SYNTHESIS OF NEW β-LACTAMS

A Thesis

by

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fulfillment of the requirements for the degree of
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Department of Chemistry
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Montreal, Quebec, Canada

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To my grandmother,

To my parents.

SYNTHESIS OF NEW β-LACTAMS

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ABSTRACT

The syntheses of nocardicin analogues 31, 52 and cephalosporin analogue 95 are described. None of the above compounds showed the significant antibacterial activity.

The reaction of azidoacetyl chloride with various Schiff bases derived from phenylpropargyl aldehyde and substituted anilines, afforded β-lactams in fair to good yields.

A new bicyclic β-lactam 162 was synthesized. The key step involved the phosphorylation of the nitrogen on an N-unsubstituted β-lactam ring.

A key intermediate 188 for the synthesis of monobactam analogue was been prepared.

SYNTHESE DE NOUVELLES β-LACTAMES

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RESUME

On décrit la synthèse des composés 31 et 52, analogues de la nocardicine, et du composé 95 analogue des céphalosporines. Aucun de ces dérivés ne présente une activité antibiotique marquée.

La réaction du chlorure d'azido-acétyle avec diverses bases de Schiff, provenant de la condensation de l'aldéhyde propargylique sur des anilines substituées, conduit à l'obtention de β-lactames avec de bons rendements.

Une nouvelle β-lactame bicyclique 162 a été synthétisée. L'étape clef met en jeu la phosphorylation de l'atome d'azote d'un cycle β-lactame N-non substitué.

Un intermédiaire clef 188 dans la synthèse d'analogues monobactams a été préparé.

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All my co-workers for their friendship and helpful discussions.

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GLOSSARY OF ABBREVIATIONS

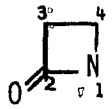
Ac	Acetate
AcOH	Acetic acid
b	Broad
Bn	Benzyl
t-BOC	t-Butyloxycarbonyl
Bu	Butyl
CI-ms	chemical ionization mass spectrometry
CSI	Chlorosulfonyl isocyanate
d	Doublet
DCC	N,N'-Dicyclohexylcarbodiimide
DMF	N,N'-Dimethylformamide
DMSO	Dimethylsulfoxide
Et	Ethyl
g	Gram
gc-ms	Gas chromatography-mass spectrum
^{1}Hmr	Proton magnetic resonance (spectrum)
ir	Infrared (spectrum)
J	Coupling constant
m	Multiplet
Me	Methyl
MIC	Minimum inhibitory concentration (µg/mL)
mL	Milliliter
mmol	Millimole

ms	Mass spectrum
MsCl	Mesyl chloride
NCS	N-chlorosuccinimide
Ph	Phenyl
pmr	Proton magnetic resonance (spectrum)
ppm	Parts per million
PPTS	Pyridinium p-toluenesulfonate
psi	Pounds per square inch
py.	Pyridine
q	quartet
s	singlet
t	Triplet
THP	Tetrahydropyran
tlc	Thin layer chromatography
WSC	1-Ethyl-3[3-(dimethylamino)-propyl]carbodiimide
ϕ	Phenyl

INTRODUCTION

PREFACE

β-Lactams are four-membered heterocyclic compounds of type 1. Though the first member was synthesized by Staudinger in 1907[1], the β-lactams as a class acquired importance since the discovery[2] of penicillin 2, which contains a β-lactam unit as an essential structural feature. Penicillin was the first microbial metabolite found to be toxic to



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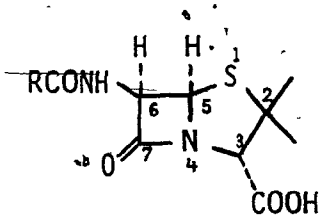

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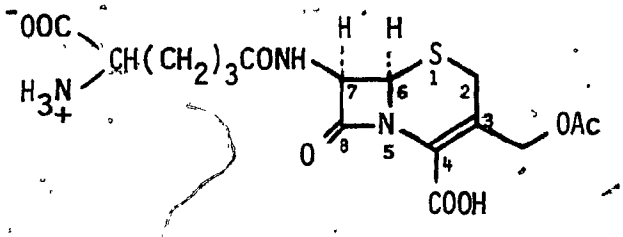
the bacteria but therapeutic to the mammalian host.

In 1955, Abraham isolated[3] a new antibiotic from *Cephalosporium sp.*, and proposed a structure[4] for cephalosporin C 3 which was later (1961) confirmed by Hodgkin[5] by means of X-ray crystallographic studies. It has



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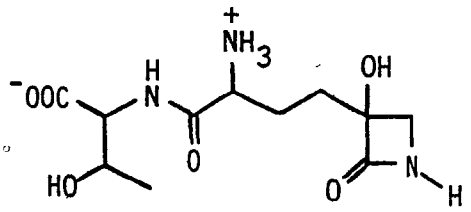
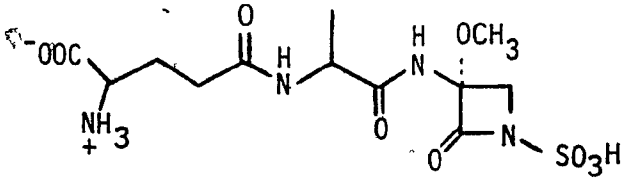
filled many of the gaps in the antibacterial spectrum of penicillin, often as a consequence of its resistance to the β-lactamases produced by bacteria in their own defence.

There has been an enormous amount of research on penicillins and cephalosporins. This effort has produced the new types of β-lactam antibiotics by the isolation from natural sources, such as: wildfire toxin[6] 4, nocardicins[7] 5, sulfazecin[8] 6, thienamycin[9] 7, olivanic acids[10] 8 and clavulanic acid[11] 9.



Wildfire toxin 4



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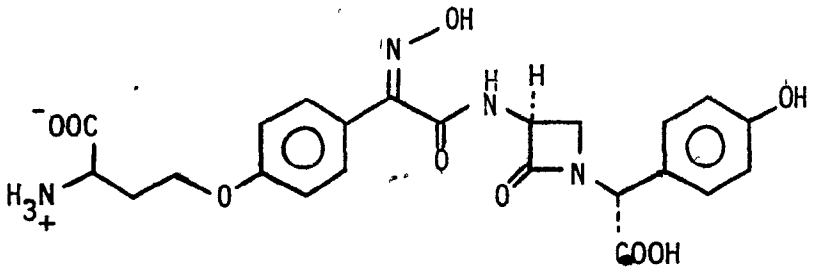
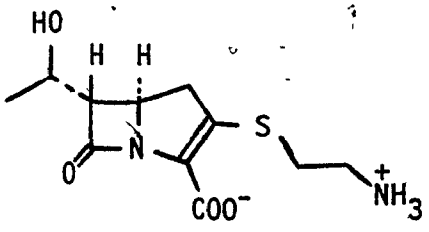
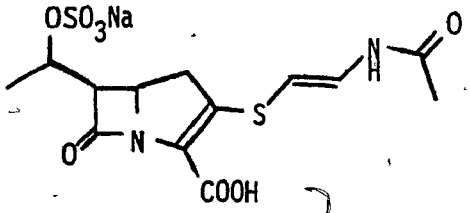


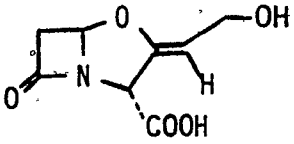
Nocardicin A 5



Thienamycin 7



Olivanic acid MM13902 8



Clavulanic acid 9

Nocardicin A is active against a wide range of Gram-negative bacteria, but it has no activity against Gram-positive bacteria[12]. The most remarkable feature of nocardicin A is that *in vivo* activity is much higher than *in vitro* activity. Sulfazecin is active against Gram-negative bacteria and weakly active against Gram-positive bacteria. Clavulanic acid is a β-lactamase inhibitor. Thienamycin and the related olivanic acid are effective against Gram-positive and Gram-negative bacteria, and show as well β-lactamase inhibitory activity[13].

MODE OF ACTION OF β-LACTAM ANTIBIOTICS

Studies on the mechanism of action of penicillin have been in progress since it was first obtained in a pure form in the 1940's. In 1945, it was suggested by Duguid[14] that penicillins affected the cell walls of bacteria. In 1965, Strominger *et al.*[15] as well as Wise and Park[16] discovered evidence in support of the view that penicillin inhibits the cross-linking reaction in the cell wall synthesis. The defective cell walls leads to the destruction of bacteria. Since animal cells do not have a cell wall, the unusually low toxicity of penicillin is easy to understand.

The bacterial cell wall is a complex, partly inelastic, structure which protects the cell from osmotic swelling and also conditions the microenvironment of the cytoplasmic membrane[17]. While the cytoplasmic membrane has a relatively consistent chemical composition and molecular architecture, the walls of Gram-positive and Gram-negative bacteria are very different in structure and chemical composition. Gram-positive walls are mainly composed of peptidoglycan (murein). Gram-negative walls

contain a thin layer of peptidoglycan and a bilayered outer membrane.

Bacterial peptidoglycan consists of a backbone of glycan chains of alternate residues of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). Each residue of MurNAc is substituted with a short peptide containing both D- and L-aminoacids. The glycan chains are meshed together to form a three-dimensional structure by cross-linking between the peptide of neighboring glycan chains. The structure of the peptidoglycan of *E. coli* is shown in Fig. 1[18]. Species specific variation of this general structure is wide spread, *e.g.* in the peptidoglycan of *S. aureus*

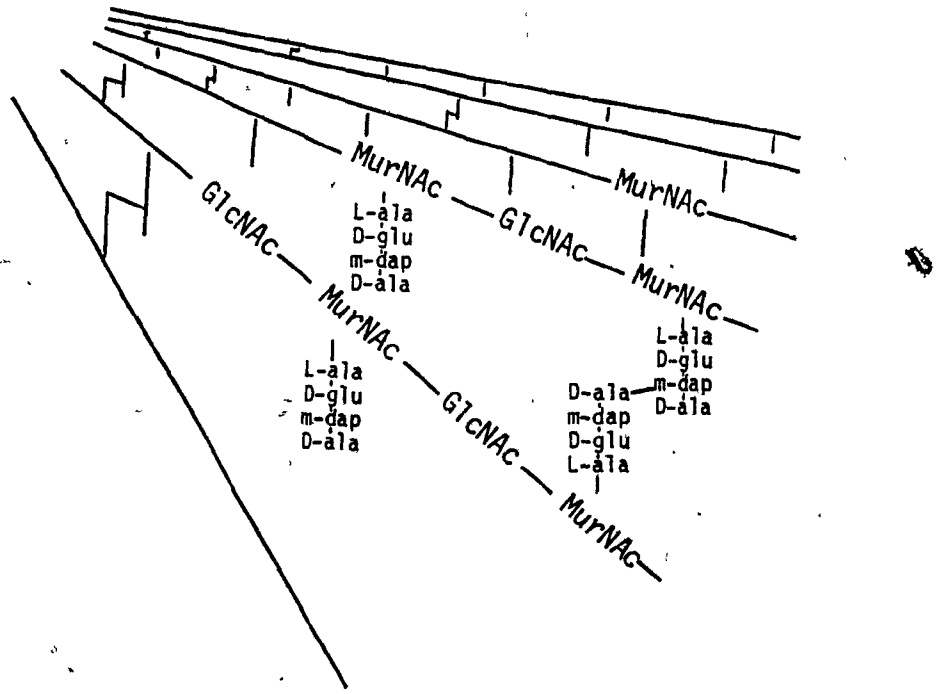


Fig. 1: The structure of part of the peptidoglycan of *E. coli*, MurNAC, N-acetylmuramic acid; GlcNAc, N-acetylglucosamine; L-ala, L-alanine; D-glu, D-glutamate; m-dap, meso-diaminopimelic acid; D-ala, D-alanine.

diaminopimelic acid is replaced by lysine and the peptide crosslinks involve an additional pentaglycine chain[19].

Tipper and Strominger have proposed a model which sharply influenced our ideas for the mechanism of the transpeptidation reaction and its inhibition by penicillin. According to this model, a transpeptidase enzyme reacts with the terminal D-alanine of the pentapeptide side chain in the case of *S. aureus*, breaking the peptide bond, with the release of the terminal D-alanine, and the formation of a D-alanyl-enzyme intermediate. Transpeptidation is then completed by the transfer of the D-alanyl residue from the enzyme to the free amino group of a neighboring peptide side chain. Penicillin was postulated to be a structural analogue of the terminal D-alanyl-D-alanine of the pentapeptide. The transpeptidase binds penicillin to its active site, where the highly strained β-lactam ring is cleaved to form a stable and inactive penicilloyl-enzyme complex, which is analogous to the transitory D-alanyl-enzyme intermediate of the normal enzyme mechanism (Fig. 2, p. 6)[20]. The antibiotic binds to the transpeptidase and inactivates it, preventing any further incorporation of nascent peptidoglycan into the cell wall. Continuing growth, in the absence of the synthesis of rigid cross-linked peptidoglycan, results in the rupture of the wall at the region of active wall growth, and the release of the cell contents.

More recent mechanism of action studies on β-lactam antibiotics now make it clear that the original proposal by Strominger is an oversimplification. Multiple binding sites for penicillin and related β-lactam antibiotics have been detected in the membrane of all bacteria that have

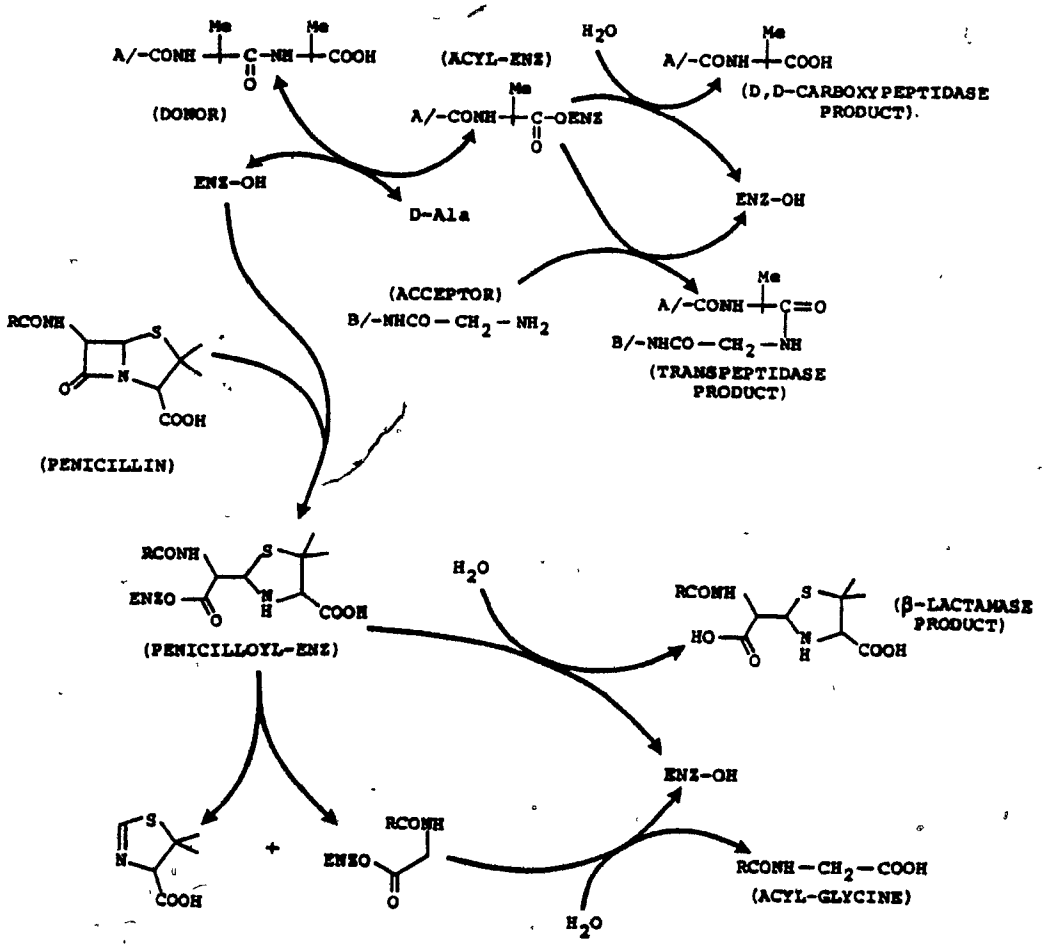


Fig. 2: Formation of acyl-enzyme intermediates in D,D-carboxypeptidase, D,D-transpeptidase, and β-lactamase action.

been examined. Several penicillin-sensitive enzymes (transpeptidase, carboxypeptidase and endopeptidases) that apparently correspond to some of the binding sites, have been identified. Six distinct pencillin binding proteins (PBP) have been identified in the inner membrane of *E. coli*[21]. They separated out according to molecular weight and have been numbered in order of decreasing size. The two smallest proteins, 5 and 6, have been

shown to be equivalent to D-alanine carboxypeptidase. Although sensitive to penicillin, this enzyme is not believed to be a normal killing site for most β-lactam antibiotics. Protein 4 is also an unlikely candidate because mutants have been reported that lack this protein but grow normally. Protein 3 is believed to be involved in septum formation and cell division. Selective inhibition of this protein causes the normally rod-shaped cell to become filamenteous. Protein 2 has been shown to be necessary for maintenance of cell shape. Mutants which lack this protein, or normal bacteria treated with an agent that selectively inhibits it, grow as osmotically stable spherical cells. Finally, protein 1A and 1B, have been identified as the peptidoglycan transpeptidase originally described by Strominger as the killing site for penicillin. Selective inhibition of this enzyme preferentially inhibits cell elongation and causes cell lysis. Although most β-lactam antibiotics bind covalently to some or all of the six proteins, there are decided differences among them in terms of their relative affinities.

At the present time it is not exactly clear how the inhibition of those enzymes is linked to the induction of bacterial lysis. A number of observations[22] suggest that inhibition of cell wall synthesis by any means triggers bacterial autolytic enzymes (an N-acetylmuramyl-L-alanineamidase) by destabilizing the endogenous complex of an autolysin inhibitor and autolytic enzyme.

BACTERIAL RESISTANCE TO β-LACTAM ANTIBIOTICS

Resistant strains of bacteria arise due to the inhomogeneity of bacterial species. Because of drug tolerance, the resistant strains can take place of antibiotic-sensitive strains through the process of natural selection. In the majority of Gram-positive organisms, the β-lactamase is usually the sole complicating factor in resistance. β-Lactamases are large proteins produced by bacteria to hydrolyze the β-lactam to the β-aminoacid derivative[23]. The β-lactamases of Gram-positive bacteria are predominantly extracellular and inducible. They often display a very high affinity for their substrates.

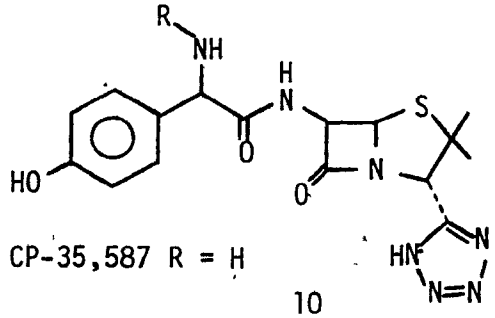
In Gram-negative organisms the main contributing factors to the highly complex defence system (protecting the cells from destruction by β-lactam antibiotics) are the permeability barrier, the β-lactamase and the interplay between the β-lactamase and the barrier. Several lines of evidence have supported the view that outer membrane of the cell wall provided a permeability barrier which makes Gram-negative bacteria less sensitive to many antimicrobial agents[24]. With few exceptions, the β-lactamases from Gram-negative bacteria are cell-bound enzymes, produced in much smaller quantities. The majority of enzymes are constitutive and their substrate affinities are low compared to the β-lactamases from Gram-positive organisms[25]. However, the β-lactamases of Gram-negative bacteria are very effective since they hydrolyze only the antibiotic that penetrates the cell wall.

STRUCTURE-ACTIVITY RELATIONSHIPS

Early work on the penicillin and cephalosporin groups of antibiotics soon established the following structure features necessary for the optimal antimicrobial activity[26]: (i) a *cis*-fused β-lactam ring, (ii) an acylamino side chain, (iii) an acidic function at C-3 of a penicillin or at C-4 of a cephalosporin and (iv) a penam or cephem system to raise the infrared spectrum of β-lactam above 1765 cm^{-1}. Replacement of the carboxyl function with a tetrazole in the penicillin series seems compatible with retention of bioactivity while conferring increased β-lactamase stability to the penam nucleus. Examples of 3-(5-tetrazolyl)-penams 10 have been reported. The 7α-methoxycephalosporin class of β-lactam antibiotics, *e.g.* cefoxitin 11, possesses the attributes of the cephalosporins combined with β-lactamase stability.

Recently, however, many β-lactam antibiotics that do not fulfill all the criteria listed above have shown significant biological activity[13]. A number of penicillins and cephalosporins having side chains other than those of the acylamino group have been prepared. Mecillinam 12 is used clinically. Naturally-occurring thienamycin 7, although it has a *trans*-fused ring and 1-hydroxyethyl side chain, shows remarkable activity. Clavulanic acid 9, which has no side chain, shows anti β-lactamase activity.

Cephalosporins with the sulfur atom replaced by an oxygen or a methylene group were found to retain the activity of the parent. Doyle *et al.* have synthesized O-2-isocephems[26], which have comparable activity. Woodward and co-workers have reported the synthesis of biologically active



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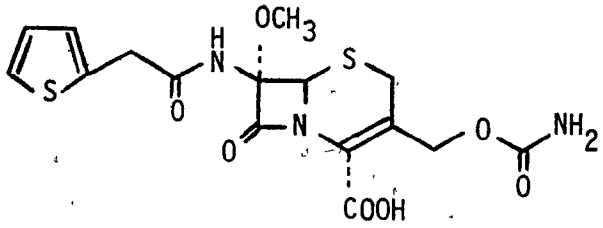
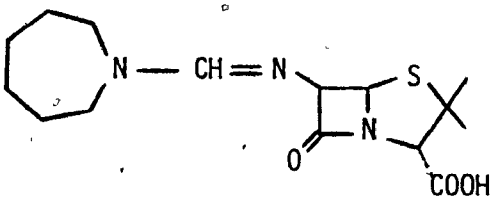
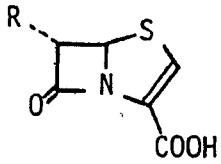


Cefoxitin 11



Mecillinam 12



13 R = H, 

penems 13[27]. Nocardicins 5 and sulfazecin 6, which do not have a bicyclic system, possess good activity against a large number of very resistant bacteria.

