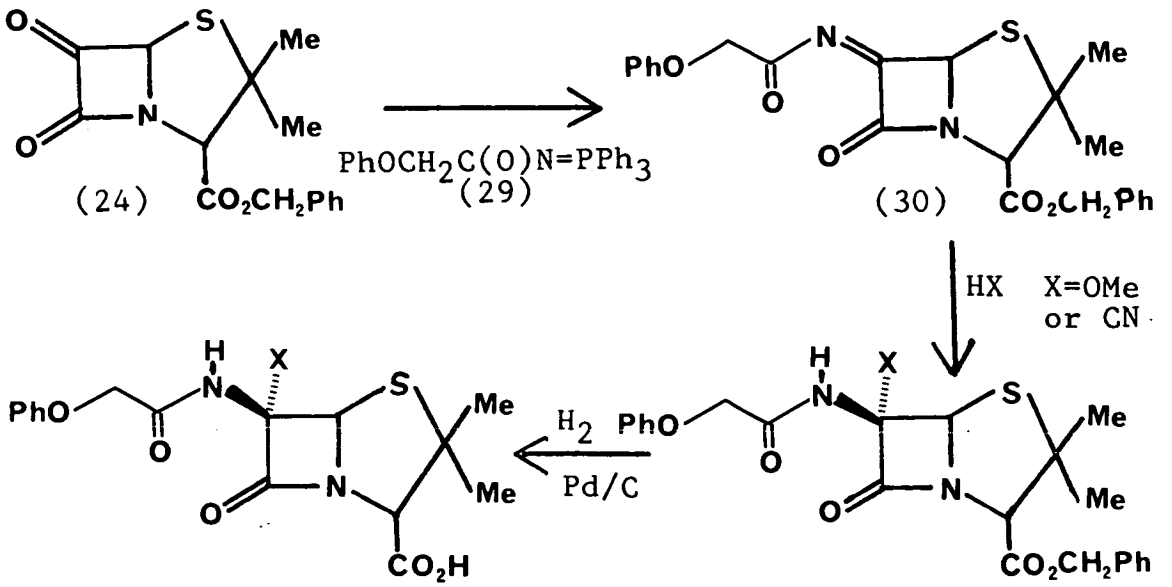
(28)

Scheme 1.10

It should be noted that Wittig reactions with β-lactam carbonyl groups have since been successful. Workers at Beecham[29] initially reported the reaction of $CH_3CO_2CHPPH_3$ with derivatives of clavulanic acid (19) and penicillin V (2). Mixtures of the E and Z-isomers were obtained in high

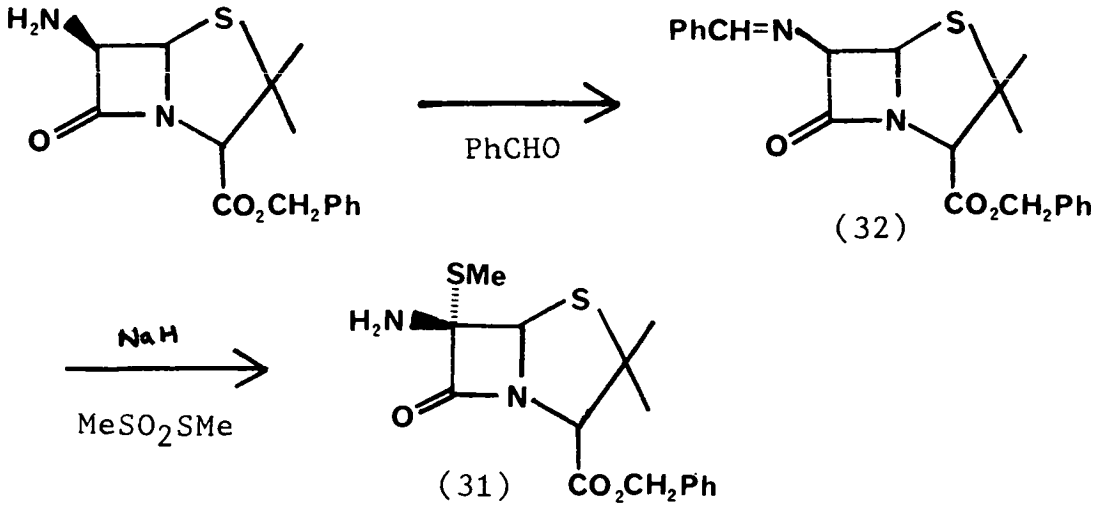
yield when the reactions were carried out in refluxing toluene. A more comprehensive study was later published in which reactions involving a wider range of β-lactam compounds and Wittig reagents were reported[30].

The synthesis of 6α-substituted penicillins from 6-oxo-penicillins was investigated soon after the discovery of the 7α-methoxycephalosporins (cephamycins) (12). These are naturally occurring compounds which were found to have a high degree of stability to β-lactamase. Benzyl 6-oxopenicillanate was treated with the nitrogen Wittig reagent, N-phenoxyacetyliminotriphenylphosphorane (29), which gave an N-acylimine intermediate (30). This was then converted to the 6α-methoxy or cyano compound by treatment with methanol or HCN respectively, with the addition in both cases occurring from the least hindered side of the double bond[31] (scheme 1.11).



Scheme 1.11 Synthesis of 6α-methoxypenicillanic acid.

Unrelated work, by T. Jen et al[32], on 6α (7α)-methoxy penicillins and cephalosporins led to an alternative method for the synthesis of 6-oxopenicillins. They synthesised both 6α (7α) -methoxy penicillins and cephalosporins by the addition of methanol in the presence of mercuric chloride to the respective 6α (7α)-methylthio compound. The methylthio intermediate (31) was synthesised from the Schiff base (32) by treatment with methyl methanethiolsulphonate (Scheme 1.12). It was found that the 6α -methylthiopenicillin derivative (31) would yield benzyl 6-oxopenicillanate (24) on treatment with mercuric chloride in the presence of DMF and water.



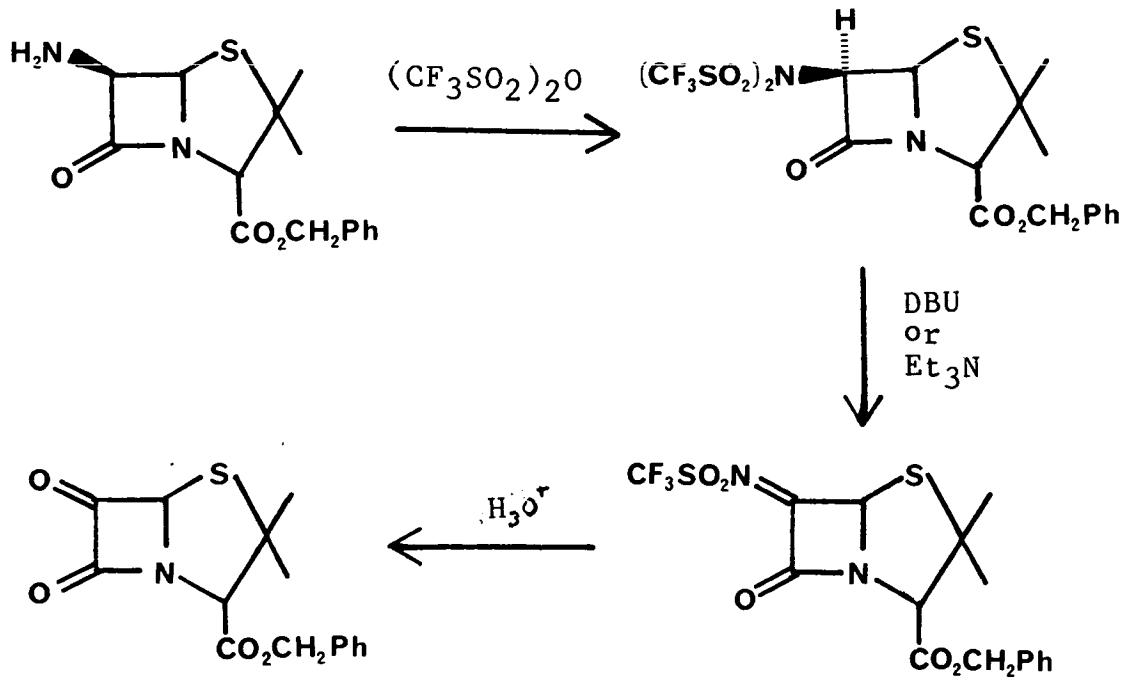
Scheme 1.12

During a study of the isomerisation of benzyl 6α - and 6β -phenoxyacetoxypenicillanates H. Vanderhaege et al[33] attempted the oxidation of benzyl 6α-hydroxy-penicillanate (25) to benzyl 6-oxopenicillanate (24). These workers reported that the Moffat oxidation of (25) using either

orthophosphoric acid or pyridinium trifluoroacetate as catalyst gave a mixture containing approximately 50% of the desired product which could not be further purified by silica gel chromatography. An improved method for this oxidation was suggested, involving the use of dimethyl sulphoxide and acetic anhydride. Using these conditions benzyl 6-oxopenicillanate (24) was obtained, in 92% yield, as a crystalline solid.

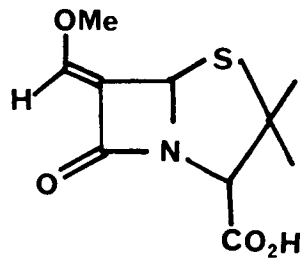
A comparison of some oxidising methods for the conversion of 6 α -hydroxypenicillanates to 6-oxo penicillanates was carried out by S. Chandrasekaran et al[34] after which they concluded that the preparation of 6-oxopenicillanates was not as straightforward as earlier reports led one to believe. Benzyl 6-oxopenicillanate (24) was synthesised in approximately 80% yield using the Moffat oxidation with dichloroacetic acid as the catalyst and in order to repeat the DMSO/acetic anhydride conditions described by Vanderhaege[33], they found it necessary to distil the acetic anhydride from either aluminium chloride or calcium carbide. The oxidation was also attempted using N-chlorosuccinimide-dimethyl sulphide[35], Jones reagent[36] and silver carbonate on Celite[37], all of which were unsuccessful. t-Butyl 7-oxo cephalosporanate (76) was also synthesised from the corresponding 7 α -hydroxycephalosporanate using dimethyl sulphoxide-DCCI, though the reaction was not as clean as for the penicillins.

An alternative route to 6-oxopenicillanic acid and 7-oxocephalosporanic acid derivatives, involving totally different methodology from previous syntheses, was introduced by D. Hagiwara et al in 1982[38]. They reacted both benzyl 6β-aminopenicillanate and benzyl 7β-aminocephalosporanate with trifluoromethanesulphonic anhydride. This gave the corresponding bis(trifluoromethyl)sulphonate derivatives, which were then converted to the imines by treatment with either DBU or triethylamine. The imines were finally hydrolysed with dilute HCl to give the 6-oxopenicillanate and 7-oxocephalosporanate derivatives in approximately 75% yield (Scheme 1.14).



Scheme 1.14 Synthesis of benzyl 6-oxopenicillanate using trifluoromethanesulphonic anhydride.

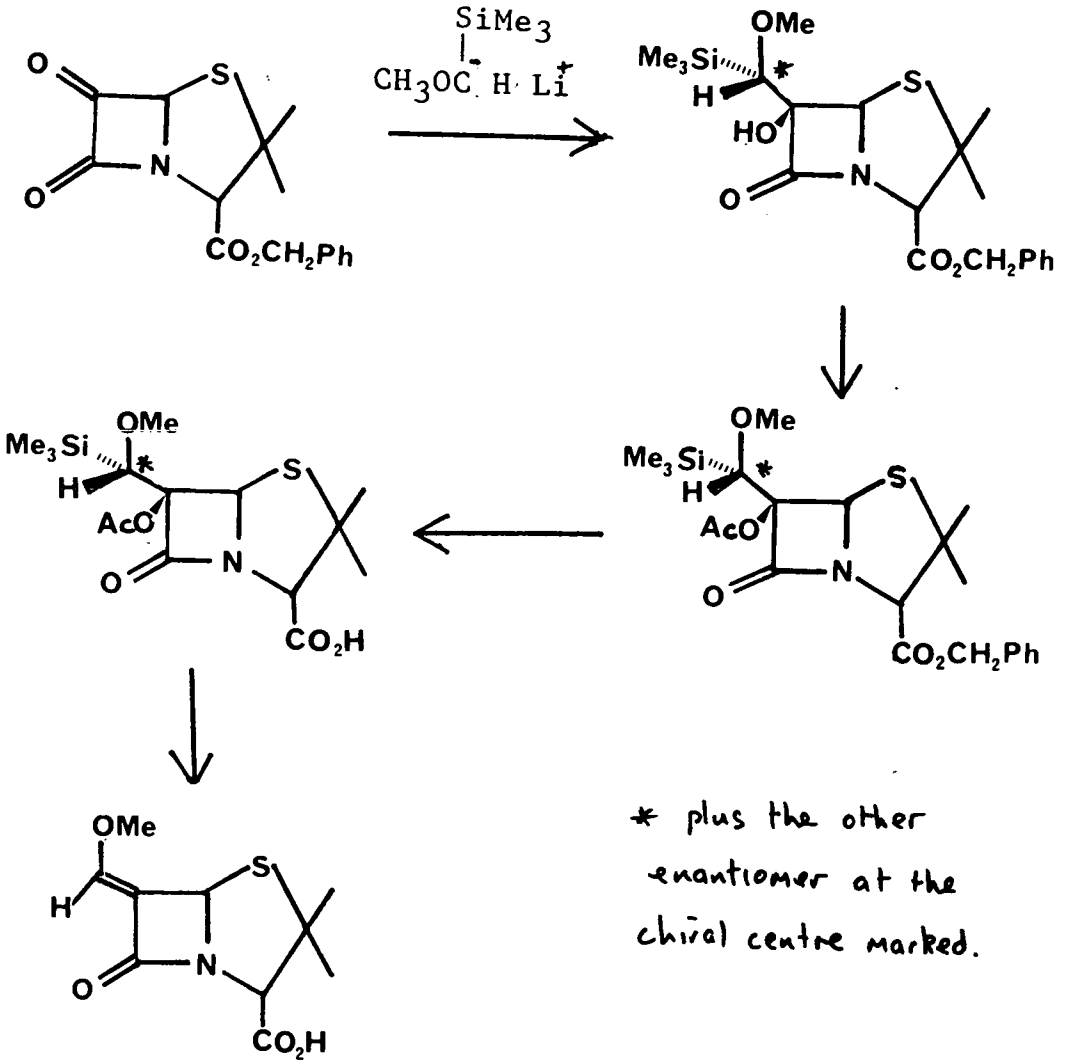
Some penicillins with a carbon-carbon double bond at the 6 position have have been reported to have β-lactamase inhibitory properties. D. G. Brenner synthesised 6-(methoxymethylene)penicillanic acid (34)[39], from benzyl 6-oxopenicillanate (24), and found the Z-isomer to be a potent β-lactamase inhibitor which behaved in a similar way to clavulanic acid (18).



(34)

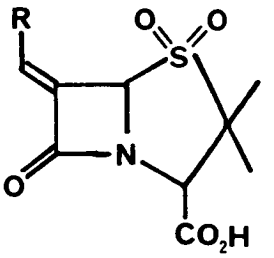
The above compound could not be synthesised by a direct Wittig reaction between benzyl 6-oxopenicillanate and the ylide generated from methoxymethyltriphenylphosphonium bromide, as the reaction was too slow at low temperatures, and increasing the temperature resulted in the loss of β-lactam functionality. A method previously employed by Magnus and Roy[40] involving the use of silicon stabilised anions was found to be successful. This involves the reaction of (methoxy(trimethylsilyl)methyl)lithium, generated from (methoxymethyl)trimethylsilane and butyllithium, with a ketone to form stable β-silylalcohol adducts. Treatment with caesium fluoride will give methyl vinyl ethers in high yield. Brenner reported that this reaction had an added advantage in the case of penicillins

in that the C-3 ester group could be removed at the intermediate stage prior to formation of the enol ether. Problems were encountered with the final stage due to desilylation competing with the desired elimination reaction. To promote the elimination the alcohol at C-6 was made into a better leaving group by acetylation (Scheme 1.15).



Scheme 1.15 Synthesis of 6-methoxymethylene-penicillanic acid.

Y. L. Chen et al[41] prepared a series of 6-(heterocyclyl)methylene penam sulphones (35) and reported that these had β-lactamase inhibitory properties.



(35)

They carried out a Swern oxidation[42] on allyl 6α-hydroxypenicillanate to give allyl 6-oxopenicillanate which was used without purification. The product of the Wittig reaction was oxidised to the sulphone using 2 equivalents of m-chloroperbenzoic acid, final removal of the allyl group gave the required compounds.

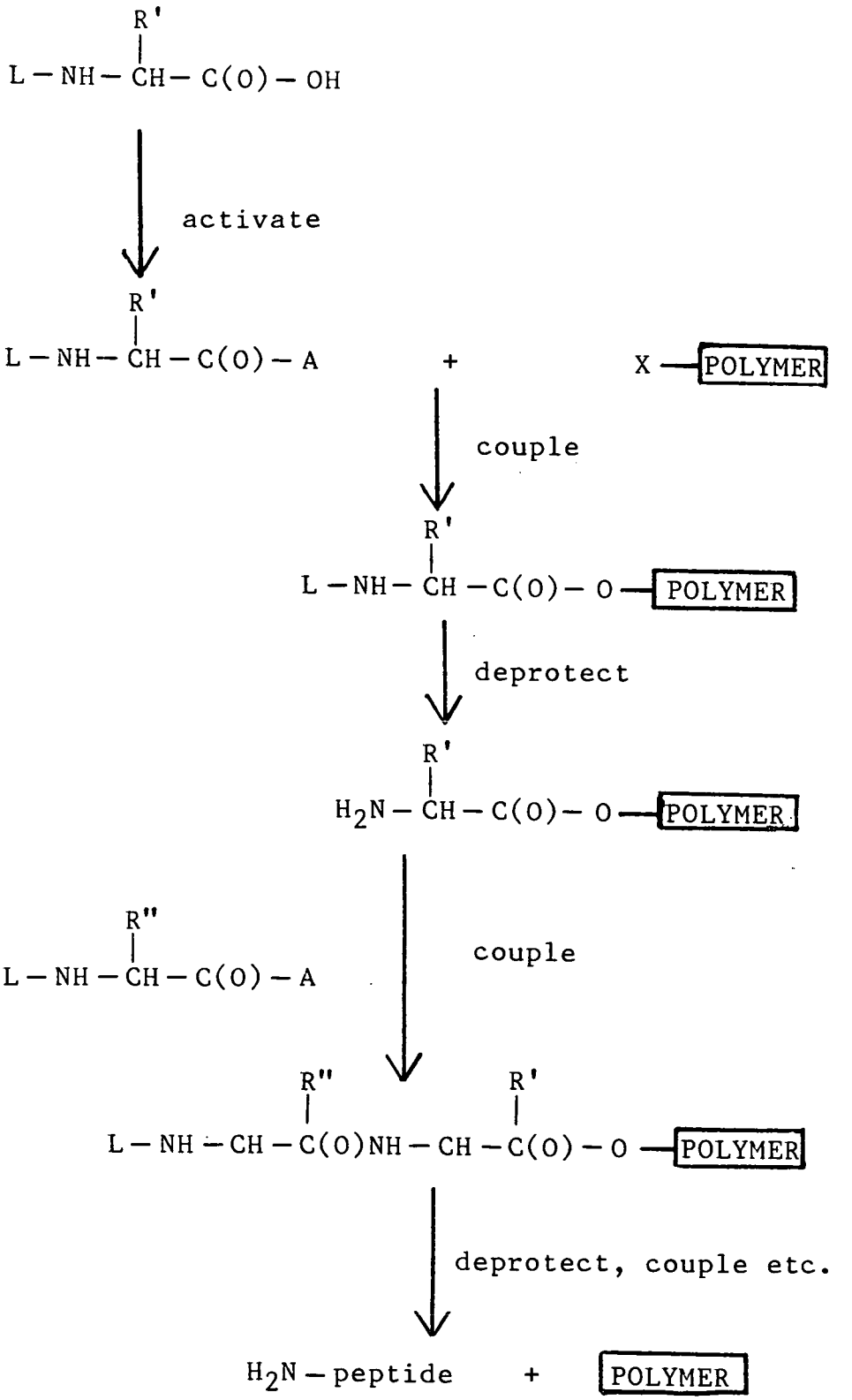
1.4 Solid phase peptide synthesis

1.4.1 Introduction.

Since the introduction of solid phase methodology by R. B. Merrifield in 1963[43], it has developed into by far the simplest and most efficient means of chemically synthesising both peptides and oligonucleotides. Merrifield later received the Nobel prize for this work[44].

The solution phase synthesis of peptides is a complicated and laborious task. Each protection, coupling and deprotection step, of which there may be many, will contribute to a significant loss of product, resulting in a poor yield of the target compound. Combined with purification problems and the decreasing solubility of the growing peptide, it is clear that conventional methodology is not ideal for this area of chemistry.

In solid phase peptide synthesis (SPPS) the first amino acid is attached to an insoluble support, a synthetic polymer called a 'resin'. Successive activation, coupling and deprotection steps eventually lead to the target peptide bound to the resin (scheme 1.16). The amino acid to be attached to the resin must be protected at the N^{α} -position. Once bound to the resin the N^{α} -protection can be removed. The nature of the protecting group must be such that its removal does not affect the amino acid-resin linkage or any other side chain protection that may be present.



Scheme 1.16 Solid phase peptide synthesis.

A major advantage of solid phase techniques is that when attached to the resin, the peptide is insoluble in all solvents. This means that excess reactants and reagents may be removed by simple filtration techniques.

Once the required peptide has been synthesised, all side chain protecting groups must be removed and the product must be cleaved from the resin. Ideally these steps should be carried out simultaneously.

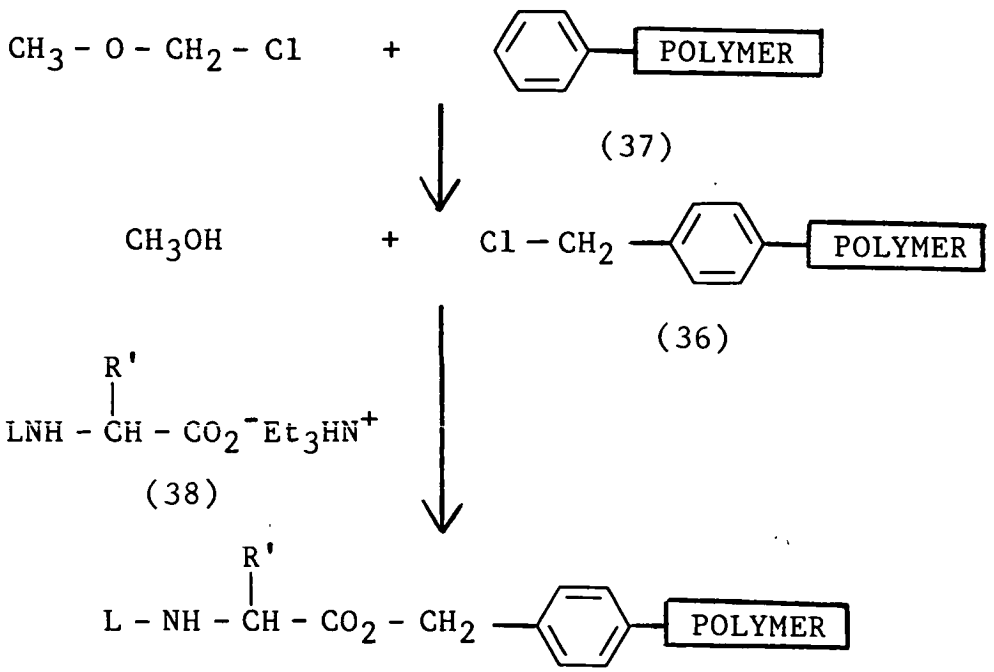
The repetitive nature of this cycle has led to the automation of SPPS. Peptides may be assembled at the rate of one amino acid residue every four hours, a vast improvement over solution phase techniques.

1.4.2 The polymer support

For a polymer to be of use as an insoluble support in SPPS it must comply with the following basic rules:

1. The size of the polymer particles must allow easy manipulation and rapid filtration.
2. The polymer must be sufficiently inert so as not to change during the synthesis.
3. The polymer must swell in appropriate solvents to allow the penetration of reagents.

The resin initially developed by Merrifield (36)[43] was prepared by the chloromethylation of a cross-linked copolymer (37) obtained from 2% divinylbenzene in styrene. This gave a chloromethylated resin to which the first amino acid could be attached by using the triethylammonium salt (38) (Scheme 1.17).



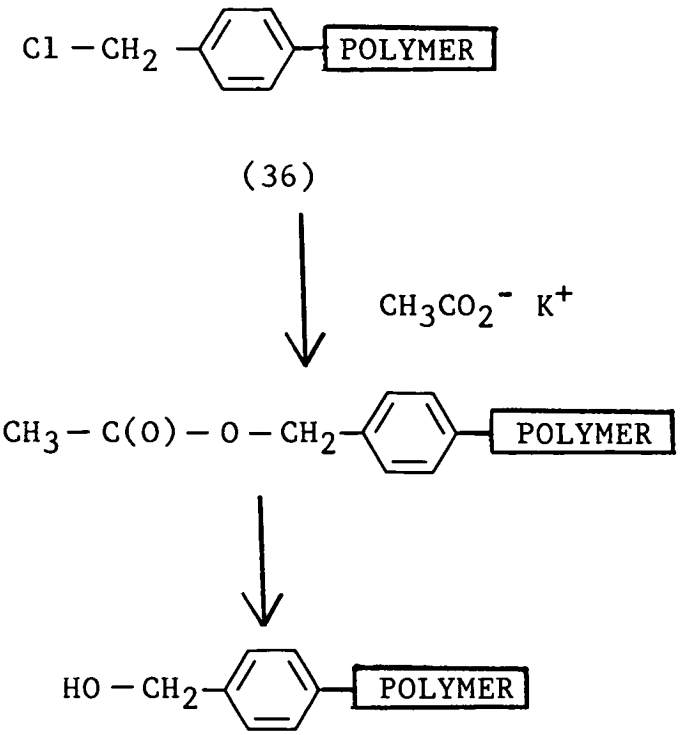
Scheme 1.17 Use of the chloromethylated resin.

The N^{α} - protecting group initially used by Merrifield was benzyloxycarbonyl (Z)[45] which required the rather harsh deprotection conditions of HBr in acetic acid. When subjected to these conditions a small amount of the growing peptide would be cleaved from the resin. This created a significant problem during the synthesis of large peptides, which was overcome to some extent with the introduction of the t-butyloxycarbonyl[46] group.

Many alterations have been made to the original resin, usually with the aim of increasing or decreasing the stability of the peptide-resin bond. It was thought that increasing the electron withdrawing effect on the benzylic position, by nitrating or brominating the aromatic nucleus of the copolymer, would result in a more stable peptide-resin bond[43]. This was found to be true, however the peptide-resin link in the nitrated example was resistant to acidolysis with HBr and HF, and the brominated example suffered from reduced swelling properties.

Other modifications were carried out to the copolymer itself. Reducing the divinylbenzene content from 2% to 1% resulted in a resin with improved swelling characteristics in both DCM and DMF[43,47]. This gave improved access to the reactive sites of the resin, especially important when amino acids with bulky side chains are involved. If less than 1% divinylbenzene is used[48] the resulting resin is very soft and there are often problems with filtration. Merrifield also reported[43] that supports with 8% and 16% crosslinking were very rigid and did not allow easy penetration of reagents.

A further improvement on the chloromethyl resin has been the development of the hydroxymethyl resin (39) (Scheme 1.18).



Scheme 1.18 Synthesis of the hydroxymethyl resin.

The hydroxymethyl resin allows the use of milder coupling reagents e.g. DCCI[49], hence the alkylation of nucleophilic side chain functions, for example in histidine and methionine, is avoided. This was often a problem when the original resin (36) was used with such amino acids.

1.4.3 The peptide-resin link

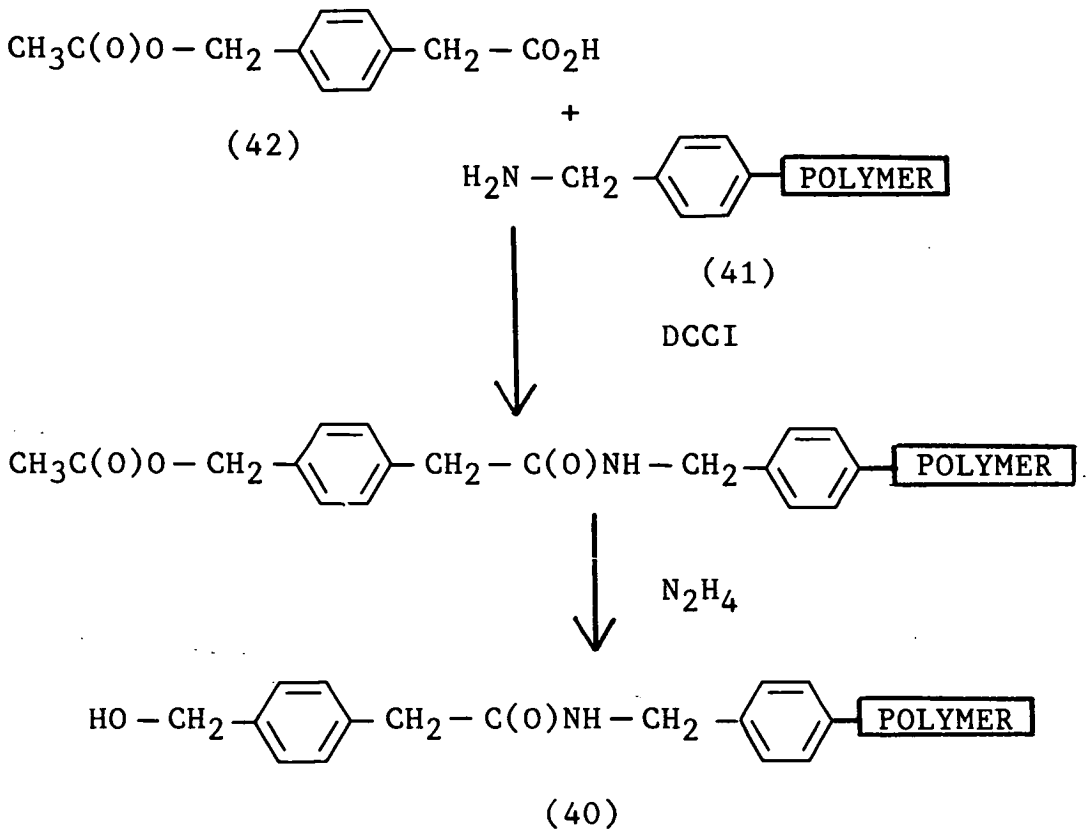
Even after the introduction of the Boc group as a means of N^{α} - protection in SPPS there were still problems with peptide-resin benzyl ester bond cleavage during deprotection. It has been reported that using 50% TFA in DCM for the removal of Boc results in the loss of approximately 1% of the peptide from the resin.

A large improvement came with the introduction of the phenylacetamidomethyl (Pam) resin (40) by Merrifield et al[50]. This involved the inclusion of a 'handle' between the peptide and the resin. The Pam resin is synthesised by the acylation of the aminomethyl resin (41) with p-acetoxymethylacetic acid (42) using DCCI[51]. The acetyl group is then removed by hydrazinolysis to the hydroxymethyl Pam resin (40) (Scheme 1.19). (41) is generated by the treatment of the chloromethyl resin (36) with potassium phthalamide then hydrazine[52].

The electron withdrawing effect of the acetamido group in the para position of the phenyl ring to which the peptide is attached increases the stability of the peptide-resin link, by virtue of deactivating any incipient carbocation intermediate during alkyl-oxygen fission of the benzyl ester.

The 'handle' also acts as a spacer, increasing the distance between the resin and the peptide. This has the

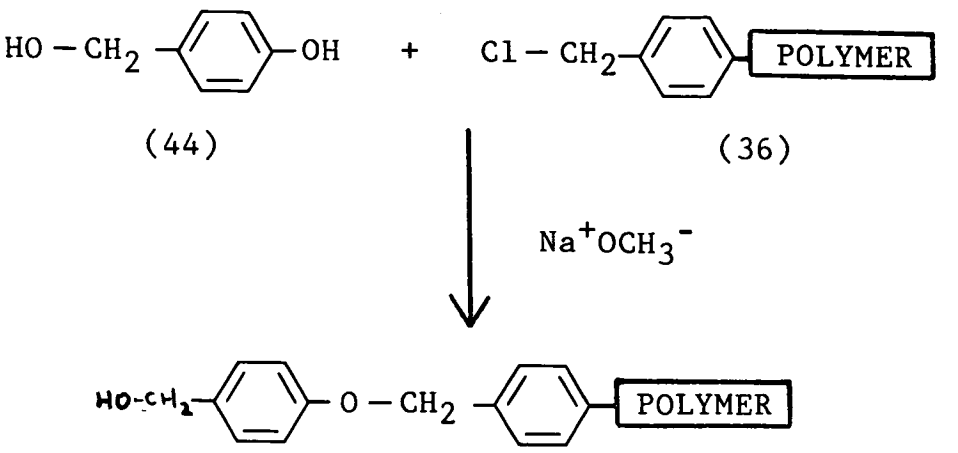
effect of making the reactive sites more accessible to reagents.