SYNTHESIS OF β-LACTAMS

Fermentation and Semi-Synthesis

Improvements in the techniques of isolation of penicillins and use of new strains of microorganisms generated through mutation induced by ultraviolet light and other agents have made penicillin G and V very cheap and abundant[28]. 6-Aminopenicillanic acid (6-APA) is now directly available through fermentation or indirectly by the scission of the side chain under enzyme action or by economic chemical manipulation. Thousands of penicillins have been prepared since then by the acylation of 6-APA

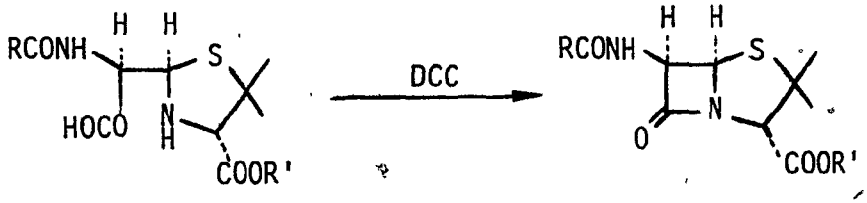
with a variety of acids. Several of these "semi-synthetic" penicillins are now in clinical use. The cephalosporins are obtained by fermentation or through a semi-synthetic procedure similar to that used for penicillins. Besides, the easy availability of penicillins G and V at an economic price has led to the extensive studies for the conversion of penams to cephems[7,28,29].

Total Synthesis

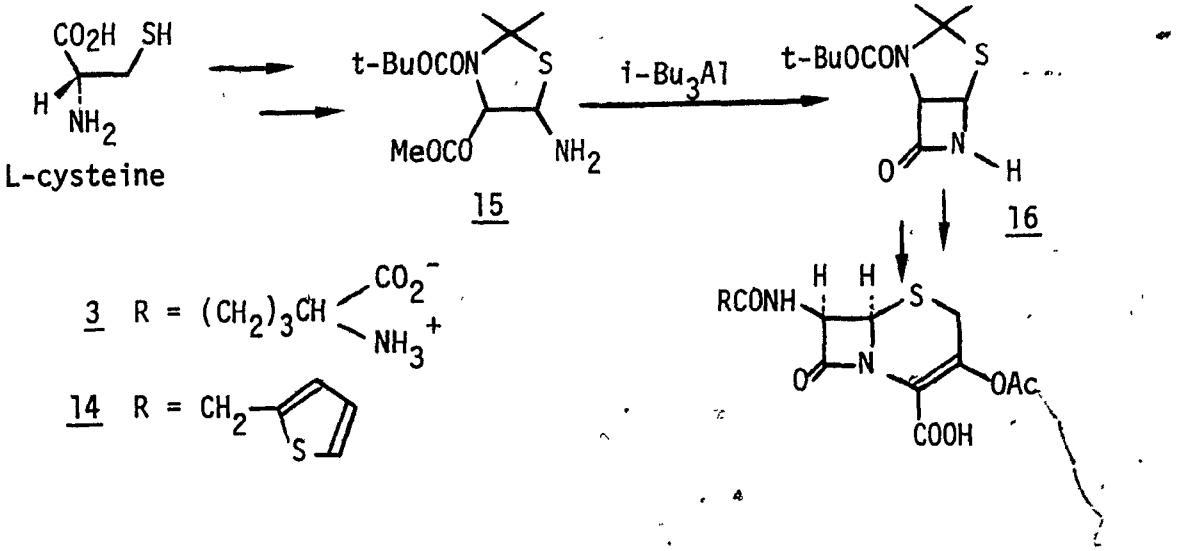
Interest in β-lactam continues unabated because of the therapeutic importance of β-lactam antibiotics. As a result, diverse refined preparative methods have been developed, culminating in the total synthesis of many interesting β-lactams. These are summarized in several review articles[29,30].

Cyclization of Suitable Acyclic Compounds

Different β-lactams have been synthesized by cyclizing β-aminoacids with the aid of such reagents as acetic anhydride, acetyl chloride, phosphorus trichloride, thionyl chloride and carbodiimides. In 1962, Sheehan reported the first total synthesis of a penicillin[31]. The formation of the β-lactam ring was achieved using dicyclohexylcarbodiimide (DCC) as a cyclizing reagent. Most recently, Ohno has applied Mukaiyama's reagent[32], triphenylphosphine and 2,2'-dipyridyl disulfide, to form β-lactams[33] from β-aminoacids. β-Aminoacids were also cyclized by conversion into acid chlorides followed by treatment with a base[33].



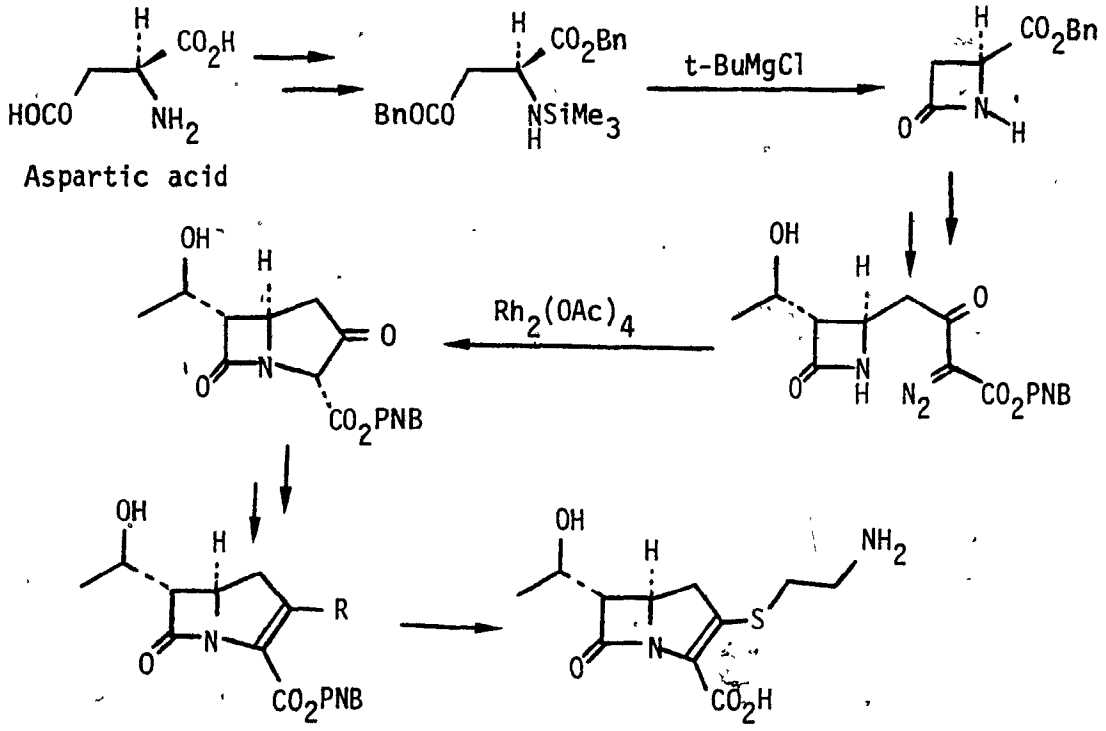
Cyclization of β-aminoacid esters was effected with Grignard reagents or triisobutylaluminum. In 1965, Woodward announced the first stereospecific synthesis of cephalosporin C 3 and cephalothin 14[34] in his Nobel prize address. Triisobutylaluminum was used for cyclizing 15 to the bicyclic lactam 16, which served as an important intermediate in the total synthesis. A Grignard reagent was first employed in the synthesis of



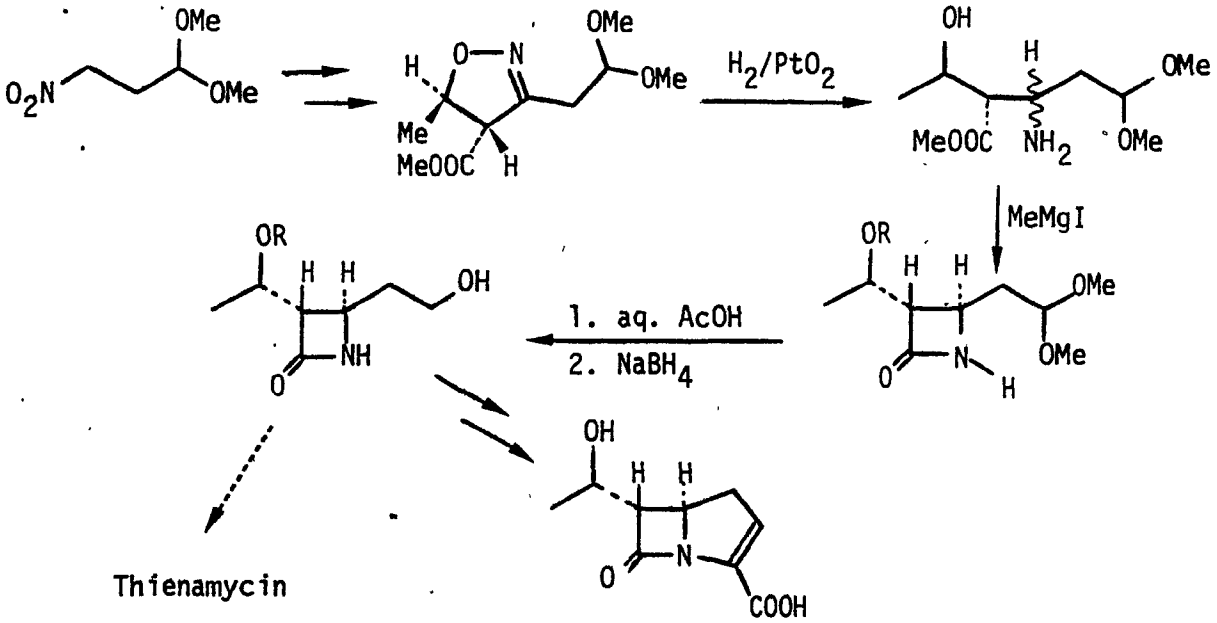
β-lactam by Breckpot[35] and has since found application in the preparation of various types of β-lactams. Lately, a key intermediate for the preparation of thienamycin 7 has been synthesized separately by two groups, Christensen[36] and Kametani[37], *via* the ring closure of the amino ester by treatment of an appropriate Grignard reagent. Their

approaches was illustrated as follows:

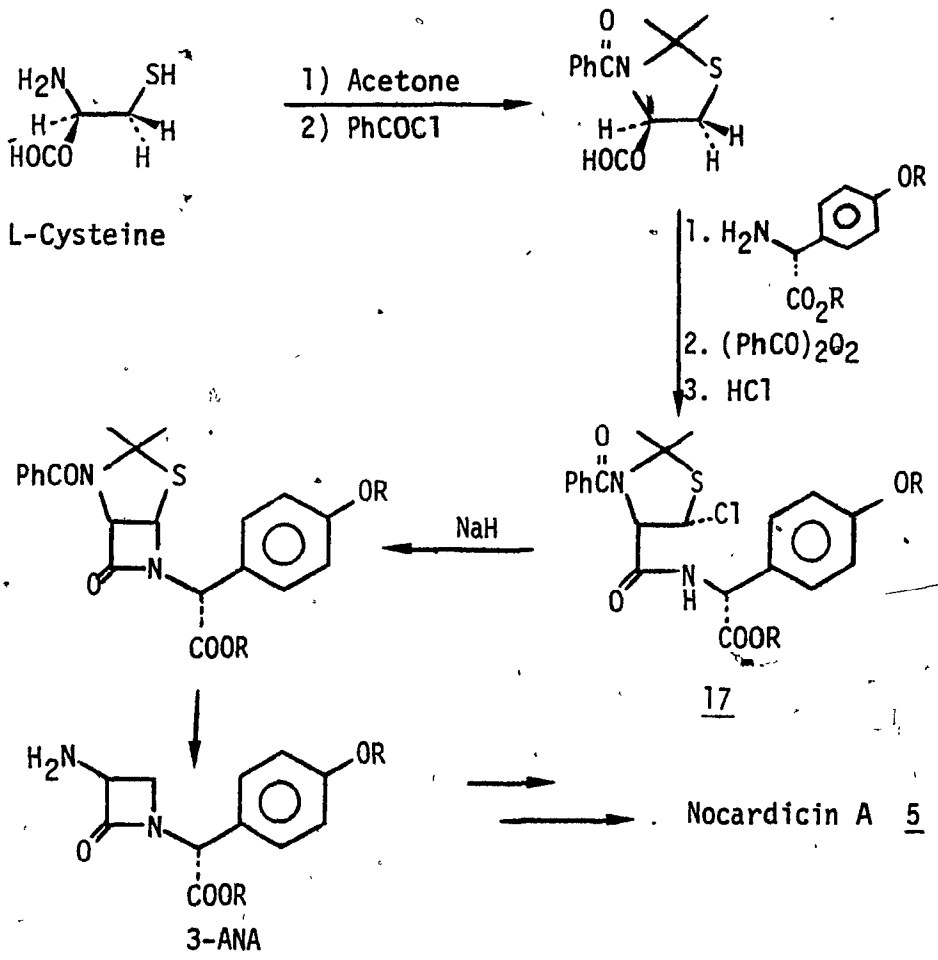
Merck.



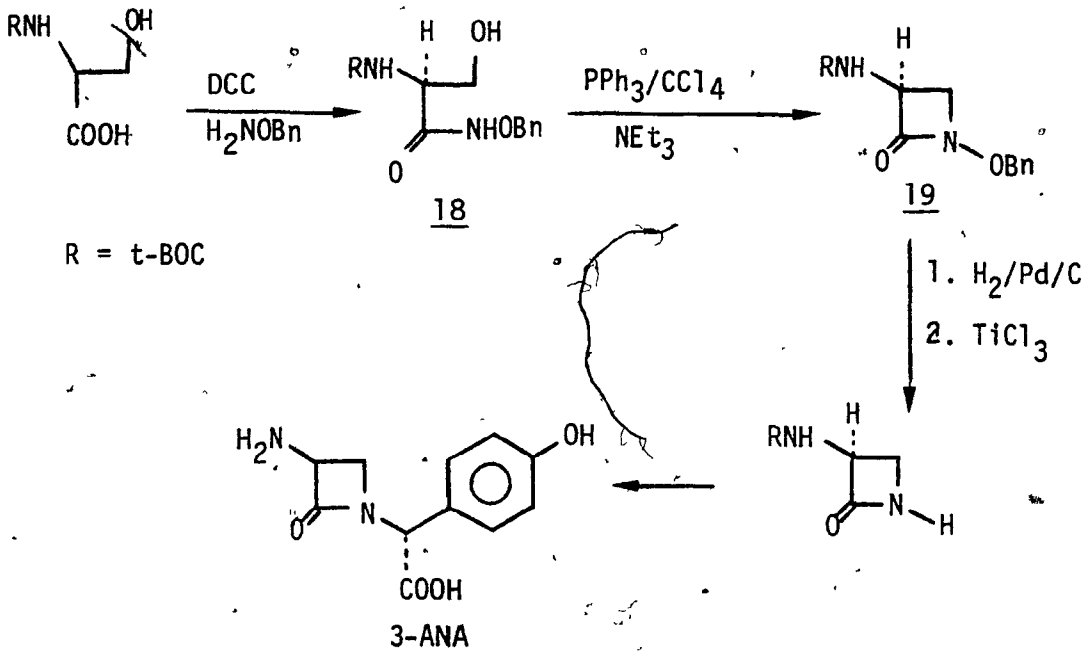
Kametani *et al.*



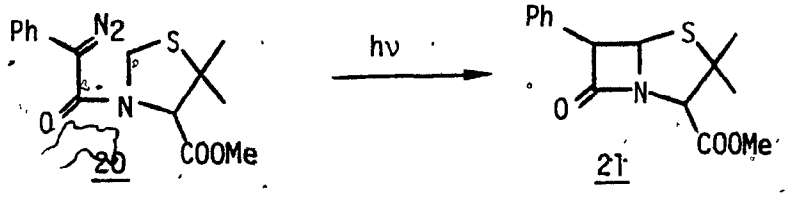
Knunyants *et al.* synthesized β-lactams *via* N-C_4 bond formation through dehydrohalogenation of 3-halopropanamides in the presence of strong bases[29]. It was found that the substitution at the nitrogen atom does not affect cyclization. The Lilly Research Laboratory[38] reported the total synthesis of nocardicin A by the cyclization of 17 with sodium hydride. Miller's approach to 3-aminonocardicinic acid (3-ANA) relied



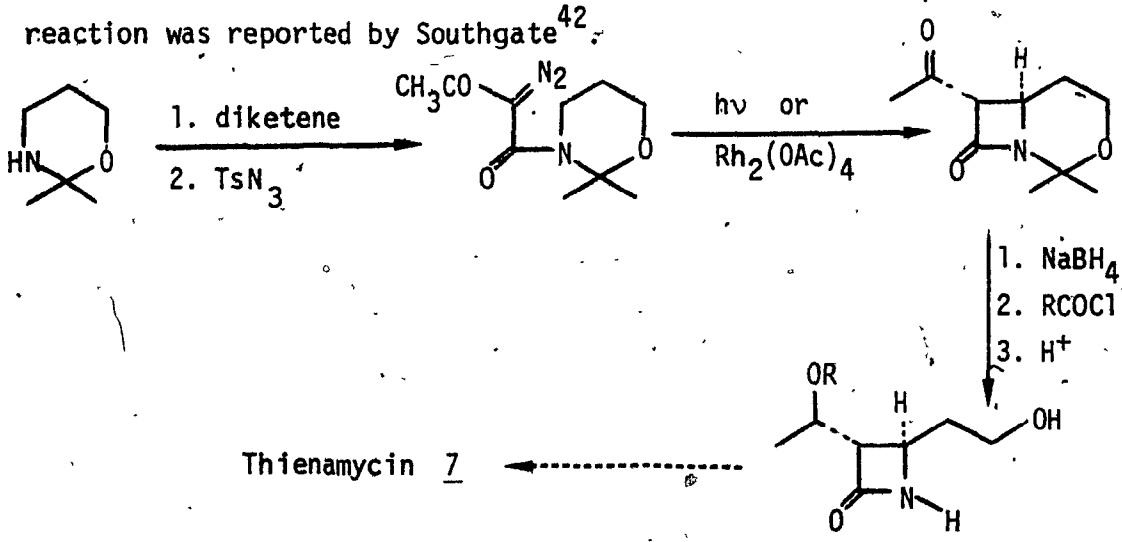
on the efficient cyclization[39] of the O-benzyl hydroxamate 18 to 19 with $PPh_3/CCl_4/NEt_3$, followed by subsequent alkylation of the β-lactam nitrogen.



Photolysis of the diazoamide 20 gave the annulated β-lactam 21[40]. This method was extended to the synthesis of several bicyclic β-lactams[41].



A synthesis of thienamycin intermediate through this carbene insertion reaction was reported by Southgate[42].

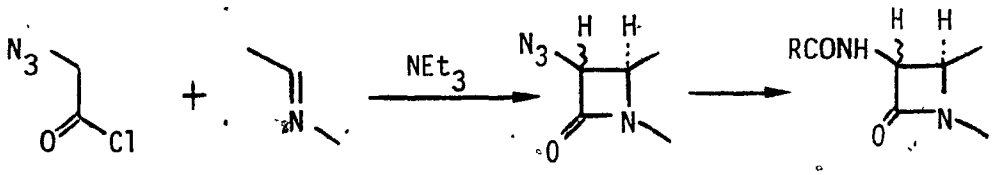


Cycloaddition reactions

Two well documented cycloaddition approaches for the construction of a β-lactam ring are the interaction of an imine with a ketene or of an isocyanate with an olefin.

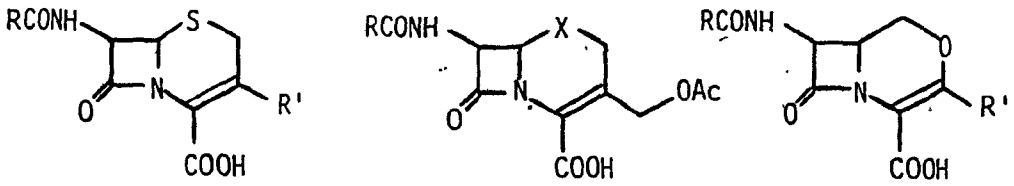
(i) Addition on imines

The first β-lactam was prepared by the ketene-imine reaction[1]. The addition of substituted acetic acid derivatives to imines in the presence of a base may be treated as an extention of this reaction. Bose carried out the addition reaction of azidoacetyl chloride 22 to the appropriate imines 23 to yield the azido β-lactam 24, which could be transformed into the desired acylamino β-lactam 25[43]. Since then, this



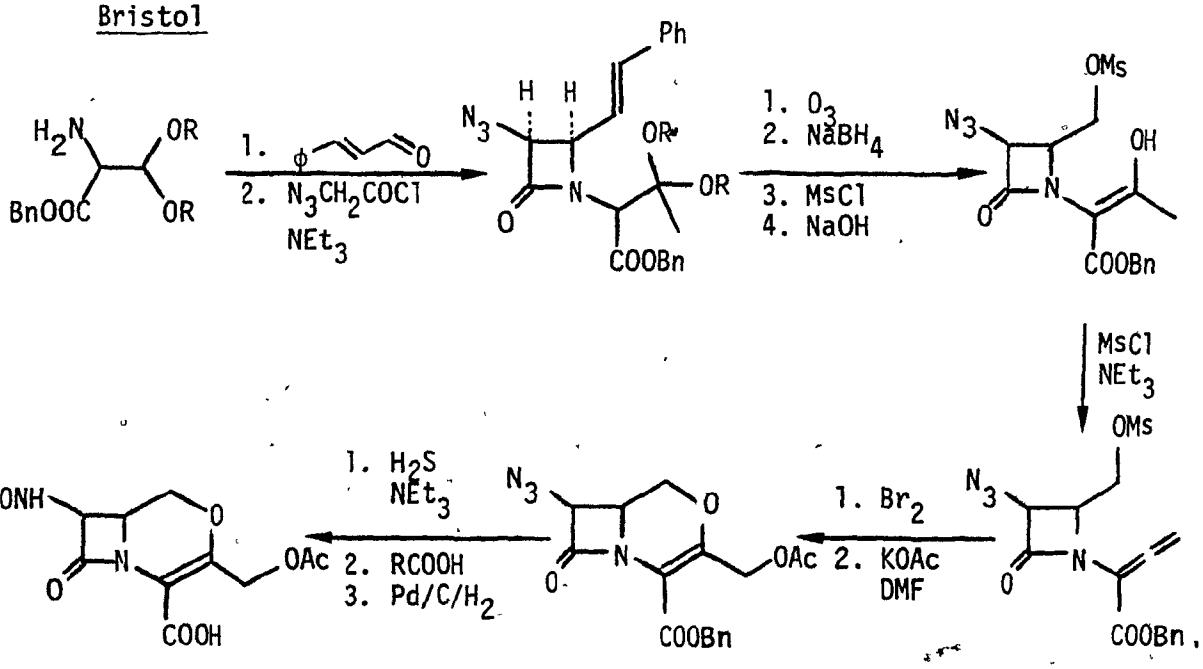
22 23 24 25

reaction has been intensively used. Christensen *et al.* at Merck Pharmaceutical Company synthesized many cephalosporin derivatives with the structure type of 26[44] and 27[45]. The Bristol[26,46] group have been involved in the synthesis of isocephems 28. All of these compounds 26, 27 and 28 showed relatively good activity. A common feature of those approaches was first to form the β-lactam ring using acetyl chloride/imine reaction and then construct the remaining ring. One of the examples is illustrated as follows:



26 27 X = CH_2,O 28

Bristol



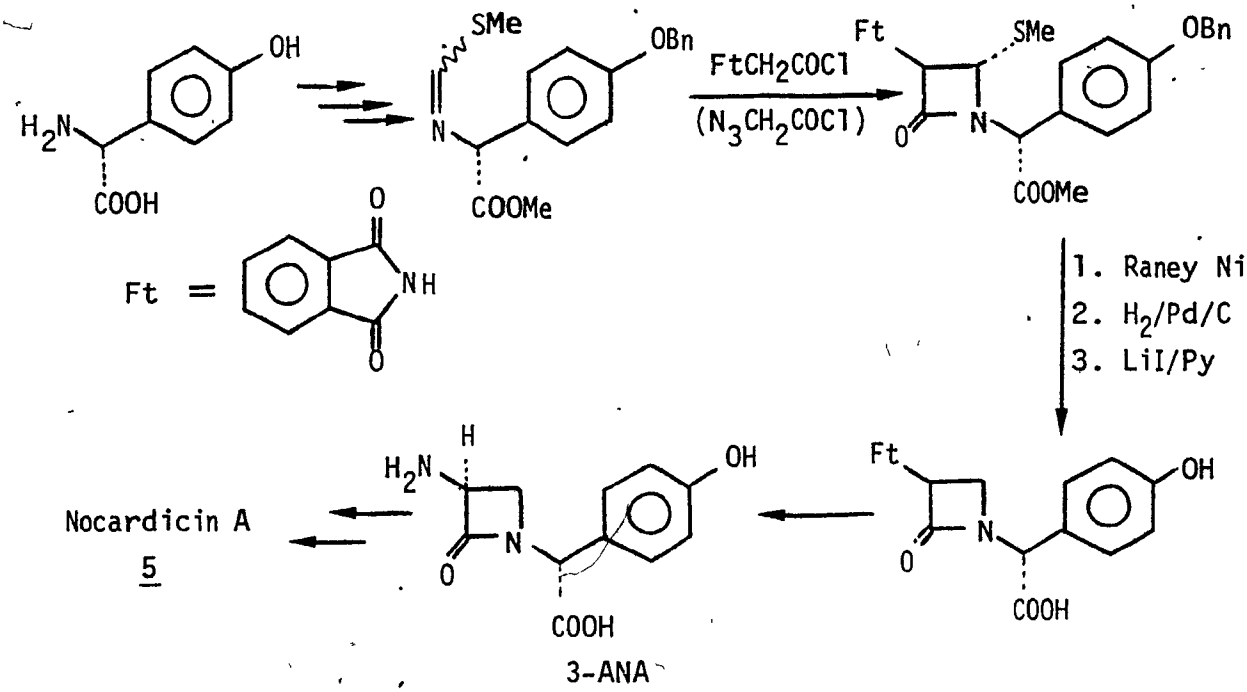
28

It is noteworthy, in this synthetic sequence, that the Bristol group have applied the addition of a cinnamylidene Schiff base with an azidoacetyl chloride to yield exclusively *cis* β-lactam[47].

Kamiya[48] at Fujisawas Pharmaceutical Company adopted the cyclo-addition between a thioimidate and a substituted acetyl chloride for the

first synthesis of nocardicins 5 as well.

Fujisawas

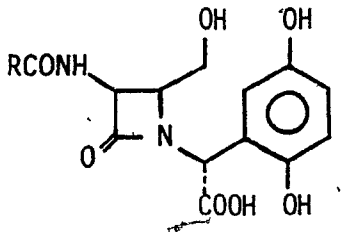


(ii) Reaction of isocyanates

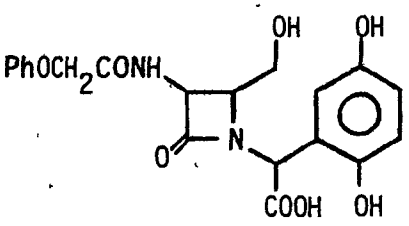
Several isocyanates[29], such as trichloroacetyl isocyanate, phenyl isocyanate, various aroyl and arenesulfonyl isocyanates, have been used to react with different olefins to produce β-lactams. The discovery of chlorosulfonyl isocyanate by Graf[49] opened an efficient pathway for the synthesis of β-lactams, particularly used in the recent synthesis of penems 13[27], thienamycin 7[50] and clavulanic acid 9[51].

DESCRIPTION OF PROJECT

This thesis consists of three sections, these dealing with the synthesis of different types of β-lactams. In the first chapter, the syntheses of nocardicin analogues 31 and 52 are described. The second chapter involves the synthesis of β-lactam 95 and some interesting β-lactam intermediates which were subjected to the biological test (p. 48). Finally, the last chapter discusses the synthetic studies toward monobactam analogues S, and the successful synthesis of bicyclic β-lactam 162.



31 R = HOOCCCHCH$_2$CH$_2$O-⟨O⟩-C(=N-OH)- (NH$_2$)



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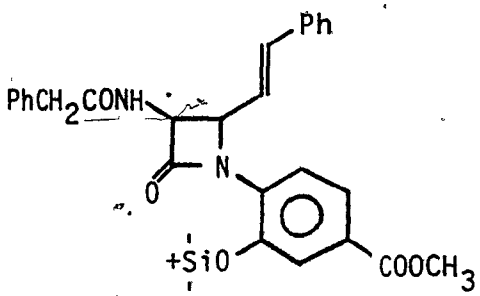

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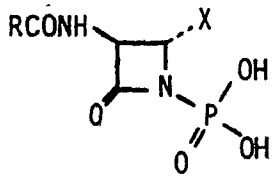
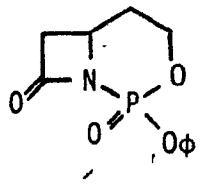


S X = H, CH$_3$

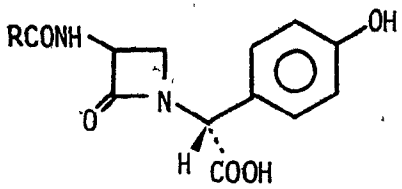


162

RESULTS AND DISCUSSION

CHAPTER I

Nocardicins 5[52], a group of novel monocyclic β-lactam antibiotics, possess relatively high antimicrobial activity and are stereochemically and biologically related to penicillins and cephalosphorins.



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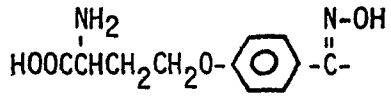
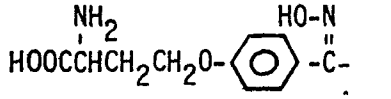
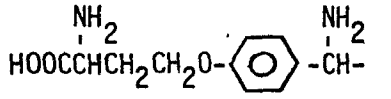
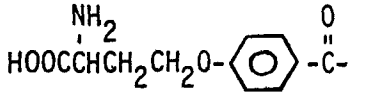
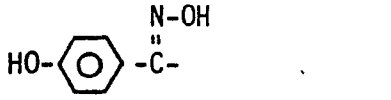
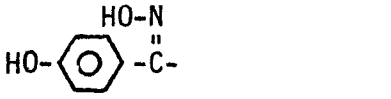
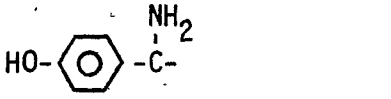
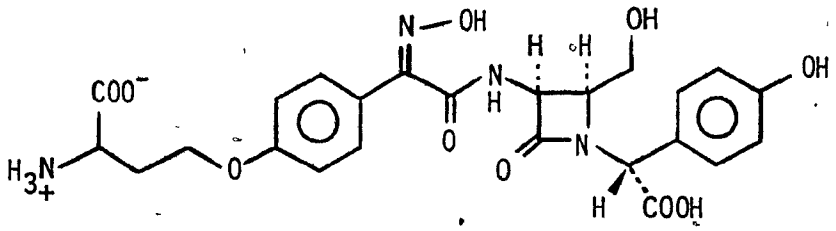
Nocardicin	R =
A	
B	
C	
D	
E	
F	
G	

Several total syntheses[38,48] of nocardicins have been reported, including a well established route for the synthesis of some nocardicin analogues accomplished in our laboratory by Dr. Hakimelahi[53,54]. One of

those analogues, compound 29 had an *in vitro* activity against *S. lutea*, which was about the same as that of natural nocardicin A.



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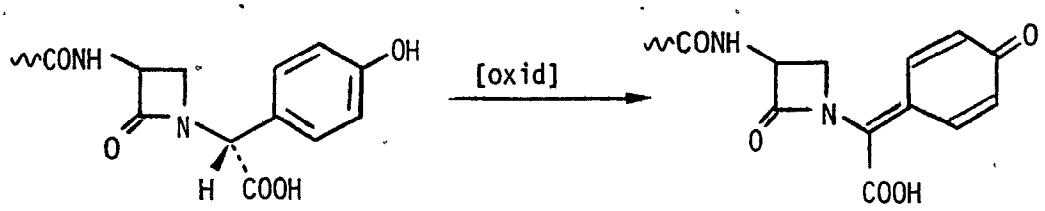
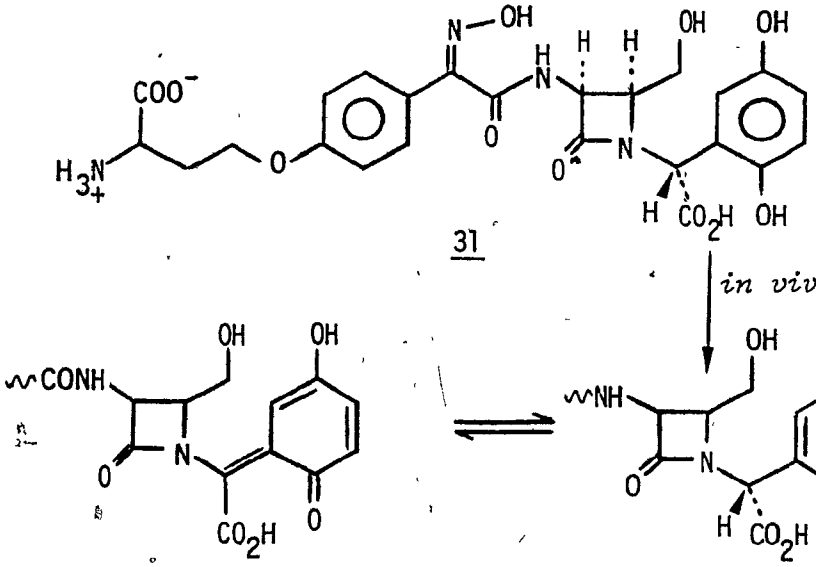
Nocardicin A shows antimicrobial activity *in vivo* rather than *in vitro*. It occurred to us that this *in vivo* activity may be linked to an oxidation to the corresponding quinonoid structure 30. The reactivity



5 30

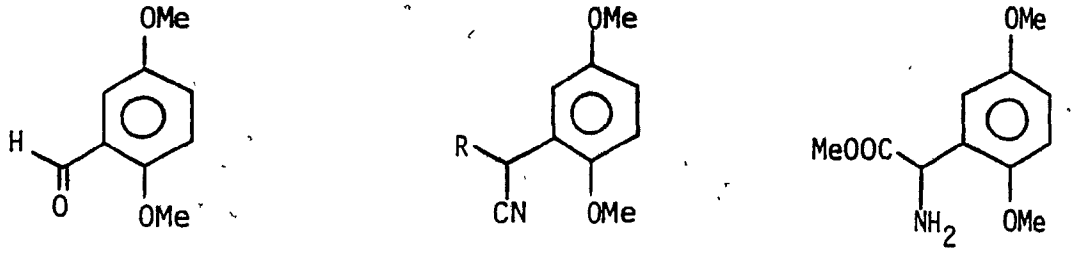
of the β-lactam ring, as reflected by the infrared frequency, should be considerably enhanced, thus resulting in an increase in its biological activity. According to the methodology developed in our laboratory, it was decided to prepare nocardicin analogue 31 bearing two para-related hydroxy groups on the aromatic ring. It was anticipated that the *in vivo* and/or *in vitro* oxidation of this compound 31 to a quinone may be more easily achieved than in compound 29. It may, as well, exist in part as the

quinone-methine tautomer which should display the enhanced β-lactam frequency mentioned above (Fig. 3).



(Fig. 3)

After analyzing the structure of the desired compound 31, we initially began our synthesis from the commercially available 2,5-dimethoxybenzaldehyde 32. This provided us with a methyl protected phenol that was stable to both acid and base.

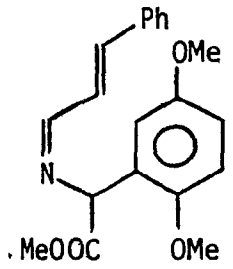


32 33 R = NH_2 34 R = OH 35

Treatment of the aldehyde 32 with sodium cyanide and ammonium chloride in methanol saturated with ammonia at room temperature for 18 h, afforded a mixture of cyanoamine 33 and cyanohydrin 34, based on the pmr

spectrum. The separation was achieved by the formation of the hydrochloride salt of cyanoamine 33, and aqueous extraction. Neutralization with sodium carbonate yielded the pure cyanoamine 33. The infrared spectrum indicated the presence of an amine with an absorption at 3300-3400 cm^{-1}, and a cyano function at 2100 cm^{-1}. The cyanoamine 33 was transformed into methyl glycinate 35 by heating with hydrogen chloride in methanol-water (95:5).

For the construction of the β-lactam ring system, we adopted the cycloaddition reaction between a cinnamylidene Schiff base and a substituted acetyl chloride which had been well developed by Doyle *et al.*[26]. Thus, amine 35 was condensed with cinnamaldehyde to afford the cinnamylidene Schiff base 36 in quantitative yield. Upon treatment with azidoacetyl chloride 22 and triethylamine in methylene chloride at -20°, the Schiff base 36 was readily converted into azido β-lactam 37. In the pmr spectrum, there



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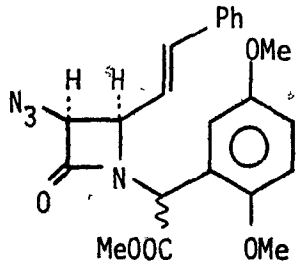
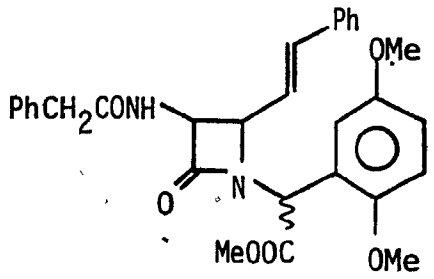


37

were two singlets at 5.91 and 5.93 ppm for the proton at the benzylic position, which clearly indicated a mixture of two diastereomers. The coupling constant between the two β-lactam protons was found to be 5 Hz, which indicated the *cis*-stereochemistry for the β-lactam ring[55]. In addition, there were absorptions in the infrared spectrum at 2100 and

1780 cm^{-1} for the azide function and the β-lactam carbonyl group respectively.

At this point deprotection of phenolic ether was attempted in order to be sure that the methyl protecting group could be cleaved in the presence of a β-lactam. Even though there are many methods[56] available for the demethylation of a phenol methyl ether in the literature, most of those described reaction conditions would easily open the acid and base sensitive β-lactam ring. Attempted removal of the protecting groups on 37 by means of trimethylsilyl iodide[57], which was considered to be a mild reagent, unfortunately also resulted in the destruction of the β-lactam ring. It was decided to transform the azido β-lactam 37 to an amide. Reduction of 37 using hydrogen sulfide-triethylamine[47] in methylene chloride and acylation with phenylacetyl chloride afforded amide 38. Unfortunately, we were not able to demethylate amide 38 with trimethylsilyl iodide as well.



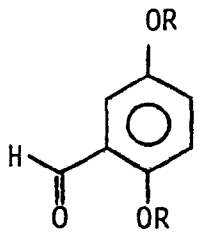
38

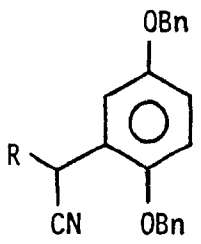
Also, several attempts to remove the methyl groups on the compound 35 using boron tribromide[58], hydrogen iodide[59], or hydrogen chloride[60] under various reaction conditions, failed to give any promising results. Therefore, the benzyl group was chosen as the protecting group.

The benzyl group has been successfully employed in the transformation

of an aldehyde to a methyl glycinate[53] and could be cleaved by hydrogenation under neutral conditions at the last stage of the synthetic scheme.

2,5-Dimethoxybenzaldehyde 32 was demethylated with boron tribromide[58] to afford 2,5-dihydroxybenzaldehyde 39 in excellent yield. Compound 39 was readily converted to 2,5-dibenzyloxybenzaldehyde 40 using benzyl chloride and potassium carbonate in absolute ethanol.





39 R = H

40 R = $CH_2C_6H_5$

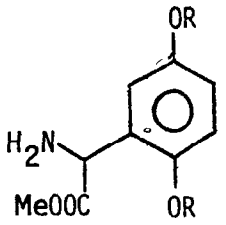
41a R = NH_2

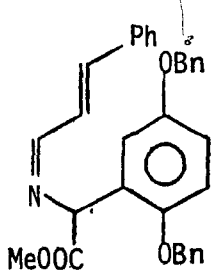
41b R = $NH_2 \cdot HCl$

42 R = OH

The cyanoamine reaction of the benzyloxybenzaldehyde 40 proceeded in methanol-tetrahydrofuran, in a manner similar to the one mentioned above. Although the pmr and infrared spectra seemed to show only one compound - cyanoamine 41a, thin-layer chromatography indicated the presence of a major and minor product, presumably, cyanoamine 41a and cyanohydrin 42 respectively. Attempted separation of the two by the formation of the cyanoamine hydrochloride salt was not successful due to the low solubility in water of the bulky hydrochloride salt 41b. Because of the similarity of R_f values between the two compounds, we decided to attempt the next reaction using the mixture directly.

The crude cyanoamine 41a, in methanol water, was saturated with hydrogen chloride gas and then refluxed for 2 h. After work-up, chromatographic purification afforded the desired aminoester 43 and some of compound 44. Obviously, under these severe acidic conditions,



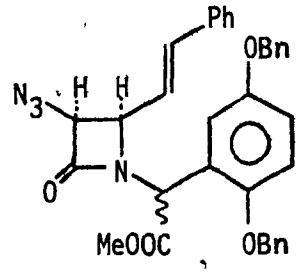


45

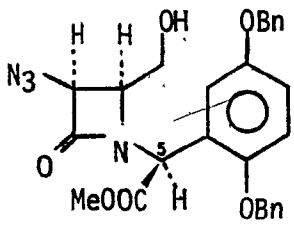
43 R = $CH_2C_6H_5$; 44 R = H, $CH_2C_6H_5$

the debenzylation of 43 took place to yield the partially hydrolyzed side product 44. The overall yield of the transformation (40 → 41a → 43) was not reproducible and ranged approximately from 5% to 45%.

After condensation of the aminoester 43 with cinnamaldehyde, Schiff base 45 was obtained. Treatment of the Schiff base 45 with azidoacetyl chloride-triethylamine at -20° afforded β-lactam 46, as a mixture of two diastereomers, in ∿ 90% yield. The ratio of the two was close to 1:1 as shown in the pmr spectrum. Its *cis*-stereochemistry was indicated by the coupling constants (5 Hz) between the two β-lactam protons.



46



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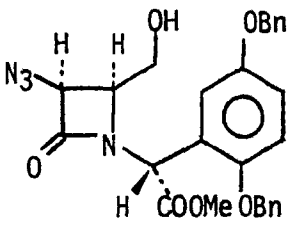


48

Ozonolysis of the azido β-lactam 46 with ozone and nitrogen[53] in methylene chloride-methanol at -78°, followed by reduction with sodium borohydride at -40° afforded β-lactam 47 and 48, in a ratio of 3:1. The reason for the change in the ratio of the two isomers from azido β-lactam 46 to β-lactams 47 and 48 is not clear. The mixture 47 and 48 could be separated using preparative layer chromatography. The relative configuration at benzylic position (C-5) of the less polar adduct 48 was assigned the same stereochemistry as the natural nocardicin A, based on the arguments discussed[53]. The ester function absorbed in the infrared spectrum at 1740 cm^{-1} (hydrogen-bonded carbomethoxy group) and the hydroxyl function at 3300-3500 cm^{-1} (hydrogen-bonded hydroxyl), with a lowering of the chemical shift of the proton α to the carbomethoxy group to 5.9 ppm. The corresponding numbers for the more polar isomer 47 were 1750 cm^{-1}, 3400-3600 cm^{-1} and 5.6 ppm. Model studies indicated that hydrogen-bonding of the ester and hydroxyl group resulted in the phenyl group being placed away from the β-lactam ring in the case of 48, whereas in isomer 47, the phenyl group would be very close to the β-lactam ring to permit hydrogen-bonding. Equilibration of the two isomers by refluxing in benzene in the presence of pyridine was unsuccessful. However, refluxing the mixture with triethylamine gave a 3:7 ratio of 47 to 48. This suggests that the hydrogen-bond between the free alcohol and the methyl ester made 48 slightly more stable than 47. The pmr spectra of β-lactams 47 and 48 are shown in Figs. 4 and 5 on page 28.