Scheme 1.19 Synthesis of the hydroxymethyl Pam resin (40).

The main problem with such a resin is that larger peptides may not be stable to the cleavage conditions required, namely HF. Therefore ideally more acid labile peptide-resin linkages are required. An example of such a resin is the p-alkoxybenzylalcohol resin (43) introduced by Wang[53]. The synthesis of this involves the treatment of the chloromethyl resin (36) with 4-hydroxybenzylalcohol (44) and sodium methoxide (Scheme 1.20)[54].

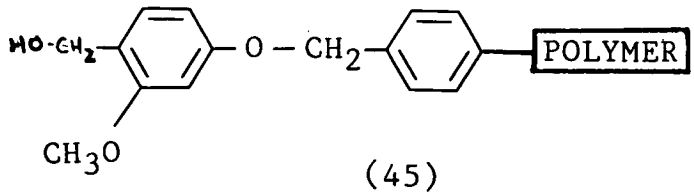
The p-alkoxy substituent enhances the lability of the ester linkage by donating electrons. A solution of 50% TFA in DCM is sufficient to cleave the final peptide from this resin. If acid labile N^{α} - protection is used it must be stable to these conditions, alternatively, base labile protection can be used e.g. 9-fluorenylmethoxycarbonyl (see section 1.4.4).



(43)

Scheme 1.20 Synthesis of the Wang resin (43).

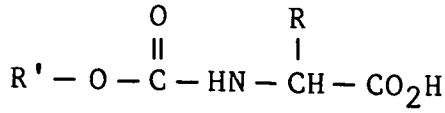
The 2-methoxy-4-alkoxybenzylalcohol resin (45)[55] (Sasrin resin) has recently been introduced as a resin that is even more acid labile than the Wang resin. Cleavage conditions of between 0.5-1.0% TFA in DCM are successful in removing peptides from this resin. Besides the increased acid lability of the peptide-resin linkage, the Sasrin resin is reported to behave in a similar manner to the Wang resin.



(45)

1.4.4 N^{α}-protecting groups

The N^{α}- position of an amino acid is usually protected by groups of the urethane type (46), as they may be removed under relatively mild condition as well as allowing the formation of amide bonds by racemisation free coupling

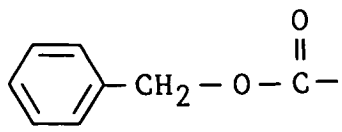


(46)

Some of the N^{α} - protecting groups used in peptide synthesis are discussed below:

Z protecting group

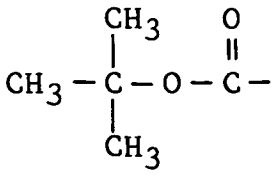
Benzyloxycarbonyl (Z) (47), the first urethane type protecting group, was introduced by Bergman and Zervas in 1932[45].



(47)

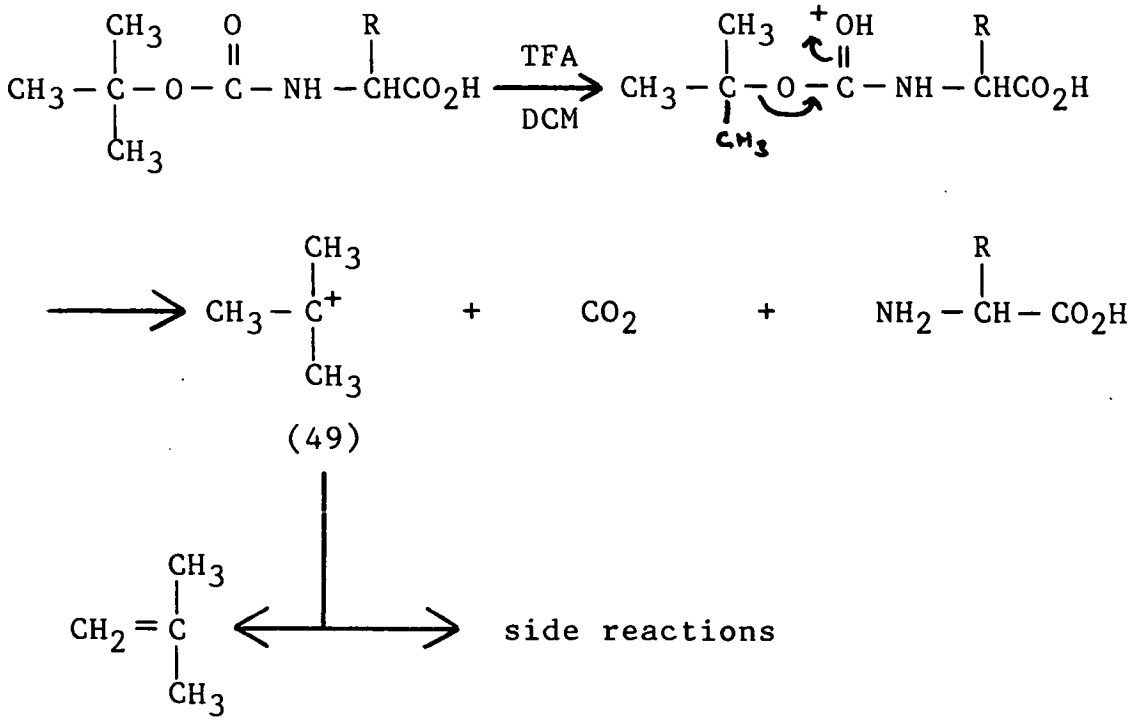
Deprotection may be achieved by hydrogenolysis or acid catalysed solvolysis using hydrobromic acid. Peptide - resin cleavage may occur with successive acidic N^{α} - deprotections.

Boc protecting group



(48)

tert.Butyloxycarbonyl (Boc) (48)[46] is the most widely used acid labile protecting group in SPPS. Boc protected amino acids are usually deprotected using 50% TFA in DCM (Scheme 1.21).

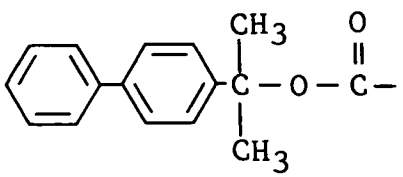


Scheme 1.21 Removal of the Boc protecting group.

The carbonium ion intermediate (49) will lose a proton to give but-2-ene, but when sulphur containing amino acids (methionine or cysteine) or the aromatic systems of tyrosine or tryptophan are present, (49) may become involved in unwanted side reactions. The addition of cation scavengers such as anisole or dimethyl sulphide[56] will sometimes prevent these side reactions.

Bpoc protecting group

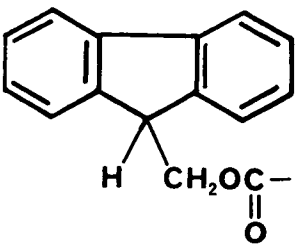
The 2-(4-biphenyl)isopropyloxycarbonyl (Bpoc) group (50)[57] is considerably more acid labile than Boc and can be deprotected using 0.2-0.5% TFA in DCM. Bpoc amino acids are not stable as free acids, and must be stored as salts.



(50)

Fmoc protecting group

The 9-fluorenylmethoxycarbonyl (Fmoc) group (51), introduced by Carpino[58], is the main base labile protecting group used in peptide synthesis. Its potential in this field was first realised by Sheppard[59].

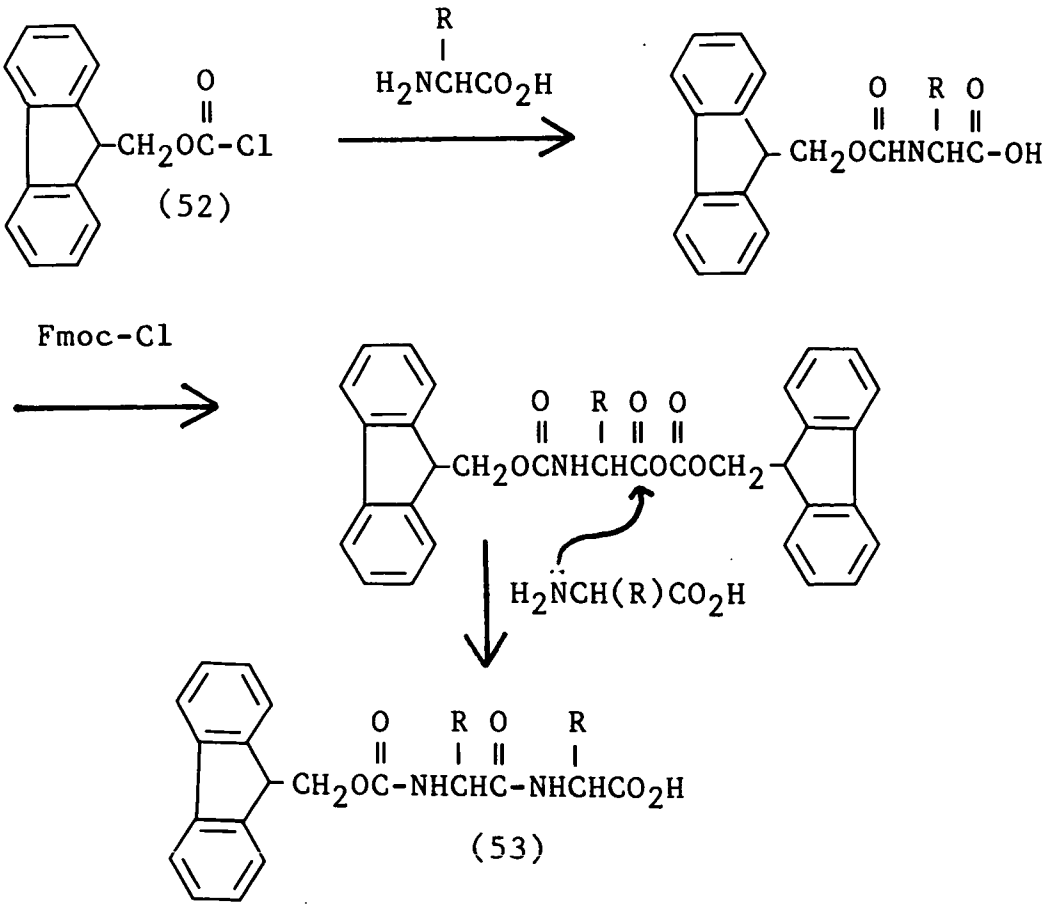


(51)

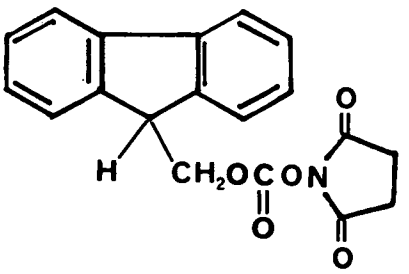
Base labile N^{α} - protection is often used in SPPS. When used in conjunction with acid labile side chain protection and peptide-resin linkage, a fully selective system is achieved allowing removal of the N^{α} - protection as the peptide is synthesised, followed by the removal of any side chain protection, and cleavage from the resin after the synthesis is complete.

Problems are often encountered when amino acids are directly acylated with Fmoc chloroformate (52)[60], due to the formation of up to 20% of an Fmoc-oligopeptide contaminant. The chloroformate reacts with the carboxyl function of the amino acid producing a mixed anhydride intermediate, which can then react with an amine group on another amino acid to give an Fmoc-dipeptide system (53) (Scheme 1.22).

This problem is overcome by using the less reactive active ester derivative, N-(9-fluorenylmethoxycarbonyloxy) succinimide (Fmoc-ONSu) (54)[61]. This is a stable, crystalline compound and is formed by the reaction of (52) with N-hydroxysuccinimide.



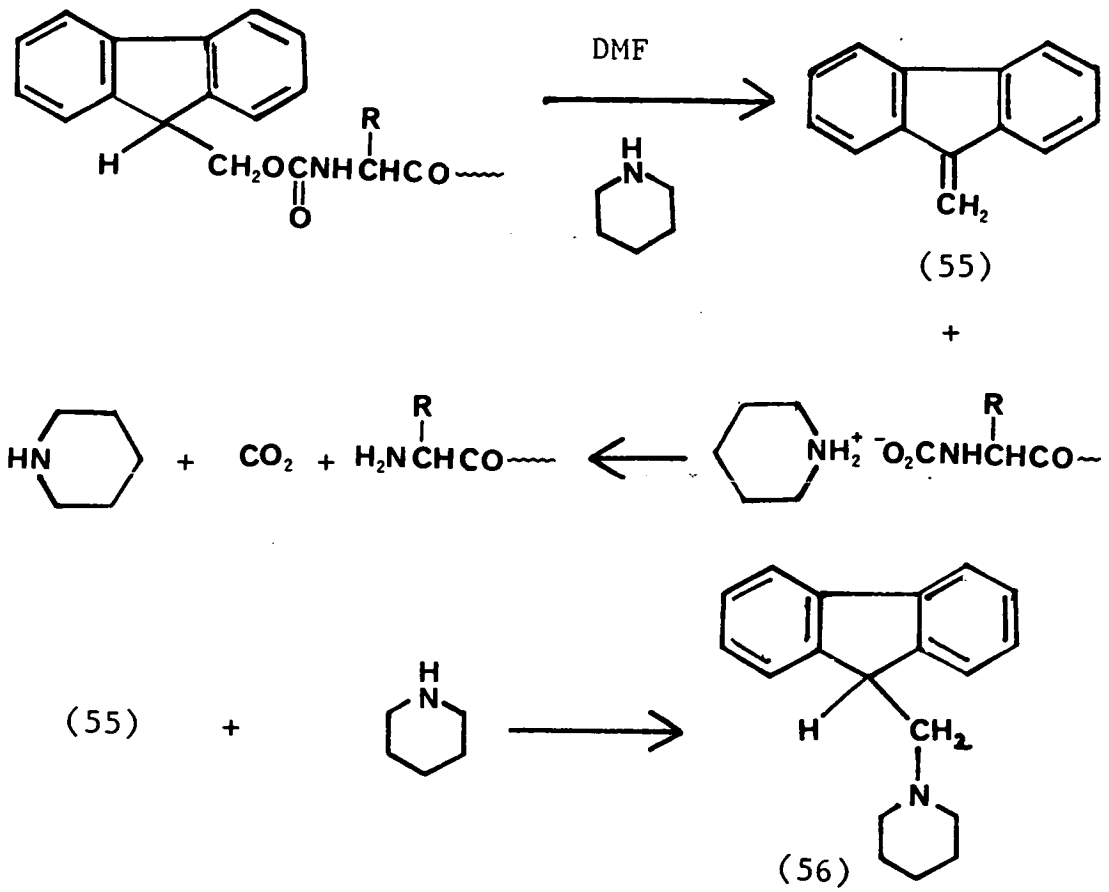
Scheme 1.22 Attempted protection of amino acids using Fmoc chloroformate (52).



(54)

The removal of Fmoc proceeds via a β-elimination mechanism and is normally brought about by the addition of 20% piperidine in DMF (Scheme 1.23). The by-products include dibenzofulvene (55) and the tertiary amine (56) formed by the addition of piperidine to the double bond.

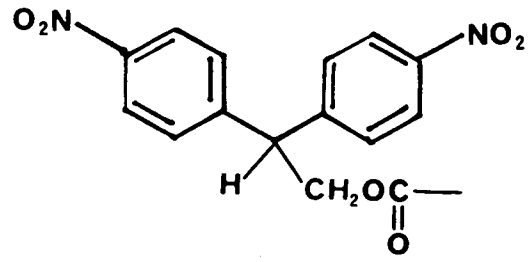
(55) and (56) are both soluble in DMF and therefore present no contamination problems. No intermediate carbonium ions are produced, so scavengers are not required. All by-products are soluble in DMF and can be removed once the deprotection is complete.



Scheme 1.23 Deprotection of Fmoc amino acids.

Bnpeoc protecting group

Fmoc is expensive; Fmoc amino acids are generally two to five times more expensive than their Boc counterparts. The 2,2-bis(4'nitrophenyl)ethoxycarbonyl (Bnpeoc) group (57) was introduced by Ramage and Florence[62], and Valentine[63] as a cheaper alternative to Fmoc.



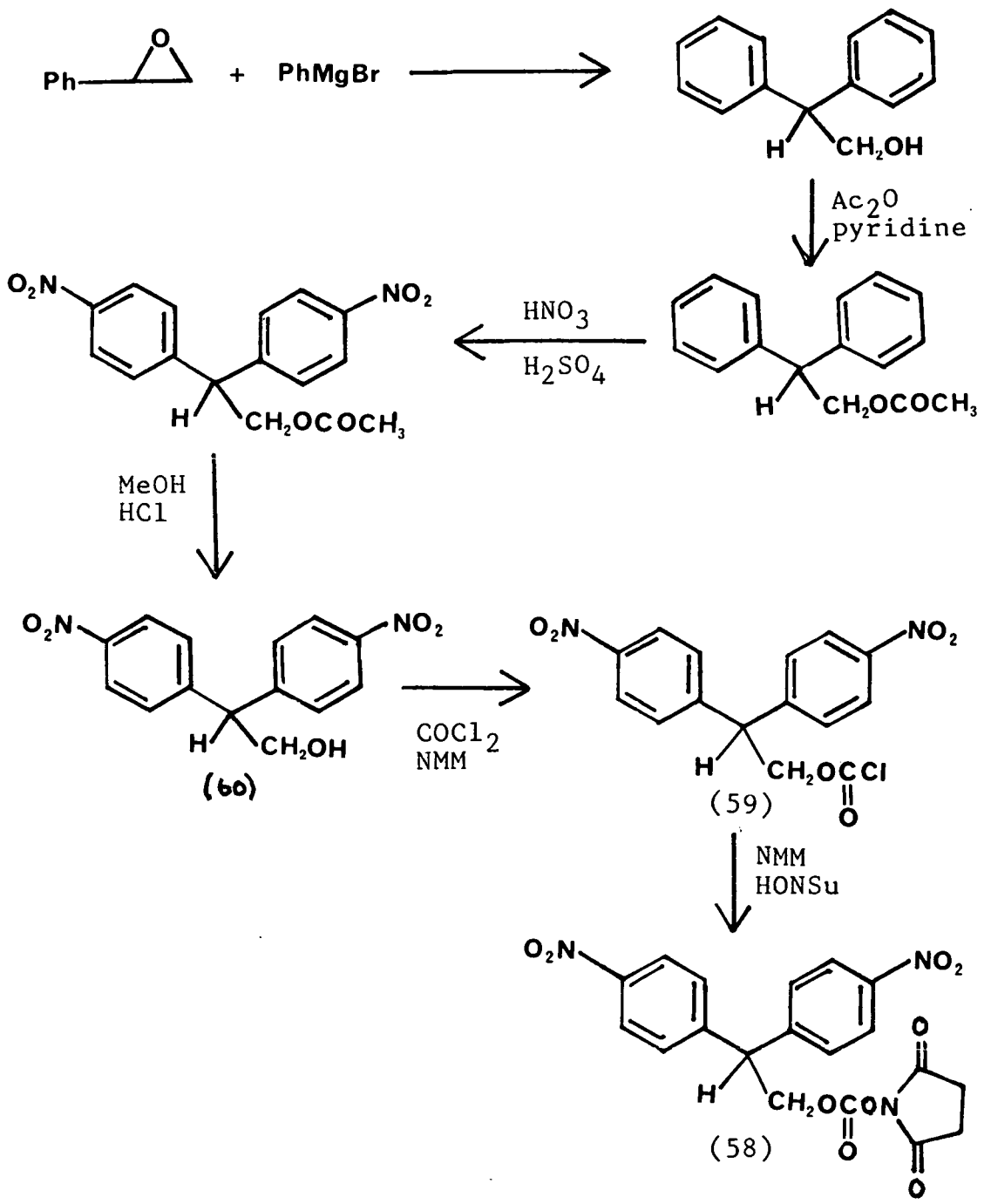
(57)

The group is introduced to amino acids using the active ester derivative (58), since the chloroformate (59) resulted in the same problems that were encountered with Fmoc chloroformate (52) (scheme 1.22).

The synthesis of Bnpeoc is based on the ring opening of styrene oxide using phenyl magnesium bromide. Karash and Clapp[64] observed that the nature of the product depended on the order of addition of the reactants. The expected secondary alcohol was obtained by adding the Grignard reagent to the oxide, but if this order was reversed then the primary alcohol would be obtained in 80% yield. Para nitration of the phenyl rings of the acetate gave 2,2-bis(4'nitrophenyl)ethanol (60), from which the chloroformate (59) and active ester derivative (58) could be synthesised in high yield (Scheme 1.24). Amino acids are then protected using either the method developed by Lapatsanis[65] or by Rich[66].

The deprotection of Bnpeoc proceeds by a similar mechanism to that of Fmoc (Scheme 1.23). Either 1,5-diazabicyclo[4.3.0]non-3-ene (DBN) or 1,8-diazabicyclo-

[5.4.0]undec-7-ene (DBU) can be used as the base for this reaction. The low nucleophilicity of these bases reduces the possibility of unwanted side reactions. The system is often buffered with acetic acid to avoid conditions of extreme pH.



Scheme 1.24 Synthesis of the Bnpeoc protecting group.

1.4.5 Side chain protection

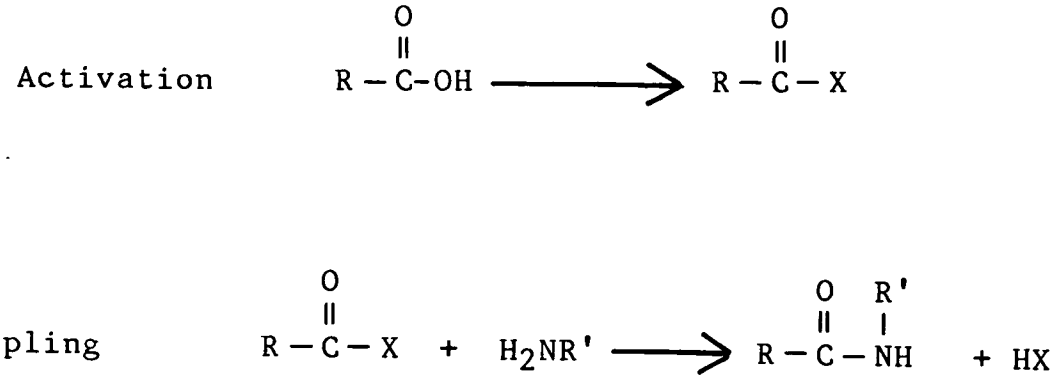
All of the reactive sites of amino acid side chains must be protected throughout the synthesis of a peptide, in order to prevent any unwanted chemical transformation of the side chain.

When acid labile N^{α}- protection is used, the side chain protection must be more stable than this, so that it is not affected during N^{α}- deprotection. Groups often used in this case are derived from the benzyl protecting group.

When the very acid labile N^{α} - protection, Bpoc (50), or base labile N^{α}- protection are used, the t.butyl protecting group is often employed to protect side chain functionality.

1.4.6 Coupling methods

The standard procedure for the formation of a peptide bond involves the activation of the carboxyl group of the incoming N^{α} -protected amino acid towards nucleophilic attack by the amine function. To achieve this activation it is necessary to replace the hydroxyl group with an electron withdrawing group (X) (Scheme 1.25).



Scheme 1.25 Coupling of amino acids.

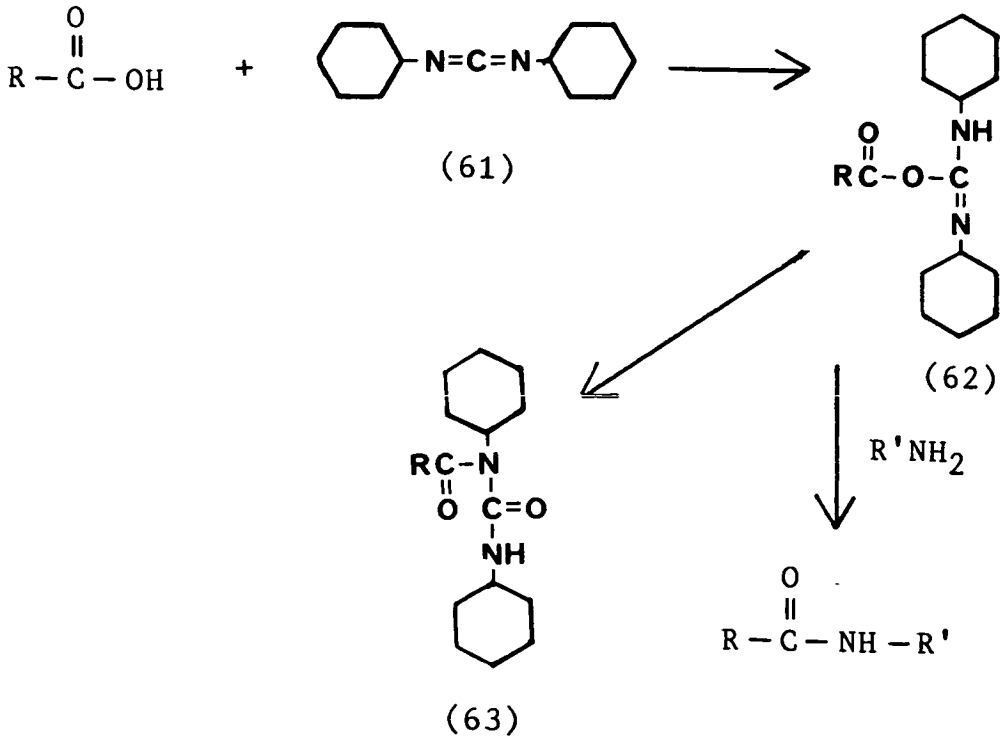
Some of the more common methods of peptide bond formation are outlined below :

The acid chloride method

This uses the chlorine atom as the electron withdrawing group (X). Protected amino acid chlorides have had limited use in peptide synthesis due to problems of racemisation (via oxazolidinone formation). Other problems encountered when using this method are the presence of unwanted side reactions, and the need to use relatively stable protecting groups in order for them to survive the conversion of a carboxylic acid to an acid chloride. Recently thionyl chloride in DCM has been used to form several Fmoc protected amino acid chlorides[67]. Less than 0.1% loss of chirality was reported[68].

The carbodiimide method

This is now the most widely used coupling method in peptide synthesis. It was first recognised by Sheehan and Hess[49] and was later used by Merrifield[43] when he first introduced SPPS. Merrifield used N,N'-dicyclohexyl carbodiimide (DCCI) (61) as the coupling reagent. (Scheme 1.26).



Scheme 1.26 Peptide bond formation using DCCI.

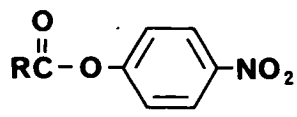
The mechanism of this reaction was first studied by Khorana[69] and more recently by De Tar et al[70]. The reaction has been shown to proceed rapidly due to the high reactivity of the O-acyl urea intermediate (62). This intermediate will however spontaneously rearrange to the unreactive N-acyl urea (63), which will result in the loss

of the N^{α}-protected amino acid from the coupling reaction. Such a rearrangement often occurs in polar aprotic solvents such as DMF. If the reaction necessitates the use of such solvents, then the minimum volume possible should be used.

Diisopropylcarbodiimide (DICI)[71] has emerged as an alternative to DCCI. Being a liquid DICI is easier to handle than DCCI, which is a waxy solid, and the corresponding urea by-product is more soluble.

The active ester method

The first synthetic peptide chemistry was carried out using amino acid methyl and ethyl esters as activating agents[72]. It was later discovered by Schwyzer et al[73] that increasing the electron withdrawing effect of the alcohol component of the ester increases the electrophilicity of the carbonyl carbon, thereby making it more susceptible to nucleophilic attack. Aryl esters, especially the p-nitro phenyl active ester (64) introduced by Bodansky[74], have shown considerable promise.

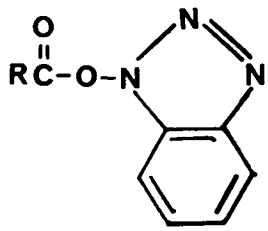


(64)

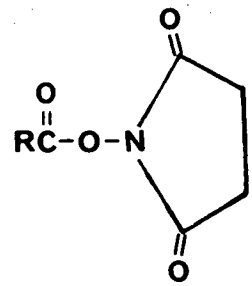
These are prepared by reacting the N^{α}-protected amino acid with either p-nitrophenyl sulphate or phosphite[75],or

by condensation with p-nitrophenol using DCCI[76].

The most successful and widely used active esters today are those of 1-hydroxybenzotriazole (HOBt) (65)[77] and N-hydroxy succinimide (HONSu) (66)[78].



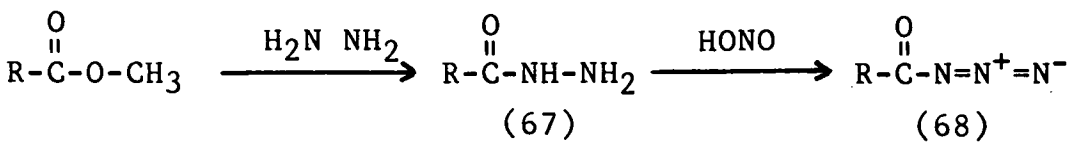
(65)



(66)

The azide method

This was first introduced in 1902 by Curtius[79]. His methodology involved the treatment of a methyl ester with hydrazine followed by the addition of nitrous acid to convert the resulting hydrazide (67) to the acid azide (68) (Scheme 1.27). An improved method is the direct conversion of carboxylic acids to acid azides using diphenylphosphonyl azide.

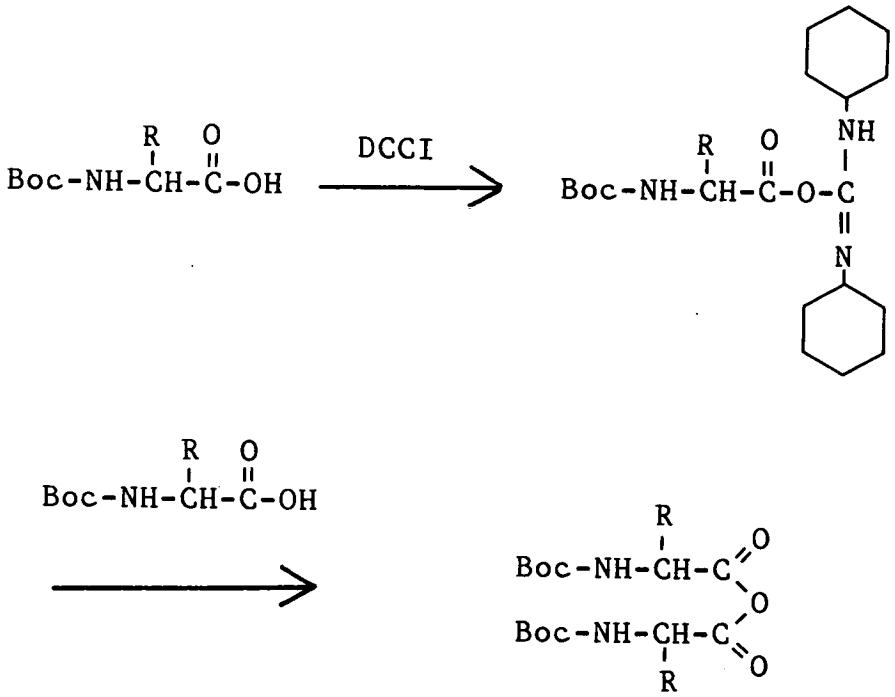


Scheme 1.27 Preparation of acid azides.

The main problem with this method is the tendency of acid azides to rearrange to isocyanates by the Curtius rearrangement. To keep this to a minimum, the reaction should be performed at low temperature using a minimum of solvent and the acid azide must be prepared immediately before it is required.

The preformed symmetrical anhydride method

Amino acid symmetrical anhydrides (69) can be prepared using carbodiimides such as DCCI (Scheme 1.28).



(69)

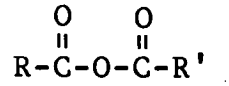
Scheme 1.28 Preparation of symmetrical anhydrides.

Boc amino acid symmetrical anhydrides are unstable and must be prepared immediately before use. This can lead to problems with automated SPPS where often the reagents are

prepared, loaded into the machine, and depending on the position in the sequence, may have to wait some time before becoming involved in the synthesis. Fmoc amino acid symmetrical anhydrides, on the other hand, are stable.

The mixed anhydride method

Mixed or unsymmetrical anhydrides (70) have been of interest to the peptide chemist for many years.