As a model, the more polar isomer of azido β-lactam 47 was reduced using hydrogen sulfide-triethylamine to yield amine 49. After protecting

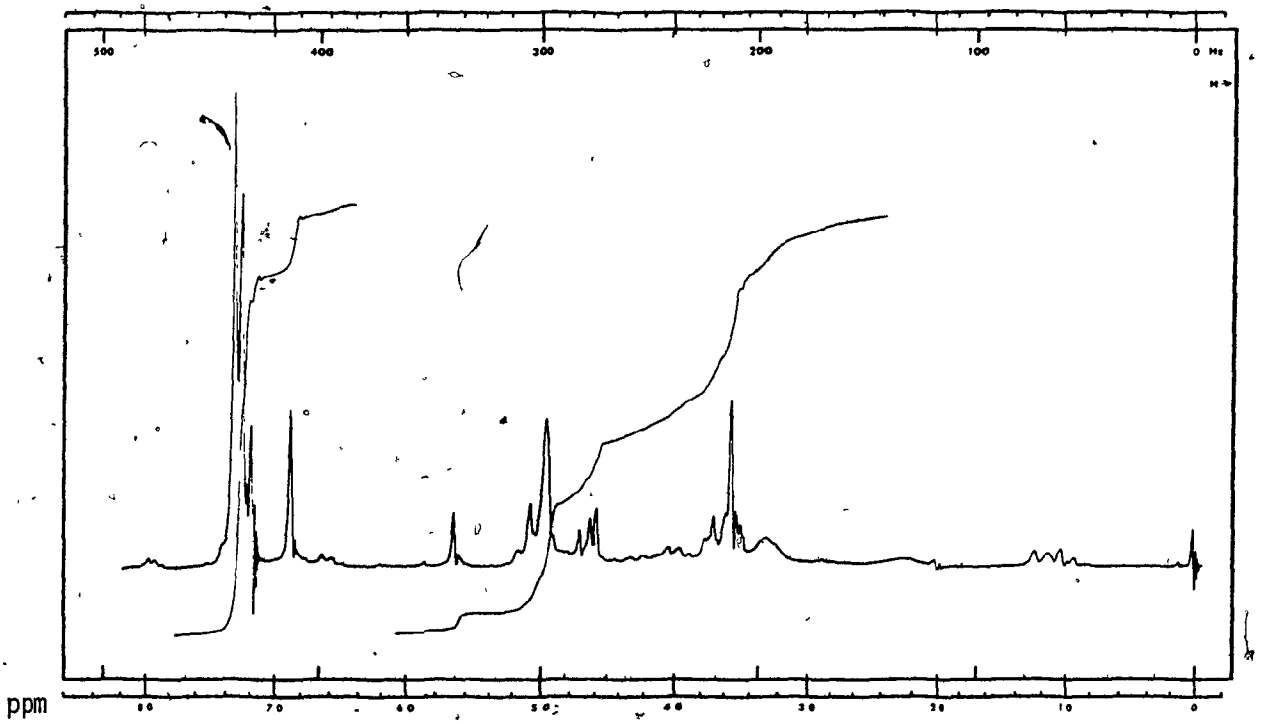


Fig. 4: 60 MHz pmr spectrum of 47, in $CDCl_3$.

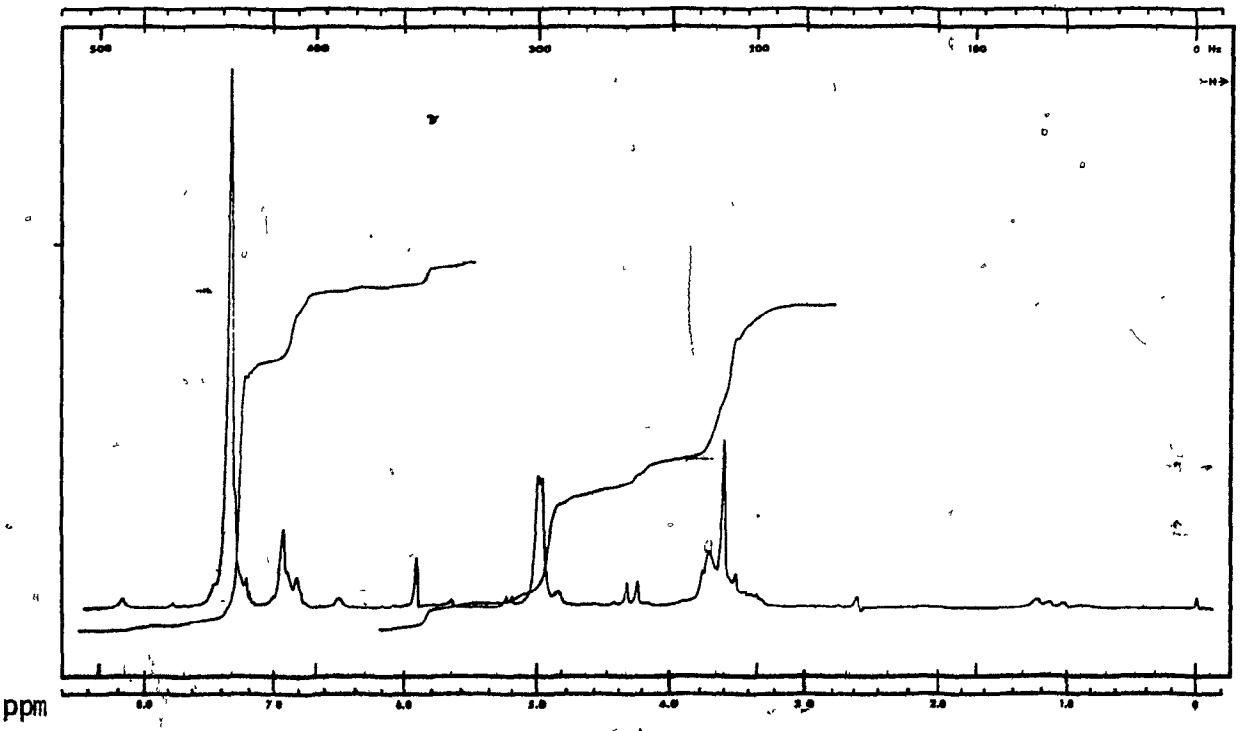
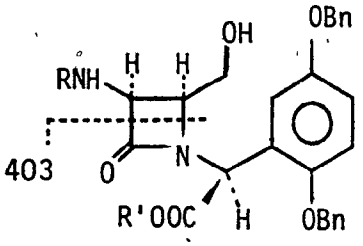
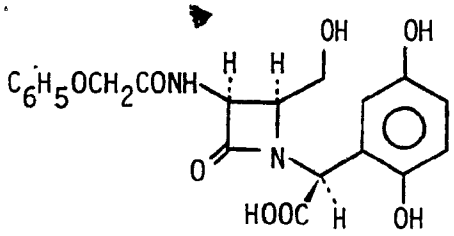


Fig. 5: 60 MHz pmr spectrum of 48, in $CDCl_3$.

the hydroxyl group of β-lactam 49 with trimethylsilyl chloride-triethylamine, acylation with phenoxyacetic acid, using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[61] as a coupling reagent, resulted in the formation of amide 50 in 78% yield. The pmr, infrared and mass spectra of the amide 50





52

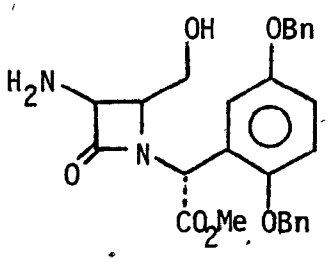
49 R = H, R' = CH_3
50 R = $C_6H_5OCH_2CO$, R' = CH_3
51 R = $C_6H_5OCH_2CO$, R' = H

were consistent with the structure assigned. The infrared spectrum displayed absorptions at 1765 and 1740 cm^{-1} for the carbonyl groups of the β-lactam and ester, respectively. The mass spectrum showed a parent ion at m/e 610 (11.56%) and a fragment at m/e 403 (42.76%) confirming structure 50.

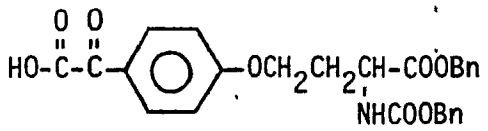
Treatment of β-lactam 50 with one equivalent of 1% sodium hydroxide in methanol afforded the corresponding acid 51. Removal of the benzyl protecting groups by hydrogenation over palladium on charcoal yielded the desired β-lactam 52. The ultraviolet spectrum showed a shoulder at 225 nm and a maximum absorption at 285 nm. The infrared spectrum measured in nujol displayed a β-lactam carbonyl absorption at 1750 cm^{-1}, an absorption of hydroxy and acid functions in the 2800-3500 cm^{-1}

region and an amide absorption at 1650 cm^{-1}. The pmr spectrum of 52 in D_2O and sodium bicarbonate[53,54,62], showed one proton doublet for C-3 at 5.5-5.6 ppm (J = 5Hz) and a singlet for the proton α to the carbomethoxy group at 5.8 ppm.

We then turned our attention to the synthesis of the nocardicin analogue 31 *via* a similar route. The less polar isomer, D,L-azido-β-lactam 48 was transformed into amine 53 by means of hydrogen sulfide and triethylamine. Amine 53 was silylated with trimethylsilyl chloride-



53



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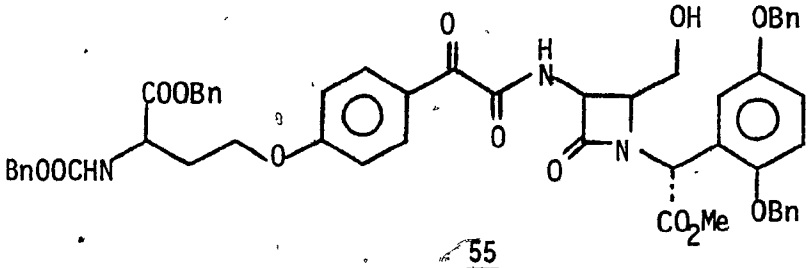


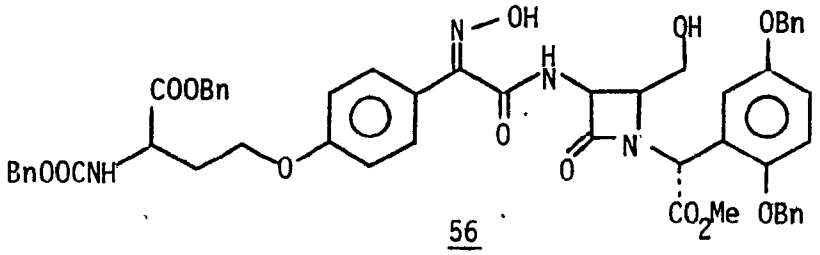
55

triethylamine *in situ*, and coupled with D,L-glyoxylic acid* 54 to afford β-lactam 55, as a mixture of unseparable diastereomeric racemates. The infrared spectrum indicated a β-lactam carbonyl function at 1765 cm^{-1}. The ultraviolet spectral data [λ_{max} (EtOH, H_2O): 228 nm and 298 nm] were

*A gift from Lederle Laboratories of the American Cyanamid Company.

consistent with the presence of an alkylated phenol and a conjugated alkoxyphenol derivative.

The next step in the synthetic sequence was the introduction of the oxyimino group. Treatment of β-lactam 55 with hydroxylamine hydrochloride in pyridine-ethanol[53,63], followed by purification on a silica gel column, afforded syn-oxime 56. Its infrared spectrum indicated the presence of β-lactam carbonyl function at 1768 cm^{-1}. The ultraviolet spectrum showed a shoulder at 230 nm and a maximum absorption at 274 nm, which was different from that of β-lactam 55.



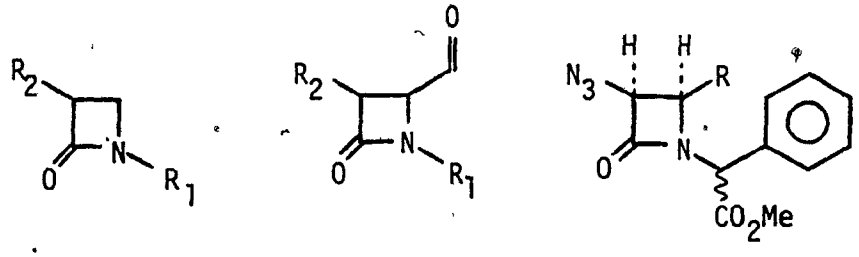
56

Hydrolysis of the methyl ester of β-lactam 56 using 1% aqueous sodium hydroxide in methanol, followed by catalytic hydrogenation over palladium on charcoal in ethanol for an hour, afforded β-lactam 31. The oxime configuration was established to be syn to the acylamino group by comparison of its ultraviolet spectrum with that obtained by Kamiya at Fujisawa Pharmaceutical Company for nocardicin A and B[64]. The ultraviolet spectrum showed a shoulder at 225 nm and a maximum at 273 nm in ethanol solution and a shoulder at 235 nm and a maximum at 283 nm in alkaline solution.

Compounds 52 and 31 did not show any notable activity against a

variety of bacteria.

In view of the structure of nocardicin 5, it would be interesting to discover if we could prepare the analogue 57 from the readily available aldehyde 58 by decarbonylation. Thus, β-lactam 59[53] was ozonized in

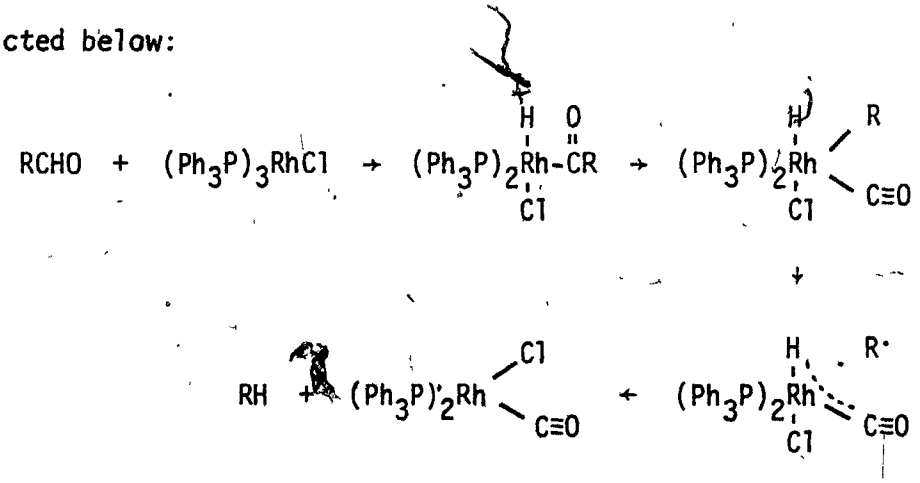


57 58 59 R = CH = CHR

60 R = CHO

methanol at -30°, followed by dimethyl sulfide reduction, and gave the expected aldehyde 60.

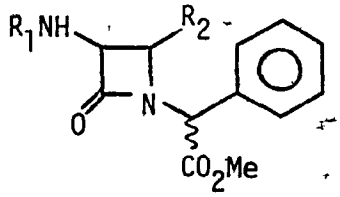
Tris(triphenylphosphine)rhodium chloride[65] is known to be a mild, selective decarbonylation reagent for aldehydes. The proposed mechanism[66] is depicted below:

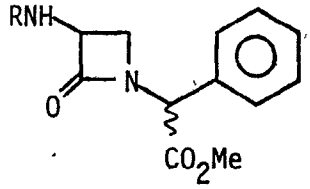


Aldehyde 60 was treated with an equimolar amount of tris-(triphenylphosphine)rhodium chloride in dry, oxygen free benzene at ∿ 70° for 3 h.

The infrared spectrum of the product indicated the absence of the azide and β-lactam carbonyl functional groups. It was therefore decided to transform the azide group into an amide, which hopefully could be removed after decarbonylation.

Reduction of β-lactam 59 with hydrogen sulfide-triethylamine afforded amine 61. Amine 61 was condensed with either di-tert-butyl





61 R_1 = H, R_2 = CH = CHPh

62 R_1 = t-BuOCO, R_2 = CH = CHPh

63 R_1 = CF_3CO, R_2 = CH = CHPh

64 R_1 = t-BuOCO, R_2 = CHO

65 R_1 = CF_3CO, R_2 = CHO

66 R = t-BuOCO

67 R = CF_3CO

68 R = H

dicarbonate or trifluoroacetic anhydride to yield amide 62 or 63 respectively. Ozonolysis and reduction of β-lactams 62 and 63, using the standard conditions, afforded aldehydes 64 and 65. Decarbonylation of aldehydes 64 and 65 were repeated under similar conditions using tris-(triphenylphosphine)rhodium chloride, and β-lactams 66 and 67 were obtained. All the spectral data were in accord with the structure proposed. Attempts to convert the trifluoroamide 67 to amine 68 using sodium bicarbonate[67] failed. However, stirring the amide 66 in a mixture of trifluoroacetic acid and methylene chloride (3:7) at room temperature for

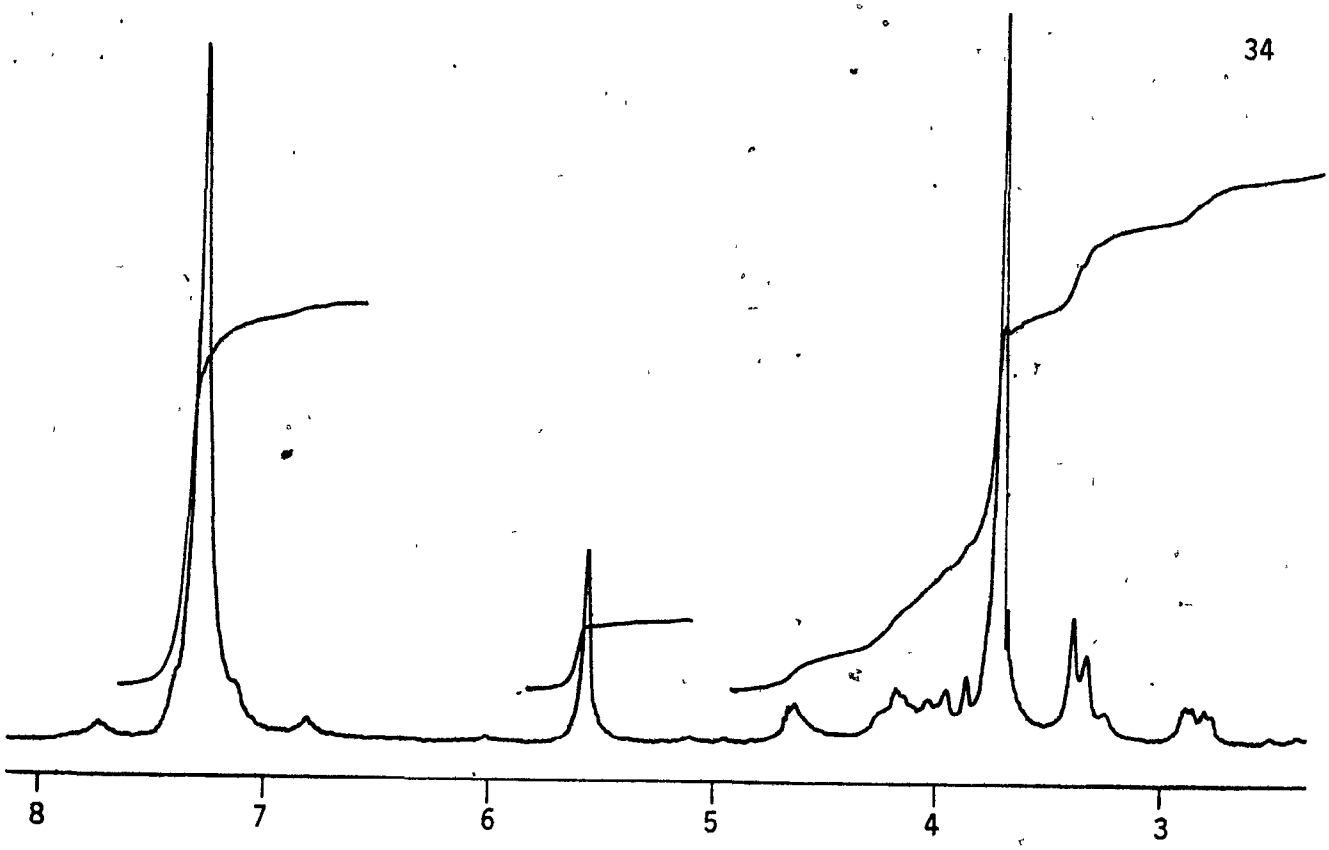


Fig. 6: 60 MHz pmr spectrum of 68, in $CDCl_3$ and D_2O.