(70)

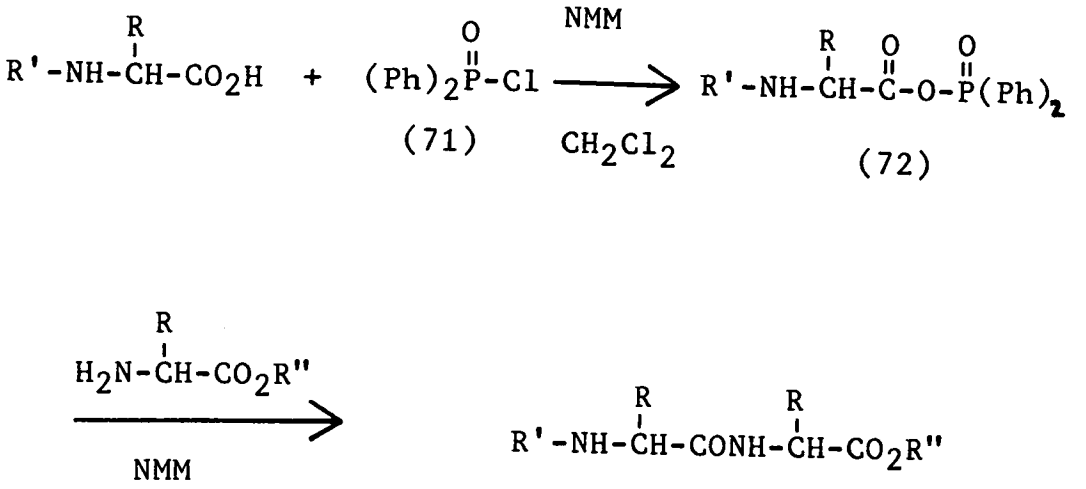
Ideally the R' group should be electron donating, good examples being the mixed anhydrides formed from isovaleric[80] and trimethylacetic acid chlorides[81]. As well as the electron donating effect, the steric bulk of the R' group promotes the reaction at the carbonyl derived from the carboxyl.

The problem with mixed anhydrides is their tendency to convert to symmetrical anhydrides[82] and the possibility of racemisation. Employing low temperatures during preparation of the anhydride will reduce the chance of the former and short coupling times will help to prevent the latter.

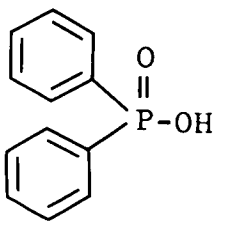
Mixed anhydrides of organophosphorus acids have recently received a lot of attention. The nature of the R' group must be such that there is minimal disproportionation to the symmetrical anhydride and also such that the reaction takes place at the desired carbonyl. Some mixed anhydrides of organophosphorus acids have been found to satisfy these requirements.

Of particular interest to the present research (see chapter 2) are phosphinic-carboxylic mixed anhydrides derived from a phosphinic acid. A method of synthesising these compounds involves the use of diphenylphosphinyl chloride (Dpp-Cl) (71)[83], a readily available reagent that was introduced as a means of preparing diphenylphosphinic -carboxylic mixed anhydrides (72) by Ramage et al[84,85] (Scheme 1.29). Dpp anhydrides have been compared to pivalic anhydrides in order to determine their relative reactivity[85]. The results of these experiments indicated that greater activation had occurred in the Dpp example.

Dpp mixed anhydrides demonstrate a number of advantages over their carboxylic counterparts, though problems are occasionally encountered due to the insolubility of the diphenylphosphinic acid byproduct (73). Dpp-Cl is also rather difficult to handle since it is a viscous liquid that will readily decompose to (73) in the presence of moisture.



Scheme 1.29 Use of Dpp-Cl in peptide synthesis.



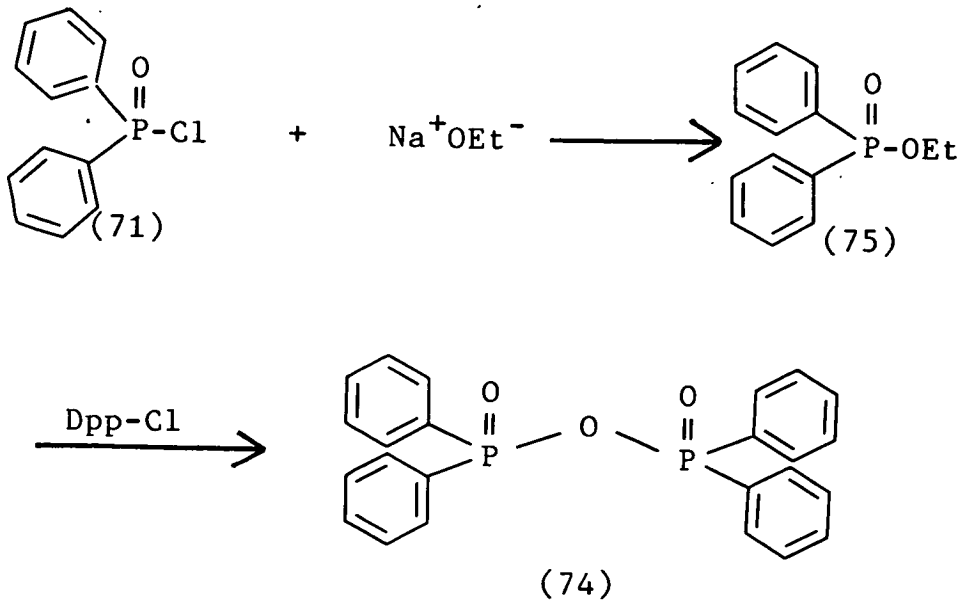
(73)

In an attempt to overcome the handling problems often encountered with DPP-Cl, diphenylphosphinic anhydride (DppODpp) (74) has recently been prepared by workers in this research group[86]. This compound is a white crystalline solid which is stable under a dry atmosphere. The synthesis of (74) was carried out using the method of Crofts et al[87], this involves the reaction of Dpp-Cl with

ethyl diphenylphosphinate (75) in the absence of solvent and in an inert environment at 200°C (Scheme 1.30). (75) was prepared by the method of Kosalapoff and Watson[88], which involves the addition of sodium ethoxide to a cooled solution of Dpp-Cl in benzene. The reaction can be conveniently monitored using ^{31}P n.m.r., the chemical shift of the phosphorus atom is different in the chloride (71), anhydride (74) and acid (73).

Diphenylphosphinic anhydride was shown to be effective as a coupling reagent in both solution and solid phase peptide synthesis. The pentapeptide, leucylisoleucyl-phenylalanylalanylglycine (H-Leu-Ile-Phe-Ala-Gly-OH) was prepared using both DppODpp and DICI/HOBt activation, with Fmoc amino acids and p-alkoxybenzylalcohol resin, in order to compare the performance of the two coupling procedures[85]. Yields of the product in both cases were found to be comparable, however with DppODpp the coupling times were found to be significantly shorter and only four equivalents of the Fmoc amino acid was required per residue compared to six with DICI/HOBt.

The byproduct of diphenylphosphinic anhydride mediated coupling reactions is also the acid derivative (73). This can be conveniently converted back to Dpp-Cl (71) by reaction with thionyl chloride, allowing the regeneration of the starting material.



Scheme 1.30 Synthesis of diphenylphosphinic anhydride.

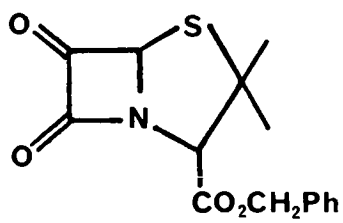
CHAPTER 2

DISCUSSION

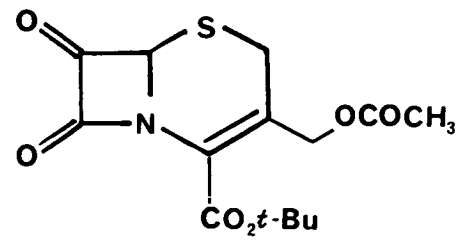
2.1 Chemistry involving 6-oxopenicillin and 7-oxocephalosporin compounds

2.1.1 Introduction

As was discussed in the previous chapter, the carbonyl group at the 6(7)- position of compounds such as benzyl 6-oxopenicillanate (24) and t.butyl 7-oxocephalosporanate (76) is highly reactive towards a number of addition reactions, making these compounds very useful precursors for the synthesis of novel β-lactam compounds.



(24)



(76)

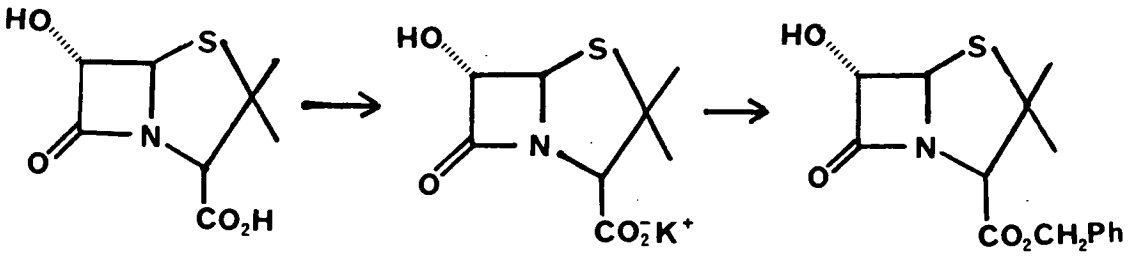
The present research has studied some of the literature methods for the synthesis of compounds of this type, and then attempted some chemical transformations, with the aim of synthesising novel, potentially biologically active compounds.

2.1.2 The synthesis of benzyl 6-oxopenicillanate (24)

This research initially undertook the task of synthesising benzyl 6-oxopenicillanate (24), in order to clarify some of the conflicting reports that appear in the literature (see chapter 1).

Infrared spectroscopy was found to be a particularly useful technique for monitoring the synthesis of this compound, and the related cephalosporin (76). The target compound has three carbonyl groups, each with a distinct absorption in the carbonyl region of the infrared spectrum. The ester carbonyl group has an absorption at approximately $1740cm^{-1}$, the β-lactam carbonyl has the usual absorption at approximately $1790cm^{-1}$ and the 6-oxo carbonyl group has a higher absorption at $1830cm^{-1}$. The presence of a peak at $1830cm^{-1}$ in the infrared spectrum is therefore a good indication that the target compound has been synthesised.

The first reaction studied was the oxidation of benzyl 6α-hydroxypenicillanate (25) which was synthesised by the method of Hauser and Sigg[26], except that a slight variation was adopted for the formation of the benzyl ester at the end of the synthesis. 6α-Hydroxypenicillanic acid (26) was converted to the potassium salt, by neutralising with potassium carbonate, this was followed by treatment with benzylbromide to give the target compound (25) in quantitative yield (scheme 2.1). The compound was obtained as an oil, which was crystallised using DCM and petroleum-ether to give the pure (25) as a white solid. Tlc indicated that no further purification was required.



(26) (25)

Scheme 2.1

The first oxidation method attempted was that of Vanderhaege et al[33], involving the use of DMSO and acetic anhydride. Chandrasekaran et al[34] had reported some difficulty in repeating this reaction, and similar problems were encountered during the current research. The reaction was only successful when acetic anhydride that had been freshly distilled from $AlCl_3$ was used, as was suggested by Chandrasekaran. Even then, the benzyl 6-oxopenicillanate was obtained as a yellow oil in 31% yield and all attempts to crystallise the product were unsuccessful. A much poorer result than that reported by Vanderhaege, who obtained a crys-talline solid in 92% yield[33].

Two further oxidation methods, involving the use of pyridinium chlorochromate (PCC), and freshly prepared manganese dioxide, were then attempted. There appeared to be no literature reports detailing the use of these reagents for this reaction. Both methods proved unsuccessful.

Attention was then directed towards the Moffat oxidation[25] which was the method used by Sheehan and Lo in the first synthesis of compounds of this type[24]. All attempts to repeat their work, using DICI, were unsuccessful. The reaction was repeated using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (77), a water soluble carbodiimide, that had previously been used in the Moffat oxidation by workers in this research group with some success[89]. This reagent also proved to be incapable of carrying out the required oxidation.

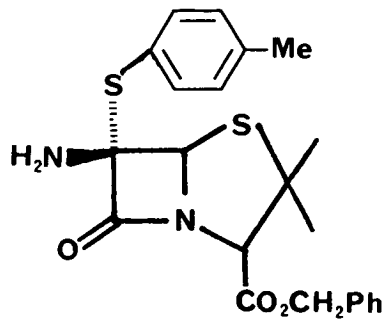
$Me_2N(CH_2)_3N=C=NEt.HCl$

(77)

Considerable difficulty was also experienced when attempting to repeat the work of Hagiwara et al[38] (Scheme 1.14), involving the use of trifluoromethanesulphonic anhydride. After the initial attempt at the reaction, the crude product consisted mainly of starting material. Further triethylamine was added, as it was thought that the problem was due to the failure to form the bistriflate intermediate. However after workup, very little of the target compound was recovered.

The final method attempted was that of T.Jen et al[32] who synthesised benzyl 6-oxopenicillanate (24) from benzyl 6α-methylthio-6β-aminopenicillanate (31) using mercuric

chloride in DMF and water. Indeed (31) was used by workers at Beecham Pharmaceuticals as a precursor to 6α - subtituted penicillins[90], and the named company were able to provide a quantity of this compound for the purpose of this research. As well as (31), a similar compound, benzyl 6α-tolylthio 6β-aminopenicillanate (78) was also provided by the same company. Both (31) and (78) had been shown to behave in a similar manner towards substitution reactions at the 6α-position.



(78)

Benzyl 6-oxopenicillanate was synthesised in high yield from both (31) and (78). The product was obtained as a yellow oil in both cases and all attempts to convert it to a solid failed. Purification was then attempted using silica gel chromatography, however when the collected fractions were monitored by silica gel tlc, impurities were still apparent. This coupled with the fact that the silica on the column turned red indicated that the compound was unstable to these purification conditions. Satisfactory nmr data were obtained from the unpurified product, which was used in future reactions without further purification.

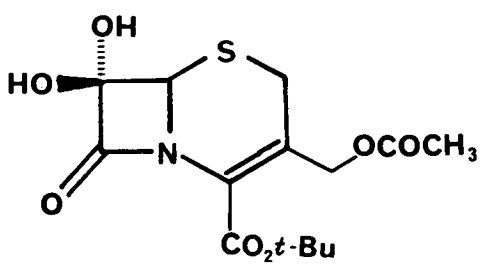
This work has demonstrated the high sensitivity of benzyl 6-oxopenicillanate which leads to considerable synthetic and purification problems. As far as the present research is concerned, the most convenient method of synthesising this compound was by the method of Jen et al[31]. The reason for this being the ready availability of the precursors (31) and (78), indeed under normal circumstances the lengthy synthesis of these precursors would significantly decrease the viability of this route. This was therefore adopted as the standard method for the synthesis of (24) for the remainder of this research.

2.1.3 The synthesis of t.butyl 7-oxocephalosporanate (76)

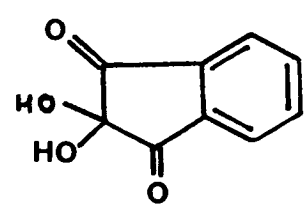
In parallel with the work involving penicillins, it was decided to study the analogous cephalosporin analogues.

Initially the method of Hagiwara et al[38] was repeated in order to synthesise (76). This involves the use of trifluoromethanesulphonic anhydride and triethylamine, and is identical to the reaction previously attempted when synthesising the penicillin derivative (24) (Scheme 1.14). Infrared spectroscopy indicated that the required compound had been synthesised and this was purified by silica gel wet flash chromatography. Some product was recovered from the column in the form of a yellow oil, however the percentage recovery from the column was low. The silica

changed to a red colour during the course of the purification, indicating that some decomposition of the product was occurring. The recovered material was crystallised using ethyl acetate and hexane which resulted in a yield of only 15% of a yellow solid. Nmr and infrared analysis of the product indicated that (76) had converted to the dihydroxy analogue (79). There were two singlets in the n.m.r spectrum at δ-6.7 and 6.9 ppm, that disappeared when D_2O was added to the n.m.r sample. It appeared that when solid, the hydrated form (79) was more stable. This is likely to be due to the electronic effects resulting from two adjacent carbonyl groups. Hydrating one of the carbonyl functions avoids two adjacent partially positively charged carbon atoms. A similar situation is found with ninhydrin (80), which has a hydrated carbonyl adjacent to two carbonyl groups. Previous literature reports of 7-oxocephalosporins have not reported the dihydroxy compound (79). We found no evidence of an analogous compound in the case of the penicillin (24).

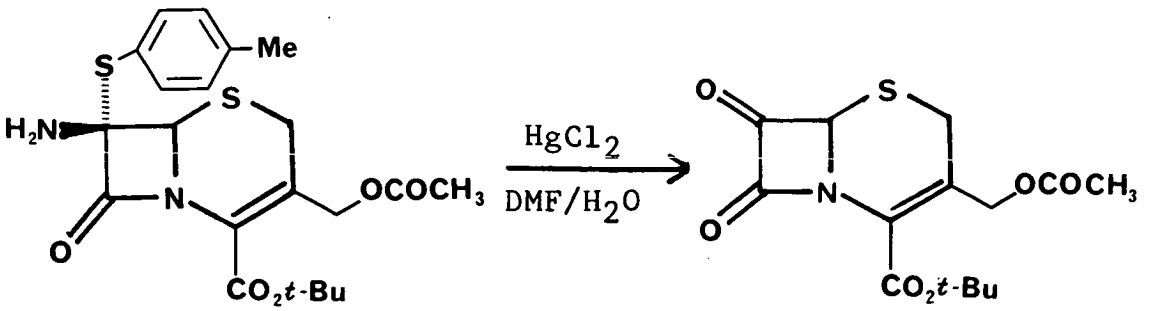


(79)



(80)

Because of previous success when the method of Jen et al[32] was used to synthesise benzyl 6-oxopenicillanate, this method was repeated with the aim of synthesising t.butyl 7-oxocephalosporanate (76) (Scheme 2.3). This reaction was not mentioned in the above report. t.Butyl 7α-tolylthio 7β-aminocephalosporanate (81), supplied by Beecham Pharmaceuticals, was reacted with mercuric chloride in the presence of water and DMF and the target compound was obtained as a yellow oil in 85% yield. This was converted to a solid using ethyl acetate and hexane, and once again hydrated compound (79) was recovered.



(81)

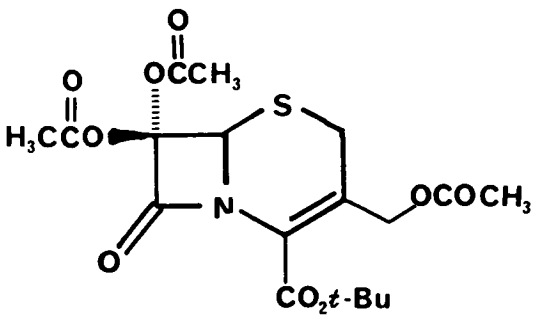
Scheme 2.3

t.Butyl 7α,7β-dihydroxycephalosporanate (79) could be converted back to t.butyl 7-oxocephalosporanate (76) by stirring with activated molecular sieves in DCM. This conversion was monitored every 5 minutes using 60 MHz ^1H nmr spectroscopy, which indicated the disappearance of the signals due to the hydrated compound (79), combined with the appearance of signals due to (76). The main differences in the n.m.r spectra of these two compounds are the absence of the hydroxy proton signals in the

spectrum of (76) and a difference in the chemical shift of the signal due to the proton attached to C-6.

The dihydroxy compound (79) was then reacted with 1 equivalent of acetyl chloride in an attempt to acetylate one of the hydroxy groups. It was thought that acetylation would occur preferably at the 7α-hydroxy group, since reaction at this site would involve attack from the least hindered side of the molecule. Only starting material was recovered from this reaction. It was then decided to attempt the reaction using excess acetyl chloride in an attempt to acetylate both hydroxyl groups. (79) was reacted with 3 equivalents each of acetyl chloride and pyridine using DCM as the solvent. The reaction mixture was stirred for 15 hours, after which tlc showed that two major components were present, neither of which had a retention time corresponding to that of the starting material. Furthermore there was a weak absorption at 1833cm^{-1} in the infrared spectrum of the crude product indicating that there was some t.butyl 7-oxocephalosporanate present. The mixture was separated using silica gel wet flash chromatography, during which the silica turned red. two compounds were isolated. The most polar of these was not identified, however the ^{1}H n.m.r spectrum was consistent with the structure being a cephalosporin nucleus with an additional CH_2 group, this being represented by an extra AB quartet between δ3.88 and 4.05 ppm. ^{1}H N.m.r spectroscopy indicated that the other product was the target compound, t.butyl 7α,7β-diacetoxycephalosporanate (83). The failure to recover the t.butyl 7-oxocephalosporanate

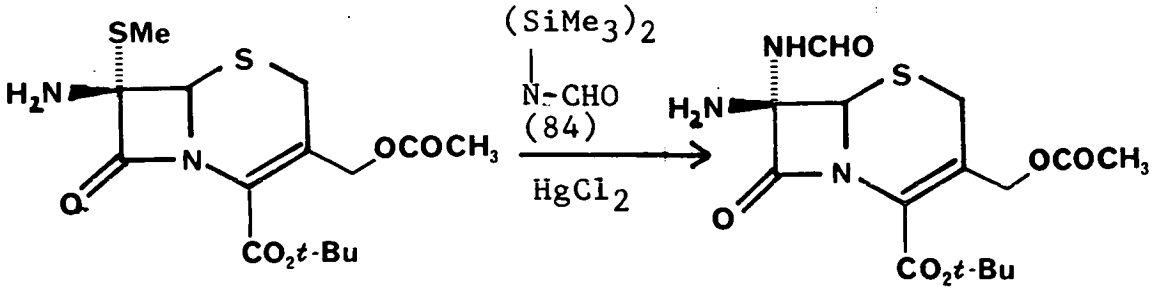
that appeared to be present in the crude mixture was probably due to the instability of this compound on silica, hence the red colouration of the column.



(83)

2.1.4 Reactions with amides

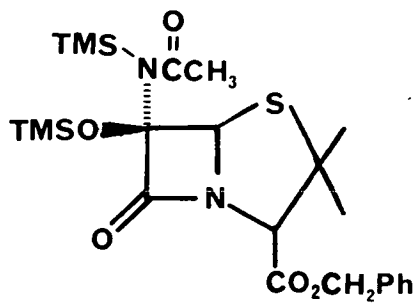
Whilst working on the synthesis of 6α (7α)-formamido penicillins and cephalosporins workers at Beecham[91] had discovered that formamide was insufficiently nucleophilic to yield the target compounds by a mercury (II) mediated substitution with 6α (7α)-methylthio substituted compounds e.g. (31). However the addition of trimethylsilyl (TMS) groups to the nitrogen atom of the amide increased its nucleophilicity, and 6α (7α)-formamido penicillins and cephalosporins were formed when N,N-bis(trimethylsilyl)-formamide (84) was used[92] (scheme 2.4).



Scheme 2.4

It was decided to investigate the reactions of similar amides with 6-oxopenicillins and 7-oxocephalosporins.

N-Trimethylsilylacetamide failed to react with benzyl 6-oxopenicillanate (24). The reaction was monitored by infrared spectroscopy, and after 4 hours there was still an absorption at 1830cm^{-1} in the infrared spectrum of the reaction mixture, indicating that starting material remained. The reaction was left for a further 16 hours. Whereas there was an amide carbonyl absorption present in the infrared spectrum of the final reaction mixture, this was absent in the spectrum of the product after workup. This indicated that the amide in the reaction mixture was unreacted, and that no reaction had taken place. The reaction was repeated using bistrimethylsilylacetamide. N.m.r, infrared, and mass spectrometry of the product indicated that the reaction had worked in this case. The product, benzyl 6α-(N-trimethylsilylacetamido)6β-trimethyl silyloxypenicillanate (85), was recovered as an oil in 88% yield.

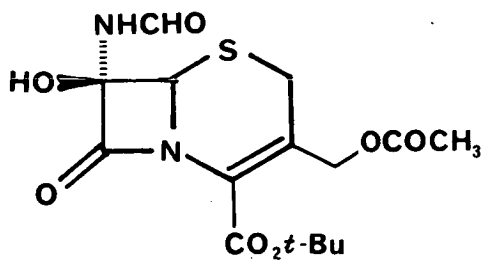


(85)

The two methods that were attempted with the aim of removing the TMS groups from (85) involved the use of tetrabutyl ammonium fluoride (TBAF) (1 eq.) and methanol (1 eq.), and water. Cleavage of the β-lactam ring resulted in both cases

No reaction was observed between t-butyl 7-oxocephalosporanate (76) and N-methyl(N-trimethylsilyl)-acetamide. This result confirms that the extra nucleophilicity of the bis-trimethylsilyl compounds is required for amides to react with 6-oxopenicillins and 7-oxocephalosporins.

The final reaction attempted was between bistrimethylsilylformamide (84) and t-butyl 7-oxocephalosporanate (76) from which two products were isolated. The major product was identified as t-butyl 7α,7β-bistrimethylsilyloxycephalosporanate, formed by the addition of trimethylsilyl groups to the hydroxy groups of the hydrated compound (79). The minor product from this reaction was the more interesting t.butyl 7α-formamido 7β-hydroxycephalosporanate (86), which was obtained after aqueous workup and purification by wet flash silica gel chromatography in 8% yield.

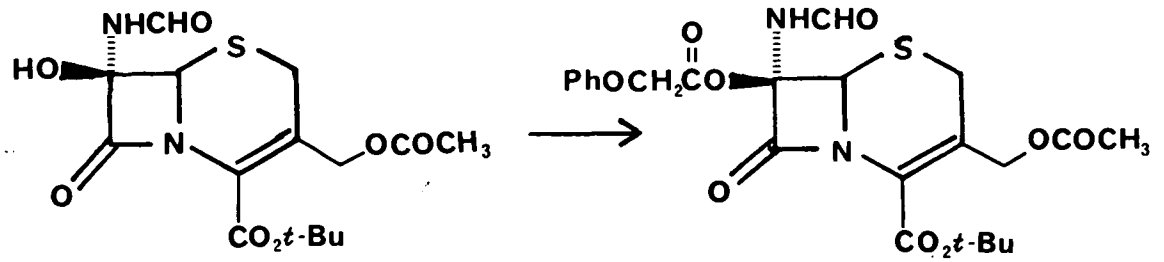


(86)

The nmr spectrum of this compound indicated that two rotameric forms were present, due to restricted rotation about the C-N bond of the formamido group. Similar observations were made by researchers at Beecham[92], who studied similar systems.

Due to the very small amounts of (86) that had been synthesised, the reaction was repeated on several occasions, in an attempt to obtain sufficient material to investigate the chemistry and biological activity of the compound. However (86) was not isolated from any of the repeated experiments, indicating the high degree of sensitivity of such systems.

Attempts were then made to react the hydroxy group of (86) with phenoxyacetyl chloride with a view of synthesising (87) (Scheme 2.7). Infrared spectroscopy indicated that the major product of this reaction was t.butyl 7-oxocephalosporante (76).

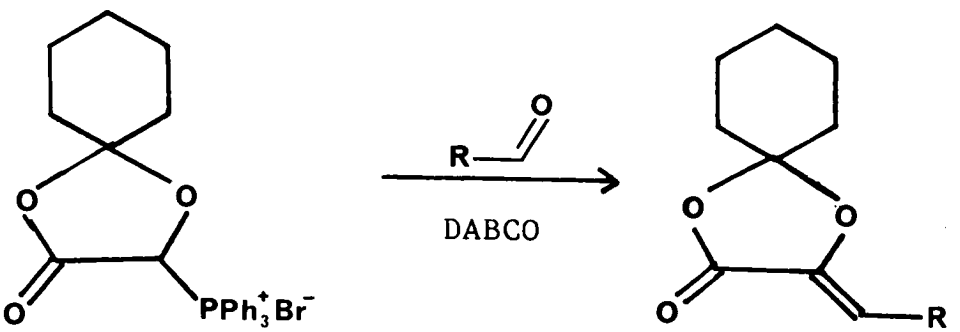


(87)

Scheme 2.7

2.1.5 Wittig reactions

Wittig reactions of benzyl 6-oxopenicillanate have previously been reported by Sheehan and Lo[28]. It was decided to investigate the reaction of both benzyl 6-oxopenicillanate and t-butyl 7-oxocephalosporanate with cyclohexanespiro-2'-(1'3'-dioxolan)-4'-yltriphenyl phosphonium bromide (88). This reagent was developed by workers in this research group as a precursor to α-ketoacid functionality, and it has been used successfully in the synthesis of compounds containing this functionality[93,94]. Reaction of (88) with an aldehyde or an activated ketone will produce an ylidene dioxolanone (89). The carbonyl group of (89) is susceptible to attack by the hydroxyl anion under mildly basic conditions, which will give the salt of a 3-deoxy-α-keto acid (90) (Scheme 2.8).

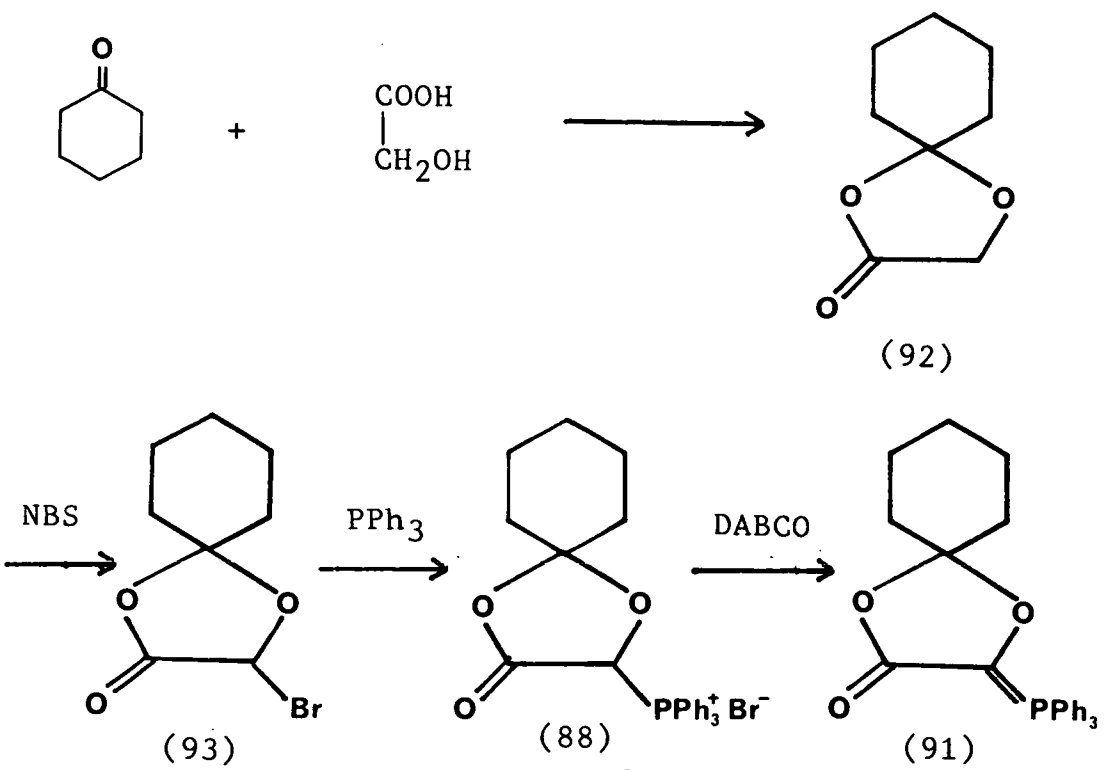


(88) (89)

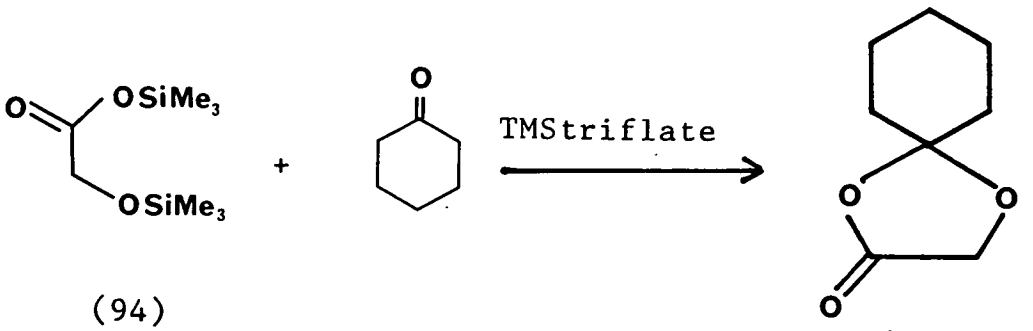
(90)

Scheme 2.8

The phosphorane (91) has been synthesised in two ways. The first method involved the condensation of glycolic acid with cyclohexanone using p-toluenesulphonic acid as a catalyst to give the dioxolanone derivative (92). This was converted to the bromide (93) using N-bromosuccinimide. Reaction of the bromide with triphenylphosphine produced the stable phosphonium bromide (88) (Scheme 2.9). Variable yields, often as low as 40%, have been reported for the synthesis of the dioxolanone using this methodology[89]. An alternative synthesis of (92), introduced by Pearson and Cheng[95], and later used by researchers in this group[89,94], results in consistent yields of approximately 95%. This involves the treatment of cyclohexanone with trimethylsilyl(trimethylsilyloxy)-acetate (94) using trimethylsilyl triflate as a catalyst (Scheme 2.10).



Scheme 2.9

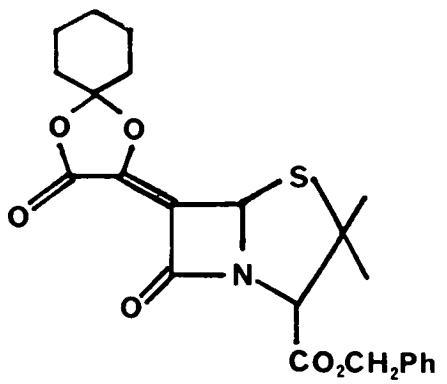


(94) (92)

Scheme 2.10

The phosphorane (91) is unstable, hence the compound must be stored in the form of the phosphonium bromide (88), which can be converted to the phosphorane in situ, using one equivalent of 1,4-diazabicyclo[2,2,2]octane.

The expected dioxolanone penicillin analogue (95) was formed when benzyl 6-oxopenicillanate and the phosphorane (91) were refluxed in toluene for 1 hour.

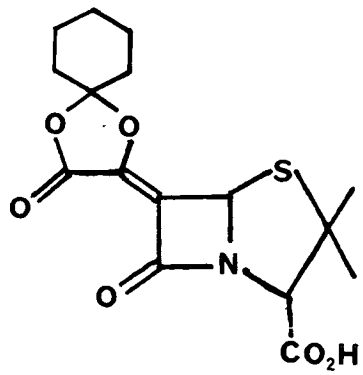


(95)

On completion of the reaction the solid residue of triphenylphosphonium oxide was removed by filtration, and the product was purified by wet flash silica gel chromatography. The target compound was obtained in a

yield in excess of 80%. The compound was analysed by ^1H nmr spectroscopy, a characteristic feature of the spectrum being a broad signal at δ-1.5 ppm due to the 10 protons on the cyclohexane ring.

It was required to remove the benzyl ester group from (95) in order that the biological activity be tested. This was accomplished using the standard conditions of hydrogenation over a palladium/carbon catalyst with tetrahydrofuran as the solvent. After the initial attempt, tlc showed that starting material remained thus fresh catalyst was added and the hydrogenation was repeated. The acid (96), which was obtained in quantitative yield, was converted to the sodium salt by stirring in one equivalent of sodium ethylhexanoate. The antibacterial activity and β-lactamase inhibitory properties of the sodium salt were tested at Beecham Pharmaceuticals research laboratories, and the compound was found to be inactive on both accounts.



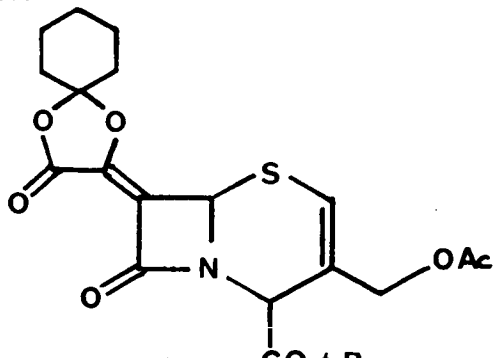
(96)

When a similar Wittig reaction was attempted on t.butyl 7-oxocephalosporanate (76), two t.butyl signals were present in the ^1H n.m.r spectrum of the product. This was further analysed by hplc, which also showed two components with similar retention times. Wet flash silica gel chromatography was used to separate these components. The ^1H n.m.r spectra of the separated components were identical, however two t.butyl signals were present in each spectrum. It was assumed that the two products that had been identified by hplc were the geometrical (E and Z) isomers at C-7, and that each one was a mixture of Δ-2 (97) and Δ-3 (98) isomers of the cephalosporin nucleus. As well as the t.butyl signal, there were other features in the n.m.r spectrum that indicated the presence of a mixture of Δ-2 and Δ-3 compounds :

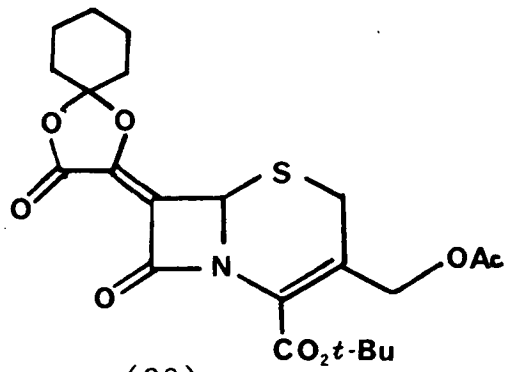
1. The presence of three AB quartets. The AB quartet at δ-3.26 and 3.55 ppm, due to the C-2 protons, had a smaller integral than would be expected if the signal represented 2 protons. The other two AB quartets, which overlap each other between δ-4.50 and 5.05 ppm, are due to slight differences in the chemical shift of the CH_2 protons adjacent to the acetoxy group in the Δ-2 and Δ-3 isomers.
2. The presence of additional signals, at δ-6.40 and 5.75 ppm, due to the single protons attached to C-2 and C-4 in the Δ-2 isomer.

It therefore appeared that this reaction had generated four products. Two geometrical isomers which, having

slightly different retention times, could be separated using chromatographic techniques. As would be expected, ^1H n.m.r spectroscopy could not be used to distinguish between these. Each geometrical isomer was present in both Δ-2 and Δ-3 forms, which would result in the four products. The Δ-2 and Δ-3 isomers had identical retention times, making their separation an impossible task, however they could both be clearly identified in the ^1H n.m.r spectrum.



(97)

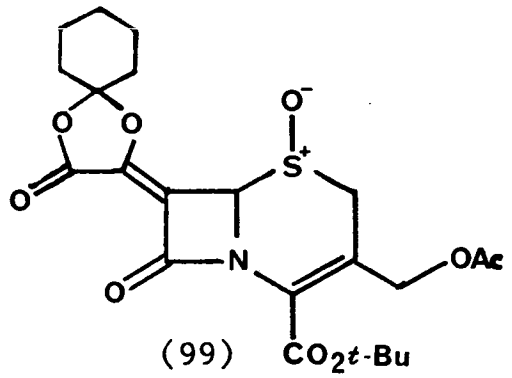


(98)

Since the formation of Δ-2 cephalosporins is generally brought about by the addition of base, it was thought that the most likely cause of the transformation in this instance was the DABCO, that was used to convert the phosphonium bromide (88) to the phosphorane (91). However, Δ-2 isomers were still formed when excess phosphonium salt (88) was used to ensure that all the DABCO was consumed prior to the addition of the cephalosporin (76).

In order to convert the product totally to the required Δ-3 isomer, the reaction product was converted to the sulphoxide (99). It is a known fact that cephalosporin

sulphoxides do not exist in the Δ-2 form, though the reason for this is not fully understood. The Δ-2/Δ-3 mixture was reacted with one equivalent of m.chloroperoxybenzoic acid in dichloromethane for 1hour. After this period the absorption of the β-lactam carbonyl in the infrared spectrum had shifted from 1770cm^{-1} to 1790cm^{-1}, indicating that the conversion had occured. This was confirmed by mass spectrometry, n.m.r, and hplc. The latter technique showed the product to be one component that was more polar than the sulphide (98). The sulphoxide (99) was reduced back to the sulphide using potassium iodide and acetyl chloride in acetone. Analysis of the product indicated the pure Δ-3 compound (98).

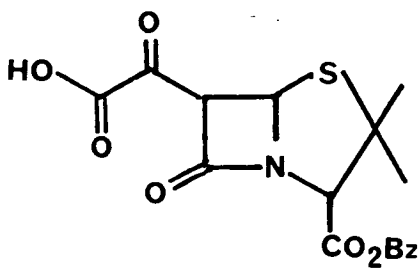


(99)

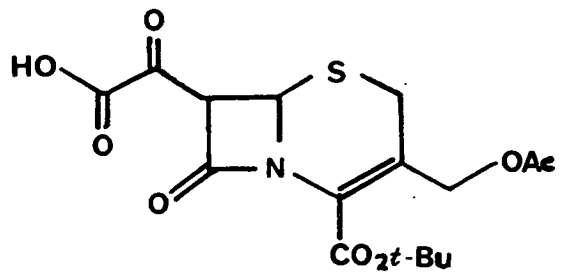
Removal of the t.butyl group of (98) was attempted using a solution of DCM/TFA (3:1). However significant impurities were observed in the product, and all purification attempts were unsuccessful.

Attempts were then made to convert the dioxolanone functionality of (94) and (98) to an α-ketoacid, in order to synthesise compounds (101) and (102). The standard

methodology for this conversion involves the use of half an equivalent of barium hydroxide, which would be expected to yield the barium salts of (101) and (102).



(101)



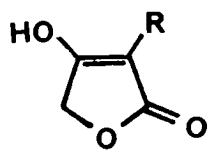
(102)

These reactions can be conveniently monitored by ultraviolet spectroscopy. Removal of the dioxolanone functionality results in a significant change in the chromophore of the molecule.

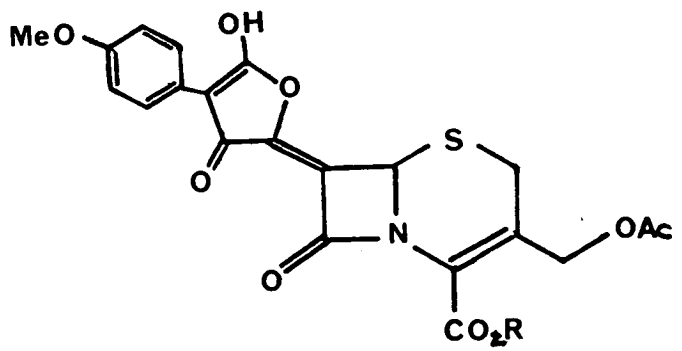
All attempts to carry out this conversion on the penicillin (94) were unsuccessful. During the reaction the maximum absorption in the ultraviolet spectrum was observed to have changed from 269nm to 293nm after one hour. After workup and lyophilisation a green coloured solid remained. The infrared spectrum of this only had a very small β-lactam carbonyl absorption at 1780cm^{-1}, indicating nearly total loss of this functionality. A similar result occured with the cephalosporin (98).

The dioxolanone derivatives have also been used as precursors to tetronic acids[96] (103). Applying similar methodology to the penicillin and cephalosporin

dioxolanone compounds (94) and (98) could yield compounds such as (104), having a tetronic acid nucleus attached directly to a β-lactam nucleus. Molecules of this type would be interesting synthetic targets since they consist of two independently biologically active nuclei joined together.



(103)



(104)

The reaction was attempted on the cephalosporin sulphoxide (99), in order to prevent any Δ-2 formation. The cephalosporin sulphoxide (99) was reacted with methyl 4-methoxyphenylacetate (105) in the presence of lithium diisopropylamide and TMEDA at -78° C for 2 hours. During the course of the reaction the reaction mixture turned dark red. The workup resulted in aqueous and organic fractions, both of which were found to have no β-lactam functionality. The β-lactam nucleus was clearly not stable to these reaction conditions.

$pCH_3OC_6H_4CH_2CO_2CH_3$

(105)

2.1.6 Further work

In hindsight, less problems may have been encountered had the work detailed in the previous section been carried out on monocyclic β-lactam compounds instead of the penicillin and cephalosporin nuclei. This would eliminate two of the major problems experienced in the current research; i.e formation of the Δ-2 cephalosporin, and removal of the ester groups. The resulting systems would be simpler, allowing the chemistry of the dioxolanone system to be investigated in detail without interference from the other functionality of the penicillin and cephalosporin nuclei. Time did not permit reactions of this type to be attempted during the course of this research.

2.2 The attachment of penicillins and cephalosporins to polymer supports

2.2.1 Introduction

The idea of synthesising peptides on insoluble polymer supports was first introduced by R. B. Merrifield in 1963[43]. Since then solid phase methodology has revolutionised the chemical synthesis of both peptides and oligonucleotides, to the extent that there are now automated systems capable of synthesising these compounds in a fraction of the time required by conventional techniques. The application of solid phase synthesis towards the preparation of peptides has been discussed in the previous chapter.

The present work investigates the potential for the use of solid phase methodology in the modif-ication of the nuclei of penicillins and cephalosporins. The structural similarity between amino acids and both 6-APA (3) and 7-ACA (9), i.e. they all have terminal amine and carboxylic acid groups, led us to believe that very little adaption of the well developed techniques for peptide synthesis would be required.

To date SPPS has only been studied in relatively small scale reactions, and the technique involves considerable expense (the polymer support alone can cost in excess of £200/25g). In view of this, it was considered that solid phase methodology could be an efficient technique for the

targeting of novel β-lactam compounds. Enough product could be recovered from the resin for initial biological studies to be carried out. If these studies gave promising results, alternative larger scale syntheses could then be sought, since for a compound to be marketed as an antibacterial agent it must be synthesised in large quantities as cheaply as possible.

The main advantages of solid phase synthesis over solution phase techniques likely to be encountered with β-lactams are :

1. Excess starting materials, reagents and byproducts may be removed by simply washing with an appropriate solvent.
2. It should reduce losses encountered during the isolation and purification of intermediates since successive reactions may be carried out in the same reaction vessel.
3. The automated techniques already developed for peptide and oligonucleotide synthesis could be adapted and used for the synthesis of modified β-lactams.

This work has been divided into 5 main sections each of which would need to be successful for the methodology to be of value. These are outlined below :

1. The preparation of suitably protected penicillin and cephalosporin derivatives. (section 2.2.2)
2. The attachment of a 6β(7β)-protected aminopenicillin or aminocephalosporin to a solid support (resin). (section 2.2.3)
3. The removal of the protecting group to give an unprotected penicillin or cephalosporin attached to a resin. (section 2.2.4)
4. The addition of an alternative group to the free amino group of the penicillin or cephalosporin. (section 2.2.5)
5. Cleavage of the final penicillin or cephalosporin from the resin. (section 2.2.6)

By carrying out the study in this order the important aspects of loading the resin and cleaving the product would be investigated, while also including a relatively simple chemical modification.

2.2.2 Preparation of 6β (7β)-protected aminopenicillins and cephalosporins

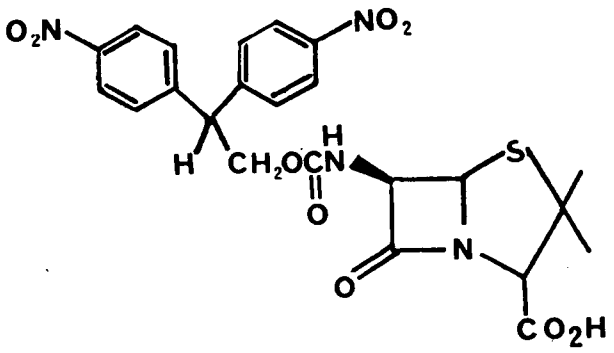
As discussed in the previous chapter, it is common practice in SPPS to use base labile N^{α}- (transient) protection combined with acid labile side chain (semi-permanent) protection and peptide-resin linkage. It was decided to protect the 6β (7β)-amino function of the

penicillin or cephalosporin with a base labile group in order that acid labile resins may be used.

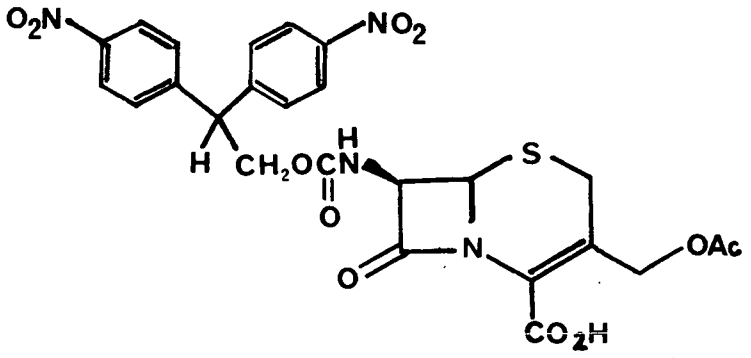
The most common base labile protecting group in use today is the 9-fluorenylmethoxycarbonyl (Fmoc) group (51). The one serious drawback to Fmoc methodology is its cost and with this in mind alternative protecting groups have been sought. One such example is the 2,2 -bis-(4'nitrophenyl)ethoxycarbonyl (Bnpeoc) group (57) introduced by workers in this research group[62].

It was decided to investigate both Fmoc and Bnpeoc protection of penicillins and cephalosporins so that a comparison of the two could be made.

Florence[62] synthesised both 6β -[2,2 -bis(4'nitrophenyl)ethoxycarbonylamino]penicillanic acid (Bnpeoc-APA) (106) and 7β -[2,2 -bis(4'nitrophenyl)ethoxycarbonylamino] cephalosporanic acid (Bnpeoc-ACA) (107) by reacting either 6-APA or 7-ACA with the chloroformate derivative of Bnpeoc (59) in the presence of 2 equivalents of 10% sodium carbonate with DMF as the solvent. He reported that, whereas with amino acids the less reactive active ester derivative of Bnpeoc (58) was required to prevent dipeptide formation, the reduced nucleophilicity of the C-6(C-7)-amino group of penicillins and cephalosporins required the more reactive chloroformate derivative (59) for a reaction to take place.

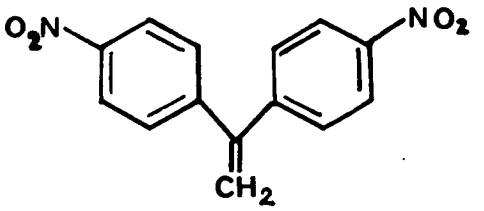


(106)



(107)

All attempts to repeat these reactions during the current research were unsuccessful. Initial reactions were carried out using 6-APA and were monitored by high performance liquid chromatography (hplc). The hplc trace of the product consisted of 5 peaks of similar intensity. The least polar component had a retention time that was identical to the retention time of a sample of Bnpeoc olefin (108) that had been run under the same conditions. (108) is the expected deprotection byproduct of Bnpeoc. The presence of this was particularly surprising as previous work[62] had indicated that Bnpeoc compounds were stable under these conditions.



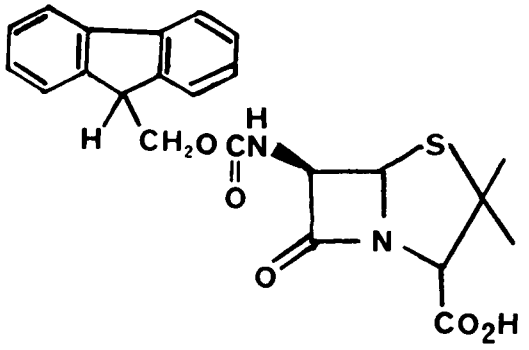
(108)

It was thought that one of the other impurities could be a Bnpeoc-APA dimer adduct, analogous to that observed when Fmoc chloroformate was used to protect amino acids (scheme 1.22) . Florence had shown this to occur when Bnpeoc chloroformate was used to protect amino acids, but had not reported any such problems during the protection of 6-APA.

A similar result was obtained from the attempted synthesis of Bnpeoc-ACA (107) using this methodology.

Attention was then diverted to the Fmoc protecting group (51). The methodology described above was repeated using Fmoc chloroformate (52) in an attempt to synthesise 6 β -[9-fluorenylmethoxycarbonylamino]penicillanic acid (Fmoc-APA) (109). This reaction also resulted in an impure product. Due to the number of components that were present, no attempt was made to isolate the target compound from this mixture. Other synthetic routes to these compounds, that would result in improved yields

combined with simpler purification , were sought.



(109)

Meanwhile an alternative method for the preparation of Fmoc and Bnpeoc amino acids had been attempted by other researchers in this group, with promising results[86]. This was a method devised by D. H. Rich[66] for the introduction of his trimethylsilylethoxycarbonyl (Teoc) protecting group to amino acids. Fmoc chloroformate (52) was reacted with 6-APA (3) using triethylamine as the base and 1,4-dioxane as the solvent. Hplc of the crude product from this reaction once again showed that significant impurities were present, however there was a major component present. It was assumed that this was the target compound (109), but attempts to isolate it by gel filtration were unsuccessful.

The reaction was repeated using the less reactive active ester derivative of Fmoc (54). Comparison of the hplc trace of the crude product from this reaction with the hplc traces of standard samples indicated the presence of the following compounds and other minor

impurities:

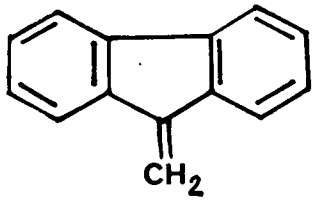
(a) triethylamine salt of 6-APA

(b) N-hydroxy succinimide

(c) Fmoc-ONSu (54)

(d) Fmoc-olefin (110)

and one other component that was assumed to be Fmoc-APA (109). The major peaks in the hplc trace were the unidentified one, and the peak attributed to Fmoc-olefin. The size of the peaks is not directly related to the quantities of these compounds present, since the chromophores differ. Two modifications of the reaction were attempted in order to reduce the amount of olefin formed. Very little difference was observed when acetonitrile was used as the solvent instead of 1,4 dioxane, and the amount of olefin increased when N-methyl morpholine was used as the base instead of triethylamine.



(110)

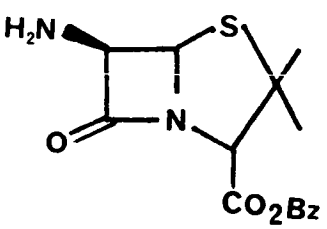
Purification of the product was attempted by forming the cyclohexylamine (CHA) salt of the crude product. This method, initially introduced by Rich[66], involves reacting the impure mixture with one equivalent of cyclohexylamine. All carboxylic acids present are converted into water

soluble cyclohexylamine salts, which allows the removal of any organic impurities. The cyclohexylamine salt is finally converted back to the carboxylic acid. Hplc analysis of the purified product showed that it consisted mainly of one component that had the same retention time as the unidentified component of the initial reaction product. However other impurities were present and further purification of this product would have been required in order for it to be used in future resin coupling experiments.

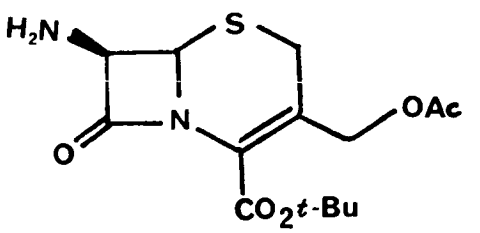
The above methodolgy was also repeated with the aim of synthesising Bnpeoc-APA (106) and Bnpeoc-ACA (107). The penicillin example resulted in very little improvement from the previous attempt. Neither chromatography using either reverse phase silica or dianion HP20SS resin, or gel filtration resulted in the pure compound. Similar problems were also experienced with the cephalosporin, after which this methodology was abandoned.

An alternative strategy for the introduction of Fmoc and Bnpeoc to penicillin and cephalosporin nuclei was sought in an attempt to try and eliminate the impurities encountered in the previous reactions. It was anticipated that esters of these compounds would be more stable than the free acids and, being considerably less polar, could be purified by standard silica gel chromatography techniques. Hence both the chloroformate and active ester

derivatives of Fmoc and Bnpeoc were reacted with esterified APA and ACA. The β-lactams selected for this work, due to their availability, were benzyl 6β-amino-penicillanate (111) and t-butyl 7β-aminocephalosporanate (112). A method for the preparation of the penicillin derivative (111) was supplied by Beecham Pharmaceuticals[91]. This involved reacting 6-APA with methyl acetoacetate and triethylamine, followed by treatment with benzyl bromide. Once the ester had been formed the free amine was liberated by treatment with p toluenesulphonic acid. The cephalosporin (112) was obtained directly from Beecham Pharmaceuticals.



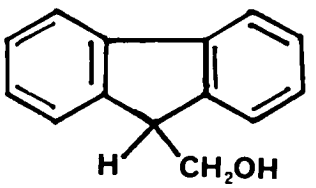
(111)



(112)

Benzyl 6β-[9-fluorenylmethoxycarbonylamino] penicillanate (Fmoc-APA-OBz) (113) was produced in high yield when Fmoc chloroformate (52) was reacted with benzyl 6β-aminopenicillanate (111). Purification of the crude reaction product using wet flash silica gel chromatography resulted in two compounds. The target compound (113) (71% yield) and also a smaller amount of an impurity which was identified by ^{1}H n.m.r as 9-fluorenylmethanol (114). This was initially thought to be due to the presence of

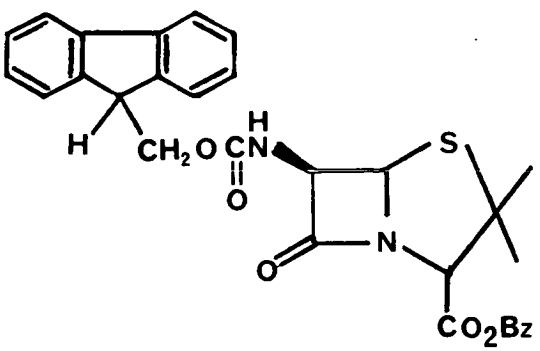
moisture in the reaction mixture, however (114) was still formed when activated molecular sieves were added to the reaction mixture.



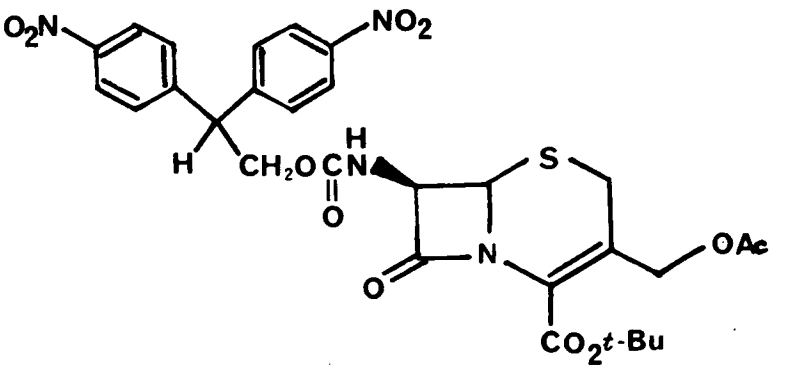
(114)

This reaction was then repeated using Fmoc-ONSu (54) instead of the Fmoc chloroformate (52), however, after a reaction time of 7 days, tlc monitoring showed that significant amounts of starting materials remained.

Similar methodology (using the chloroformate derivatives) resulted in high yields of t.butyl 7β-[2, 2 -bis-(4'nitrophenyl)ethoxycarbonylamino]cephalosporanate (Bnpeoc-ACA-OtBu) (115), Benzyl 6β-[2,2 -bis-(4'nitrophenyl)ethoxycarbonylamino]penicillanate (Bnpeoc-APA-OBz) (116) and t.butyl 7β-[9-fluorenylmethoxycarbonylamino]-cephalosporanate (Fmoc-ACA-OtBu) (117).



(113)



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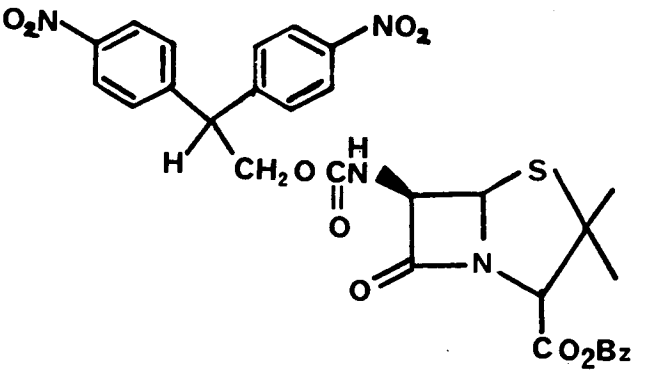
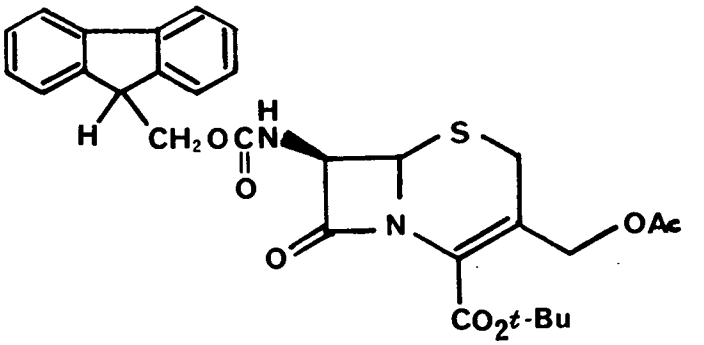


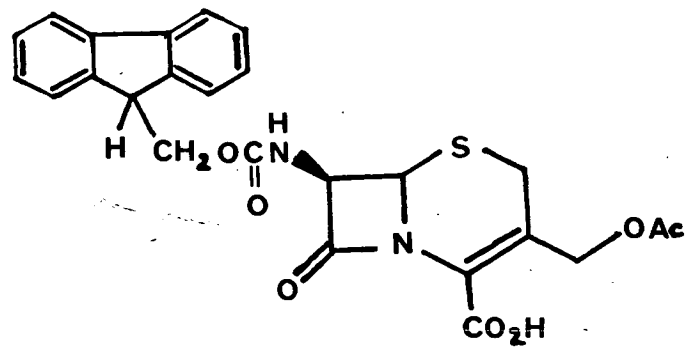
(116)



(117)

The next step was to convert the esters (113), (115), (116) and (117) to the corresponding carboxylic acid derivatives. The t-butyl group was successfully removed from the cephalosporins by stirring the ester in TFA for 15 minutes. The TFA was removed by evaporation under vacuum to yield the corresponding free acid (107) or (118)

in quantitative yield. Standard methodology was also employed for the removal of the benzyl ester from the Fmoc penicillin derivative (113); this involved hydrogenation over a palladium/carbon catalyst with THF as the solvent. The hydrogenation reaction was left overnight after which it was checked by tlc. This indicated that some ester remained, so fresh catalyst was added and after a further period of hydrogenation, the reaction was then found to have gone to completion. The tlc plates were developed by spraying with a solution of 4,4'bis(dimethylamino)phenyl carbinol (0.5 g) in acetone (500 ml) (Mary's reagent). The presence of a carboxylic acid on a tlc plate is indicated by an immediate deep blue coloration after the plate is sprayed with this solution. The solvent system used during these experiments (ethyl acetate/hexane 1:1) was insufficiently polar for the acids to run up the tlc plate, hence a successful reaction was indicated by the presence of baseline material that turned blue with Mary's reagent.



(118)

It was envisaged that hydrogenation of the Bnpeoc penicillin derivative (116) would also hydrogenate the nitro groups of the protecting group. Because of this the method of Olah et al[99], involving the use of trimethylsilyl chloride and sodium iodide, was attempted in order to remove the benzyl group from this compound. Even though tlc of the reaction mixture indicated that some acid was formed (Mary's reagent), hplc showed that the product was not pure. Considering the problems that had previously been experienced while attempting to purify this compound, no purification attempts were carried out and all future penicillin work involved the use of the Fmoc protected compound (113).

Since the compounds prepared by this method were considerably purer than those prepared by the direct method described earlier, this was adopted as the preferred method for the synthesis of (106), (107) and (118) throughout the remainder of this research.

2.2.3 Loading penicillins and cephalosporins onto a polymer support

Much work has been carried out over the years to modify the original chloromethyl resin (36), developed by Merrifield[43], with the aim of changing the lability of the peptide-resin bond and hence altering the cleavage conditions. The lability is mainly affected by the linking

agent between the molecule and the polymer chain. Some of the resins available to the peptide chemist have been discussed in the previous chapter.

In order to cleave an acid sensitive β-lactam molecule from a resin, a labile coupling is essential. The resin chosen for this study was the p.alkoxybenzylalcohol resin (43) introduced by Wang[55]. Electron donation from the p.alkoxy substituent enhances the acid lability of the ester linkage towards alkyl-oxygen fission. Standard conditions for the cleavage of peptides from this resin are 50% TFA in DCM. The Wang resin used in this research had a functionality of 0.79 mmol/g, i.e. if all the reactive sites of the resin were occupied, then there would be 0.79 mmol of substituent per gram of resin.

The coupling method chosen for this work involved the formation of a mixed carboxylic-phosphinic anhydride using diphenylphosphinic anhydride (74). This compound has been shown to be a very effective agent for the coupling of amino acids, and the methodology devised for this purpose[86] has been used in the course of the current research. It involves adding the mixed anhydride to the swelled resin in the presence of N-methyl morpholine and a catalytic amount of DMAP.

All reactions involving resin samples were agitated by placing the reaction vessel in an ultrasonic bath. This

being much preferable than stirring the reaction mixture, as some of the resin would adhere to the sides of the reaction vessel above the reactants.

Qualitative monitoring of these reactions was achieved by KBr disc infrared spectroscopy of the product resin; a carbonyl absorption at 1790 cm^{-1} indicating the presence of a β-lactam carbonyl group. Two further absorptions in the infrared spectrum of interest, specifically relevent to Bnpeoc protected compounds, are the nitro group stretching frequencies of 1350 and 1520 cm^{-1}. The coupling reactions were quantitatively monitored by one of two methods:

(i) Deprotection of a small amount of the resin, followed by ultraviolet spectroscopy of the resulting solution. This procedure was developed in this research group as a method for monitoring the synthesis of peptides[97]. A known amount of the resin was deprotected to give either the Bnpeoc olefin (108) or the Fmoc olefin (110) and the ultraviolet absorbance of the resulting solution was measured at a chosen wavelength. This was compared with a previously measured standard in order that the amount of olefin present be ascertained. BASIC computer programs for use on an Apple II microcomputer are available to calculate the results and present the information in the form of a printout[97]. This test is very sensitive to sample mass and had to be repeated to ensure that a reliable result was obtained.

(ii) Determination of the percentage nitrogen in the product resin by CHN analysis, and comparing this value to the theoretical maximum if the resin was 100% loaded.