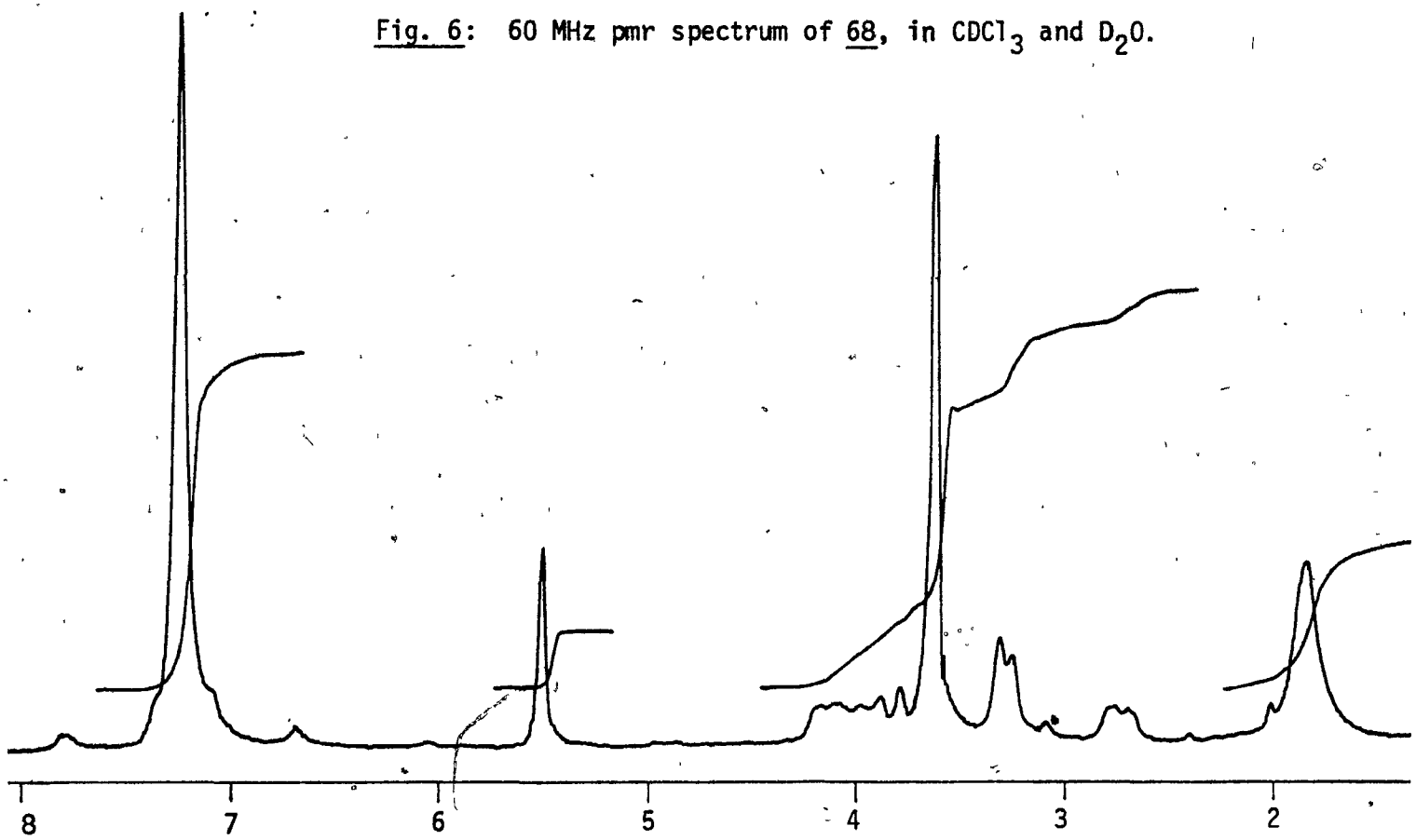
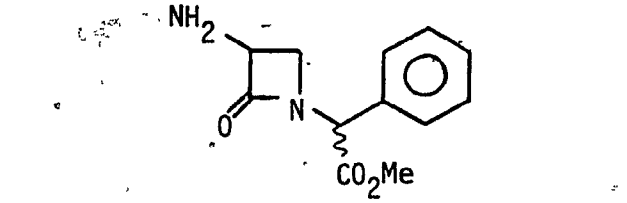


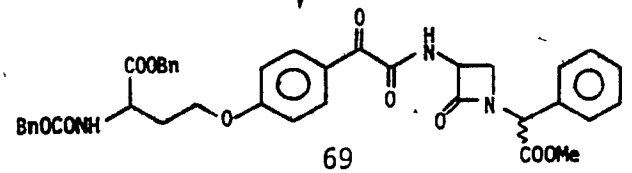
Fig. 7: 60 MHz pmr spectrum of 68, in $CDCl_3$.

an hour afforded the desired amine 68, in good yield. The pmr spectrum showed in Figs. 6 and 7 (p. 34). The elemental analysis was within acceptable limits.

β-Lactam 63 was also transformed into 71 by the same sequence described before.



68



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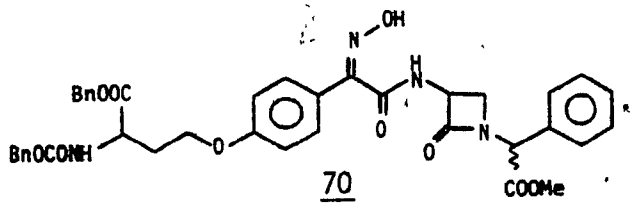
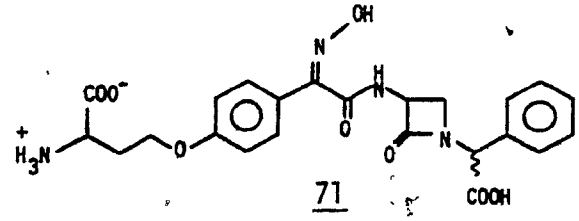


70

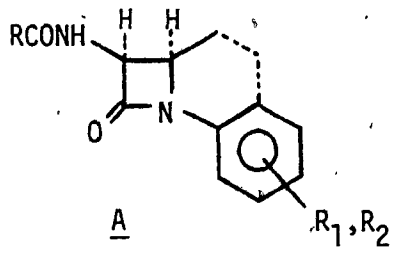


71

This work was not pursued, due to the unfavorable results from the biological tests and the low yield of the reaction (40 → 43). Nevertheless, some of the β-lactam intermediates (72a and 72b), synthesized by A. Ugolini (see Chapter II), showed interesting antibacterial activities which led us to continue to the next project.

CHAPTER II

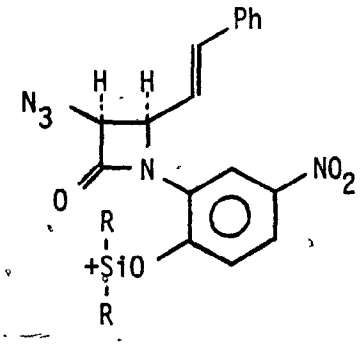
During the last few years we have been involved in the synthesis of penicillin and cephalosporin analogues with the general structure A[68-70].



A

These analogues have a *cis*-fused β-lactam ring and an acylamino side chain. The strain of the β-lactam bond may be enhanced by electron-withdrawing groups attached to the benzene ring, or by fusing the aromatic and β-lactam moiety through an additional ring. We hoped this modification would increase the β-lactam infrared frequency, which we expected would correspond to an increase in the antimicrobial activity.

Routine testing of some of the intermediate β-lactams 72a and 72b, synthesized in this laboratory, unexpectedly showed noticeable activity



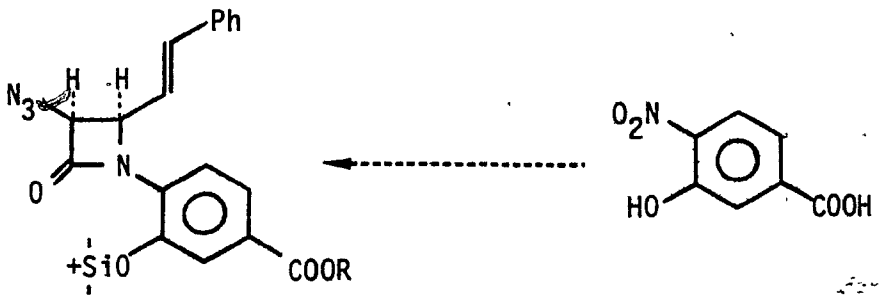
72a R = CH_3

72b R = Ph

toward a variety of bacteria[70]. The activity of β-lactam 72 may arise from the following features; an easily deblocked silyl group with the formation of an acidic phenol which is isosteric with the carbonyl group of classical β-lactam antibiotics, a *cis*-substituted β-lactam ring similar to that of penicillins, and a hydrophobic styryl group. In order to understand more clearly the effect of the styryl group, and functional groups attached to the aromatic ring necessary for the antibacterial activity, an extension of the project was undertaken.

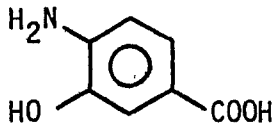
It should be noted that the nitro groups of 72a and 72b are *para* with respect to the t-butyldimethyl or t-butyldiphenylsilyloxy group, making the latter very susceptible to hydrolysis, and this class of compounds very difficult to work with. We already knew that a nitro group *para* to the β-lactam nitrogen would result in the formation of a *trans* β-lactam[70]. Therefore, the first and simplest variation would be to replace the nitro group by an ester function *para* or *meta* to the nitrogen of the β-lactam. It was hoped that this electron-withdrawing ester would provide a *cis* β-lactam with better stability.

For the synthesis of β-lactam 73, we chose the commercially available 3-hydroxy-4-nitrobenzoic acid 74 as a starting material.

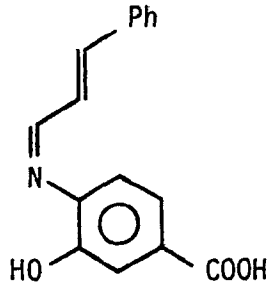


73 74

Benzoic acid 74 was reduced to amine 75 by catalytic hydrogenation using platinum oxide-hydrogen. Treatment of 75 with cinnamaldehyde under various conditions failed to produce any of the expected Schiff base 76. We thought that the low solubility of 75, due to the free carboxylic acid

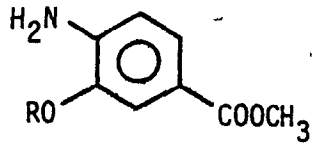


75



76

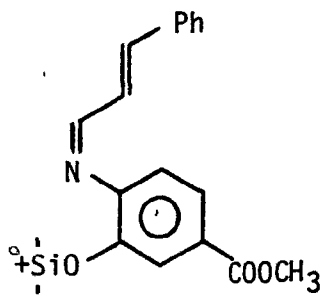
and hydroxyl groups, was responsible for the difficulty in the formation of the Schiff base. Therefore, it was decided to protect the two groups first. Methylation of 75, with diazomethane, afforded methyl 3-hydroxy-4-aminobenzoate 77. Treatment of 77 with t-butyldimethylsilyl chloride in N,N-dimethylformamide[71] gave the protected alchol 78 in good yield. Amine 78 was transformed into its Schiff base 79 by refluxing with cinnamaldehyde,



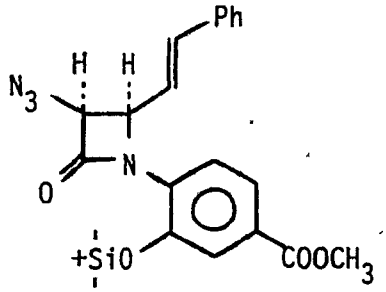
77 R = H

78 R = t-BuSiMe$_2$

reacted with azidoacetyl chloride-triethylamine to give a difficult-to-separate mixture containing *cis* β-lactam 80 in approximately 15% yield.

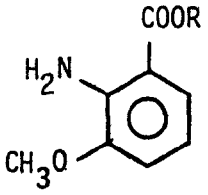


79



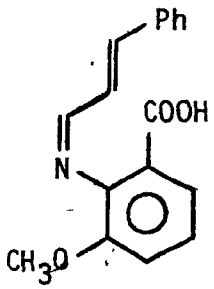
80

Recently, Dr. R. Zamboni in this laboratory had shown that the formation of a Schiff base from 81 was unsuccessful. However, he found that the free amino acid 82 could easily be converted to its Schiff base 83[72]. We hoped using the free acid 84 might improve the yield of the β-lactam, in our case as well. Silylation of amine 75 with t-butyldimethylsilyl chloride afforded 85, which was hydrolyzed with 1% aqueous hydrogen

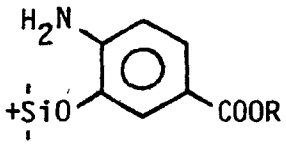


81 R = CH_3

82 R = H



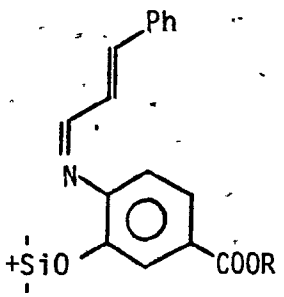
83

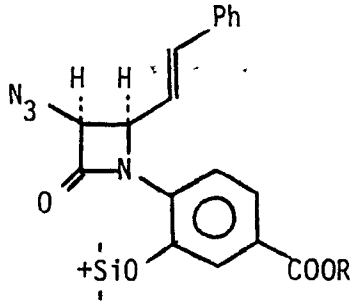


84 R = H

85 R = t-$BuSiMe_2$

chloride in methanol to afford the desired amino acid 84. Heating 84 for 1.5 h with cinnamaldehyde in refluxing benzene afforded the Schiff base 86 as a yellow solid in quantitative yield. The infrared spectrum showed two strong absorptions at 1730 and 1670 cm^{-1} for the carboxylic acid and imine, respectively. Schiff base 86 was silylated with trimethylsilyl





86 R = H
87 R = $(CH_3)_3Si$

88 R = H
80 R = CH_3

chloride-triethylamine *in situ*, to afford 87. Treatment of 87 with azidoacetyl chloride-triethylamine gave *cis* β-lactam 88, in 52% yield, after flash chromatography. Methylation of 88 with diazomethane afforded β-lactam 80, m.p. 90-91°. The infrared spectrum showed an azide absorption at 2100 cm^{-1} and two carbonyl absorptions of ester and β-lactam at 1720 and 1765 cm^{-1}, respectively. The mass spectrum of 80 displayed peaks for the loss of a nitrogen molecule and a t-butyl group, from the parent, at m/e 450 and 393. The pmr spectrum (Fig. 8, p. 42) clearly indicates the *cis*-stereochemistry of two β-lactam protons (J = 5 Hz).

Desilylation of β-lactam 80 with tetra-n-butylammonium fluoride[73] or other fluoride salts turned out to be a problem. The desilylation reaction was not examined further since we were successful in synthesizing β-lactam 89 in very good yield, using the easily removable trimethylsilyl protecting group. Silylation of 3-hydroxy-4-aminobenzoic acid 75 with trimethylsilyl chloride and hydrolysis afforded amine 90. As was described for 88, compound 90 was converted to β-lactam 91. Without purification, the crude β-lactam 91 was hydrolyzed, in methanol containing hydrochloric

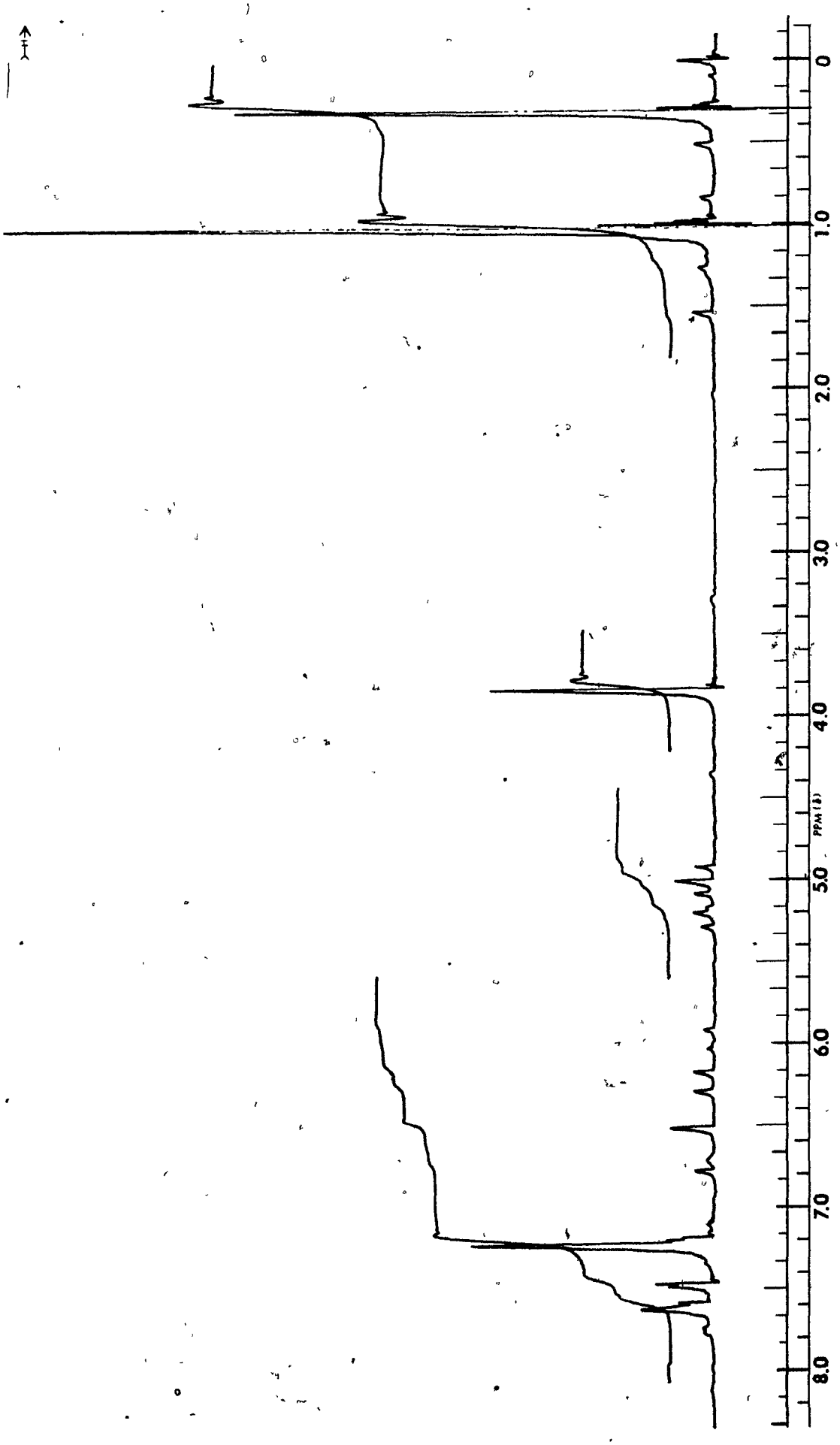
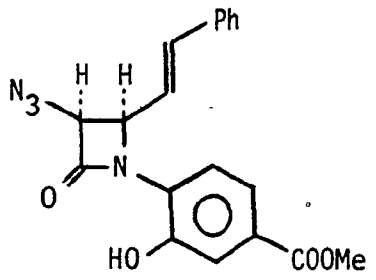
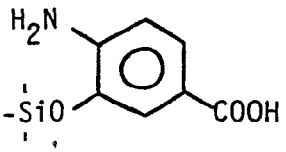


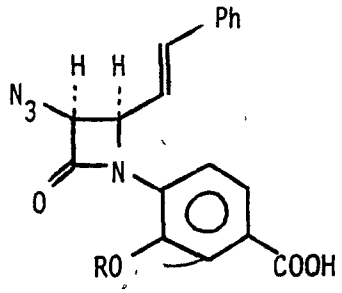
Fig. 8: 60 MHz pmr spectrum of 80, in $CDCl_3$.



89



90



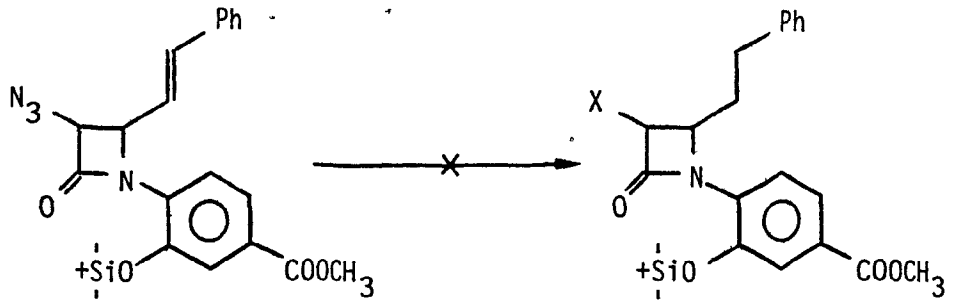
91 R = $(CH_3)_3Si$

92 R = H

acid, to phenol 92, which was methylated with ethereal diazomethane to afford the corresponding methyl ester 89, in 60% yield based on amine 75.

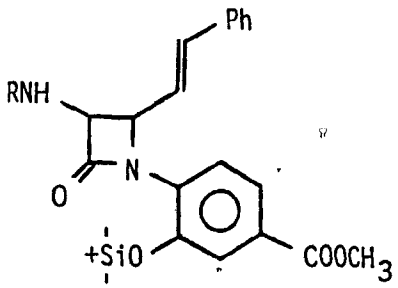
Biological testing revealed that compounds 80, 88 and 89 were mildly active against some bacteria (see Table 1, p. 48). The ester group attached to the aromatic ring, *para* to the nitrogen of the β-lactam, did show approximately the same effect as the nitro function group did. In addition, it had better stability, as expected. We therefore were interested in the preparation of a number of compounds by varying the styryl group.

Attempts to convert β-lactam 80 to saturated β-lactam 93 by a catalytic amount of palladium on charcoal, in a standard manner, failed to yield any desired products. Since it was very possible that the azide group on β-lactam 80 would be easily reduced at this stage, we transformed the azide into an amide group. Reduction of 80 with hydrogen sulfide-triethylamine gave amine 94, which was directly acylated with phenylacetyl



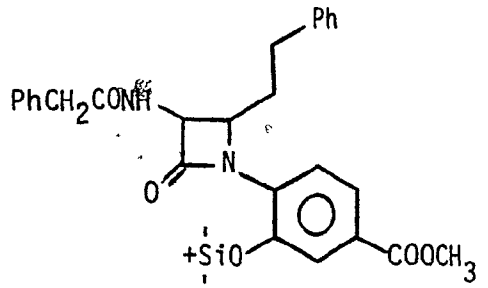
80

93 X = N_3, NH_2



94 R = H

95 R = $PhCH_2CO$



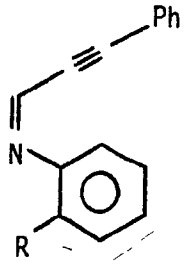
96

chloride affording amide 95 as a white solid, m.p. 168-170°. Amide 95 was hydrogenated at 40 psi with platinum oxide in absolute ethanol for 2 h. After flash chromatography, β-lactam 96 was isolated in 14% yield. There were strong absorptions in the infrared spectrum at 1750 and 1720 cm^{-1} for the carbonyl groups of the β-lactam and ester, respectively. Since β-lactam 96 was not active against bacteria, we did not attempt to improve the yield of this reaction further.

Instead of cinnamaldehyde, phenylpropargyl aldehyde, β-phenylcinnamaldehyde and o-nitrocinnamaldehyde were also used to synthesize some interesting β-lactam intermediates.

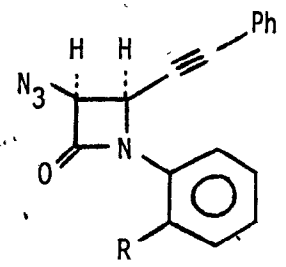
The Schiff base 97 was obtained by refluxing for 2.5 h a solution of aniline in benzene with one equivalent of phenylpropargyl aldehyde and

removing the water formed with a Dean-Stark trap. Treatment of 97 with azidoacetyl chloride-triethylamine in methylene chloride at -20° afforded a good yield of exclusively *cis* β-lactam 98; m.p. 85-86.5°. There were two doublets at 4.80 and 5.01 ppm (J = 5 Hz) for the two protons of the β-lactam 98 in the pmr spectrum. The Schiff base 99, prepared in a similar manner as described before, was silylated with trimethylsilyl chloride-triethylamine. The silylated Schiff base was subjected to treatment



97 R = H

99 R = OH

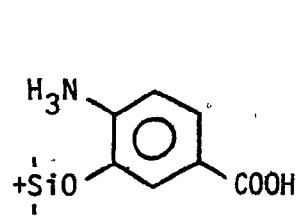


98 R = H

100 R = OH

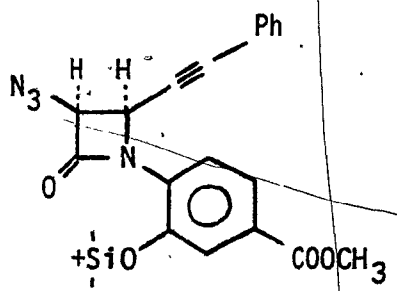
with azidoacetyl chloride-triethylamine affording *cis* β-lactam 100. In the infrared spectrum, the β-lactam carbonyl group absorbed at 1730 cm^{-1}, which was 35 cm^{-1} lower than that of β-lactam 98.