Quantitative analysis of the product resin after the initial coupling reaction indicated a coupling of approximately 10%, i.e. β-lactam molecules occupied 10% of the resin's reactive sites. This result is considerably lower than that which would be expected for amino acids, and is likely to be due to increased steric hindrance caused by the bulkier penicillin and cephalosporin nuclei. Experiments indicated that maximum possible coupling was obtained after repeating the coupling reaction three times, after which couplings of approximately 40% were obtained. Initially each loading reaction involved the use of 2 equivalents of penicillin or cephalosporin, resulting in a total of 6 equivalents of β-lactam to achieve a coupling of only approximately 0.3 mmol/g of resin. In later experiments the amount of β-lactam was reduced to 1.5 equivalents for the first two loading reactions and 1 equivalent for the final reaction. This resulted in no apparent reduction in the final loading of the resin. Clearly this still involves excessive use of starting material, and to be of commercial use cheap methods of regenerating the β-lactam would need to be devised.

Bnpeoc-ACA (107), Fmoc-ACA (118) and Fmoc-APA (109) all

gave similar results in these coupling reactions. Chemically no differences were observed between the Fmoc and the Bnpeoc protected couplings. However the presence of the nitro absorptions in the infrared spectrum of the Bnpeoc example did provide an early indication of the success of the reaction.

2.2.4 The deprotection of Fmoc and Bnpeoc

The deprotection of both Fmoc and Bnpeoc, which proceeds via a β-elimination mechanism, has been discussed in the previous chapter. The olefin by-products (110) and (108) have strong ultraviolet absorptions, hence ultraviolet spectroscopy can be conveniently used to monitor these reactions.

Test deprotection reactions were carried out on the protected penicillin and cephalosporin ester derivatives (113), (115) and (117) in an attempt to optimise conditions before using the resin samples.

The standard conditions for the deprotection of Bnpeoc amino acids involves the use of 1,8 diazabicyclo-[5.4.0]undec-7-ene (DBU) / acetic acid. When these conditions were used with Bnpeoc-ACA-O<u>t</u>Bu (115), opening of the β-lactam ring resulted. This was shown by the absence of a β-lactam carbonyl absorption in the infrared spectrum of the product. However full deprotection occurred in 30 minutes, with no apparent loss

of β-lactam functionality, when piperidine (1 equivalent) was added to a solution of (115) in DMF. This was indicated by the presence of a ninhydrin positive spot on a tlc plate. The absence of absorptions at 1520 and 1350 cm^{-1} in the infrared spectrum of the product also indicated that the deprotection was complete. The β-lactam functionality of (115) was also found to be intact after stirring in solutions of 20% and 50% piperidine in DMF for 10 minutes.

The deprotection reaction was then attempted on the resin sample. In this case very little deprotection had occurred after 1 hour when 2 equivalents of piperidine in DMF were used. When the concentration of piperidine was increased to a 20% solution in DMF, full deprotection was achieved in 5 minutes.

These reactions were monitored either by infrared spectroscopy or by the Kaiser test[98]. For the Bnpeoc deprotection, the absence of the nitro group absorptions at 1350 and 1520 cm^{-1} in the infrared spectrum indicated a successful deprotection. Infrared spectroscopy also provided information regarding the β-lactam functionality. The Kaiser test is used to confirm the presence of free amine functionality, and involves the addition of equal volumes of three solutions to a sample of the resin, which is then heated for 10 minutes. The solutions used in the Kaiser test are :

Solution 1 : 76% w/w phenol in ethanol

Solution 2 : 0.002M potassium cyanide in pyridine

Solution 3 : 0.28M ninhydrin in ethanol.

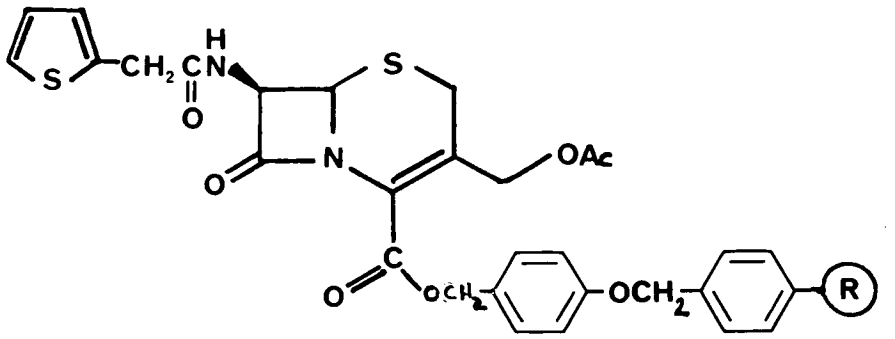
A positive test is indicated by a purple colouration.

When 20% piperidine in DMF was used to deprotect Fmoc-APA-OBz (113), infrared spectroscopy indicated that β-lactam ring opening had occurred after a reaction time of 1 hour. A preferable method for the removal of Fmoc from this compound was found to be the use of DBU in DCM. Complete deprotection was achieved when (113) was stirred in DCM with 1.2 equivalents of DBU for 10 minutes. Furthermore the β-lactam was still intact after 1 hour. These conditions were also successful in cleaving Fmoc from the sample that was attached to the resin, with no apparent loss of β-lactam functionality.

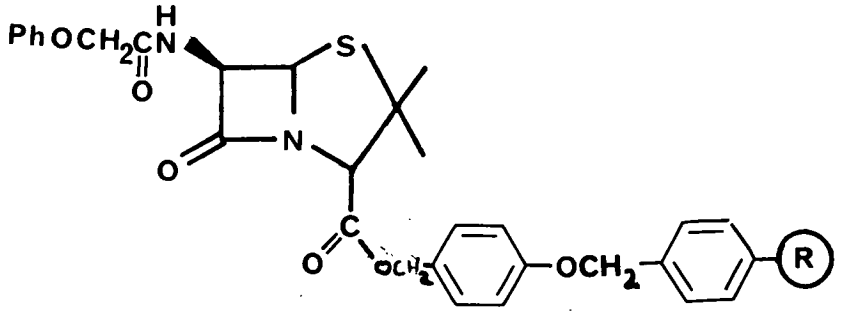
2.2.5 The coupling of a group to the amino functionality of a penicillin or cephalosporin nucleus attached to a resin

It was decided to carry out a simple reaction at the site of the free amine of the penicillin and cephalosporin nuclei while they were attached to the resin. Groups were chosen that would yield common, well characterised, compounds, so that once cleaved there would be no problems in identifying the products and consequently assessing the effectiveness of the methodology. In the case of the cephalosporin, the 2-thiopheneacetyl group was added to

the amine at the 7-position. The aim of this was to synthesise (119), which would yield 7β-(2-thiopheneacetyl) aminocephalosporanic acid (cephalothin) (10) as the cleaved product. The phenoxyacetyl group was added to the penicillin example, with the view of synthesising (120) and hence 6 β -phenoxyacetylaminopenicillanic acid (penicillin V) (2).



(119)



(120)

The coupling reactions were carried out using the respective acid chlorides in the presence of DCM and pyridine. The reactions were monitored using the Kaiser test, with a positive test indicating that further coupling was required. The coupling reactions were generally carried out twice, using 2 equivalents of the

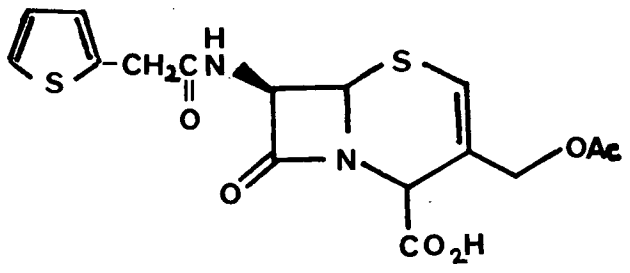
acid chloride each time, in order that a negative Kaiser test be obtained.

2.2.6 Cleaving the final products from the resin

The standard method for the cleavage of peptides from the Wang resin involves the use of a 50% mixture of trifluoroacetic acid (TFA) in DCM. Because of the sensitivity of β-lactam systems to acidic conditions the amount of TFA was reduced to 10%. The reactions were all carried out in an ultrasonic bath for 10 minutes. After this time, the resin was filtered and washed with DCM and toluene. The filtrate was then evaporated under vacuum to yield the crude product in the form of a yellow oil.

The hplc trace of the product obtained when the cephalosporin sample (119) was subjected to these conditions showed that two major components were present (figure 2.1). This hplc trace was compared to a trace of a previously characterised sample of cephalothin which had been run under the same conditions. This sample was synthesised by coupling 7-ACA with 2-thiopheneacetyl chloride using DCCI. One of the peaks in the hplc trace of the sample that had been cleaved from the resin was found to have an identical retention time to the characterised sample. Preparative hplc was then used to purify the product and pure cephalothin was obtained. The other component was not characterised, however the similarity in

retention time to the product peak suggested that it could be the Δ-2 cephalosporin analogue of cephalothin (121). This transformation is usually brought about in basic media, and it was assumed that the basic conditions used for the removal of the Bnpeoc and Fmoc were responsible in this case. Indeed when a sample of cephalothin was subjected to 20% piperidine in DMF for 15 minutes, nearly complete conversion of the cephalothin to a slightly more polar compound (hplc) occurred. The transformation to Δ-2 analogues can be prevented by using cephalosporin sulphoxide derivatives in all reactions involving basic conditions, or alternatively the Δ-2/Δ-3 mixture can be converted to the sulphoxide. This will convert all Δ-2 to Δ-3 (see section 2.2.7).



(121)

The same conditions were used to cleave 6β-phenoxyacetylamino penicillanic acid (penicillin V) (3) from the resin. The hplc trace of the product in this case indicated that the compound was unstable (figure 2.2). This was confirmed when a sample of previously characterised penicillin V was found to decompose under

similar conditions. It was clear that the less acid stable penicillin compounds could not be subjected to 10% TFA in DCM. Only slight improvement resulted when the penicillin V was subjected to 1% TFA in DCM, however these conditions would not cleave the product from the Wang resin.

2.2.7 Formation of cephalosporin sulphoxide

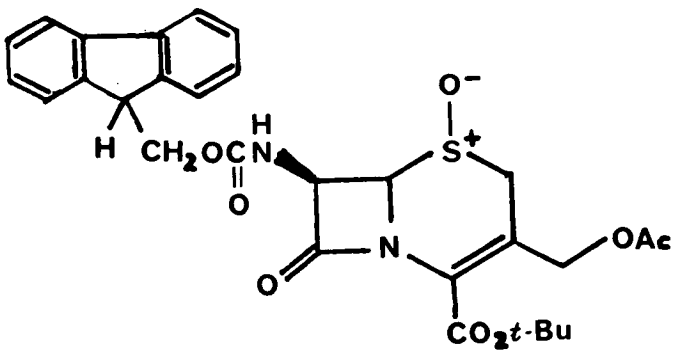
Initially alternative deprotection conditions were attempted to see if the formation of the Δ-2 adduct could be avoided. The use of piperidine in dimethylsulphide instead of DMF resulted in no improvement. A solution of 2% piperidine in DMF removed Bnpeoc from the resin sample in 30 minutes. However an equal mixture of two isomers resulted when this resin sample was treated as before (hplc).

Finally the resin was added to solutions of 5%, 10% and 20% morpholine in DMF. It was hoped that morpholine, being a weaker base than pyridine, would not encourage the formation of the Δ-2 adduct. These reactions were considerably slower than when pyridine was used. The 5% example gave a negative Kaiser test after 4 hours indicating that no deprotection had occurred. The other two examples resulted in some deprotection but the products were a mixture of isomers (hplc).

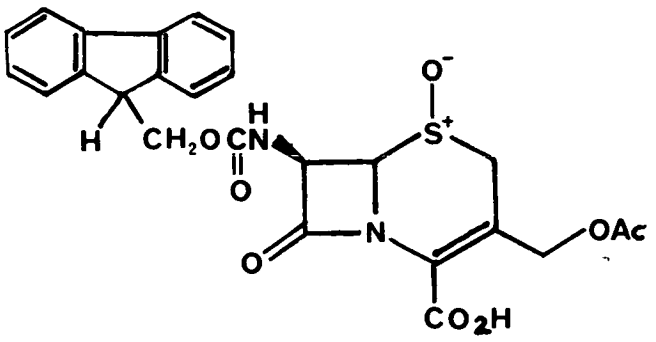
It was therefore concluded that in order to prevent the formation of a Δ-2 cephalosporin during the deprotection

of Bnpeoc or Fmoc, the cephalosporin must be converted to the sulphoxide. It is a known fact that cephalosporin sulphoxides do not rearrange to the Δ-2 analogues.

Two different methods were attempted. Fmoc-ACA-O<u>t</u>Bu (117) was oxidised to the sulphoxide (122) using 1.2 equivalents of <u>m</u>.chloroperoxybenzoic acid. Following removal of the <u>t</u>.butylester as before, the sulphoxide (123) was loaded onto the resin using the methodology described earlier. This resulted in a loading of only 25%, significantly lower than that obtained with the sulphide (118). The frequency of the infrared β-lactam carbonyl absorption of the resulting resin was at 1805 cm^{-1}, i.e. had increased slightly, as would be expected for a sulphoxide.



(122)



(123)

Due to the apparent decrease in the loading of the resin, it was decided to attempt the conversion to the sulphoxide after the cephalosporin had been coupled to the resin, deprotected, and reacted with the 2-thiopheneacetyl chloride. A sample of the resin (119) was swelled in DCM and mcpba (1.2 equivalents) was added. After this reaction some of the resin was cleaved and hplc indicated that the conversion had occurred. The product consisted of a single component that was more polar than either cephalothin or the Δ-2 compound. The remainder of the resin was reacted with phosphorous trichloride in order to reduce the sulphoxide back to the sulphide. The hplc trace of this product after cleavage from the resin consisted of one major peak that had an identical retention time to the sample previously characterised. This confirms that the extra peak observed was due to the Δ-2 analogue, and that this problem can be avoided by oxidation and reduction steps prior to the removal of the product from the resin.

2.2.8 Use of the 'Sasrin' resin (45)

During the course of this research, the 'Sasrin' resin (45) was introduced as a highly acid labile resin; 'Sasrin' stands for 'super acid sensitive resin'. Having had problems in cleaving penicillin derivatives from the Wang resin, it was considered worthwhile to repeat the work using the Sasrin resin.

The slightly modified linking agent of the Sasrin resin causes a more acid labile peptide-resin bond, which allows the use of 0.5 or 1% TFA in DCM to cleave products.

A sample of the Sasrin resin, (obtained from Bachem), was loaded with Fmoc-APA (113) using the methodology that had already been proved successful with the Wang resin. As before, the loading reaction was repeated three times, after which the resin was only 23% loaded. This lower coupling could be due to the increased steric hindrance caused by the bulkier linking agent of the Sasrin resin. The Fmoc protection was removed using DBU (1.2 equivalents), and phenoxyacetyl choride was coupled to the free amino group.

Both 0.5% and 1% TFA in DCM were then used to cleave the resulting penicillin V (3) from the resin. The reactions were sonicated for periods ranging from 15 to 45 minutes. The reaction mixture was observed to change colour after approximately 5 minutes and, after recovery, the resin was a violet colour; an observation that had been reported on the product information sheet supplied by the manufacturers. Hplc traces of the products obtained from these experiments indicated only a slight improvement from the previous experiments with the Wang resin.

The stability of penicillin V was then tested in other

weakly acidic solutions. Stirring penicillin V in 10% 2M HCl/1,4-dioxane and 10% 0.5M HCl/1,4-dioxane resulted in some decomposition, however improved stability was observed in a solution of trichloroacetic acid (5 equivalents) and DCM. A sample of the resin was then subjected to these conditions. Trichloroacetic acid (5 eq.) was added to a sample of the resin that had been swelled in DCM. The reaction was left in an ultrasonic bath for 15 minutes, after which filtration and evaporation of the filtrate yielded the crude product. Hplc showed that the major component of the product had a retention time identical to that of some previously characterised penicillin V, other minor impurities were also present (figure 2.2). The product was purified by preparative hplc and pure penicillin V (3) resulted.

2.2.9 Conclusions

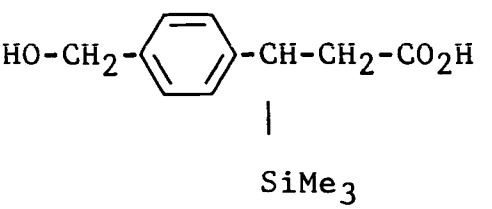
This research has demonstrated that β-lactam compounds will behave in a similar manner to peptides, and may be coupled to insoluble polymer supports. The increased bulk of the β-lactam molecules, however, leads to a lower percentage of the resin's active sites being coupled.

The synthesis of suitably protected penicillin and cephalosporin analogues proved to be a more complex task than an earlier report[57] had suggested, however the use of penicillin or cephalosporin ester derivatives allowed the protection of the 6β (7β)-amino functionality in high

yield. No significant differences were noted between the Fmoc and Bnpeoc protection strategies, however infrared spectroscopy provided a good indication of the success of the protection and deprotection reactions in the case of Bnpeoc.

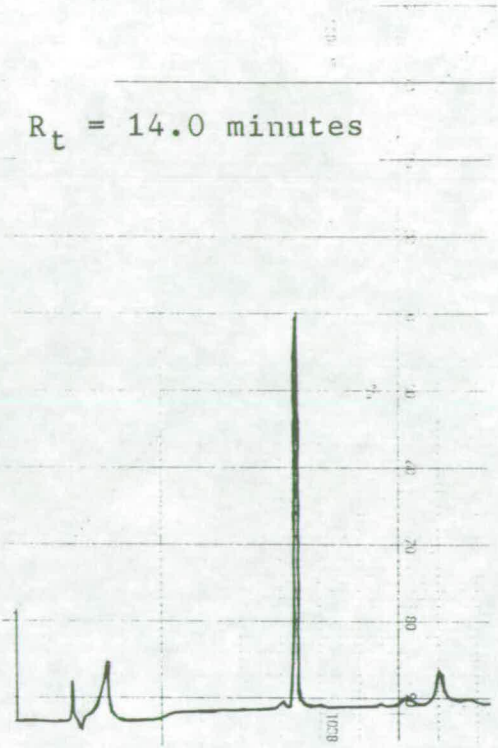
Care must be taken when using basic conditions to deprotect cephalosporin derivatives in order to prevent the formation of Δ-2 analogues. This problem may be avoided by formation of the cephalosporin sulphoxide.

During the course of this research, the most serious problems were encountered when cleaving the final product from the resin. Ideally, alternative cleavage conditions are required, to which the sensitive penicillin and cephalosporin nuclei will be more stable. This will probably involve further modification of the linking agent between the β-lactam and the resin. One possible solution could be the use of 2-(4-hydroxymethylphenyl)-2-trimethylsilylpropanoic acid group (124). This is a fluoride-labile handle that was introduced by workers in this research group, and proved to be successful in the synthesis of a number of peptides[97].

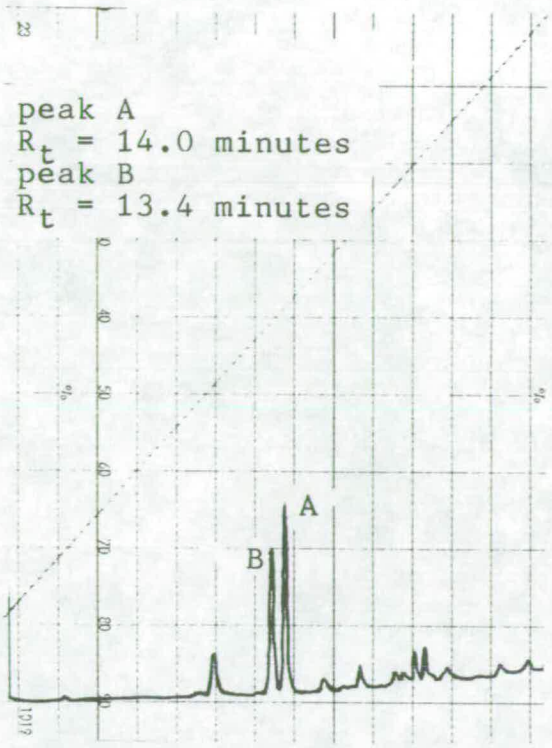


(124)

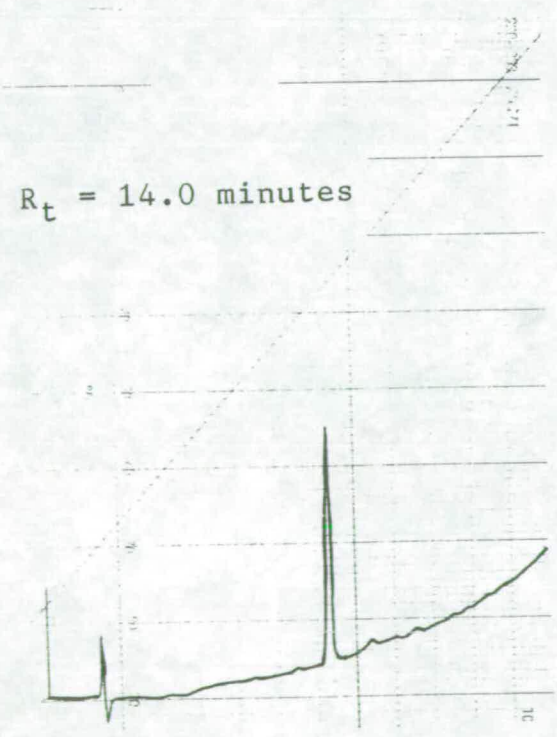
After cleaving the penicillin and cephalosporin from the resin, purification was still required. This considerably reduces any advantage likely to be gained from the use of this methodology for short syntheses. However during longer, more complicated, syntheses avoiding the necessity to purify each intermediate could lead to a considerable time saving.



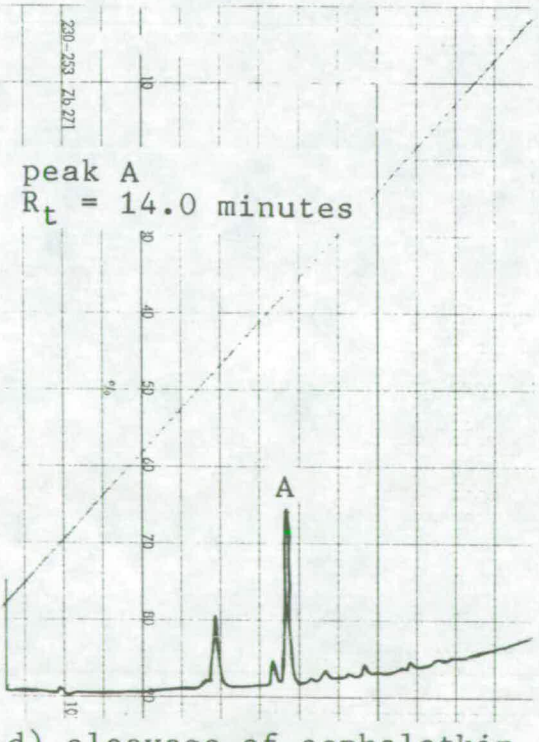
a) cephalothin standard



b) cleavage of cephalothin from Wang resin

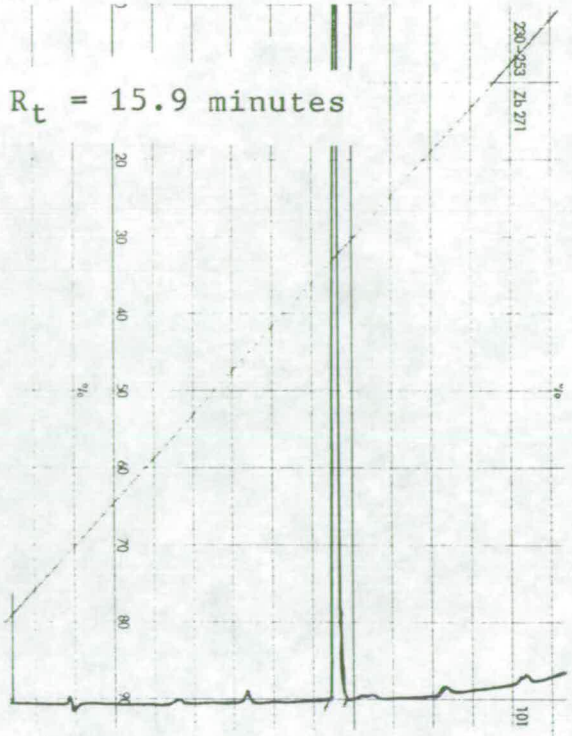


c) sample from Wang resin after preparative hplc

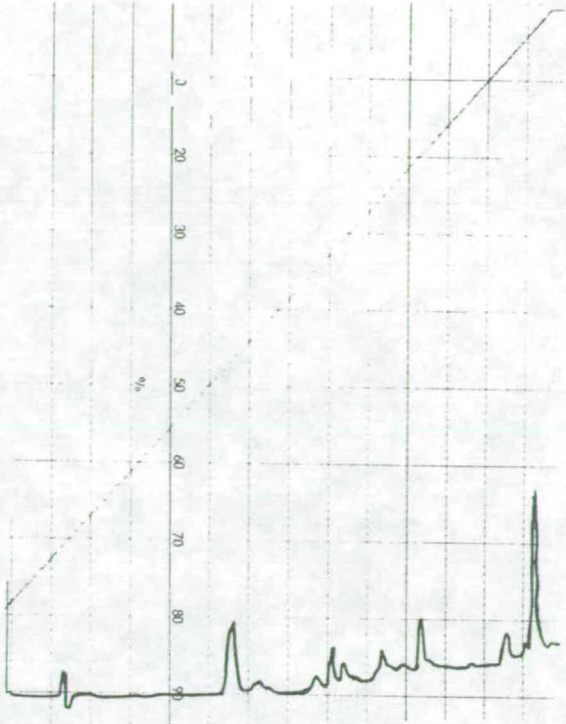


d) cleavage of cephalothin from Wang resin after sulphoxide formation

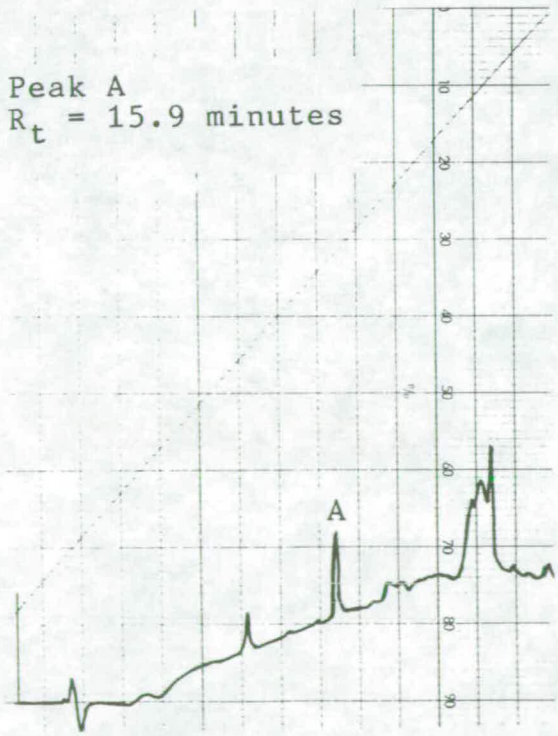
Figure 2.1



a) penicillin V standard



b) attempted cleavage of penicillin V from Wang resin using 10% TFA/DCM



c) cleavage of penicillin V from Sasrin resin using 5 eq. TCA in DCM

Figure 2.2

CHAPTER 3

EXPERIMENTAL

Melting points were recorded in open capillary tubes on an electrically heated Buchi 510 melting point apparatus and are uncorrected. Thin layer chromatography (tlc) was carried out on commercially available plastic sheets coated with silica gel 60 Gf-254 (Merck) in the solvent systems quoted in the text. Visualisation of the compounds was achieved by a suitable combination of the following methods: UV absorption at 254 nm, potassium permanganate, bromophenol blue, 4,4'-bis(dimethylamino)phenylcarbinol (Mary's reagent), and ninhydrin sprays. Wet flash chromatography was performed using Merck silica gel 60. High performance liquid chromatography (hplc) was carried out either using a Waters system consisting of 2 x 600A pumps, a U6K injector, a 680 automatic gradient controller, a model 441 ultraviolet detector, and a 308 computing integrator, or an Applied Biosystems system consisting of 2 x 1406A solvent delivery systems, a 1480A injector/mixer, and a 1783A detector/controller. Analytical separations were performed using the columns and gradients of water/acetonitrile (+ 0.05% TFA) that are listed below, with a flow rate of 1 ml/min. The elution of samples was monitored by ultraviolet absorption at 254 nm:

system 1 Spherisorb C_{18}, 80/20 to 0/100 (28 minutes)

system 2 Spherisorb C_{18}, 50/50 to 0/100 (16 minutes)

system 3 Spherisorb C_8, 50/50 to 0/100 (28 minutes)

system 4 Spherisorb C_{18}, 50/50 to 0/100 (28 minutes)

system 5 Spherisorb C_8, 90/10 to 40/60 (25 minutes)

Infrared spectra were recorded on a Perkin Elmer 781 double beam spectrophotometer in the solvent indicated, or by the KBr disc technique. Polystyrene (1603 cm^{-1}) was employed as the standard. Ultraviolet spectra were recorded on a Pye-Unicam SP8-400 spectrophotometer, in the solvent indicated. High and low resolution fast atom bombardment (FAB) spectra were measured on a Kratos MS 50TC machine. Proton n.m.r were recorded on a Jeol FX-60 (60 MHz), a Brucker WP80 (80 MHz), a Brucker WP200 (200 MHz), or a Brucker WM 250 (250 MHz) machine in the solvent indicated , using tetramethylsilane (TMS) as the external standard (δ= 0.000 ppm). Carbon-13 n.m.r spectra were recorded on a Brucker WP200 machine operating at 50.3 MHz. Elemental analysis was carried out on a Carlo Erba elemental analyser (model 1106).

All solvents were distilled before use, and the following were dried, using the reagents listed, when required: benzene (sodium wire), chloroform (phosphorous pentoxide), dichloromethane (calcium hydride), diethyl ether (sodium wire), toluene (sodium wire), and tetrahydrofuran (sodium). N,N-dimethylformamide (hplc grade) was commercially supplied by Applied Biosystems, Rathburn, or Aldrich. This was stored over 4A molecular sieves and was used without distillation. Petroleum ether refers to the fraction with boiling point between 40°C and 60°C.

6α-Hydroxypenicillanic acid (26)

The compound was prepared by the general method of Hauser and Sigg[26]

6-aminopenicillanic acid (3) (1.08 g, 5.00 mmol) was dissolved in 1M p.toluenesulphonic acid (13.5 ml) and was cooled to 0°C. To this stirred solution was added a solution of sodium nitrite (0.5 g, 7,25 mmol) in water (6 ml) over a period of 10 minutes. The reaction was left stirring for 5 minutes. The product was then extracted into ether (3 x 50 ml). The organic layer was washed with water (2 x 25 ml) and dried over Na_2SO_4. Removal of the solvent in vacuo gave the product as a pale green foam (0.82 g, 76%): δ_H(80MHz, CD_3COCD_3) 9.55 (1H,s,acid H), 5.18 (1H, d ,J 1.2 Hz, 5-H), 4.9 (1H, b, OH), 4.75 (1H, d, J 4Hz, 6-H), 4.42 (1H, s, 3-H), 1.60 (3H, s, CH_3), 1.55 (3H, s, CH_3); ν_{max} (THF) 1790 (β-lactam C=O), 1750 (C=O) cm^{-1}.

Benzyl 6α-hydroxypenicillanate (25)

(26) (1.736 g, 8.00 mmol) was dissolved in a mixture of acetone (5 ml) and water (5ml). This was neutralised using 1M potassium carbonate. The neutralised solution was lyophilised which left the potassium salt as a brown solid. The potassium salt (1.785 g, 7 mmol) was dissolved in dimethylacetamide (5 ml). A solution of benzyl bromide (2.90 g, 1.40 mmol) in dimethylacetamide (5 ml) was then added and the reaction mixture was stirred at room temperature for 1.5 hours. The solvent was then removed

in vacuo and ethyl acetate (50 ml) was added. The resulting solution was washed with water (1 x 50 ml), saturated $NaHCO_3$ (1 x 50 ml) and water (1 x 50 ml). The organic phase was then dried over Na_2SO_4 and the solvent removed in vacuo to give the crude product as a yellow oil. This was recrystallised from DCM and petroleum ether to afford the **title compound** (1.89 g, 88%) as a white, crystalline solid: m.p. 158-160°C (lit.[26] 157-160°C); δ_H (80 MHz, $CDCl_3$) 7.38 (5H, s, aromatic H), 5.25 (1H, bd, 5-H), 5.30 (2H, s, benzyl CH_2), 4.82 (1H, bd, 6-H), 4.50 (1H, s, 3-H), 3.32 (1H, bd, OH), 1.52 (3H, s, CH_3), 1.48 (3H, s, CH_3); ν_{max} (CH_2Cl_2) 1775 (β-lactam C=O), 1742 (ester C=O), 1200, 1178 cm^{-1}.

Benzyl 6-oxopenicillanate (24)

A. Oxidation of benzyl 6α-hydroxypenicillanate (25)

(i) Using DMSO and acetic anhydride

The method of Vanderhaege et al[33] was repeated.

Benzyl 6α-hydroxypenicillanate (0.338 g, 1.1 mmol) was dissolved in DMSO (2ml). To this was added acetic anhydride (0.6 ml) that had been freshly distilled from $AlCl_3$. The reaction mixture was stirred at room temperature for 48 hours, after which it was poured into a mixture of ice-water (5 ml) and $KHCO_3$ (5 ml). The suspension was extracted with benzene (3 x 10 ml) and the combined organic phase was washed with water (5 x 20 ml) and dried (Na_2SO_4). The solvent was removed in vacuo, which left the target compound as a yellow oil (0.104 g,

31%). All attempts to recrystallise the product failed: ν_{max} (CH_2Cl_2) 1830 (6-oxo C=O), 1790 (β -lactam C=O), 1742 (ester C=O), 1415, 1200, 1180, 1160, 1110 cm^{-1}.

(ii) Using pyridiniun chlorochromate

Pyridinium chlorochromate (0.053 g, 0.244 mmol) was dissolved in DCM (5 ml). To this solution was added benzyl 6 α -hydroxypenicillanate (25) (0.050 g, 0.16 mmol) that had been dissolved in DCM (2 ml). The resulting solution was stirred for 12 hours. Ether (20 ml) was then added and the solid residue that was formed was removed by filtration through celite. The filtrate, which was green coloured, was evaporated in vacuo. Infrared spectroscopy of the residue indicated that starting material had been recovered: ν_{max} (CH_2Cl_2) 1775 (β-lactam C=O), 1745 (ester C=O), 1200, 1178 cm^{-1}.

(iii) Using manganese dioxide

Manganese dioxide was freshly prepared for this reaction: A solution of manganese sulphate (22.2 g, 0.147 mol) in water (30 ml), and a solution of 40% NaOH (23.4 ml) were added simultaneously over 0.25 hour to a solution of potassium permanganate (19.2 g, 0.121 mol) in water (120 ml). A brown solid was precipitated soon after the start of the reaction. After 1 hour the solid was collected using a centrifuge and washed with water until the washings became colourless. The solid was finally dried in an oven at 120°C, and the target compound was

obtained as a brown powder (8.31 g, 65%).

Freshly prepared manganese dioxide (0.13 g, 1.6 mmol) was added to acetonitrile (1ml). To this was added benzyl 6 α-hydroxypenicillanate (25) (0,05 g, 0.16 mmol) that had also been dissolved in acetonitrile (1 ml). The reaction was stirred for 6 hours. The solid residue was then removed by filtering the reaction mixture through celite. Finally the solvent was removed in vacuo. Infrared spectroscopy indicated that starting material (25) had been recovered: ν_{max} 1775 (β-lactam C=O), 1742 (ester C=O), 1200, 1178 cm^{-1}.

(iv) Moffat oxidation using diisopropylcarbodiimide

The general methodology of Sheehan and Lo[24] was repeated, with some modifications that were introduced by Vanderhaege et al[33].

Benzyl 6 α -hydroxypenicillanate (0.050 g, 0.16 mmol) was dissolved in dimethyl sulphoxide (1 ml). To this was added anhydrous orthophosphoric acid (0.008 g, 0.08 mmol) and diisopropylcarbodiimide (0.061 g, 0.48 mmol). The reaction was stirred at room temperature for 2.5 hours. The excess carbodi-imide was then destroyed using oxalic acid dihydrate (0.5 g) in dimethyl sulphoxide (5 ml), after which the mixture was poured into ice-water (100 ml). The precipitated diisopropyl urea was removed by filtration and the filtrate was extracted with ethyl acetate (3 x 50 ml). The organic layer was washed with water (2 x 50 ml) and dried (Na_2SO_4) and the solvent

removed *in vacuo*. Tlc (EtOAc/hexane 1:1) indicated that mainly starting material remained.

(v) Moffat oxidation using 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (77)

A solution of benzyl 6α-hydroxypenicillanate (25) (0.05 g, 0.16 mmol) and dry dimethylsulphoxide (1 ml) in toluene (1 ml) was treated with (77) (0.705 g, 0.37 mmol) that had been dissolved in pyridine (0.044 g, 0.56 mmol) and toluene (0.5 ml). Dichloroacetic acid (0.010 g, 0.08 mmol) in toluene (0.5 ml) was added and the reaction mixture was stirred under nitrogen at room temperature for 24 hours. The reaction mixture was then washed with citric acid (2 x 20 ml), saturated $NaHCO_3$ (1 x 20 ml) and brine (1 x 20 ml). The organic phase was dried (Na_2SO_4) and the solvent removed *in vacuo*. Infrared spectroscopy indicated that mainly starting material had been recovered: ν_{max} (CH_2Cl_2) 1775 (β-lactam C=O), 1745 (ester C=O), 1200, 1172 cm^{-1}.

B. From benzyl 6β-aminopenicillanate using trifluoromethanesulphonic anhydride

The methodology of Hagiwara *et al*[38] was repeated.

To a solution of (25) (0.910 g, 2.90 mmol) in DCM (10 ml) at -78°C was added trifluoromethanesulphonic anhydride (2.51 g, 8.9 mmol) and triethylamine (0.899 g, 8.9 mmol). This was allowed to warm to room temperature with stirring over 2 hours. The reaction was then added to ethyl acetate

(50 ml) and washed with ice water (3 x 30 ml). The organic layer was dried (Na_2SO_4) and the solvent removed in vacuo to leave the residue as a brown oil (1.62 g, 2.8 mmol). DBU (0.693 g, 4.2 mmol) was then added and the resulting mixture was stirred at -78°C for 1 hour. This was then added to 0.5M HCl (50 ml) and ethyl acetate (50 ml). The organic layer was washed with brine (1 x 50 ml) and dried (Na_2SO_4). Removal of the solvent in vacuo left a brown oil. Tlc (EtOAc/hexane 1:1) and infrared spectroscopy showed this was mainly starting material: R_f 0.65; ν_{max} (CH_2Cl_2) 1775 (β-lactam C=O), 1745 (ester C=O) 1200, 1172 cm^{-1}.

C. From benzyl 6α-methylthio 6β-aminopenicillanate (31)

The general methodology of Jen et al[32] was repeated.

A stirred solution of (31) (1.40 g, 4.00 mmol) in DMF (60 ml) and water (20 ml) at -20°C (CCl_4/acetone bath) was treated with mercuric chloride (1.20 g, 4.40 mmol). After 15 minutes the reaction mixture was warmed up to 0°C and was stirred at this temperature for a further 30 minutes. Ether (100 ml) was then added. The precipitate was removed by filtration through celite and the filtrate was washed with water (2 x 100 ml). The organic phase was dried (Na_2SO_4) the solvent was removed in vacuo to give the **title compound** as a yellow oil (0.854 g, 70%): ν_{max} (CH_2Cl_2) 2965, 1833 (6-oxo C=O), 1790 (β-lactam C=O), 1740 (ester C=O), 1375, 1240, 1200, 1095 cm^{-1}; δ_H(200 MHz, $CDCl_3$) 7.37 (5H, s, aromatic H), 5.80 (1H, s, 5-H), 5.25

(2H, s, benzyl CH_2), 4.82 (1H, s, 3-H), 1.50 (3H, s, CH_3), 1.45 (3H, s, CH_3).

D.From benzyl 6α-tolylthio 6β-aminopenicillanate (78)

The method described above was repeated using (78) instead of (31). The reaction between (78) (1.188 g, 3.00 mmol) and mercuric chloride (0.90 g, 3.30 mmol) resulted in the target compound (24) (0.549 g, 60%).

t.butyl 7α,7β -dihydroxycephalosporanate (79) (Attempted preparation of t.butyl 7-oxocephalosporanate (76))

A. From t.butyl 7β-aminocephalosporanate using trifluoromethanesulphonic anhydride.

A solution of t.butyl 7β-aminocephalosporanate (0.79 g, 2.5 mmol) in DCM (10 ml) was cooled to -65°C. Triethylamine (0.725 ml, 5.00 mmol) and trifluoromethanesulphonic anhydride (0.858 ml, 5.00 mmol) were added. The reaction mixture was stirred for 2 hours, allowing the temperature to reach -10°C over this period. Tlc (EtOAc/hexane 3:7) showed that all starting material had been consumed: R_f (starting material) 0.05, R_f (reaction mixture) 0, 0.35, 0.56. The solution was re-cooled to -60°C and triethylamine (0.367 ml, 2.5 mmol) was added, and the resulting reaction mixture was allowed to warm up to -30°C. The solution was then poured into ethyl acetate (20 ml) and 1M HCl (20 ml). The organic phase was separated, washed with brine (2 x 20 ml) and dried

($MgSO_4$). The solvent was then removed <u>in vacuo</u> to give a yellow oil (0.449 g, 55%). Purification was attempted using silica gel chromatography, during which some decomposition of the product seemed to occur. Some product containing fractions were recovered, and the solvent was removed <u>in vacuo</u>. Recrystallisation of the residue using ethyl acetate and hexane resulted in a pale yellow solid (0.111 g, 15%): m.p. 130-132°C; ν_{max} (KBr disc) 3020 (OH), 3140 (OH), 1770 (β-lactam C=O), 1710 (ester C=O), 1700 (ester C=O), 1360, 1260, 1150, 1115, 1030, 810 cm^{-1}; δ_H (200 MHz, CD_3COCD_3) 6.88 (1H, s, OH), 6.74 (1H, s, OH), 4.97 and 4.70 (2H, ABq, 3-CH_2), 4.89 (1H, s, 6-H), 3.53 and 3.40 (2H, ABq, 2-H), 2.05 (3H, s, OCH_3), 1.53 (9H, s, <u>t</u>.butyl H).

B. From <u>t</u>.butyl 7α-tolylthio 7β-aminocephalosporanate (81)

A solution of (81) (6.75 g, 15 mmol) in DMF (75 ml) and water (15 ml) was cooled down to -5°C (ice/NaCl bath), and mercuric chloride (3.255 g, 15 mmol) was added. After 30 minutes, more DMF (30 ml) was added and the reaction mixture was filtered through celite to remove the solid residue. The solvent was then removed <u>in vacuo</u> which left a yellow oil (4.399 g, 85%). This was then recrystallised using ethyl acetate and hexane, and the **title compound** (3.778 g, 73%) was obtained as a pale yellow solid: m.p. 130-132°C; λ_{max} (DMF) 268, 247 nm; ν_{max} (KBr disc) 3460 (OH), 3290 (OH), 1773 (β-lactam C=O), 1715 (ester C=O), 1700 (ester C=O), 1360, 1260, 1150, 1120, 1030, 815 cm^{-1};

δ_H(200 MHz, CD_3COCD_3) 6.85 (1H, s, OH), 6.70 (1H, s, OH) 4.95 and 4.72 (2H, ABq, 3-CH_2), 4.87 (1H, s, 6-H) 3.67 and 3.40 (2H, ABq, 2-H), 2.05 (3H, s, OCH_3), 1.53 (9H, s, t.butyl H); δ_C(50 MHz, CD_3COCD_3) 169.5, 163.9, 160.0 (3 x C=O), 127.0 (C-7), 121.2 and 103.5 (C=C), 82.0 (t.butyl quaternary C), 65.5 (C-6), 62.0 (C-2), 26.5 (3 x t.butyl CH_3) 25.2 (3-CH_2), 19.2 (OCH_3); m/z (FAB) 328 (MH^+), 311, 292, 202, 156.

Conversion of (79) to t.butyl 7-oxocephalosporanate (76)*

(79) (0.10 g, 0.29 mmol) was dissolved in CD_3COCD_3 (5 ml): *δ_H (60 MHz) 6.40 (1H, b, OH), 6.24 (1H, s, OH), 4.60 and 4.30 (2H, ABq, 3-CH_2), 4.48 (1H, s, 6-H), 3.10 (2H, d, 2-H), 1.62 (3H, s, OCH_3), 1.80 (9H, s, t.butyl H).

Activated molecular sieves (4A) (0.10 g) were then added to this and the solution was left to stand at room temperature for 2 minutes: *δ_H (60 MHz) 6.38 (1.5H, b, 2 x OH), 5.32 (0.5H, s, 6-H(76)), 4.50 (0.5H, s, 6-H(79)), 4.70 and 4.30 (2H, 2 x ABq, 3-CH_2), 3.40 (1H, d, 2-H(76)), 3.15 (1H, d, 2-H(79)), 1.70 (3H, s, OCH_3), 1.20 and 1.22 (9H, 2 x s, t.butyl H).

Fresh activated molecular sieves were added and the solution was left to stand for a further 5 minutes. The ^1H n.m.r spectrum (60 MHz) indicated that the solution was still a mixture, but more conversion to (76) had occurred.

Fresh activated molecular sieves (0.10g) were added and the solution was left for 1 hour. The ^1H n.m.r spectrum (60MHz) of this indicated that the conversion was

complete: ν_{max} (CH_2Cl_2) 1833 (7-oxo C=O), 1790 (β-lactam C=O), 1745 (ester C=O), 1730 (ester C=O), 1344, 1230, 1158, 1045 cm^{-1}; δ_H(200 MHz, CD_3COCD_3) 5.30 (1H, s, 6-H), 5.10 and 4.85 (2H, ABq, J 13.5 Hz, 3-CH_2), 3.64 and 3.43 (2H, ABq, J 18.7 Hz, 2-H), 2.12 (3H, s, OCH_3), 1.55 (9H, s, t.butyl H).

* ^{1}H nmr chemical shifts indicated are not accurate. The spectrometer was not calibrated relative to TMS for this experiment.

Attempted preparation of t.butyl 7α-acetoxy 7β-hydroxy-cephalosporanate

Acetyl chloride (0.026 ml, 0.37 mmol) and pyridine (0.029 ml, 0.37 mmol) were added to a solution of t.butyl 7α,7β-dihydroxycephalosporanate (79) (0.127 g, 0.37 mmol) in DCM (15 ml). The reaction mixture was stirred under argon for 1 hour, after which it was added to ethyl acetate (30 ml) and dilute HCl (30 ml). The organic phase was separated and washed with water (2 x 50 ml), saturated $NaHCO_3$ (1 x 50 ml), and brine (1 x 50 ml). The organic phase was finally dried ($MgSO_4$) and the solvent removed in vacuo. Tlc (ethyl acetate/hexane 1:1) indicated that starting material remained: R_f (79) 0.26, R_f (recovered material) 0.26.

Preparation of t.butyl 7α,7β-diacetoxycephalosporanate (83)

Acetyl chloride (0.085 ml, 1.2 mmol) and pyridine (0.097 ml, 1.2 mmol) were added to a solution of t.butyl 7α,7β-dihydroxycephalosporanate (79) (0.138 g, 0.4 mmol) in DCM (10ml). The reaction mixture was stirred at room temperature for 15 hours, after which tlc (EtOAc/ hexane 1:1) indicated that all starting material had been consumed: R_f (79) 0.26, R_f (reaction mixture) 0, 0.36, 0.50, 0.54. The reaction mixture was then added to ethyl acetate (30 ml) and dilute HCl (30 ml). The organic phase was washed with water (2 x 50 ml) and brine (2 x 50 ml) and dried ($MgSO_4$), after which the solvent was removed in vacuo. Infrared spectroscopy indicated that some t.butyl 7-oxocephalosporanate was present: ν_{max} (CH_2Cl_2) 1833, 1790, 1740, 1170, 1220, 1160, 1120 cm^{-1}. Wet flash silica gel chromatography (EtOAc/hexane 1:1) afforded two products. N.m.r indicated that the least polar (R_f 0.54) product was the **title compound** (83) (0.015 g, 9%): δ_H(200 MHz, $CDCl_3$) 5.10 (1H, s, 6-H), 5.05 and 4.82 (2H, ABq, J 12 Hz, 3-CH_2), 3.55 and 3.34 (2H, ABq, J 17.8 Hz, 2-H), 2.40 (3H, s, 7-$COCH_3$), 2.24 (3H, s, 7-$COCH_3$), 2.10 (3H, s, 3-$COCH_3$), 1.59 (9H, s, t.butyl H).

The other recovered compound was not identified: δ_H(200 MHz, $CDCl_3$) 5.05 (1H, s, 6-H), 5.07 and 4.83 (2H, ABq, J 13.3 Hz, 3-CH_2), 4.05 and 3.88 (2H, ABq, J 17.1 MHz, ?-H), 3.58 and 3.42 (2H, ABq, J 18.5 Hz, 2-H), 2.10 (3H, s, $COCH_3$).

Attempted preparation of benzyl 6α-acetamido 6β-trimethylsilyloxypenicillanate

Benzyl 6-oxopenicillanate (0.148 g 0.485 mmol) was dissolved in DCM (10 ml) and N-trimethylsilylacetamide (0.64 g, 0.485 ml) was added. The reaction mixture was stirred under nitrogen for 30 minutes, after which the progress of the reaction was checked using infrared spectroscopy: ν_{max} (CH_2Cl_2) 3415 (amide NH), 2860, 1833 (6-oxo C=O), 1790 (β-lactam C=O), 1745 (ester C=O), 1670 (amide C=O), 1420, 1200, 890 cm^{-1}. The reaction was also checked after 3 and 4 hours and a similar infrared spectrum was obtained. Additional N-trimethylsilyl-acetamide (0.636 g, 0.485 mmol) was added 4 hours after the reaction was commenced, and the reaction mixture was stirred under the same conditions for a further 18 hours. After this time, infrared spectroscopy showed that the 6-oxo carbonyl group was no longer present, and that there was a strong amide carbonyl absorbance.: ν_{max} (after 22 hours) (CH_2Cl_2) 3520, 3410, 2960, 1790 (β-lactam C=O), 1740 (ester C=O), 1670 (amide C=O) 1595, 1375, 1200, 1185, 850 cm^{-1}. The reaction mixture was then added to ethyl acetate (50 ml), and washed with dilute HCl (2 x 30 ml), water (1 x 30 ml) and brine (1 x 30 ml). The organic phase was then dried (Na_2SO_4) and the solvent removed in vacuo, which left an oily residue: μ_{max} 1790 (β-lactam C=O), 1740 (ester C=O), 1200, 1185, 850 cm^{-1}. The absence of an

amide carbonyl absorbance in this spectrum indicated that the reaction had failed.

Benzyl 6α-(N-trimethylsilylacetamido)6β-trimethylsilyl oxypenicillanate (85)

To a solution of benzyl 6-oxopenicillanate (24) (0.358 g, 1.17 mmol) in DCM (15 ml), was added bistrimethylsilylacetamide (0.238 g, 1.17 mmol). The reaction mixture was stirred at room temperature for 1 hour, after which infraspectroscopy indicated that all of (24) had been consumed. Removal of the solvent in vacuo gave the **title compound** (0.512 g, 88%) as an oil: ν_{max} (CH_2Cl_2) 3420, 2960, 1780 (β-lactam C=O), 1740 (ester C=O), 1660 (amide C=O), 1420, 1305, 1180, 845 cm^{-1}; δ_H(80 MHz, CD_2Cl_2) 7.38 (5H, s, aromatic H), 5.58 (1H, s, 5-H), 5.19 (2H, s, benzyl CH_2), 4.44 (1H, s, 3-H), 1.98 (3-H, s, amide CH_3), 1.55 (3H, s, CH_3), 1.40 (3H, s, CH_3), 0.22 and 0.10(18H, 2 x s, TMS-H); m/z (FAB) 510 (MH^+), 483, 437, 409, 250, 132.

Attempted removal of trimethylsilyl groups from (85)

A. Using TBAF and methanol

A solution of (85) (0.031 g, 0.06 mmol) in dry THF (15 ml) was cooled down to -78°C. Methanol (0.0024 ml, 0.06 mmol) and TBAF (0.0189 g, 0.06 mmol) in THF (2 ml) were added. The reaction mixture was stirred at this temperature for 3 hours, after which ethyl acetate (15 ml) was added. The resulting solution was washed with water

(3 x 15 ml) and the organic phase was dried (Na_2SO_4). Finally the solvent was removed in vacuo, which left a yellow oil. The infrared spectrum of this showed that the β-lactam ring had cleaved.

B. Using water

A sample of (85) was shaken with water. The infrared spectrum of the product showed that β-lactam ring cleavage had occurred.

Attempted preparation of t.butyl 7α-N-methylacetamido 7β-trimethylsilyloxycephalosporanate

N-methyl N-trimethylsilylacetamide (0.55 g, 0.38 mmol) was added to a solution of t.butyl 7-oxocephalosporanate (0.119 g, 0.38 mmol) in chloroform (25 ml). The reaction mixture was stirred under argon for 4 hours. During this time the progress of the reaction was monitored every 30 minutes using infrared spectroscopy, which showed that no reaction was taking place: ν_{max} ($CHCl_3$) 3450, 1833 (7-oxo C=O), 1790 (β-lactam C=O), 1745 (ester C=O), 1730 (ester C=O), 1660 (amide C=O), 1344, 1230, 1158, 1045 cm^{-1}.

Reaction of t.butyl 7α,7β-dihydroxycephalosporanate with bistrimethylsilylformamide (84)

Bistrimethylsilylformamide (0.043 g, 0.19 mmol) was added to a solution of (76) (0.062 g, 0.19 mmol) in DCM (5

ml), and the resulting reaction mixture was stirred at room temperature for 2.5 hours. The infrared spectrum of the crude product showed that all of the starting material had reacted: ν_{max} (CH_2Cl_2) 3400, 2990, 1790 (β-lactam C=O), 1745 (ester C=O), 1730 (ester C=O), 1670, 1610 cm^{-1}.

The reaction mixture was then added to ethyl acetate (25 ml) and washed with water (2 x 20 ml). The organic phase was dried (Na_2SO_4) and the solvent removed in vacuo. Tlc (EtOAc/hexane 1:1) indicated that the product consisted of three components: R_f 0.89, 0.36, 0.05. Purification of this mixture was afforded by wet flash silica gel chromatography using the same solvent system. Some of the product decomposed on the silica, and the silica turned red. Two compounds were isolated:

A. 7α,7β-bistrimethylsilyloxycephalosporanate (0.026 g, 29%): R_f (EtOAc /hexane 1:1) 0.89; ν_{max} (CH_2Cl_2) 2990, 1785 (β-lactam C=O), 1740 (ester C=O); δ_H (250MHz $CDCl_3$) 5.00 and 4.75 (2H, ABq, J 13.0 Hz, 3-CH_2), 4.67 (1H, s, 6-H), 3.48 and 3.25 (2H, ABq, J 18.0 Hz, 2-H), 2.09 (3H, s, $COCH_3$), 1.57 (9H, s, t.butyl-H), 0.26 and 0.27(18H, 2 x s, TMS-H).

B. 7α-formamido 7β-hydroxycephalosporanate (86) (0.006 g, 8%) was crystallised using ether/petether and obtained as a white solid: R_f (EtOAc/hexane 1:1) 0.36; ν_{max} 3410, 1785 (β-lactam C=O), 1740 (ester C=O), 1670 (amide C=O), 1610 cm^{-1}; δ_H(250 MHz, CD_3COCD_3) (major rotamer only) 8.43 (1H, b, NH), 8.30 (1H, s, CHO), 6.72 (1H, s, OH), 5.09 (1H, s,

6-H), 5.01 and 4.74 (2H, ABq, J 12.9 Hz, 3-CH_2), 3.40 and 3.70 (2H, ABq, J 18.1 Hz, 2-H), 2.05 (3H, s, $COCH_3$), 1.55 (9H, s, t.butyl-H).

Benzyl 6-[2'-cyclohexanespiro-1',3'-dioxolan-4'-one-5'-ylidene] penicillanate (95)

The phosphonium bromide (88) (1.67 g, 3.36 mmol) was dissolved in toluene (30 ml), and 1,4-diazabicyclo[2,2,2]-octane (0.337 g, 3.36 mmol) was added. The reaction mixture was stirred at 60°C for 10 minutes. During this period the reaction mixture turned yellow. Benzyl 6-oxopenicillanate (24) (1.025 g, 3.36 mmol) was then added. The reaction mixture was stirred at the same temperature for a further 15 minutes and then allowed to cool down to room temperature over 30 minutes. The progress of the reaction was then checked by tlc (EtOAc/hexane 1:1), which showed that all of (24) had been consumed: R_f (24) 0.26; R_f (reaction mixture) 0, 0.09, 0.48. The solid triphenylphosphine oxide residue was removed by filtration and the solvent was removed in vacuo. This left a red coloured oil which was purified using wet flash silica gel chromatography (EtOAc/hexane 1:1). This resulted in a yellow oil (1.205 g, 81%). All attempts to recrystallise the product failed: R_f (EtOAc/hexane 1:1) 0.48; ν_{max} (CH_2Cl_2) 2960, 1795 (dioxolanone C=O), 1770 (β-lactam C=O), 1740 (ester C=O), 1175, 1140, 930 cm^{-1}; λ_{max} (MeOH) 274 nm, δ_H(200 MHz, $CDCl_3$) 7.30 (5H, s, aromatic H), 5.94

(1H, s, 5-H), 5.12 (2H, s, benzyl CH_2), 4.49 (1H, s, 3-H), 1.65 (10H, bdm, cyclohexane H), 1.50 and 1.33 (6H, 2s, methyl H); m/z (FAB) 444 (MH^+), 400, 354, 250, 114; hrms, found 444.14808, $C_{23}H_{26}NO_6S$ requires 444.14807 (< 1 ppm).

Sodium 6-[2'cyclohexanespiro-1',3'-dioxolan-4'-one-5'-ylidene] penicillanate

Sodium salt of (96)

Activated palladium over charcoal (3.00 g) was added to a solution of the benzyl ester (3.00 g, 6.77 mmol) in THF (50 ml). This was then stirred under an atmosphere of hydrogen for 15 hours after which the progress of the reaction was checked by tlc (EtOAc/hexane 1:1). This indicated that no starting material remained: R_f (starting material) 0.48; R_f (reaction mixture) 0 (baseline). The catalyst was removed by filtration through celite and the solvent removed in vacuo which left a pale yellow foam (1.58 g, 4.63 mmol). The residue was dissolved in acetone (75 ml) and a solution of sodium ethylhexanoate (0.845 g, 5.09 mmol) in methylisobutylketone (75 ml) was added. The resulting mixture was stirred for 30 minutes at room temperature, after which it was added to ethyl acetate (50 ml) and water (50 ml). The aqueous phase was lyophilised which left an off-white solid (1.668 g, 65%): ν_{max} (KBr disc) 1795 (dioxolanone C=O), 1770 (β-lactam C=O), 1615, 1270, 1200, 930 cm^{-1}; λ_{max} (H_2O) 270 nm; δ_H (200 MHz, D_2O) 6.00 (1H, s, 5-H), 4.29 (1H, s, 3-H), 1.55 and 1.49 (6H, 2s, methyl H), 1.00 - 2.00 (10H, b,

cyclohexane H); m/s (-veFAB) 352 (M+), 332, 310, 291, 255, 210, 107; hrms, found 352.08550, $C_{16}H_{18}NO_6S$ requires 352.08547 (< 1 ppm).

Attempted preparation of benzyl 6β-(1'-one-2'-carboxyl) penicillanate barium salt (101)

0.0579M Barium hydroxide (1.95 ml, 0.113 mmol) was added to a solution of (95) (0.10 g, 0.226 mmol) in DMF (10 ml). The reaction mixture immediately went dark yellow. The reaction mixture was stirred for 1 hour at room temperature, after which it was added to ethyl acetate (20 ml) and water (20 ml). The organic phase was removed, washed with water (1 x 20 ml), and the combined aqueous phases were lyophilised. A solid remained that was analysed by infrared spectroscopy. This indicated that there was no β-lactam C=O present: ν_{max} (KBr disc) 3450, 2990, 1740, 1330, 1150, 1010 cm^{-1}.

Attempted preparation of t.butyl 7-[2'-cyclohexanespiro-1',3'dioxolan-4'-one-5'-ylidene] cephalosporanate (98)

The phosphonium bromide (88) (4.70 g, 9.47 mmol) was dissolved in toluene (50 ml) and 1,4-diazabicyclo[2,2,2]-octane (1.06 g, 9.47 mmol) was added. This mixture was stirred at 60°C for 10 minutes, which was followed by the addition of t.butyl 7-oxocephalosporanate (76) (3.09 g, 9.47 mmol) in toluene (50 ml). The reaction mixture was

stirred at 60°C for 10 minutes and then allowed to cool down to room temperature over a further 20 minutes. After this, tlc (EtOAc/hexane 1:1) indicated that all starting material had been consumed: R_f (76) 0.29; Rf (reaction mixture) 0, 0.33, 0.38. The triphenylphosphine oxide residue was removed by filtration through celite and the solvent was removed in vacuo which gave the crude product (3.57 g, 87%) as a brown oil. This was purified using wet flash silica gel chromatography (EtOAc/Hexane 3:2). Some of the column fractions contained material that was only one spot on tlc: R_f (EtOAc/hexane 1:1) 0.38, and others contained a mixture of products: R_f (EtOAc/hexane 1:1) 0.38, 0.33. The similar fractions were bulked together and the solvent was removed in vacuo. The pure product (A) (1.98 g, 45%) and the mixture (B) (1.27 g, 29%) were both obtained as yellow foams.

(A): ν_{max} 1800, 1770 (β-lactam C=O), 1740 (ester C=O), 1370, 1225, 1150, 1090 cm^{-1}; λ_{max} (CH_3CN) 275 nm; δ_H(80 MHz, $CDCl_3$) (intensities not given for signals that are different in the Δ-2 and Δ-3 isomers) 6.40 (s, 2-H Δ-2), 5.75 (s, 4-H Δ-2), 5.32 (s, 6-H Δ-2), 5.00 (s, 6-H, Δ-3), 5.12-4.48 (2H, 2ABq, 3-CH_2 Δ-2 and Δ-3) 3.60 and 3.27 (ABq, 2-H Δ-3), 2.05 (3H, s, $COCH_3$), 1.90 (10H, bd, cyclohexane H), 1.55 and 1.48 (9H, 2s, t.butyl H Δ-2 and Δ-3); hplc R_t (system 1) 25.1 minutes.

(B): ν_{max} 1800, 1770 (β-lactam C=O), 1740 (ester C=O), 1370, 1225, 1150, 1090 cm^{-1}; λ_{max} (CH_3CN) 275 nm; δ_H(80 MHz, $CDCl_3$) (intensities not given for signals that are

affected by the mixture of isomers) 6.40 (bd, 2-H Δ-2), 5.71 (s, 4-H Δ-2) 5.36, 5.22 and 4.94 (3s 6-H Δ-2 and Δ-3), 5.10-4.45 (2ABq, 3-CH_2 Δ-2 and Δ-3), 3.58 and 3.25 (ABq, 2-H D-3), 2.05 (3H, s, $COCH_3$), 1.80 (10H, bd, cyclohexyl H), 1.50 and 1.47 (9H, 2s, t.butyl H Δ-2 and Δ-3); hplc R_t (system 1) 24.8, 25.1 minutes.

t.Butyl 7-[2'-cyclohexanespiro-1',3'dioxolan-4'-one-5'-ylidene]cephalosporanate sulphoxide (99)

To a solution consisting of a mixture of Δ-2 and Δ-3 sulphides (97) and (98) (see above) (1.00 g, 2.15 mmol) in 1,4-dioxane (30 ml) and pH 7 buffer (10 ml) was added m.chloroperoxybenzoic acid (0.445 g, 2.15 mmol). The reaction mixture was stirred at room temperature for 1 hour, after which it was added to chloroform (30 ml) and water (30 ml). The organic phase was separated, washed with water (2 x 50 ml) and brine (1 x 50 ml), and dried (Na_2SO_4). Removal of the solvent in vacuo gave the crude product (0.807 g, 78%), which was purified by dry flash silica gel chromatography (EtOAc/hexane 3:2). This resulted in the **title compound** (0.724 g, 70%): R_f (EtOAc/hexane 1:1) 0.10; ν_{max} (CH_2Cl_2) 2960, 1785, 1735, 1368, 1220, 1150 cm^{-1}; λ_{max} (CH_3CN) 275 nm; δ_H(200 MHz, $CDCl_3$) 5.10 (1H, s, 6-H), 5.22 and 4.63 (2H, ABq, J 13.5 Hz, 3-CH_2), 3.80 and 3.24 (2H, ABq, J 18.4 Hz, 2-H), 2.02 (3H, s, $COCH_3$), 2.00-1.60 (10H, bd, cyclohexyl-H), 1.52 (9H, s, t.butyl-H); δ_C(50 MHz, $CDCl_3$) 170.2, 160.5, 159.0,

155.0 (4 x C=O), 135.5, 128.5, 117.5, 108.5, 112.5 (4 C=C and cyclohexyl quaternary C), 84.0 (t.butyl quaternary C), 67.2 (C-6), 63.5 (3-CH_2), 46.2 (C-2), 36.0, 35.5, 23.5, 22.5 (cyclohexyl CH_2), 27.5 (t.butyl-CH_3), 20.5 (acetyl-CH_3); hplc R_t (system 1) 22.7 minutes; m/z (FAB) 482 (MH^+), 458, 426, 366, 240, 156; hrms, found 482.14849, $C_{22}H_{28}NO_9S$ requires 482.14846 (< 1 ppm).

t.butyl 7-[2'-cyclohexanespiro-1',3'-dioxolan-4'-one-5'-ylidene] cephalosporanate (98)

Potassium iodide (0.249 g, 1.5 mmol) and acetyl chloride (0.064 ml, 0.9 mmol) were added to a solution of the sulphoxide (99) (0.145 g, 0.3 mmol) in acetone (20 ml). The reaction mixture was stirred at 0°C, under argon for 45 minutes, after which tlc (EtOAc/hexane 7:3) showed that no sulphoxide remained: R_f (sulphoxide) 0.37; R_f (reaction mixture) 0, 0.65. Ethyl acetate (30 ml) was then added and the reaction mixture was washed with 2% sodium metabisulphite in brine (2 x 50 ml), brine (1 x 50 ml), saturated $NaHCO_3$ (1 x 50 ml), and brine (1 x 50 ml). The organic phase was dried (Na_2SO_4) and the solvent removed in vacuo, which left a yellow oil (0.418 g, 60%). This was purified using wet flash silica gel chromatography (EtOAc/hexane 1:3). This resulted in the **title compound** (0.391 g, 56%) as a yellow solid: m.p. 108-110°C (Dec); R_f (EtOAc/hexane 1:1) 0.38; ν_{max} (CH_2Cl_2) 1775, 1740, 1370, 1225, 1150, 1090, 1045 cm^{-1}; λ_{max} (CH_3CN) 275 nm;

δ_H(200 MHz, $CDCl_3$) 5.40 (1H, s, 6-H), 5.01 and 4.74 (2H, ABq, J 13.0 Hz, 3-CH_2), 3.56 and 3.27 (2H, ABq, J 18.3 Hz, 2-H), 2.75 (3H, s, $COCH_3$), 2.00-1.50 (10H, bd, cyclohexyl-H), 1.55 (9H, s, t.butyl-H); δ_C(50 MHz, $CDCl_3$) 170.5, 160.2, 160.1 and 156.1 (4 C=O), 135.4, 129.0, 120.2, 117.0 and 116.6 (4 C=C and cyclohexyl quaternary C), 83.7 (t.butyl quaternary C), 63.1 (3-CH_2), 54.8 (C-2), 36.3, 35.9, 23.6, 22.5 and 22.0 (cyclohexyl-CH_2), 27.6 (t.butyl-CH_3), 20.6 (acetyl-CH_3); m/z (FAB) 466 (MH^+), 433, 406, 350, 283, 252; hrms, found 466.15354, $C_{22}H_{28}NO_8S$ requires 466.15355 (< 1ppm).