β-Lactam 101 was prepared from the carboxylic acid 84. The infrared spectrum of 101 showed the absorption frequency of the carbonyl groups at 1780 and 1720 cm^{-1} for the β-lactam and methyl ester, respectively.



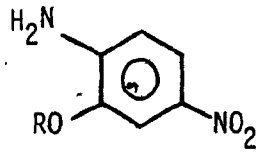
84

1. $C_6H_5C{\equiv}C\text{-}CHO$
2. $(CH_3)_3SiCl$, NEt_3
3. N_3CH_2COCl, NEt_3
4. CH_2N_2

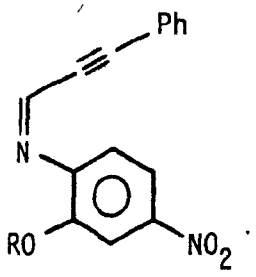


101

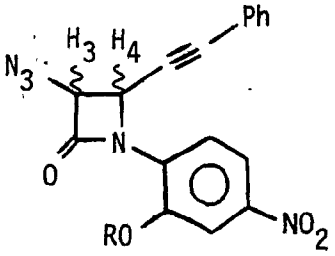
To our surprise, the reaction of phenylpropargylidene Schiff base 103, obtained by refluxing amine 102[74] and phenylproparglyaldehyde in benzene overnight, with azidoacetyl chloride-triethylamine afforded approximately a 1:1 mixture of *cis* β-lactam 104 and *trans* β-lactam 105 in 60% yield after purification. In the pmr spectrum (Fig. 9, p. 47) of



102 R = t-BuSiPh$_2$



103 R = t-BuSiPh$_2$



104 *cis* R = t-BuSiPh$_2$

105 *trans* R = t-BuSiPh$_2$

cis β-lactam 104, protons H_3 and H_4 appeared as two doublets (J = 5 Hz) at 4.98 and 5.54 ppm. In the case of the *trans* β-lactam 105 (Fig. 10, p. 47) the coupling constant of H_3 and H_4 was about 2 Hz.

In addition, β-lactam 106, 107, 108 and 109 were also synthesized from the corresponding amine and β-phenylcinnamaldehyde or o-nitrocinnamaldehyde.

Some of the intermediates in the sequences, compounds 94, 96, 101, 106, 108 and 109 were totally devoid of antibacterial activity. Some were mildly active against several bacteria, which is shown in Table 1 (p. 48).

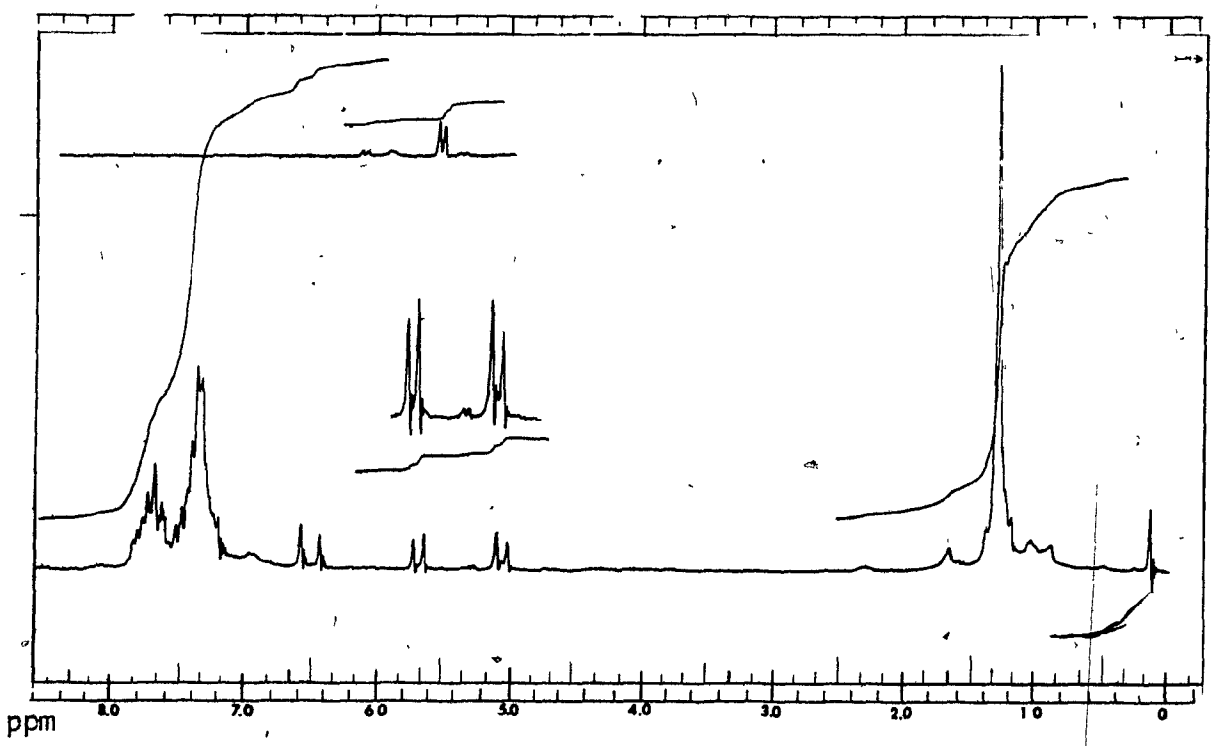


Fig. 9: 60 MHz pmr spectrum of 104, in $CDCl_3$.

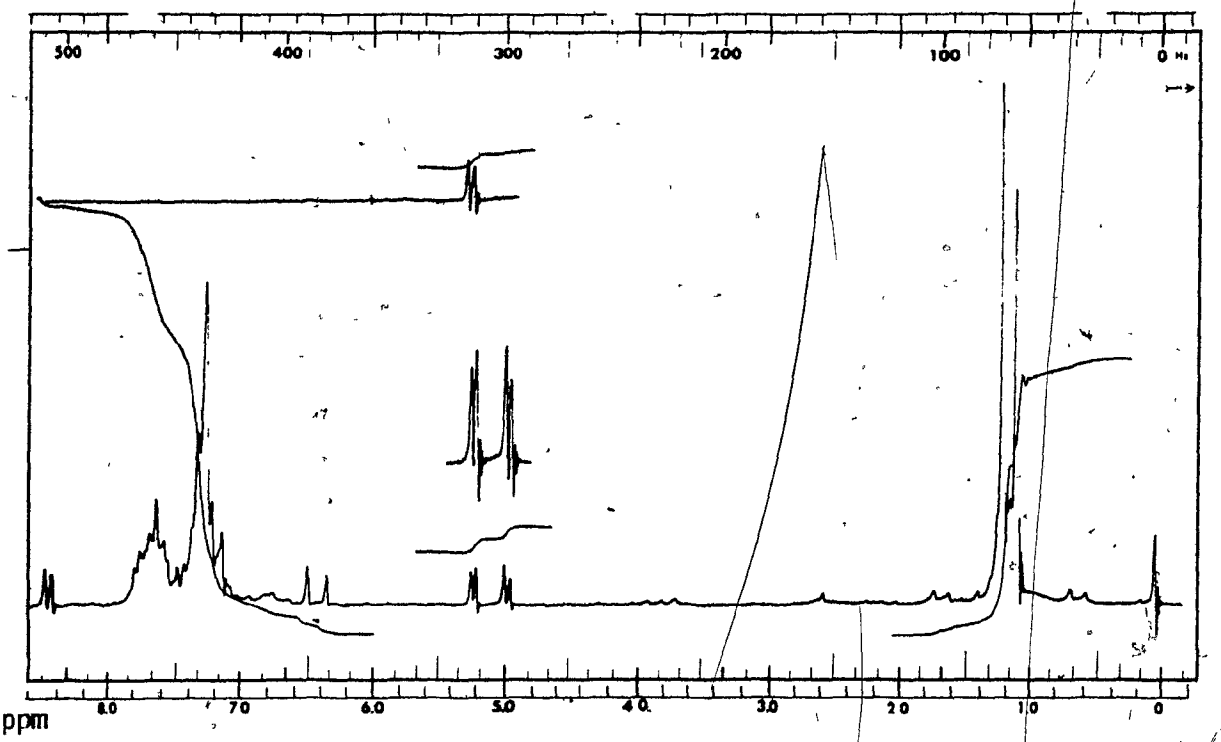


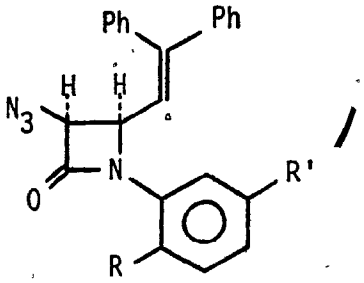
Fig. 10: 60 MHz pmr spectrum of 105, in $CDCl_3$.

Table 1. *In vitro* testing results* for some β-lactam intermediates.

Organisms	MINIMAL INHIBITORY CONCENTRATION (MIC μg/mL)												
	72a	72b	80	88	89	95	98	100	104	105	107	Cefo.	Pip.
E. Coli ESS 22-31	16	16	64	>>	128	-	256	256	32	32	128	≤ .03	≤.06
E. cloac. MA 75-1	>	>	64	>>	128	-	256	128	32	32	-	-	>128
Enteroc. OSU 75-1	32	32	-	-	-	>64	-	-	-	-	128	128	2
Enteroc. SM 77-15	32	32	128	>>	>>	>64	>>	>>	>>	64	128	128	2
Micro. lutea PCI 1001	8	8	-	>>	128	-	>>	>>	32	32	2	.06	≤.06
Staph. aureus Q 74-1	8	8	32	64	64	-	64	64	32	16	-	-	.25
Staph. aureus Q 74-6	-	-	32	128	128	-	128	128	32	16	-	-	.5
Staph. FU 79-19-2 β^+	-	-	-	-	-	>64	-	-	-	-	2	4	32
Staph. smith	-	-	-	-	-	-	-	-	-	-	2	2	.5
Staph. SSC 79-18 β^-	-	-	-	-	-	>64	-	-	-	-	2	1	.5

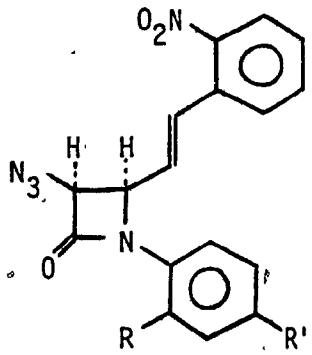
Note: >> present > 256, Cefo. Cefotaxime, Pip. Piperacillin.

*Antibacterial testing was performed at Lederle Laboratories of the American Cyanamide Company.



106 R = R' = H

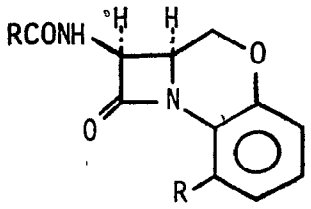
107 R = O-t-BuSiPh$_2$
R' = NO_2



108 R = R' = H

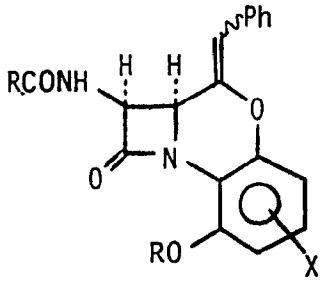
109 R = O-t-BuSiPh$_2$
R' = NO_2

As mentioned in the beginning of this chapter, the synthesis of compounds B[70] and C[72] had been accomplished in our laboratory.



B R = OH

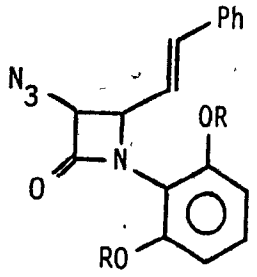
C R = COOH



D

Although the carboxylic acid C showed no activity toward a variety of bacteria, phenol B with a lower β-lactam infrared absorption frequency (1760 cm^{-1}) displayed weak activity toward bacteria. With this knowledge in mind and the other factors described on page 37 , we thought it would be interesting to synthesize compounds with the general structure D and to examine their biological activity.

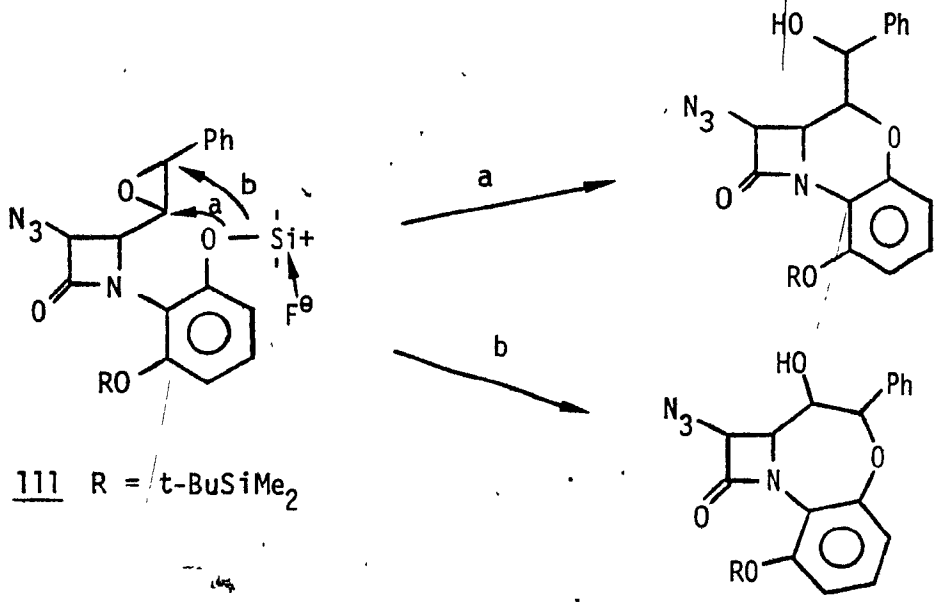
As a model, the relatively readily available β-lactam 110[75] was used as starting material. This starting material also has the advantage that one could introduce a destabilizing nitro group at the end[70]. Epoxidation of 110 with m-chloroperbenzoic acid afforded in 90% yield



110 R = t-BuSiMe$_2$

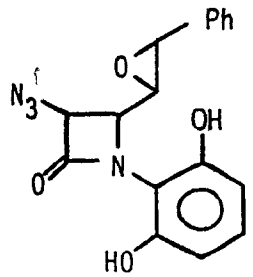
two isomers of 111, which were not completely separable by flash chromatography. We anticipated that treatment of 111 with one equivalent of fluoride anion should desilylate one of the silyl ether and then open the epoxide ring to form either a six- or seven-membered ring (Scheme 1). Reaction of the epoxide mixture 111 with one equivalent

Scheme 1

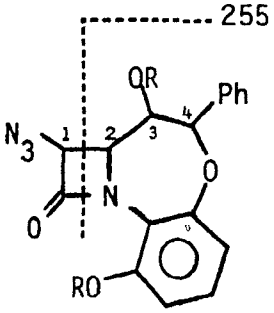


111 R = t-BuSiMe$_2$

of potassium fluoride in the presence of 18-crown-6 at 0° or tetra-n-butylammonium fluoride at -20° failed to give either of the expected compounds. However, desilylation was finally achieved by the reaction of 111 with two equivalents of tetra-n-butylammonium fluoride and three equivalents of acetic acid at 0° to afford phenol 112. After several unsuccessful attempts with different acidic or basic reagents, phenol 112 was cyclized using camphorsulfonic acid in methylene chloride. A 5.5% yield of one isomer having structure 113 was isolated. The latter resulted from attack of the phenolic hydroxy group at the benzylic position. The mass spectrum of 113 showed a parent ion at m/e 338 (6%) and peaks at m/e 310 (28.5%) and 255 (6.7%) for the loss of a nitrogen molecule and the fragment as indicated on the structure 113, respectively. The 200 MHz pmr spectrum of 113 displayed a doublet of doublets at 3.9 ppm for H_2 (J = 5,8 Hz), a triplet (doublet of doublets) at 4.21 ppm for H_3 (J = 8,8 Hz) and a doublet at 4.44 and at 5.14 ppm for H_4 (J = 8 Hz) and H_1 (J = 5 Hz). Acetylation of 113 with acetic anhydride afforded the acetate 114. The structure of

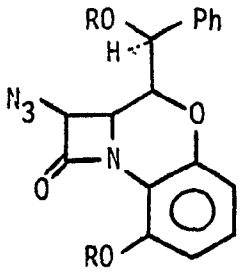


112



113 R = H

114 R = OAc



R

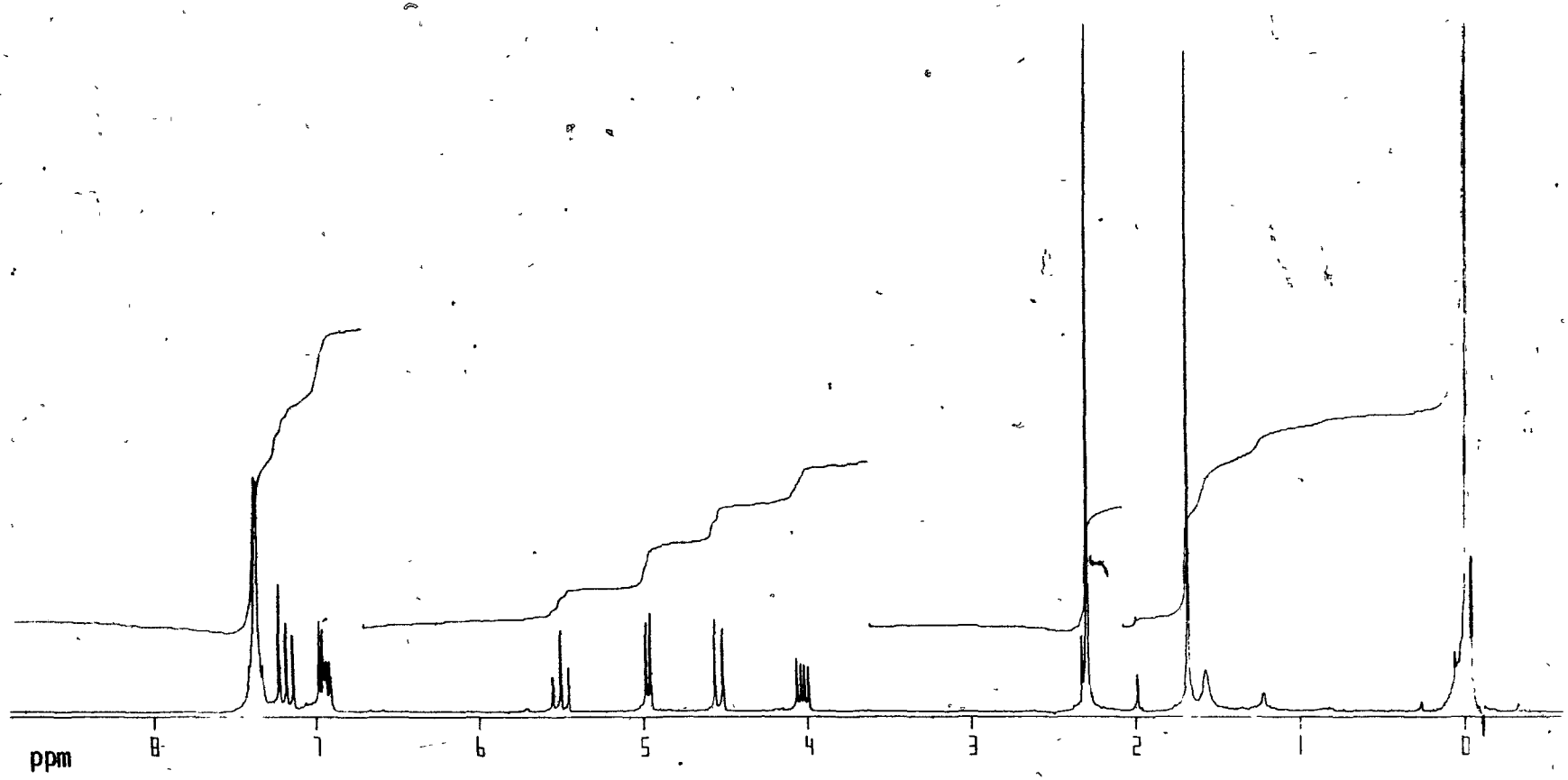
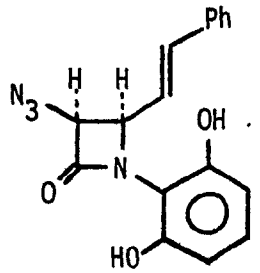


Fig. 11: 200 MHz pmr spectrum of 114, in $CDCl_3$.

113 was further confirmed by the pmr spectrum of acetate 114 (Fig. 11, p. 52). In particular, acetylation resulted in the shift to lower field (5.50 ppm) of the proton (H_3) α to a secondary aliphatic hydroxy group. However, if a six-membered ring of the structure E were the adduct, we would expect, after acetylation, that the proton of the benzylic alcohol should shift to a much lower field.

Some other methods were also used to attempt cyclization in the desired manner, but no six-membered ring was ever detected. For example, attempted bromination of the styryl double bond on 110 led to the bromination of the aromatic ring and partial desilylation of the silyl ether groups. The reaction of β-lactam 108 with N-bromosuccinimide and water in dimethyl sulfoxide[76] or hydrogen bromide-acetic acid[77] was unsuccessful as well. After desilylation of β-lactam 110, treatment of β-lactam 115 with phenylselenenyl chloride according to the method of Nicolaou[78] also failed. It is suspected that the phenylselenenyl electrophile attacked the electron-rich



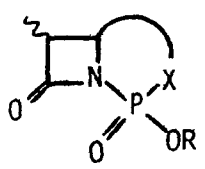
115

aromatic ring, rather than the styryl double bond.