Attempted preparation of 7-[2'-cyclohexanespiro-1',3'-dioxolan-4'-one-5'-ylidene] cephalosporanic acid

The t.butyl ester (98) (0.20 g, 0.43 mmol) was dissolved in DCM (6 ml) and trifluoroacetic acid (2 ml). The reaction mixture was stirred at room temperature for 15 minutes, after which the progress of the reaction was checked by hplc: R_t (system 2) (98) 16.7 minutes; R_t (system 2) (reaction mixture) 4.1, 6.4, 10.1, 12.2 minutes. This indicated that significant impurities were present. All attempts to purify the mixture failed.

Attempted preparation of t.butyl 7-(1'-one-2'-carboxyl) cephalosporanate barium salt (102)

A solution of barium hydroxide (0.034 g, 0.107 mmol) in water (10 ml) was added to (98) (0.100 g, 0.215 mmol) that had been dissolved in methanol (1 ml). On addition of the barium hydroxide, a precipitate appeared. More methanol (4 ml) was added until the precipitate redissolved. The reaction mixture was stirred at room temperature for 1 hour, after which tlc showed that no starting material remained. The reaction was washed with ethyl acetate (2 x 20 ml), and the aqueous phase was lyophilised. Infrared spectroscopy of the solid residue showed that there was no β-lactam carbonyl present: ν_{max} (KBr disc) 3440, 2995, 1740, 1410, 1210, 1050 cm^{-1}.

Attempted preparation of the 7- substituted cephalosporin tetronic acid (104)

Lithium diisopropylamide.THF (1.5M solution in cyclohexane) (0.29 ml, 0.435 mmol) was added to THF (3 ml) and cooled down to -78°C. To this solution was added methyl 4-methoxyphenylacetate (0.069 ml, 0.435 mmol) and tetramethylethylenediamine (0.066 ml, 0.435 mmol). The resulting mixture was stirred at -78°C for 30 minutes. The cephalosporin sulphoxide (99) (0.20 g, 0.416 mmol) in THF (2 ml) was then added dropwise. The reaction mixture, which immediately turned dark red, was stirred at -78°C for 3 hours, and then allowed to warm up to room

temperature over a further 2 hours. Ether (10 ml) and water (10 ml) were then added. The aqueous phase was extracted with ether (2 x 30 ml), and then acidified to pH 1.5 using 2M HCl. This was once again extracted with ethyl acetate (2 x 50 ml) and the combined organic phases were dried (Na_2SO_4). Removal of the solvent <u>in vacuo</u> left a dark coloured oil. Infrared spectroscopy showed that there was no β-lactam C=O present in the product: ν_{max} (CH_2Cl_2) 1740, 1612, 1520, 1150 cm^{-1}. The ether phase from the extraction was then dried (Na_2SO_4) and evaporated <u>in vacuo</u>. There was no β-lactam carbonyl absorption in the infrared spectrum of this residue: ν_{max} (CH_2Cl_2) 1740, 1615, 1518, 1230, 1160, 1040 cm^{-1}.

Attempted preparation of 6β-[2,2 -bis(4'nitrophenyl)-ethoxycarbonylamino]penicillanic acid (Bnpeoc-APA) (106) direct from 6-aminopenicillanic acid (3)

A. Using Bnpeoc-chloroformate (59)

6-Aminopenicillanic acid (0.259 g, 1.20 mmol) was dissolved in 10% aqueous Na_2CO_3 (2.55 ml, 2.40 mmol) and DMF (2.5 ml). Water (1 ml) was added, so that a clear solution was obtained. This solution was cooled down to 0°C and Bnpeoc-chloroformate (59) (0.351 g, 1.20 mmol) was added. The reaction mixture was stirred at room temperature for 30 minutes, after which it was added to ether (50 ml) and water (50 ml). This was extracted, and the aqueous phase was washed with ether (2 x 50 ml) and ethyl acetate (2 x 50 ml). The aqueous phase was then acidified to pH 1.5 using 2M citric acid. The expected precipitate did not appear at this stage. The aqueous phase was extracted with ethyl acetate (3 x 100 ml) and the combined organic phases were washed with brine (1 x 100 ml) and dried ($MgSO_4$). Removal of the solvent in vacuo left the crude product. This was shown, by hplc, to consist of 5 significant components: R_t (system 3) 6.8, 10.0, 14.0, 18.2, 20.9 minutes. No attempt was made to purify this product, since improved methodology was sought.

B. Using Bnpeoc-ONSu (58)

The general procedure of Rich[66] was followed.

Triethylamine (0.271 g, 2.33 mmol) in 1,4-dioxane (2 ml) was added to 6-aminopenicillanic acid (3) (0.503 g, 2.33 mmol) that had been suspended in water (10 ml). When the 6-APA had dissolved, Bnpeoc-ONSu (58) (1.00 g, 2.33 mmol) was added. The reaction mixture was stirred at room temperature for 2 hours, after which the Bnpeoc ONSu still appeared to be suspended in solution. More 1,4-dioxane (6 ml) was added, after which the reaction mixture was stirred for a further 10 hours. Water (50 ml) was added and the reaction mixture was acidified to pH 3 using saturated potassium hydrogen sulphate. This was then extracted into ethyl acetate (3 x 25 ml). The combined organic phases were dried (Na_2SO_4) and the solvent was removed in vacuo. This left an oil, which was converted into a white solid upon trituration with ether/petroleum ether. Hplc showed that the reaction product was impure, with the major components having similar retention times as the product from method A (above): R_t (system 3) 6.8, 10.4, 14.0, 18.5, 21.0 minutes. Three purification techniques; chromatography using both reverse phase silica gel and dianion HP20SS resin (supplied by Beecham Pharmaceuticals), and gel filtration, were attempted on the reaction product, but the **title compound** was not isolated in a pure form.

Attempted preparation of 6β-[9-fluorenylmethoxycarbonylamino]penicillanic acid (Fmoc-APA) (109) direct from 6-aminopenicillanic acid (3)

A. Using Fmoc-chloroformate (52)

6-Aminopenicillanic acid (0.10 g, 0.463 mmol) was dissolved in 10% sodium carbonate (1.00 ml, 0.926 mmol) and acetone (2 ml). Fmoc-chloroformate (52) (0.120 g, 0.463 mmol) was added to this. The reaction mixture was stirred for 24 hours, after which it was acidified to pH 3 using 2M potassium hydrogensulphate, and added to ether (20 ml) and water (20 ml). The organic phase was separated and the aqueous layer washed with ether (1 x 20 ml) and ethyl acetate (1 x 20 ml). The combined organic phases were then washed with brine (1 x 30 ml), dried (Na_2SO_4) and the solvent removed in vacuo. A yellow oily residue remained, which hplc showed to be impure: R_t (system 3) 6.2, 7.8 (strong), 10.2 (strong), 18.2, 20.8 (strong).

B. Using Fmoc-chloroformate (52)

The general procedure of Rich[66] was followed.

Triethylamine (0.701 g, 6.94 mmol) in 1,4-dioxane (10 ml) was added to 6-aminopenicillanic acid (1.00 g, 4.63 mmol) that had been suspended in water (10 ml). When the 6-APA had dissolved, Fmoc-Cl (52) (1.795 g, 4.63 mmol) was added. The reaction mixture was stirred at room temperature for 10 hours, after which it was acidified to pH 3 using 2M potassium hydrogensulphate. Ethyl acetate (50 ml) and water (50 ml) were then added and the organic

phase was separated. The aqueous phase was washed with ethyl acetate (2 x 50 ml) and the combined organic phases were dried (Na_2SO_4). Removal of the solvent <u>in vacuo</u> left a white solid. Hplc of this showed that impurities were present: R_t (system 3) 7.9, 10.3, 16.1, 18.1, 21.0 minutes. The peak at 10.3 minutes was considerably larger than the rest. Purification was attempted using gel filtration, but even though the gel filtration trace indicated that some separation had been achieved, hplc of the recovered material indicated that very little improvement had resulted.

C. Using Fmoc-ONSu (54)

The procedure described above, (B), was repeated using the active ester derivative of Fmoc (54) instead of Fmoc-chloroformate (52). 6-aminopenicillanic acid (0.20 g, 0.962 mmol) was reacted with Fmoc-ONSu (0.312 g, 0.962 mmol) and triethylamine (0.140 g, 1.443 mmol), in water (3 ml) and 1,4-dioxane (3 ml). Following workup, a white solid was obtained. This was analysed by hplc: R_t (system 3) 6.0, 9.0, 10.1, 15.8, 18.0 minutes. Previous studies had indicated that, under similar hplc conditions, Fmoc-olefin (110) had a retention time of 18.0 minutes, Fmoc-ONSu a had retention time of 15.9 minutes, N-hydroxysuccinimide had a retention time of 8.9 minutes and that the triethylamine salt of 6-APA had a retention time of 6.0 minutes.

D. Using Fmoc-ONSu (54)

The reaction described above, (C), was repeated using acetonitrile as the solvent instead of 1,4-dioxane. Hplc of the crude reaction product indicated that less Fmoc-olefin was produced in this case. However significant amounts were still present.

E. Using Fmoc-ONSu (54)

The reaction described above, (D), was repeated using N-methylmorpholine as the base instead of triethylamine. Hplc of the crude reaction product indicated that the amount of olefin produced had increased compared to the reaction with triethylamine.

Attempted purification of Fmoc-APA (109) by formation of the cyclohexylamine salt

Cyclohexylamine (0.40 g, 4.04 mmol) was added to a solution of the crude reaction product (109) (1.76 g, 4.04 mmol), that had been prepared by method C above, in ethyl acetate (15 ml). A white precipitate appeared within a few minutes. The reaction mixture was stirred at room temperature for 1.5 hours. The precipitate was then removed by filtration and washed thoroughly with ethyl acetate, which on drying, left a white solid (1.062 g)

The solid formed above was suspended in ethyl acetate (10 ml), and citric acid (10 ml) was added. The reaction mixture was stirred at room temperature for 2 hours, then

allowed to stand for 5 minutes before removing the organic phase. This was washed with citric acid (1 x 20 ml), water (1 x 20 ml) and brine (1 x 20 ml), and dried (Na_2SO_4). Removal of the solvent in vacuo left a white solid (0.454 g) which was recrystallised using ether/petroleum ether. Hplc showed that the product consisted of one major constituent and four minor constituents: R_t (system 3) 6.0, 8.8, 10.0 (major), 11.9, 15.8. Infrared spectroscopy indicated that a β-lactam C=O group was present: ν_{max} (KBr disc) 3420, 1790 (β-lactam C=O), 1730, 1520, 1240, 1180 cm^{-1}.

Attempted preparation of 7β -[2,2 -bis(4'nitrophenyl) ethoxycarbonylaminocephalosporanic acid (Bnpeoc-ACA) (107) direct from 7-aminocephalosporanic acid (9)

A. Using Bnpeoc-chloroformate (59)

7-Aminocephalosporanic acid (0.10 g, 0.37 mmol) was dissolved in 10% aqueous sodium carbonate (0.7 ml, 0.74 mmol) and acetonitrile (10 ml). The solution was cooled down to 0°C, and Bnpeoc-chloroformate (0.150 g, 0.42 mmol) in acetonitrile (2 ml) was added. The reaction mixture was stirred at 0°C for 30 minutes and then allowed to warm up to room temperature over a further 30 minutes. The reaction mixture was then washed with ethyl acetate and the resulting aqueous phase was lyophilised with the intent of recovering the sodium salt of the **title**

compound. ^1H N.m.r of the recovered solid indicated that the reaction had not worked.

B. Using Bnpeoc-ONSu

To a solution of Bnpeoc-ONSu (0.167 g, 0.4 mmol) in acetonitrile (8 ml), a solution of 7-aminocephalosporanic acid (0.10 g, 0.367 mmol) in water (4 ml) and triethylamine (0.073 g, 0.55 mmol) was added dropwise over 15 minutes. The reaction mixture was then stirred at room temperature and the progress of the reaction was followed by hplc: R_t (system 3) Bnpe-olefin (108) 16.5 minutes, Bnpeoc-ONSu 113.5 minutes, N-hydroxysuccinimide 3.3 minutes, 7-ACA in H_2O/Et_3N 3.1 minutes; R_t (system 3) Reaction mixture, after 30 minutes 3.1, 3.3, 9.4 (small), 10.2 (small), 12.2, 13.4 minutes, after 2 hours 3.0, 3.3. 9.5 (small), 10.3 (small), 12.3, 13.4 minutes, after 6 hours 3.1 (small), 3.3, 9.3, 9.8, 10.1, 12.1 (major), 13.1 (small) minutes. In view of the purification problems experienced with the penicillin reactions, no attempt was made to isolate the product from this reaction.

Benzyl 6β-[2,2 -bis(4'nitrophenyl)ethoxycarbonylamino] penicillanate (Bnpeoc-APA-OBz) (116)

Bnpeoc-chloroformate (1.60 g, 5.10 mmol) and pyridine (0.40 g, 5.10 mmol) were added to a solution of benzyl 6β-aminopenicillanate (1.40 g, 4.60 mmol) in DCM (50 ml) at 0°C. The reaction mixture was stirred at this

temperature for 15 minutes and allowed to warm up to room temperature over a further 30 minutes. Tlc after this showed that all of the starting material had been consumed: R_f (EtOAc/hexane 1:1) (benzyl 6β-amino-penicillanate) 0.20, (reaction mixture) 0.35, 0.46 (major), 0.70. The reaction mixture was then added to ethyl acetate (100 ml) and 0.5M HCl (100 ml). The organic phase was separated and washed with saturated $NaHCO_3$ (1 x 100 ml) and brine (1 x 100 ml). After drying (Na_2SO_4), the solvent was removed <u>in vacuo</u> which left a yellow oil. This was purified using wet flash silica gel chromatography (EtOAc/hexane 1:1). Removal of the solvent <u>in vacuo</u>, from the fractions containing material with R_f 0.46, left a white solid (2.567g, 90%): m.p. 73-75°C; ν_{max} (CH_2Cl_2) 3420, 1790 (β-lactam C=O), 1740 (ester C=O), 1520 (Bnpeoc NO_2), 1350 (Bnpeoc NO_2), 1240, 1050 cm^{-1}; δ_H(80 MHz, $CDCl_3$) 8.17 (4H, d, J_{AB} 8.7 Hz, Bnpeoc aromatic H), 7.27 (4H, d, J_{AB} 8.7 Hz, Bnpeoc aromatic H), 7.34 (5H, s, benzyl aromatic H), 5.38 (2H, b, NH and 5-H), 5.27 (2H, s, benzyl CH_2), 4.65 (3H, m, Bnpeoc CH and CH_2), 4.40 (1H, s, 3-H), 1.55 (3H, s, CH_3), 1.38 (3H, s, CH_3); m/z (FAB) 621 (MH^+), 575, 502, 486, 350; hrms, $C_{30}H_{29}N_4O_9S$ requires 621.64054, found 621.64057 (< 1 ppm).

Attempted preparation of 6 β-[2,2 -(4'nitrophenyl)ethoxy-carbonylaminopenicillanic acid (Bnpeoc-APA) (106) from the benzyl ester (Bnpeoc-APA-OBz) (116)

The method of Olah et al[99] was attempted.

Trimethylsilyl chloride (0.05 ml, 0.378 mmol) was added to a solution of Bnpeoc-APA-OBz (116) (0.117 g, 0.189 mmol) and sodium iodide (0.056 g, 0.378 mmol) in acetonitrile (10 ml). The reaction mixture was stirred at room temperature, and the progress was monitored by tlc. After 45 minutes only a small amount of material corresponding to the starting material remained, and there was a strong polar component that turned blue when sprayed with Mary's reagent: R_f ($MeOH/CHCl_3$ 1:9) (starting material) 0.60; (reaction mixture) 0.15 (+ve Mary's reagent), 0.37, 0.67 (weak). Water (20 ml) was then added and the product was extracted into ether (2 x 30 ml). The organic phase was washed with water (1 x 30 ml) and saturated sodium thiosulphate (1 x 30 ml) and then dried (Na_2SO_4), Removal of the solvent in vacuo left a white solid which was analysed by hplc: R_t (system 3) 12.1, 14.5, 16.4, 20.4 (weak), 21.6 (weak), 23.5 (weak) minutes. No attempts were made to purify this mixture in view of the problems that were experienced in earlier experiments.

Benzyl 6β -[9-fluorenylmethoxycarbonylamino]penicillanate (Fmoc-APA-OBz) (113)

A similar procedure to that described above for the synthesis of Bnpeoc-APA-OBz (116) was followed, using Fmoc-chloroformate instead of the Bnpeoc chloroformate. Benzyl 6 β -aminopenicillanate (1.021 g, 3.02 mmol) was reacted with pyridine (0.24 ml, 3.02 mmol) and Fmoc-chloroformate (0.861 g, 3.02 mmol) in DCM (30 ml). After workup, a yellow oil was obtained. This was purified using wet flash silica gel chromatography (EtOAc/hexane 1:3) and two compounds were isolated.

A. **title compound** (1.242 g, 71%): R_f (EtOAc/hexane 1:1) 0.59; ν_{max} (CH_2Cl_2) 3420, 1790 (β-lactam C=O), 1733 (ester C=O, urethane C=O), 1510, 1220, 1180 cm^{-1}; λ_{max} (MeOH) 265 nm; δ_H(200 MHz, $CDCl_3$) 7.80-7.25 (13H, m, Fmoc aromatic H and benzyl aromatic H), 5.52 (3H, b, NH, 5-H and 6-H), 5.20 (2H, s, benzyl CH_2), 4.47 (1H, s, 3-H), 4.43 (2H, d, J_{AB} 6.3 Hz, Fmoc CH_2), 4.22 (1H, t, J_{AB} 7.1 Hz, Fmoc CH), 1.64 (3H, s, CH_3), 1.43 (3H, s, CH_3); δ_C(50MHz, $CDCl_3$) 173.5 and 167.3 (β-lactam C=O, ester C=O), 154.8 (urethane C=O), 143.4 and 141.1 (Fmoc quaternary aromatic C's), 134.6 (Benzyl quaternary C), 128.5-119.9 (Fmoc aromatic CH's and benzyl aromatic CH's), 70.4, 68.0 and 60.5 (C-3, C-5 and C-6), 67.6 and 67.3 (Fmoc CH_2, benzyl CH_2), 64.7 (C-2), 46.9 (Fmoc CH), 31.7 and 26.8 (2 x CH_3); m/z (FAB) 529 (MH^+), 551 (MNa^+), 410, 351, 291; hrms, $C_{30}H_{29}N_2O_5S$ requires 529.17970, found 529.17968 (< 1 ppm); hplc, R_t (system 1) 25.0 minutes.

B. 9-fluorenylmethanol: m.p 99-100°C (ref[100] 100-101°C); R_f (EtOAc/hexane 1:1) 0.40; δ_H (80MHz, $CDCl_3$) 7.83-7.21 (8H, m, Fmoc aromatic H), 4.50 (2H, s, CH_2), 1.60 (1H, s, CH).

Attempted synthesis of benzyl 6β-[9-fluorenylmethoxycarbonylamino]penicillanate (Fmoc-APA-OBz) (113) using Fmoc-ONSu (54)

Benzyl 6β-aminopenicillanate (0.217 g, 0.71 mmol), Fmoc-ONSu (0.183 g, 0.71 mmol) and pyridine (0.06 ml, 0.71 mmol) were stirred in DCM (30 ml) at room temperature. The reaction was monitored using tlc for 7 days, after which much starting material remained and the reaction was terminated.

6β-[9-Fluorenylmethoxycarbonylamino]penicillanic acid (Fmoc-APA) (109)

Fmoc-APA-OBz (113) (1.171 g, 2.29 mmol) in THF (15 ml) was hydrogenated in the presence of 10% palladium-charcoal catalyst (1.171 g) for 12 hours at room temperature and pressure. After this the progress of the reaction was checked by tlc (EtOAc/hexane 1:1) which showed that starting material remained: R_f 0.59. The spent catalyst was removed by filtration through celite and fresh catalyst (1.171 g) was added. Following hydrogenation for a further 12 hours, tlc (EtOAC/hexane

1:1) showed that only baseline material remained. The reaction mixture was filtered through celite and the solvent removed in vacuo, which left the **title compound** as a colourless oil. This was converted to a solid (0.830 g, 86%) using ether/petether: m.p 121-123°C (Dec); ν_{max} (KBr disc) 3420, 1790 (β-lactam C=O), 1730 (urethane C=O), 1695 (acid C=O), 1510, 1220, 1180 cm^{-1}; δ_H(200MHz, CD_3COCD_3) 8.15-7.55 (8H, m, Fmoc aromatic H), 5.80 (2H, bd, 5-H and 6-H), 4.73 (1H, s, 3-H), 4.70-4.50 (3H, m, Fmoc CH, CH_2), 1.64 (3H, s, CH_3), 1.45 (3H, s, CH_3); δ_C(50 MHz, CD_3COCD_3) 174.2-155.1 (3 C=O), 143.3, 141.1 (Fmoc quaternary aromatic C's), 128.8 and 128.0 (C=C), 127.6-120.2 (Fmoc aromatic C's), 70.5 64.4 and 60.1 (C-3, C-5 and C-6), 67.7 (Fmoc CH_2), 46.7 (Fmoc CH), 26.9 and 25.3 (2 x CH_3); m/z (FAB) 439 (MH^+), 261, 179; hrms, $C_{23}H_{23}N_2O_5S$ requires 439.13276, found 439.132762 (<1 ppm); hplc (system 1) R_t 19.5 minutes.

t.Butyl 7β -[2,2 -bis(4'nitrophenyl)ethoxycarbonylamino cephalosporanate (Bnpeoc-ACA-OtBu) (115)

Pyridine (0.88 ml, 1.1 mmol) and Bnpeoc-chloroformate (0.351 g, 1.0 mmol) were added to a solution of t.butyl 7β-aminocephalosporanate (112) (0.328 g, 1.0 mmol) in DCM (20 ml) at 0°C. The reaction mixture was stirred for 1 hour at this temperature, after which tlc (EtOAc/hexane 1:1) indicated that all starting material had been consumed: R_f (112) 0.15; R_f (reaction mixture) 0.33

(major). The reaction mixture was added to ethyl acetate (30 ml) and 0.5M HCl (30 ml). The organic phase was separated and washed with water (1 x 50 ml), saturated $NaHCO_3$ (1 x 50 ml) and brine (1 x 50 ml). Drying of the organic phase (Na_2SO_4) followed by removal of the solvent in vacuo gave a yellow oil which was purified by wet flash silica gel chromatography to give the **title compound** as a colourless oil (0.558 g, 79%): ν_{max} (CH_2Cl_2) 3425, 1790 (β-lactam C=O), 1730 (2 ester C=O, urethane C=O), 1520 (NO_2), 1350 (NO_2), 1225, 1155, 1050 cm^{-1}; δ_H(200 MHz, $CDCl_3$) 8.20 (4H, d, J_{AB} 8.7 Hz, Bnpeoc aromatic H), 7.40 (4H, d, J_{AB} 8.7 Hz, Bnpeoc aromatic H), 5.52 (2H, b, 7-H and NH), 5.05 and 4.79 (2H, ABq, J 13.2 Hz, 3-CH_2), 4.92 (1H, d, J_{AB} 4.4 Hz, 6-H), 4.74-4.60 (3H, m, Bnpeoc CH, CH_2), 3.56 and 3.34 (2H, ABq, J 18.4 Hz, 2-H), 2.06 (3H, s, acetyl CH_3), 1.50 (9H, s, t.butyl H); δ_C(50 MHz, $CDCl_3$) 170.0-154.7 (4 x C=O), 146.5 (Bnpeoc quaternary aromatic C's), 128.8 and 123.4 (Bnpeoc aromatic CH's), 126.4 and 123.6 (C=C), 83.0 (t.butyl quaternary C), 65.9 62.5 and 59.8 (Bnpeoc CH_2, C-2 and 3-CH_2), 60.5 and 56.9 (C-6 and C-7), 48.9 (Bnpeoc CH), 27.1 (t.butyl-CH_3), 20.1 (acetyl-CH_3); hplc R_t (system 4) 14.0 minutes.

7β-[2,2 -(4'Nitrophenyl)ethoxycarbonylamino-cephalosporanic acid (Bnpeoc-ACA) (107)

Trifluoroacetic acid (1.5 ml) was added to Bnpeoc-ACA-OtBu (115) (0.205 g, 0.32 mmol). The resulting solution was stirred at room temperature for 30 minutes, after which tlc (EtOAc/hexane 1:1) showed that all starting material had been consumed. Toluene (5 ml) was added and the solvent was removed in vacuo. This resulted in the **title compound** as a pale yellow solid (0.184 g, 98%): ν_{max} (KBr disc) 3420, 1790 (β-lactam C=O), 1740 (ester C=O, urethane C=O), 1520 (NO_2), 1350 (NO_2), 1220 cm^{-1}; δ_H(200 MHz, $CDCl_3$) 8.16 (4H, d, J_{AB} 8.6 Hz, Bnpeoc aromatic H), 7.40 (4H, d, J_{AB} 8.6 Hz, Bnpeoc aromatic H), 6.61 (1H, b, OH), 5.84 (1H, d, J_{AB} 9.7 Hz, NH) 5.54 (1H, bm, 7-H), 4.96 (1H, d, J_{AB} 4.3 Hz, 6-H), 5.11 and 4.92 (2H, ABq, J 13.9 Hz, 3-CH_2), 4.85-4.60 (3H, m, Bnpeoc CH_2, CH), 3.59 and 3.37 2H, ABq, J 18.6 Hz, 2-H), 2.06 (3H, s, acetyl-CH_3); δ_C(50 MHz, $CDCl_3$) 171.0-155.1 (4 x C=O), 147.0 and 146.6 (Bnpeoc quaternary aromatic C's), 129.1 and 123.9 (Bnpeoc aromatic CH's), 127.6 and 124.9 (C=C), 66.6 and 63.0 (Bnpeoc CH_2 and 3-CH_2), 60.7 and 57.3 (C-6 and C-7), 49.4 (Bnpeoc CH), 26.1 (C-2), 20.5 (acetyl-CH_3); m/z (FAB) 587 (MH^+), 609 (MNa^+), 528; hrms, $C_{25}H_{23}N_4O_{11}S$ requires 587.10839, found 587.10838 (< 1 ppm); hplc (system 1) R_t 20.3 minutes.

t.Butyl 7β-[9-fluorenylmethoxycarbonylamino cephalosporanate (Fmoc-ACA-OtBu) (117)

The procedure described above for the synthesis of Bnpeoc-ACA-OtBu (115) was repeated using Fmoc-chloroformate instead of Bnpeoc-chloroformate. t.Butyl 7-aminocephalosporanate (1.00 g, 3.05 mmol) was reacted with Fmoc-chloroformate (0.80 g, 3.35 mmol) in the presence of pyridine (0.27 ml, 3.35 mmol) with DCM (20 ml) as the solvent. Purification of the crude product by wet flash silica gel chromatography left a colourless oil which was triturated from ether/petether to give the **title compound** as a white solid (1.342 g, 80%): m.p. 103-104°C (dec); R_f (EtOAc/hexane 1:1) 0.67; ν_{max} (CH_2Cl_2) 3420, 1790 (β-lactam C=O), 1730 (2 ester C=O, urethane C=O), 1510, 1223, 1155 cm^{-1}; λ_{max} (MeOH) 264 nm; δ_H(200MHz, $CDCl_3$) 7.79-7.25 (8H, m, Fmoc aromatic H), 5.70-5.52 (2H, m, 7-H and NH), 5.09 and 4.82 (2H, ABq, J 13.2 Hz, 3-CH_2), 5.00 (1H, d, J_{AB} 4.6 Hz, 6-H), 4.42 (2H, d, J_{AB} 6.4 Hz, Fmoc CH_2), 4.24 (1H, t, J_{AB} 6.7 Hz, Fmoc CH), 3.59 and 3.38 (2H, ABq, J 18.5 Hz, 2-H), 2.10 (3H, s, acetyl CH_3), 1.54 (9H, s, t.butyl CH_3); δ_C(50 MHz, $CDCl_3$) 170.4-155.4 (4 x C=O), 143.3 and 141.0 (Fmoc quaternary aromatic C's), 127.6 126.9 124.9 and 119.8 (Fmoc aromatic CH's), 127.3 and 123.6 (C=C), 83.6 (t.butyl quaternary C), 67.6 and 62.9 (Fmoc CH_2 and 3-CH_2), 61.0 and 57.4 (C-6 and C-7), 46.7 (Fmoc CH), 27.6 (t.butyl-CH_3), 26.2 (C-2), 20.6 (acetyl-CH_3); m/z 551 (MH^+), 494, 450, 435, 407, 257, 166; hrms, $C_{29}H_{31}N_2O_7S$ requires 551.18518, found 551.18519 (< 1 ppm);

hplc (system 1) 26.0 minutes.

7β -[9-Fluorenylmethoxycarbonylamino]cephalosporanic acid (Fmoc-ACA) (118)

Trifluoroacetic acid (10 ml) was added to Fmoc-ACA-O<u>t</u>Bu (117) (0.424 g, 0.77 mmol) and the resulting solution was stirred at room temperature for 30 minutes. Toluene (10 ml) was added and the solvent was removed <u>in vacuo</u> which left the **title compound** as a yellow oil. Trituration using ether/petroleum ether resulted in a pale yellow powder (0.332 g, 87%): ν_{max} (KBr disc) 3300, 1790 (β-lactam C=O), 1730 (ester C=O, urethane C=O), 1690 (acid C=O), 1530, 1330, 1235, 1050 cm^{-1}; λ_{max} (MeOH) 265 nm; δ_H(200 MHz, CD_3SOCD_3) 8.63 (1H, d, J_{AB} 8.6 Hz, NH), 7.94-7.29 (8H, m, Fmoc aromatic H's), 5.56 (1H, q, J 4.6 Hz, 7-H), 5.12 (1H, d, J_{AB} 4.8 Hz, 6-H), 5.01 and 4.70 (2H, ABq, J 12.8 Hz, 3-CH_2), 4.40-4.25 (3H, m, Fmoc CH, CH_2), 3.68 and 3.48 (2H, ABq, J 18.1 Hz, 2-H), 2.02 (3H, s, acetyl-CH_3); δ_C(50MHz, CD_3SOCD_3) 170.4-156.1 (4 x C=O), 143.8 and 140.9 (Fmoc quaternary aromatic C's), 127.9 127.3 125.5 and 120.3 (Fmoc aromatic CH's), 126.6 and 123.4 (C=C), 66.6 and 62.9 (Fmoc CH_2 and 3-CH_2), 61.3 and 57.9 (C-6 and C-7), 46.7 (Fmoc CH), 25.8 (C-2), 20.7 (acetyl-CH_3); m/z (FAB) 495 (MH^+), 435; hrms, $C_{25}H_{23}N_2O_7S$ requires 495.12258, found 494.12259 (< 1 ppm); hplc (system 4) R_t 4.0 minutes.

t.Butyl 7β-aminocephalosporanate

Removal of Bnpeoc from Bnpeoc-ACA-OtBu (115)

A. Using DBU and acetic acid

Acetic acid (0.009 ml, 0.158 mmol) and DBU (0.023 ml, 0.158 mmol) were added to a solution of Bnpeoc-ACA-OtBu (115) (0.050 g, 0.078 mmol) in DMF (5 ml). The reaction mixture was stirred at room temperature for 30 minutes and the solvent was removed in vacuo. Infrared spectroscopy showed that the β-lactam ring had cleaved: ν_{max} (CH_2Cl_2) 2950, 1710, 1675, 1645, 1520 (NO_2), 1350 (NO_2) cm^{-1}.

B. Using piperidine

Piperidine (0.007 ml, 0.078 mmol) was added to a solution of Bnpeoc-ACA-OtBu (115) (0.050 g, 0.078 mmol) in DMF 5 ml. The reaction was stirred for 30 minutes after which tlc (EtOAc/hexane 1:1) indicated that no starting material remained: R_f (115) 0.33; R_f (reaction mixture) 0,24, 0.58. The more polar component of the reaction mixture went purple when sprayed with ninhydrin, indicating the presence of a free amine group. The solvent was removed from the reaction mixture in vacuo, and infrared spectroscopy identified the presence of a β-lactam C=O group in the residue: ν_{max} 3420, 1790 (β-lactam C=O), 1740, 1675, 1520, 1350, 1230 cm^{-1}.

A similar result was obtained when solutions of 20% and 50% piperidine in DMF were used.

Benzyl 6β-aminopenicillanate

Removal of Fmoc from Fmoc-APA-OBz (113)

A. Using 20%piperidine/DMF

Fmoc-APA-OBz (113) (0.020 g, 0.037 mmol) was stirred in 20% piperidine/DMF for 15 minutes. The solvent was then removed in vacuo and the infrared spectrum of the residue showed a considerable decrease in the strength of the β-lactam C=O group absorption. Additional 20% piperidine/DMF (5 ml) was added to the residue and the deprotection was stirred for a further 45 minutes, after which the solvent was removed in vacuo. There was no β-lactam C=O absorption in the infrared spectrum of the residue recovered after this period: ν_{max} 3320, 1730, 1670 (s) (DMF C=O), 1495, 1380, 1090 cm^{-1}.

B. Using DBU

DBU (0.004 ml, 0.023 mmol) was added to a solution of Fmoc-APA-OBz (113) (0.010g, 0.019 mmol) in DCM (3 ml). The reaction mixture was stirred at room temperature for 10 minutes after which tlc (EtOAc/hexane 1:1) showed that no starting material remained: R_f (113) 0.59; R_f (reaction mixture) 0, 0.14, 0.70. The component with R_f 0.14 turned purple when the tlc plate was sprayed with ninhydrin, indicating the presence of a free amine group. The stability of the β-lactam system under these conditions was then further tested by adding additional DBU (0.010 ml, 0.057 mmol) and running an infrared spectum of the reaction mixture after a further 20 minutes and after a

total time of 1 hour: ν_{max} (CH_2Cl_2) (after 1 hour) 2915, 1780 (β-lactam C=O), 1740, 1610, 1312, 1185, 960 cm^{-1}.

The attachment of 7β-[2,2-(4'nitrophenyl)ethoxycarbonyl amino]cephalosporanic acid (107) onto the p-benzyloxybenzylalcohol resin

(preparation of Bnpeoc ACA-(O-$CH_2C_6H_4$-OR))

N-methylmorpholine (0.093 ml, 0.85 mmol) was added to a solution of Bnpeoc-ACA (107) (0.500 g, 0.85 mmol) in DCM (5 ml). This was stirred for 1 minute after which diphenylphosphinic anhydride (0.355 g, 0.85 mmol) was added. The resulting solution was stirred at room temperature for 10 minutes and then added to p-benzyloxybenzylalcohol resin (Bachem, 0.79 mmol/g) (0.717 g, 0.57 mmol) which had been swelled in DMA (5 ml) in the presence of DMAP (0.004 g, 0.03 mmol). NMM (0.093 ml, 0.85 mmol) was added and the reaction mixture was agitated for three hours in an ultrasonic bath at room temperature. The resin was filtered, washed with DMA (3 x 30 ml) and DCM (3 x 30 ml), and dried. Infrared spectroscopy showed that some coupling had taken place: ν_{max} (KBr disc) 3300, 2920, 1790 (w) (β-lactam C=O), 1750 (ester C=O), 1605, 1510, 1350 (w) (NO_2), 1240, 1030 cm^{-1}. The coupling yield was found to be 20% (0.158 mmol/g) (Calculated from %N of the resin, obtained from CHN analysis).

The resin was swelled in DMA (5 ml) in the presence of DMAP (0.004 g, 0.03 mmol) and a solution of Bnpeoc-ACA

(107) (0.441 mg, 0.75 mmol), N-methylmorpholine (0.083 ml, 0.75 mmol), and diphenylphosphinic anhydride (0.315 mg, 0.75 mmol), prepared as described above, was added. Following the addition of N-methylmorpholine (0.83 mmol, 0.75 mmol), the reaction mixture was agitated in an ultrasonic bath for 3 hours at room temperature. The resin was then filtered, washed with DMA (3 x 30 ml) and DCM (3 x 30 ml), and dried. The infrared spectrum of the resin had a stronger β -lactam C=O absorption indicating that further coupling had taken place. The coupling yield after this reaction was 42% (calculated from %N of the resin).

The coupling reaction was repeated once more using Bnpeoc-ACA (107) (0.200 g, 0.34 mmol), N-methylmorpholine (0.037 ml, 0.34 mmol) and diphenylphosphinic anhydride (0.142 g, 0.34 mmol). The infrared spectrum of the resin after this reaction had a strong β-lactam C=O absorption: ν_{max} (KBr disc) 3300, 2920, 1790 (β-lactam C=O), 1740 (ester C=O), 1600, 1510, 1350 (NO_2), 1230, 1030 cm^{-1}. A coupling yield of 47% (0.37 mmol/g) was calculated from the %N of the final resin: %N 1.44%, %N for 100% loaded resin 3.06%.

Removal of Bnpeoc from Bnpeoc-ACA-(O-$CH_2C_6H_4$-OR)

(preparation of 7-ACA-(O-$CH_2C_6H_4$-OR))

A. Using 1% and 2% piperidine in DMF

A sample of the loaded resin (0.500 g) was added to a

solution of 1% piperidine in DMF (10 ml). The reaction mixture was agitated in an ultrasonic bath for 5 minutes at room temperature. The resin was filtered, washed with DMF (3 x 30 ml) and DCM (3 x 30 ml), and dried. The dried resin was analysed by infrared spectroscopy: ν_{max} (KBr disc) 3225, 2920, 1790 (β-lactam C=O), 1740, 1600, 1510, 1350 (NO_2), 1220, 1030 cm^{-1}. The presence of the absorbance at 1350 cm^{-1} indicated that full deprotection had not occured. A further quantity of 1% piperidine in DMF (10 ml). The reaction mixture was agitated under the same conditions for 10 minutes after which the resin was washed and dried as before. A qualitative Kaiser test of the final resin indicated that the deprotection had not ocurred. A solution of 2% piperidine in DMF (10 ml) was then added to the resin. This was agitated under the same conditions for 1 hour and the resin recovered as before. The infrared spectrum of the resin after this showed a significant decrease in the strength of the β-lactam C=O absorbance: ν_{max} (KBr disc) 3025, 2920, 1790 (w), 1740, 1605, 1510, 1450, 1220, 1030 cm^{-1}.

B. Using 20% piperidine in DMF

A sample of the loaded resin (0.100 g) was added to a solution of 20% piperidine in DMF (5 ml). The reaction mixture was agitated in an ultrasonic bath for 5 minutes at room temperature. The resin was filtered, washed with DMF (3 x 30 ml) and DCM (3 x 30 ml), and dried. The final resin gave a positive qualitative Kaiser test and infrared

spectroscopy indicated that the β-lactam ring was still intact: ν_{max} (KBr disc) 3025, 2920, 1790 (β-lactam C=O), 1740, 1605, 1510, 1450, 1220, 1025 cm^{-1}.

C. Using 5%, 10% and 20% morpholine in DMF

The loaded resin (0.100 g) was added to a solution of 5% morpholine in DMF (10 ml). The reaction mixture was agitated in an ultrasonic bath for 1 hour at room temperature, after which the resin was filtered, washed with DMF (2 x 30 ml) and DCM (3 x 30 ml), and dried. The final resin gave a negative qualitative Kaiser test, indicating that the deprotection had not taken place. A solution of 10% morpholine in DMF (15 ml) was then added to the same resin sample and the reaction mixture was left, under the same conditions as above, for a further 2 hours. The resin was recovered by filtration, and washed and dried as before. There was still an absorbance at 1350 cm^{-1} in the infrared spectrum of the resin, indicating that Bnpeoc was still present: ν_{max} (KBr disc) 3020, 2920, 1790, 1740, 1605, 1510, 1450, 1350 (NO_2),1215, 1030 cm^{-1}. Finally a solution of 20% morpholine in DMF (15 ml) was added to the same resin sample, and the reaction mixture was agitated under the same conditions as before for a further 2 hours. The intensity of the β-lactam C=O at 1790 cm^{-1} in the infrared spectrum of the resin had decreased significantly after this. Indicating that, as well as removing the protecting group, some β-lactam ring opening was taking place: ν_{max} (KBr disc) 3020, 2920, 1790 (w),

1740, 1605, 1510, 1450, 1215, 1030 cm^{-1}.

Coupling of 2-thiopheneacetylchloride and 7-ACA-(O-$CH_2C_6H_4$-OR)

(preparation of cephalothin-(O-$CH_2C_6H_4$-OR) (119))

DCM (20 ml) was added to the deprotected resin (xx) (0.37 mmol/g) (0.218 g, 0.08 mmol). To this was added 2-thiopheneacetylchloride (0.025 ml, 0.161 mmol) and pyridine (0.02 ml, 0.161 mmol). The reaction mixture was agitated in an ultrasonic bath for 4 hours at room temperature. The resin was then filtered and washed with DCM (3 x 50 ml). The reaction was repeated, using the same quantities of solvents and reagents, to ensure that complete coupling had taken place. The final dried resin gave a negative qualitative Kaiser test and the infrared spectrum had an additional C=O absorption at 1690 cm^{-1}: ν_{max} (KBr disc) 3030, 2920, 1790 (β-lactam C=O), 1740, 1690, 1605, 1510, 1450, 1230, 1030 cm^{-1}.

Cleaving 7β-(2-thiopheneacetyl)aminocephalosporanic acid (cephalothin) (10) from the Wang resin

To the loaded resin (0.570 g, 0.189 mmol) was added a solution of 10% TFA in DCM (15 ml). The reaction mixture was agitated in an ultrasonic bath for 15 minutes at room temperature, after which the resin was removed by filtration and washed with DCM (3 x 30 ml) and toluene (3

x 30 ml). The combined filtrates were evaporated in vacuo which left the crude product as a yellow oil. This was converted into a white powder (0.045 g, 61%) by trituration using ether. Hplc of the crude product indicated that two main components were present, one of which corresponded to a standard sample of cephalothin which was run under the same conditions: R_t (reaction mixture) (system 1) 13.4, 14.0 (major); R_t (10) (system 1) 14.0 minutes. The close proximity of the peaks at 14.0 and 13.4 minutes suggested that they could be due to Δ-2 and -3 cephalosporin analogues. The mixture was purified using preparative hplc, and a sample consisting of a single peak (R_t (system 1) 14.0 minutes) was obtained: ν_{max} ($CHCl_3$) 1780, 1740, 1680, 1503, 1140, 1020 cm^{-1}; δ_H(200MHz, CD_3COCD_3) 8.07 (1H, b, NH), 7.30-6.95 (3H, m, Thiophene ring H's), 5.88 (1H, ABq, J 4.7 Hz, 7-H), 5.15 (1H, d, J_{AB} 4.7 Hz, 6-H), 5.10 and 4.82 (2H, ABq, J 13.8 Hz, 3-CH_2), 3.90 (2H, s, thiophene-CH_2), 3.72 and 3.53 (2H, ABq, J 18.4 Hz, 2-H); m/z (FAB) 397 (MH^+), 337, 293, 274.

7β-(2-Thiopheneacetyl)aminocephalosporanic acid (10)

N,N-Dicyclohexylcarbodiimide (0.412 g, 2.0 mmol) was added to a mixture 2-thiopheneacetic acid (0.284 g, 2 mmol) and hydroxybenzotriazole (0.306 g, 2.26 mmol) in DCM (40 ml). The resulting solution was stirred at room temperature for 5 hours. The solid residue was removed by

filtration, and the filtrate was added to a solution of 7-aminocephalosporanic acid (0.545 g, 2.0 mmol) and triethylamine (1.40 ml, 10.0 mmol) in DCM (10 ml). This was stirred at room temperature for 3 hours, after which the solvent was removed in vacuo. Water (50 ml) was added to the residue and the solution was acidified to pH2 with 10% citric acid. The product was then extracted with ethyl acetate (2 x 50 ml). The combined organic phases were washed with water (2 x 50 ml) and dried (Na_2SO_4). Removal of the solvent in vacuo left a yellow residue which was converted into a pale brown powder (0.610 g, 77%) by trituration with ether: ν_{max} (CH_2Cl_2) 3680, 1790 (β-lactam C=0), 1740, 1690, 1510, 1210, 1140 cm^{-1}; δ_H(200 MHz, CD_3COCD_3) 7.33-6.95 (3H, m, thiophene ring H'S), 5.84 (1H, d, J_{AB} 4.9 Hz, 7-H), 5.13 (1H, d, J_{AB} 4.8 Hz, 6-H), 5.10 and 4,83 (2H, ABq, J 13.1 Hz, 3-CH_2), 3.90 (2H, s, thiophene-CH_2), 3.72 and 3.53 (2H, ABq, J 18.2 Hz, 2-H); m/z (FAB) 397 (MH^+), 337, 225; hplc (system 1) R_t 14.0 minutes.

Investigating the stability of 7β-(2-thiophenacetyl)-aminocephalosporanic acid (10) in 20% piperidine/DMF

7β-(2-Thiopheneacetyl)aminocephalosporanic acid (0.050 g) was stirred in 20% piperidine/DMF (10 ml) for 15 minutes at room temperature. The solvent was then removed in vacuo, and the residue was analysed by hplc. This was compared to hplc traces of the starting material, and of a

sample of the product that had been cleaved from the resin, that had been obtained under the same conditions: R_t (10) (system 5) 21.4 minutes; R_t (product from resin) (system 5) 20.7, 21.4 minutes; R_t (reaction residue) (system 5) 20.7 minutes.

t.Butyl 7β-[9-fluorenylmethoxycarbonylamino] cephalosporanate 1-oxide (121)

Fmoc-ACA-OtBu (117) (4.000 g, 7.27 mmol) and m.chloroperoxybenzoic acid (1.880 g, 10.90 mmol) were stirred in DCM (100 ml) for 10 minutes at room temperature. After this time, tlc indicated that no starting material remained: R_f (EtOAc/hexane 1:1) (reaction mixture) 0.10, 0.34; R_f (117) 0.67. The solvent was then removed in vacuo, and ethyl acetate (50 ml) was added to the residue. This solution was washed with saturated $NaHCO_3$ (2 x 50 ml), the organic phase was dried (Na_2SO_4), and the solvent removed in vacuo. Purification of the crude product was carried out by dry flash silica gel chromatography to give the **title compound** (2.75 g, 66.8%): R_f (EtOAc/hexane 1:1) 0.34; ν_{max} 3400, 1805 (β-lactam C=O), 1735 (2 ester C=O, urethane C=O), 1510, 1223, 1048 cm^{-1}; λ_{max} (MeOH) 265 nm; δ_H(200MHz, $CDCl_3$) 7.75-7.25 (8H, m, Fmoc aromatic H's), 6.22 (1H, bd, 6-H), 5.78 (1H, bm, 7-H), 5.32 and 5.73 (2H, ABq, J 13.7 Hz, 3-CH_2), 4.50-4.15 (3H, m, Fmoc CH, CH_2), 3.77 and 3.20 (2H, ABq, J 18.6 Hz, 2-H), 2.05 (3H, s, acetyl CH_3); δ_C(50 MHz, $CDCl_3$) 170.3-155.4 (4 x C=O),

143.3 and 141.0 (Fmoc quaternary aromatic C's), 127.6-119.8 (Fmoc aromatic CH's), 118.3 (C=C), 84.1 (t.butyl quaternary C), 67.9 and 63.1 (3-CH_2 and Fmoc CH_2), 66.7 and 61.3 (C-6 and C-7), 46.6 (Fmoc CH), 45.7 (C-2), 27.6 (t.butyl-CH_3), 20.5 (acetyl-CH_3); hplc (system 1) R_t 24.0 minutes.

7 β -[9-Fluorenylmethoxycarbonylamino]cephalosporanic acid 1-oxide (122)

7 β -[9-Fluorenylmethoxycarbonylamino]cephalosporanate 1-oxide (121) (2.10 g, 3.71 mmol) was stirred in TFA (15 ml) for 10 minutes at room temperature. Toluene (10 ml) was added to the reaction mixture and the solvent was removed in vacuo. The **title compound** was isolated as a solid (1.88 g, 99%) after triturating with ether/petroleum ether: ν_{max} 3400, 1805 (β-lactam C=O), 1735, 1690, 1530, 1225, 1040 cm^{-1}; λ_{max} (MeOH) 265 nm; δ_H(200 MHz, CD_3SOCD_3) 8.94-7.60 (8H, m, Fmoc aromatic H's), 5.76 (1H, bq, 7-H), 4.97 (1H, b, 6-H), 5.26 and 4.70 (2H, ABq, J 13.1 Hz, 3-CH_2), 4.35 (3H, m, Fmoc CH, CH_2), 3.98 and 3.65 (2H, ABq, J 18.8 Hz, 2-H), 2.05 (3H, s, acetyl-CH_3); δ_C(50 MHz, CD_3SOCD_3) 170.3-155.7 (4 C=O), 143.8 and 140.9 (Fmoc aromatic quaternary C's), 127.9 127.3 125.6 125.5 and 120.3 (Fmoc aromatic CH's), 126.0 and 119.1 (C=C), 66.9 and 63.2 (Fmoc CH_2 and 3-CH_2), 66.5 and 60.7 (C-6 and C-7), 46.6 (C-2), 45.5 (Fmoc CH), 20.8 (acetyl CH_3); hplc (system 1) R_t 18.1 minutes.

The attachment of 7β -[9-fluorenylmethoxycarbonylamino] cephalosporanic acid 1-oxide (122) onto the p-benzyloxy-benzylalcohol resin

(preparation of Fmoc-ACA(1-oxide)-(O-$CH_2C_6H_4$-OR))

7 β -[9-Fluorenylmethoxycarbonylamino]cephalosporanic acid 1-oxide (122) (0.750 g, 1.47 mmol) and N-methylmorpholine (0.161 ml, 1.47 mmol) were added to DMF (5 ml). This was stirred for 1 minute at room temperature after which diphenylphosphinic anhydride (0.614 g, 1.47 mmol) was added. After stirring this solution for 10 minutes at room temperature, it was added to the resin (Bachem, 0.79 mmol/g) (0.930, 0.74 mmol) which had been swelled 1n DMF (10 ml) in the presence of DMAP (0.009 g, 0.07 mmol). N-methylmorpholine (0.161 ml, 1.47 mmol) was added and the reaction mixture was agitated for 3 hours in an ultrasonic bath at room temperature. The resin was then filtered, washed with DMF (3 x 30 ml) and DCM (3 x 30 ml), and dried. The coupling yield of the resin was found to be 9% (UV deprotection study[97]).

The reaction was repeated using the same procedure and quantities of reagents as outlined above. This resulted in the resin being 16% loaded (UV deprotection study[97]).

A third reaction was then carried out on the resin using only half the amount of reagents that were used previously (i.e. 1 equivalent). The final resin was 25% loaded (UV deprotection study[97]) and infrared spectroscopy identified the presence of β -lactam functionality: ν_{max} (KBr disc) 3020, 1804 (β-lactam C=O), 1740, 1605, 1450,

1220, 1030 cm^{-1}.

Oxidation of cephalothin-(O-CH_2-C_6H_4-OR) (119) to cephalothin 1-oxide-(O-CH_2-C_6H_4-OR) followed by reduction back to cephalothin-(O-CH_2-C_6H_4-OR) (119)

The loaded resin (0.710 g, 0.258 mmol) was swelled in DCM (10 ml), and m.chloroperoxybenzoic acid (0.058 g, 0.309 mmol) was added. The reaction mixture was agitated in an ultrasonic bath for 3 hours at room temperature, after which the resin was filtered, washed with DCM (3 x 30 ml), and dried. Infrared spectroscopy indicated that the conversion had taken place: ν_{max} (KBr disc) 3020, 2925, 1803 (β-lactam C=O), 1740, 1680, 1450, 1220, 1030 cm^{-1}. A sample of the resin (0.010 g) was cleaved at this stage. 10% TFA in DCM (1 ml) was added to the resin and the reaction mixture was agitated in an ultrasonic bath for 15 minutes. The resin was removed by filtration and washed with toluene (30 ml). The solvent was removed from the filtrate in vacuo, and the residue was analysed by hplc: R_t (starting material) (system 1) 10.1 (impurity), 13.2 (Δ-2), 14.0 (Δ-3); R_t (reaction residue) (system 1) 9.6 (sulphoxide), 10.1 (impurity).

The remaining loaded resin (0.665 g, 0.24 mmol) was swelled in DCM and phosphorus trichloride (0.043 g, 0.312 mmol) was added. The reaction mixture was agitated in an ultrasonic bath for 1.5 hours at room temperature. The resin was then filtered and washed with DCM (3 x 50 ml).

The product was then cleaved from the resin using 10% TFA in DCM (15 ml), in a similar manner to that described above. Hplc of the residue showed that only a very small amount of the Δ-2 adduct was present, and that the major peak had the same retention time as a sample of cephalothin (10) that had previously been characterised by n.m.r: R_t (10) (system 1) 14.0 minutes; R_t (reaction residue) (system 1) 10.1 (impurity), 14.0 (cephalothin).

The attachment of 6β-[9-fluorenylmethoxycarbonylamino-penicillanic acid (109) onto the p-benzyloxybenzylalcohol resin

(preparation of Fmoc-APA-(O-CH_2-C_6H_4-OR))

N-methylmorpholine (0.17 ml, 1.58 mmol) was added to a solution of Fmoc-APA (109) (0.692 g, 1.58 mmol) in DCM (10 ml). The resulting mixture was stirred for 1 minute, after which diphenylphosphinic anhydride (0.660 g, 1.58 mmol) in DMF (10 ml) was added. After the reaction mixture had been stirred for 10 minutes at room temperature, it was added to the benzyloxybenzylalcohol resin (Bachem, 0.79 mmol/g) (1.00 g, 0.79 mmol) that had been swelled in DMF (5 ml) in the presence of DMAP (0.005 g. 0.04 mmol). N-methyl-morpholine (0.17 ml, 1.58 mmol) was added and the reaction mixture was agitated in an ultrasonic bath for 3 hours at room temperature. The resin was filtered, washed with DMF (3 x 30 ml) and DCM (3 x 30 ml), and dried.

The reaction was then repeated on the same resin, using

the same procedure and quantities of reagents. After this, a coupling yield of 46% (0.348 mmol/g) was obtained (UV deprotection study[97]). This was considered to be sufficient for future work. Infrared spectroscopy identified the presence of β-lactam C=O functionality on the resin: ν_{max} (KBr disc) 3020, 2920, 1790 (β-lactam C=O), 1740, 1605, 1450, 1030 cm^{-1}.

Removal of Fmoc from 6 β-Fmoc-APA-(O-$CH_2C_6H_4$-OR)

(preparation of 6-APA-(O-$CH_2C_6H_4$-OR))

DBU (0.015 ml, 0.083 mmol) was added to the loaded resin (0.200 g, 0.069 mmol) that had been swelled in DCM (15 ml). The reaction mixture was agitated in an ultrasonic bath for 15 minutes at room temperature. The resin was then filtered, washed with DCM (3 x 30 ml) and dried. The final resin gave a positive qualitative Kaiser test, and infrared spectroscopy identified the presence of β-lactam C=O functionality: ν_{max} (KBr disc) 3020, 2920, 1780 (β-lactam C=O), 1740, 1605, 1450, 1170, 1030 cm^{-1}.

Coupling of phenylacetyl chloride and 6-APA-(O-$CH_2C_6H_4$-OR)

(preparation of penicillin V-(O-$CH_2C_6H_4$-OR) (120))

The deprotected resin (0.166 g, 0.060 mmol) was swelled in DCM (10 ml). To this was added pyridine (0.010 ml, 0.12 mmol) and phenoxyacetyl chloride (0.020 ml, 0.12 mmol). The reaction mixture was agitated in an ultrasonic bath

for 2 hours at room temperature, after which the resin was filtered, washed with DCM (3 x 30 ml), and dried. A qualitative Kaiser test on the final resin proved negative, and infrared spectroscopy indicated the presence of an extra C=O absorption at 1700 cm^{-1}: ν_{max} (KBr disc) 3025, 2930, 1795 (β-lactam C=O), 1740, 1700, 1605, 1455, 1170, 1030 cm^{-1}.

Investigating the stability of penicillin V (2) in TFA/DCM

Penicillin V (0.10 g, 0.28 mmol) was stirred in 10% TFA/DCM (15 ml) for 10 minutes at room temperature, after which toluene (10 ml) was added and the solvent was removed in vacuo. The residue was analysed by hplc. The hplc traces were compared to traces obtained from authentic samples that had been run under the same conditions: R_t (system 1) (2) 15.9 minutes; R_t (system 1) (reaction product) 10.0, 12.5, 15.9 (major), 20.8 minutes.

The stability of penicillin V in 1% TFA/DCM was also investigated. Penicillin V (0.10 g, 0.28 mmol) was stirred in 1% TFA/DCM for 15 minutes at room temperature. Toluene (10 ml) was added and the solvent was removed in vacuo. The residue was analysed using the same hplc system as in the previous experiment: R_t (system 1) (reaction product) 10.0, 12.5, 15.9 (major).

Investigating the stability of penicillin V (2) in HCl/1,4-dioxane

Penicillin V (0.100 g, 0.28 mmol) was stirred in a solution of 2M hydrochloric acid (0.5 ml) in 1,4-dioxane (4.5 ml) for 15 minutes at room temperature. The reaction mixture was then added to ethyl acetate (20 ml) and water (20 ml). The resulting aqueous phase was washed with ethyl acetate (3 x 30 ml). The combined organic phases were dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was analysed by hplc.: R_t (system 1), (reaction product) 9.8 (major), 14.5, 15.9 (penicillin V), 20.2.

The stability of penicillin V in a solution of 0.5M hydrochloric acid (0.5 ml) in 1,4-dioxane (4.5 ml) was then investigated. Penicillin V (0.050 g, 0.14 mmol) was stirred in this solution (5 ml) for 15 minutes at room temperature. The product was obtained in a similar manner to that described above, and was analysed by hplc: R_t (system 1) (reaction product) 12.2, 15.9 (penicillin V), 21.1, 24.0.

Investigating the stability of penicillin V (2) in trichloroacetic acid (TCA)/DCM

Trichloroacetic acid (0.035 g, 0.215 mmol) was added to a solution of penicillin V (0.015 g, 0.043 mmol) in DCM (5 ml). The reaction mixture was agitated in an ultrasonic bath for 15 minutes at room temperature after which it was added to ethyl acetate (20 ml) and water (20 ml). The

organic layer was washed with water (3 x 20 ml), dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was analysed by hplc: R_t (system 1) (reaction product) 15.9 minutes.

Attempted cleavage of penicillin V from the Wang resin (120)

The loaded resin (120) (0.010 g, 0.004 mmol) was added to 10% TFA/DCM (2 ml), and the reaction was agitated in an ultrasonic bath for 10 minutes at room temperature, after which the resin was removed by filtration. Toluene (10 ml) was added to the filtrate, and the solvent was removed *in vacuo*. Hplc indicated that extensive decomposition of the product had occured. R_t (system 1) 11.4, 15.9 (small), 20.8, 27.0.

The reaction was repeated using one equivalent of TFA. TFA (0.007 ml, 0.04 mmol) was added to the resin (0.100 g, 0.04 mmol) that had been swelled in DCM (15 ml). The reaction mixture was agitated in an ultasonic bath for 30 minutes at room temperature after which the resin was removed by filtration. Toluene (5 ml) was added to the filtrate, and the solvent was removed *in vacuo*. No residue was visible after removal of the solvent, indicating that the reaction had not taken place.

The attachment of 6β-[9-fluorenylmethoxycarbonylamino] penicillanic acid (109) onto the 2-methoxy-4-alkoxybenzyl alcohol resin (Sasrin resin)

(preparation of FmocAPA-(O-CH_2-C_6H_3(OMe)-OR))

Fmoc-APA (109) (0.468 g., 1.07 mmol) in DCM (10 ml) and N-methylmorpholine (0.12 ml, 1.07 mmol) were added to diphenylphosphinic anhydride (0.447 g, 1.07 mmol) in DMF (20 ml). The reaction mixture was stirred for 10 minutes, after which it was added to the 2-methoxy-4-alkoxybenzylalcohol resin (Bachem, 1.07 mmol/g) (0.50 g, 0.535 mmol) that had been swelled in DMF (10 ml) in the presence of DMAP (0.003 g, 0.027 mmol). N-methylmorpholine (0.12 ml, 1.07 mmol) was added and the reaction mixture was agitated in an ultrasonic bath for 3 hours at room temperature. The resin was then filtered and washed with DMF (3 x 30 ml) and DCM (3 x 30 ml).

The reaction was then repeated on the same resin, using the same conditions and quantities of reactants as before. After this the resin was dried, and was shown to be 23% loaded (UV deprotection study[97]): ν_{max} (KBr disc) 3020, 2920, 1790 (β-lactam C=O), 1735, 1605, 1450, 1150, 1025 cm^{-1}.

The removal of Fmoc from FmocAPA-(O-CH_2-C_6H_3(OMe)-OR)

(preparation of 6-APA-(O-CH_2-C_6H_3(OMe)-OR)

The loaded resin (0.467 g, 0.115 mmol) was swelled in DCM (15 ml) and DBU (0.023 ml, 0.138 mmol) was added. The

reaction mixture was agitated in an ultrasonic bath for 15 minutes at room temperature. The resin was then filtered, washed with DCM (3 x 30 ml), and dried. The final resin gave a positive qualitative Kaiser test: ν_{max} (KBr disc) 3020, 2920, 1785, 1745, 1450, 1153, 1025 cm^{-1}.

Coupling of phenoxyacetyl chloride and 6-APA-(O-$CH_2C_6H_3$-(OMe)-OR)

(preparation of penicillin V-(O-$CH_2C_6H_3$(OMe)-OR)

Phenoxyacetyl chloride (0.027 ml, 0.20 mmol) and pyridine (0.016 ml, 0.20 mmol) were added to the deprotected resin (0.410 g, 0.10 mmol) that had been swelled in DCM (10 ml). The reaction mixture was agitated in an ultrasonic bath for 2 hours at room temperature. The resin was then filtered, washed with DCM (3 x 30 ml), and dried.

The reaction was then repeated using the same conditions and quantities of reagents, to ensure that full coupling had occured. The final resin gave a negative qualitative Kaiser test: ν_{max} (KBr disc) 3025, 2900, 1790 (β-lactam C=O), 1740, 1700, 1605, 1453, 1160, 1030 cm^{-1}.

The cleavage of penicillin V from penicillin V-(O-$CH_2C_6H_3$(OMe)-OR)

Trichloroacetic acid (0.022 g, 0.123 mmol) in DCM (5 ml) was added to the loaded resin (0.100 g, 0.02 mmol)

that had been swelled in DCM (5 ml). The reaction mixture was agitated in an ultrasonic bath for 15 minutes at room temperature. The resin was then removed by filtration, and the filtrate was added to ethyl acetate (20 ml) and water (20 ml). The organic phase was separated, washed with water (2 x 20 ml) and dried (Na_2SO_4). The solvent was removed in vacuo which left the crude product: Hplc, R_t (system 1) (reaction product) 11.5, 15.9, 24.0 minutes; R_t (system 1) (penicillin V) 15.9 minutes. The product was purified using preparative hplc and pure penicillin V (0.021 g , 48%) was obtained as a white solid: δ_H(80MHz, CD_3COCD) 7.65 (1H, bd, NH), 7.48-6.90 (5H, m, phenoxy aromatic H's), 5.77-5.56 (2H, m, 5-H and 6-H), 4.63 (2H, s, acetyl CH_2), 4.42 (1H, s, 3-H), 1.61 and 1.57 (6H, 2s, 2 x CH_3); R_t (system 1) 15.9 minutes.

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Courses attended

Organic research seminars - various speakers, University of Edinburgh.

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X-ray crystallography - Dr. R. O. Gould and Dr. A. J. Blake, University of Edinburgh.

N.m.r spectroscopy - Dr. I. H. Sadler, University of Edinburgh

Mass spectroscopy - Prof. K. R. Jennings, Warwick University

Medicinal chemistry - various speakers, I.C.I. and Beecham.

Medicinal chemistry - Prof. P. G. Sammes, S. K. & F.

Cell biology - Dr. J. Phillips, Biochemistry department, University of Edinburgh.