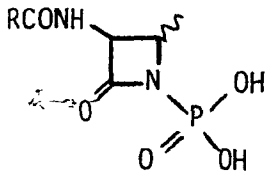
Since we were unable to devise a method to construct the six-membered ring in all our attempts, this project was not continued.

CHAPTER III

This chapter will describe synthetic studies toward β-lactams having structure F and monobactam analogues of the type G. Several different



F



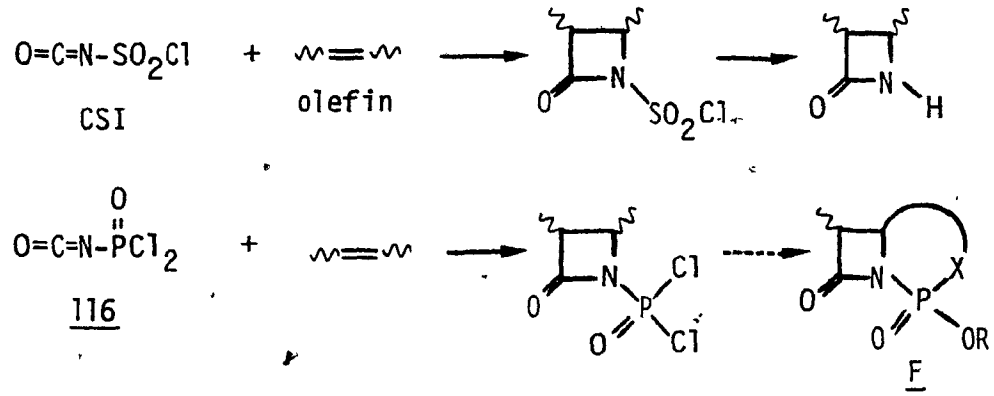
G

approaches have been investigated and will be discussed.

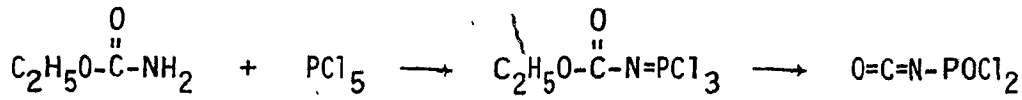
ADDITION OF N-PHOSPHORISOCYANATE TO OLEFINS

The cycloaddition reaction of an isocyanate with an olefin has been used extensively for the synthesis of various substituted β-lactams. Chlorosulfonyl isocyanate (CSI) is the most reactive, and some other isocyanates have also been used[80]. The reactivity towards condensation with olefins is greatly diminished by the presence of groups less electron-withdrawing than chlorine, on the sulfonyl moiety. Addition of isocyanates have been performed on various olefins, and it was found that less reactive isocyanates required reactive olefins to produce β-lactams[81]. Based on this methodology, we thought it might be possible to synthesize compound F *via* a similar route as shown in Scheme 2.

Scheme 2

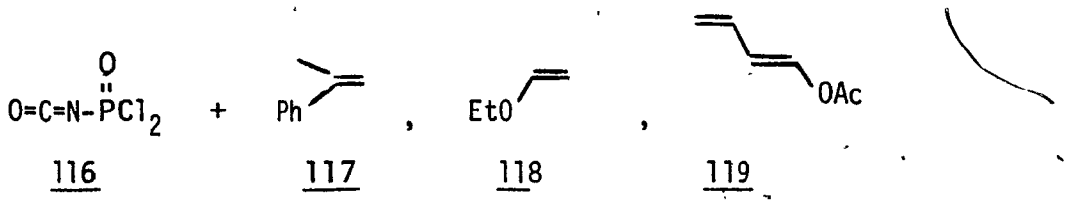


The phosphorisocyanatidic dichloride 116 was prepared by addition of ethyl urethane to phosphorus pentachloride in dichloroethane, according to Kirsanov's method[82].



It is known that, to a high degree, the speed of the cycloaddition reaction depends on the electrophilicity of the carbon-carbon double bond, and on the polarity of the solvent. Therefore, we chose the most reactive olefin often used in CSI cycloaddition, α-methylstyrene 117[83], as starting material. Attempts to react 116 with α-methylstyrene under various conditions were unsuccessful: in anhydrous ether at room temperature or refluxing, in methylene chloride at room temperature or refluxing, in nitromethane at room temperature. The reaction mixture showed no absorption within the 1800-1700 cm^{-1} region in the infrared, and from the pmr spectrum

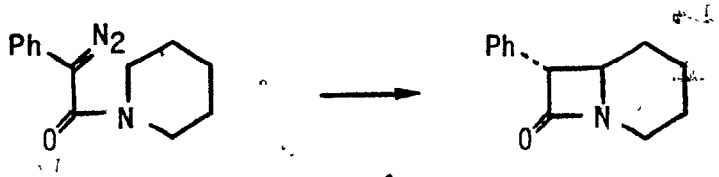
most of the α-methylstyrene 117 was found to be unreacted. Furthermore, Dr. D. Dugat, in our laboratory, attempted to react 116 with ethyl vinyl ether 118 or 1-acetoxy-1,3-butadiene 119. However, no β-lactam-containing products were obtained.



116 117 118 119

ANNULATION THROUGH CARBENE INSERTION

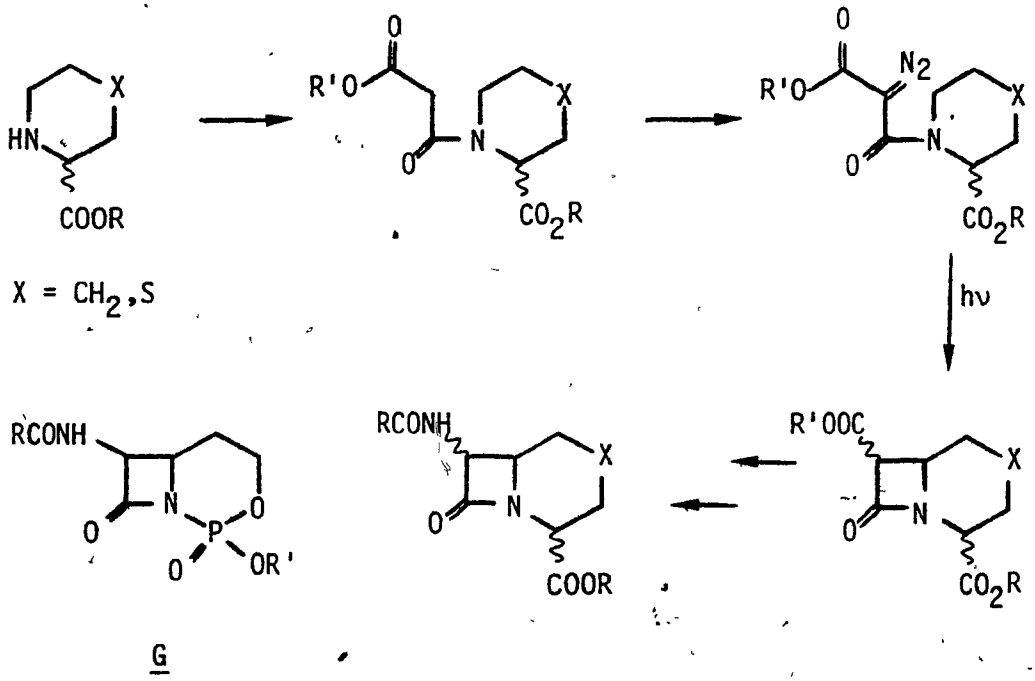
We then decided to study an alternate approach used extensively by G. Lowe *et al.*, for the formation of β-lactam *via* the cyclization of the corresponding intermediate carbene. This method was first employed by Corey and Felix[84], who showed that photolysis of the diazo-amide 120 gave a single β-lactam, the *trans* isomer 121. Lowe[85] extended the method to the synthesis of several bicyclic β-lactams, containing a carboxylic



120 121

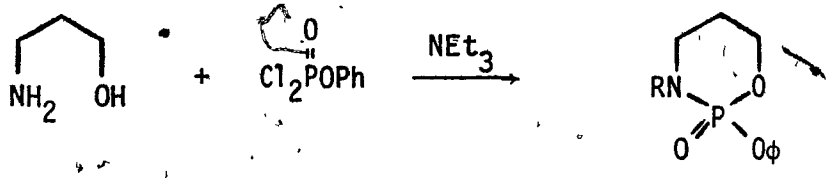
acid function α to the β-lactam carbonyl, which was later converted into an amino group, through a Curtius reaction (Scheme 3). Based on this methodology, we planned to construct the desired compound G.

Scheme 3



G

Thus, phenyl cyclophosphoramidate 122 was prepared from 3-amino-1-propanol 123 and commercially available chlorophosphate 124.

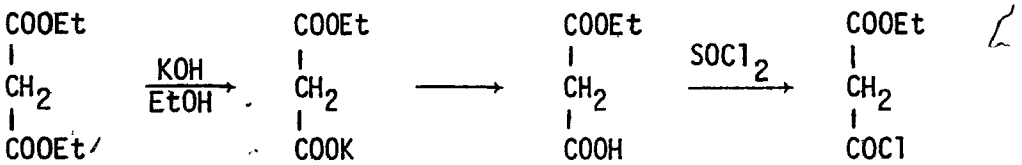


123 124 122 R = H

125 R = C_6H_5CO

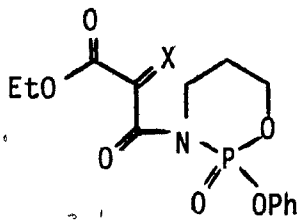
Treatment of 122 with one equivalent of butyllithium and benzoyl chloride afforded 125, in 90% yield. The synthesis of the required ethyl malonate fragment was accomplished using the method of Breslow[86]. Diethyl malonate

was readily converted into its monopotassium salt. The free acid obtained



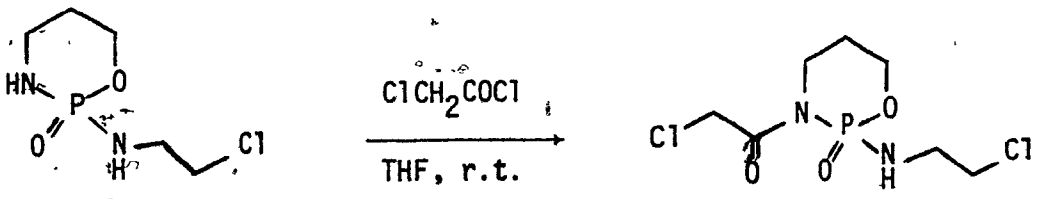
126

from this salt was transformed into the corresponding acid chloride 126 by treatment with thionyl chloride. Unfortunately, addition of ethyl malonyl chloride 126 to the cyclophosphoramidate anion of 122, generated with butyllithium, did not give any of the expected amide 127. Perhaps,



127 X = H_2

130 X = N_2

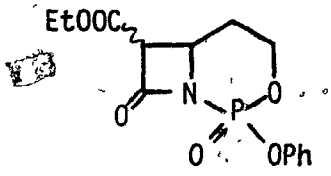


128 129

deprotonation of the methylene group on compound 126, and/or formation of a ketene, was responsible for the failure of this reaction.

In 1979, Stec[87] reported that reaction of cyclic phosphoramide 128 with an equivalent amount of chloroacetyl chloride in tetrahydrofuran at room temperature gave the corresponding chloroacetylamide 129, in 73% yield. In a similar manner, we readily transformed cyclic phosphoramide 122 in excellent yield into amide 127 which was obtained as a colorless oil. Amide 127 underwent smooth base catalyzed diazo-exchange with p-toluenesulfonyl azide[88] and triethylamine, to afford compound 130. The infrared spectrum showed a strong absorption at 2135 cm^{-1} and the mass spectrum displayed a molecular ion at m/e 353. The pmr spectrum of 130 (Fig. 12, p. 60) showed a broad triplet at 3.2-3.8 ppm for one of the protons α to the amide nitrogen, in addition to all other appropriate signals.

The following transformation, namely a carbene insertion, would be the key step in our sequence, to form a fused bicyclic β-lactam system 131. Irradiation of the α-diazo-amide 130 at 0° in carbon tetrachloride



131

using a Rayonet photochemical reactor, with maximum output at 350 mμ, led to a complicated reaction mixture. The infrared spectrum did not show any diazo or β-lactam absorptions at 2200 or ∿ 1750 cm^{-1}, respectively.

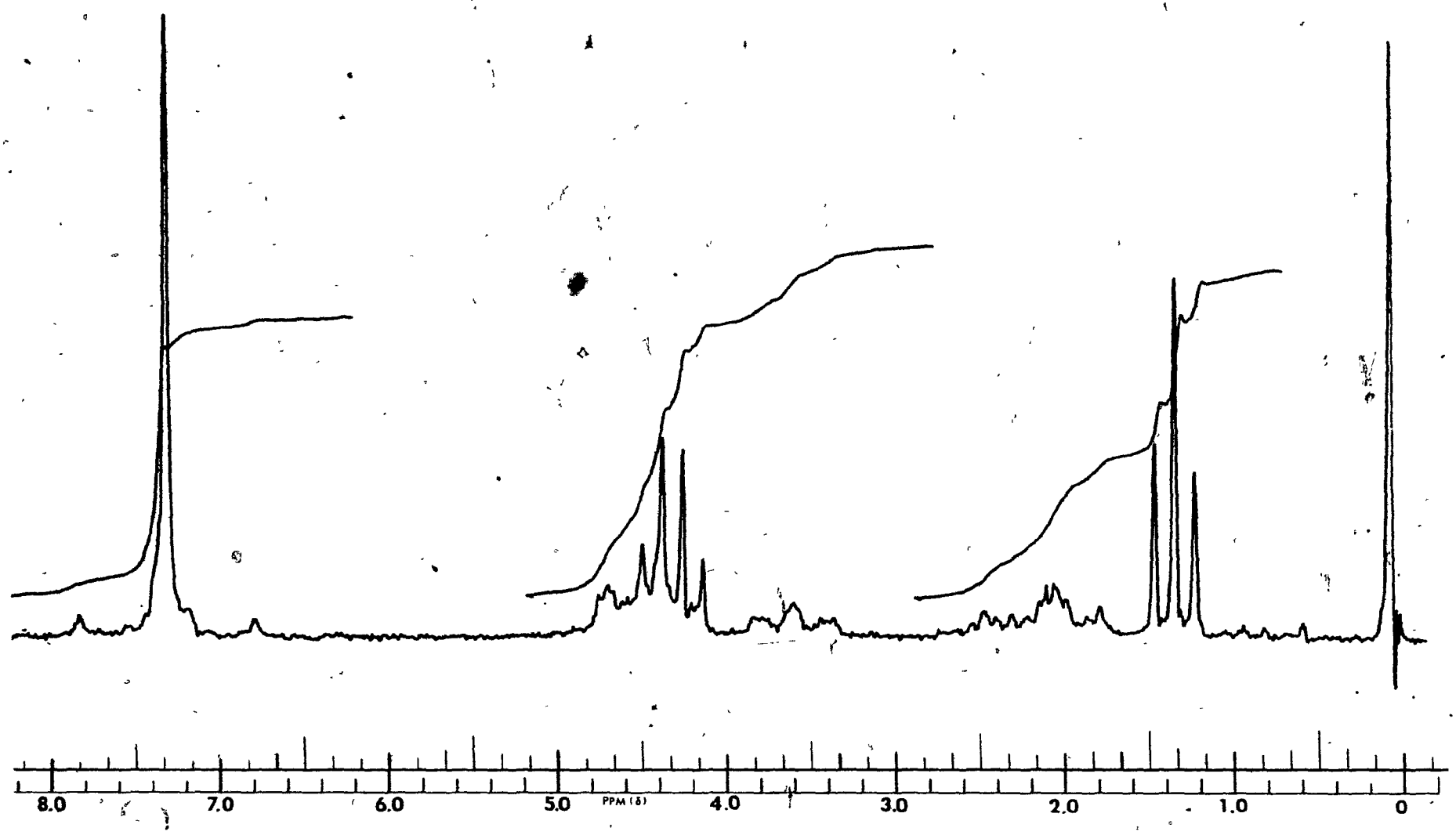
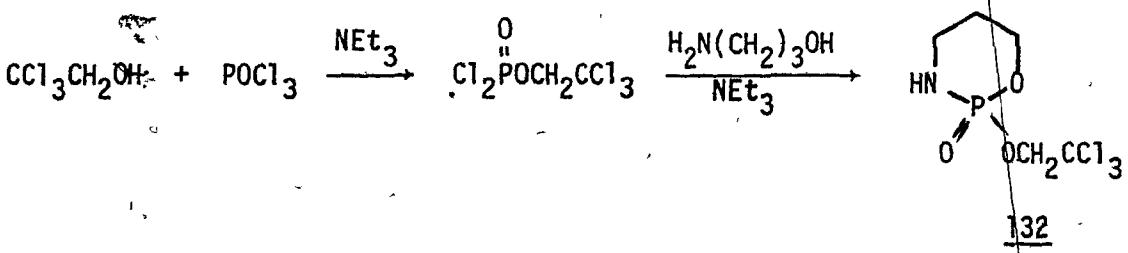


Fig. 12: 60 MHz pmr spectrum of 130, in $CDCl_3$.

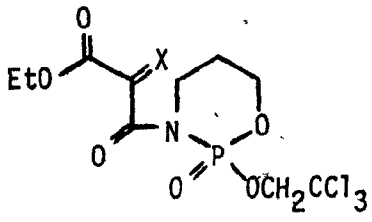
Using flash chromatography, one of the less polar spot isolated, proved to be phenol. Since phenol absorbs in ultraviolet spectrum at 210 mμ and 270 mμ, we suspected that it might interfere with the carbene insertion reaction under the photolysis condition. We therefore decided to replace the phenoxy moiety by a trichloroethyl group. This protecting group can be removed in the presence of a β-lactam[34], and should not absorb light.

Addition of 3-amino-1-propanol and triethylamine to trichloroethyl dichlorophosphate, prepared *in situ* by reaction of trichloroethyl alcohol and triethylamine with phosphorus oxychloride, afforded the cyclophosphoramidate 132, in 93% yield, after flash chromatography. The mass spectrum of 132 showed the isotopic pattern for the existence of the three chlorine atoms. Phosphoramidate 132 was coupled, as before, with ethyl malonyl chloride 126 to give amide 133. The diazo compound 134 was obtained



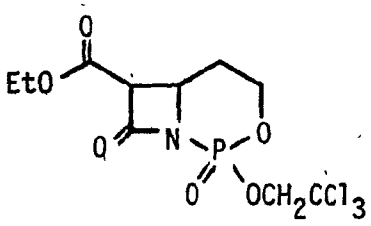
132

in a manner analogous to that of its corresponding phenyl derivative 130.



133 X = HH

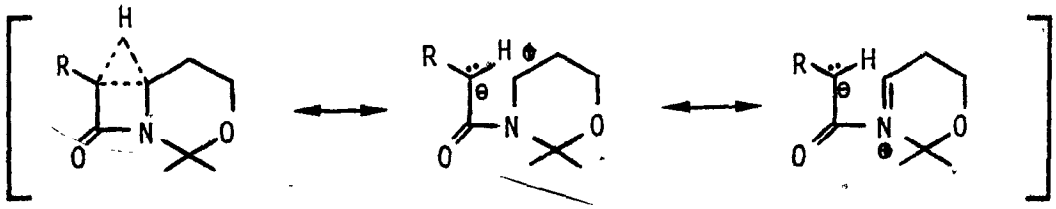
134 X = N_2



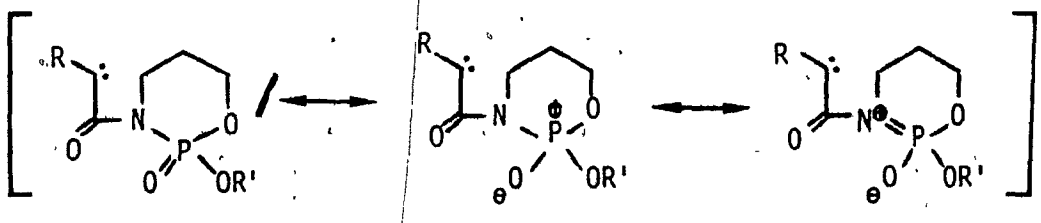
135

Attempts to photolyze 134 using a Rayonet photochemical reactor in methylene chloride did not give any of the desired β-lactam 135, but afforded instead a complex mixture of products. Recently, Southgate[42] prepared an intermediate for the synthesis of thienamycin, in very good yield, *via* either photochemical or metal catalyzed cyclizations of a carbene (see also Introduction, p. 15). Diazoester 134, dissolved in toluene in the presence of a catalytical amount of copper was recovered unreacted, after stirring at room temperature for 4 hours. The reaction mixture was then refluxed for 18 h, during which 134 gradually disappeared, to be converted into a complicated reaction mixture. This mixture lacked any absorption within the 1700-1800 cm^{-1} region in the infrared spectrum. Attempts to cyclize 134 using rhodium (II) acetate in methylene chloride also failed, giving unidentifiable products.

The preference for the insertion of carbene into the α-CH bond of nitrogen has been rationalized in terms of polar resonance structures of the transition state[89]. Therefore, there is a possibility that the



undesired resonance form caused by phosphorus moiety might inhibit the insertion reaction. One could also consider that the electron-rich oxygen on the phosphate might somehow react with electron deficient carbene to



interfere with the desired transformation. Due to the failure of the carbene insertion at this critical stage in the synthesis, this approach was abandoned.

PHOSPHORYLATION OF N-UNSUBSTITUTED β-LACTAMS ON NITROGEN

A survey of literature revealed that numerous compounds with the general structure $R'CO\text{-}NH\text{-}P(O)(OR)_2$ had been synthesized. Usually, primary amides reacted with phosphorus pentachloride to form N-acyl-trichlorophosphinimides H[90]. The reaction of three equivalents of alcohol with trichlorophosphinimides H resulted in the formation of the phosphoramidates I. However, examples of the synthesis of phosphoramidates from a chloro-

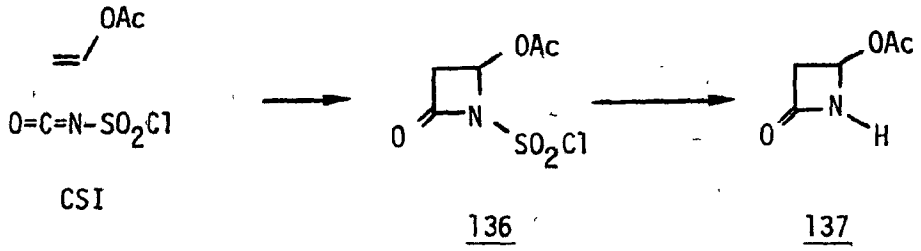
$$RCO\text{-}NH_2 + PCl_5 \longrightarrow \underset{\underline{H}}{RCO\text{-}N{=}PCl_3} + 2HCl$$

$$RCO\text{-}N{=}PCl_3 + 3R'OH \longrightarrow \underset{\underline{I}}{RCO\text{-}NH\text{-}P(O)(OR')_2}$$

phosphate and an amide, especially a secondary amide, could not be found. Therefore, in order to be able to attach a phosphate to the nitrogen on a

β-lactam ring, we decided to first explore the reaction on a model.

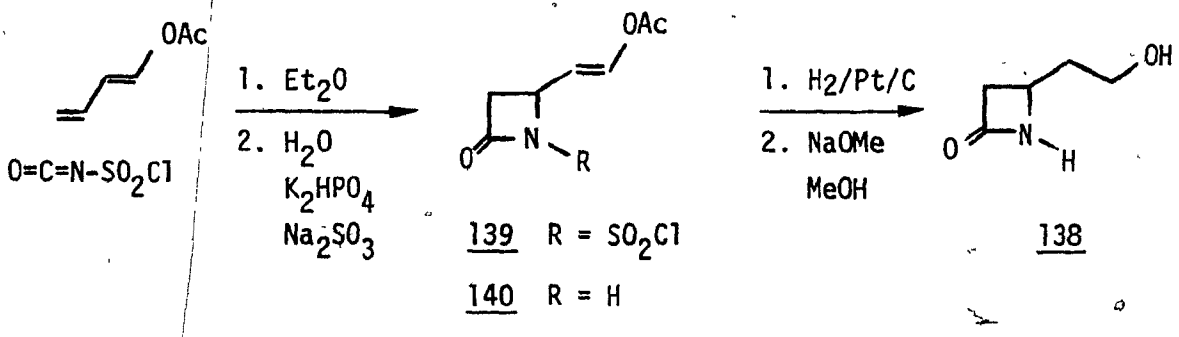
Hence, azetidinone 136 was synthesized from the condensation of CSI and vinylacetate[91]. Reductive hydrolysis of the chlorosulfonyl group using sodium sulfite and sodium bicarbonate yielded acetoxyazetidinone 137. Reaction of β-lactam 137 and diethyl chlorophosphate, in tetrahydro-



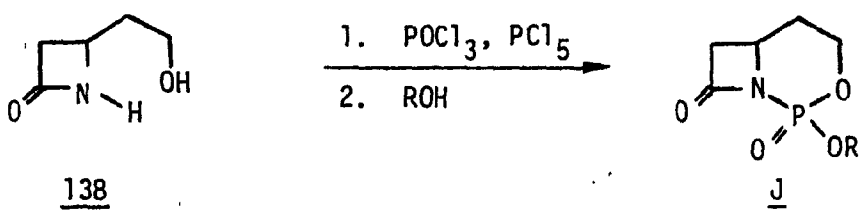
furan with or without triethylamine at room temperature, or in methylene chloride with triethylamine at room temperature or at reflux afforded unreacted starting material 137. Addition of diethyl chlorophosphate to the anion of β-lactam 137, generated by sodium hydride in tetrahydrofuran, gave some unidentifiable products, lacking absorptions for the carbonyl groups of the acetate and β-lactam in the infrared spectrum. Treatment of β-lactam 137 with phosphorus pentachloride or phosphorus oxychloride and triethylamine resulted in its decomposition. In view of those results we suspected that under such conditions β-lactam 137 was probably not an appropriate model owing to the lability of the acetate group.

Unable to prepare the N-phosphorylated derivative of β-lactam 137, we decided to attempt similar condensation on β-lactam 138 instead of 137. Christensen *et al.*[50] published the first total synthesis of thienamycin

in 1978. They found that the cycloaddition of 1-acetoxybutadiene 119 and CSI gave β-lactam 139. Reductive hydrolysis (H_2O, K_2HPO_4, Na_2SO_3, 0°) of 139 afforded the crystalline acetoxyvinylazetidinone 140 in 42% overall yield, based on isocyanate. Hydrogenation, followed by deacetylation using a catalytical amount of sodium methoxide in methanol at 0° afforded alcohol 138 in excellent yield. After much experimentation, we were able



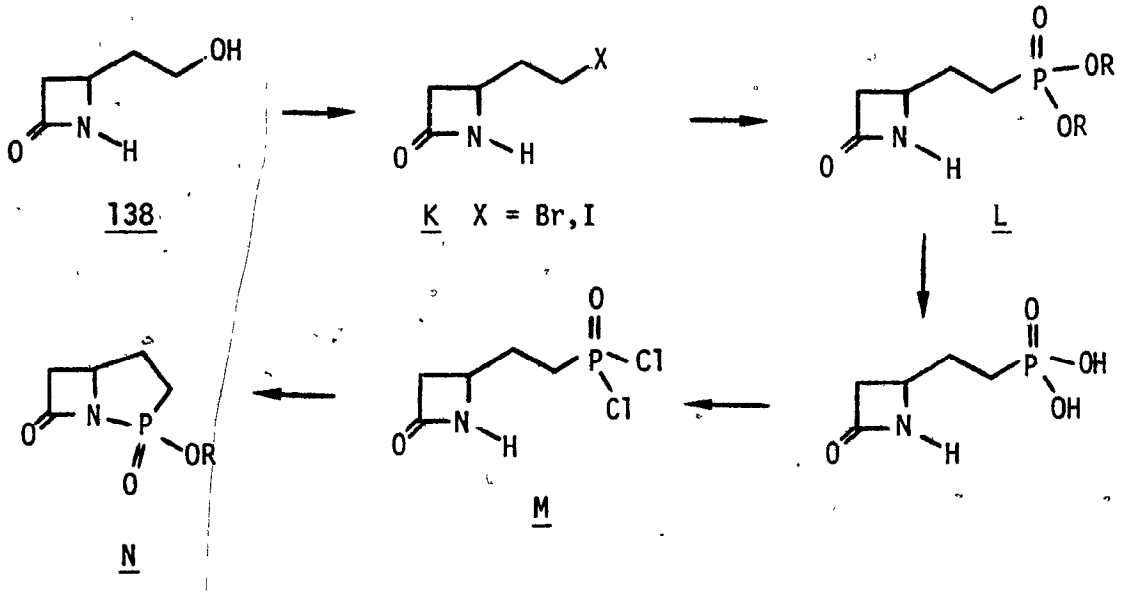
to repeat the above cycloaddition reaction obtaining at best a 25% yield of 140. Subsquently, hydrogenation and deacetylation produced the required starting material 138. We hoped that the reaction of β-lactam 138 with phosphorus oxychloride, followed by quenching with water or alcohol, would afford bicyclic β-lactam J, in one operation. β-Lactam 138 was reacted with phosphorus pentachloride or phosphorus oxychloride in methylene



chloride, in pyridine and in methylene chloride containing triethylamine (or pyridine). Unfortunately, all of the above attempts to prepare J were unsuccessful. Due to the difficulties encountered, another approach was initiated.

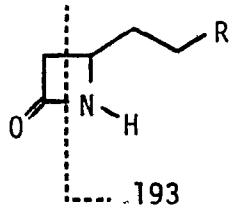
We planned to convert alcohol 138 to β-lactam K, form phosphonate L from halide K through an Arbuzov reaction, deprotect phosphonate L and transform it into the dichlorophosphonate M, and finally cyclize the resulting compound (Scheme 4).

Scheme 4



Reaction of alcohol 138 with N-bromosuccinimide and triphenylphosphine in N,N-dimethylformamide afforded bromide 141 and triphenylphosphine oxide. Because of the R_f values of both products being very similar, purification was difficult. Thus, the Arbuzov reaction was

attempted on crude compound 141, hoping that the purification would be possible at this stage. The Arbuzov reaction was performed by heating crude bromide 141 and commercially available triethyl phosphite at 120° for 18 h. Following flash chromatography of the reaction mixture β-lactam 142 was isolated, in 21% yield. In the pmr spectrum of 142 (Fig. 13, p. 68) the ethyl groups appeared as a triplet at 1.15 ppm and a quartet at 4.0 ppm.



141 R = Br

142 R = $P(O)(OEt)_2$

A broad multiplet was found at 1.4-2.0 ppm corresponding to the two methylene groups next to the phosphonate. The signals and coupling constants for the three β-lactam ring protons were very similar to those for the same protons in alcohol 138. The mass spectrum showed a molecular ion and a very intense peak at m/e 193 for the fragment (loss of ketene) shown on structure 142.

There are several methods[92,93] in the literature for the preparation of alkylphosphonyl dichlorides from phosphonic acids or phosphonates. Okamoto[93] recently reported the most mild and efficient method *via* the silyl ester. Bis(trimethylsily) phosphonates, which were prepared from the dialkyl phosphonates with chlorotrimethylsilane-sodium iodide, were transformed in high yield into the corresponding phosphonyl dichlorides on

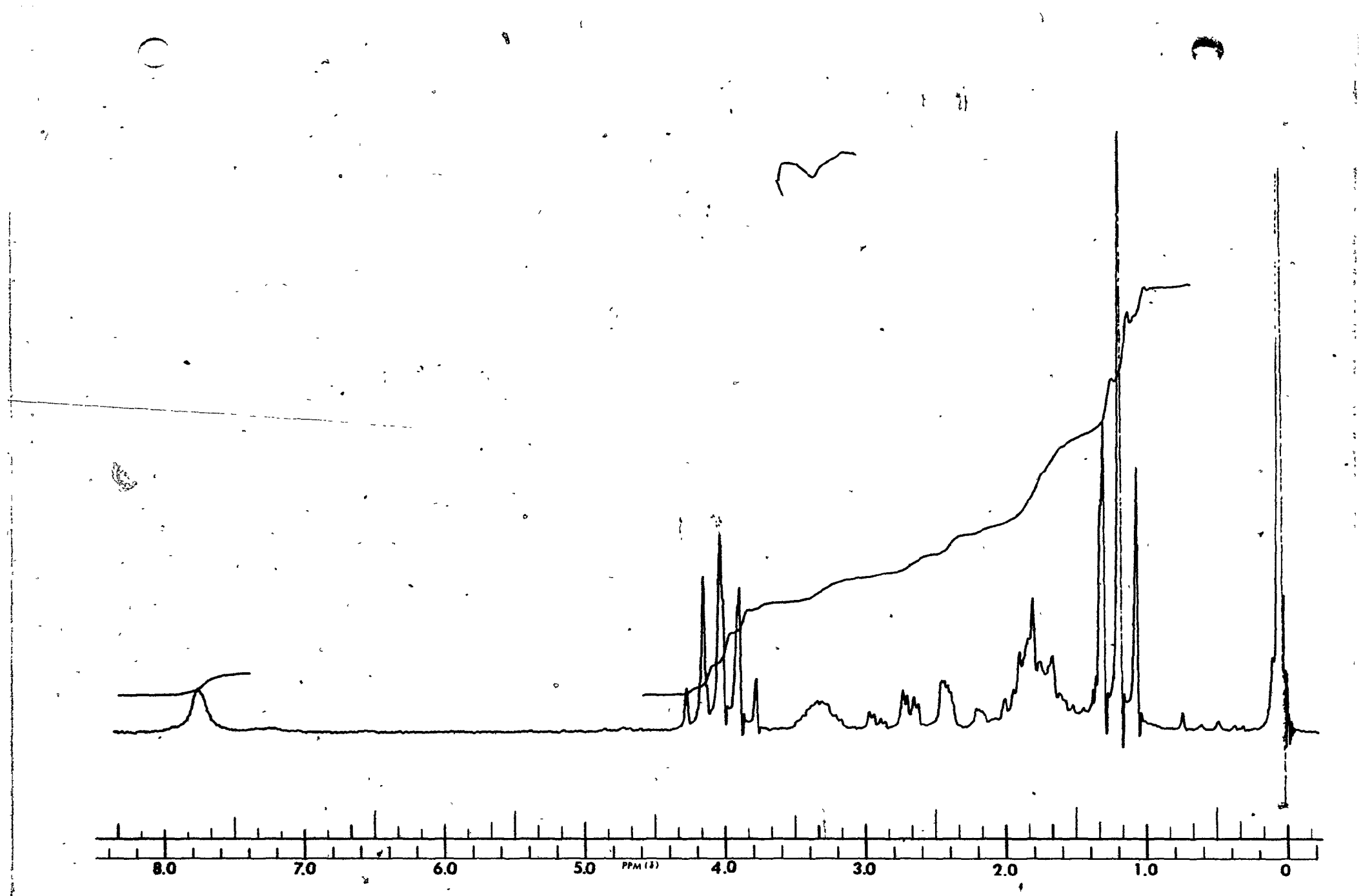
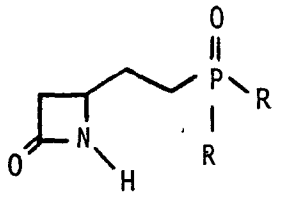


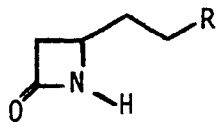
Fig. 13: 60 MHz pmr spectrum of 142, in $CDCl_3$.

the treatment with phosphorus pentachloride at 20-30° in carbon tetrachloride. Subjection of phosphonate 142 to similar conditions failed to give dichloride 143 and resulted in the destruction of the β-lactam ring. Attempts to dealkylate phosphonate 142 with an equivalent amount of chlorotrimethylsilane in the presence of sodium iodide[94] did not afford any of the desired phosphonic acid 144, but gave instead complicated reaction products with no β-lactam carbonyl absorption in the infrared spectrum. In



143 R = Cl
144 R = OH
145 R = OMe

view of the fact that it was difficult to remove the ethyl group of the phosphonate 142 smoothly, and that formation of 142 was a low yield reaction, we chose to use trimethyl group instead of triethyl phosphite for the Abruzov reaction. We expected that a methyl[95] group should be more easily removed than the ethyl group. Crude bromide 141 was reacted with trimethyl phosphite at 110° for 16 h. However, an inseparable mixture containing methylphosphonate 145, in very low yield, was obtained. We therefore turned our attention to the synthesis of iodide 146. We hoped that the better leaving group ability of iodide compared with bromide would improve the Arbuzov reaction. Therefore, alcohol 138 was transformed into its mesylate 147 in the usual manner. Refluxing 147 with sodium iodide in acetone for 7 h afforded iodide 146, in excellent yield. However, reaction

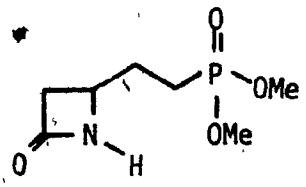


146 R = I

147 R = OMs

of iodide 146 with trimethyl phosphite failed as with the bromide. Since it is known that the yields of Arbuzov reactions are usually not very high[96], we thus sought an alternate route to synthesize the desired phosphonate 144.

The sodium salt of dimethyl phosphite, generated with sodium hydride, was reacted with mesylate 147 in dry tetrahydrofuran at 0°. After stirring at room temperature or refluxing for several hours, only the starting mesylate 147 was recovered, after work-up. Addition of 147 to the anion of dimethyl methylphosphonate, prepared using sodium hydride, at room temperature for 18 h, did not afford any phosphonate 148. Instead unreacted mesylate 147 was recovered. We thought that perhaps a stable N-sodium salt was being formed. Hence, mesylate 147 was silylated with dimethyl-t-butylsilyl chloride and triethylamine, in the usual manner, to afford the silylated β-lactam 149. To the lithium salt of dimethyl



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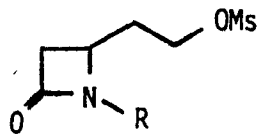


149

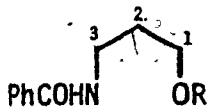
R = t-BuSiMe$_2$

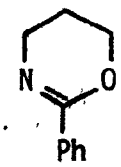
methylphosphonate, prepared using n-butyllithium at -78° in tetrahydrofuran was added a solution of silylated β-lactam 149. After stirring at room temperature for 3 days, thin-layer chromatography still showed the existence of most of β-lactam 149. This reaction was not pursued further, and this scheme was abandoned.

AMIDE-MODEL STUDY

Because of the low yield of formation of β-lactam 140 and the troublesome preparation of the starting alcohol 138, a simple amide was chosen as a model to investigate the reaction of an amide nitrogen with a chlorophosphate.

To a solution of aminopropanol 123 in 5 N sodium hydroxide was added benzoyl chloride and 5 N sodium hydroxide. Neutralization and extraction afforded amide 150 as a colorless oil. Reaction of amide 150 with phosphorus





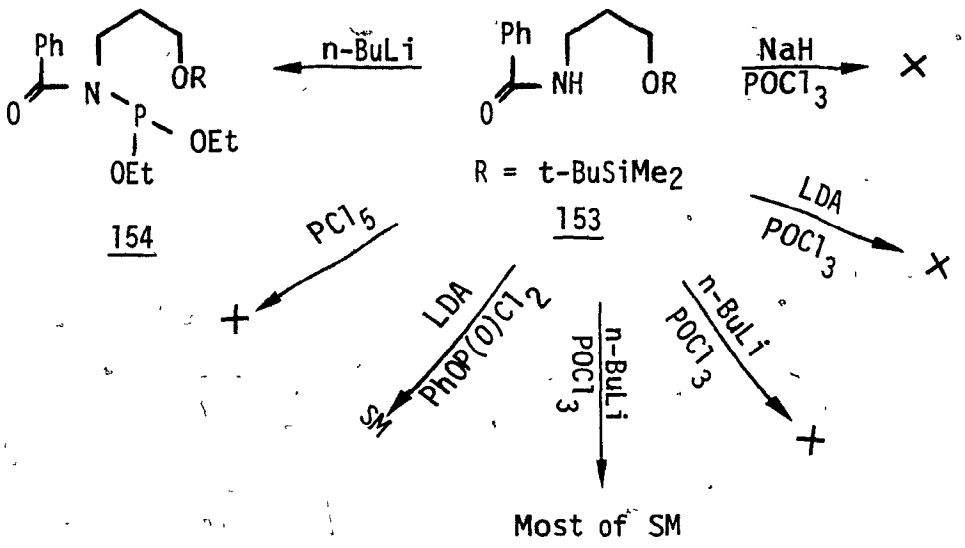
150 R = H
151 R = $POCl_2$

152

oxychloride and one equivalent of triethylamine in dry tetrahydrofuran at

-78° for 3 h, afforded triethylamine hydrochloride and a product, presumably, dichlorophosphate 151. The pmr spectrum of 151 showed a multiplet at 2.1-2.6 ppm for $2H_2$, a broad triplet at 3.6-4.0 ppm for $2H_3$, a triplet at 4.72-5.08 for $2H_1$, and a multiplet 7.3-8.2 ppm for the five protons of aromatic ring and the proton of the amide. In the infrared spectrum, there were absorptions at 1660, 1380, 1300 and 1110 cm^{-1}, but no absorption at 3500-3100 cm^{-1} for the hydroxyl group. Treatment of 151 with sodium methoxide in methanol, produced, after flash chromatography, compound 152. Meanwhile, compound 152 was also obtained by the reaction of 150 and phosphorus oxychloride with two equivalents of triethylamine. The pmr, infrared, mass spectra and analysis were in perfect accordance with the structure of 152.

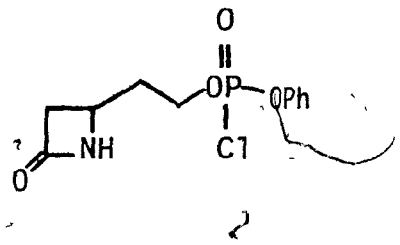
In order to avoid this side-reaction, amide 150 was silylated with dimethyl-t-butylsilyl chloride to give 153. Several reaction conditions were then attempted in order to prepare the desired N-phosphorylated derivative. Treatment of silyl ether 153 with sodium hydride or n-butyllithium, followed by the addition of phosphorus oxychloride resulted in the formation of unidentifiable products. Reaction of the anion of amide 153, obtained by treatment with lithium diisopropylamine, with phosphorus oxychloride afforded a complicated reaction mixture along with some starting material, while the reaction of this amide anion with phenyl dichlorophosphate or diphenyl chlorophosphate for 18 h led to the recovery of most of the initial amide 153. In a last attempt to effect the desired condensation, amide 153 was treated with n-butyllithium,



followed by addition of diethyl chlorophosphite at -78°. The N-phosphorylated amide 154 was obtained in 20-35% yield after purification.

It thus appeared through the model study, and also according to the literature[97], that the reaction of a nitrogen of an amide with chlorophosphate was not facile. The reasons for the difficulty of the reaction, perhaps, are due to the relative low nucleophilicity of the amide anion towards the chlorophosphate, and/or the instability of the nitrogen-phosphate bond in this type of compounds. On the other hand, the hydrogen on the nitrogen of a β-lactam is more acidic than that of an amide, and the chemical reactivity (nucleophilicity) of a β-lactam nitrogen lies between that of an amine and an amide[39,98]. We therefore attempted to react a chlorophosphate with a β-lactam again. Thus, addition of phenyl dichlorophosphate to a solution of alcohol 138 and two equivalents of triethylamine (or pyridine) in dry methylene chloride at 0°. afforded most probably

chlorophosphate 155 as an intermediate. This was supported by the pmr and



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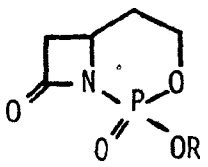


156 R = Ph

157 R = Me

infrared evidence only. Prolonged reaction did not produce any bicyclic β-lactam 156, rather opening of the β-lactam ring was observed, based on the infrared spectrum. Treatment of 138 with two equivalents of n-butyllithium and dimethyl chlorophosphate did not yield any β-lactam 157 either. We thought that it might be safer to perform this reaction in a stepwise fashion, that is, to form the N-P bond by treatment with an appropriate base after initially protecting the alcohol.

Thus, alcohol 138 and dihydropyran in dry methylene chloride containing pyridium p-toluenesulfonate[99] (PPTS) was stirred for 8 h at room temperature to afford THP ether 158. Treatment of the THP ether 158 with one equivalent of n-butyllithium at -78° in dry tetrahydrofuran, followed by addition of diethyl chlorophosphate, afforded the adduct 159, in good yield. The carbonyl infrared absorption frequency of β-lactam 159, as expected, increased from 1750 cm^{-1}, in 158, to 1790 cm^{-1}. The mass spectrum of 159 showed a base peak at m/e 180 for the fragment indicated in structure 159. The 200 MHz pmr of 159 (Fig. 14, p. 75) showed the

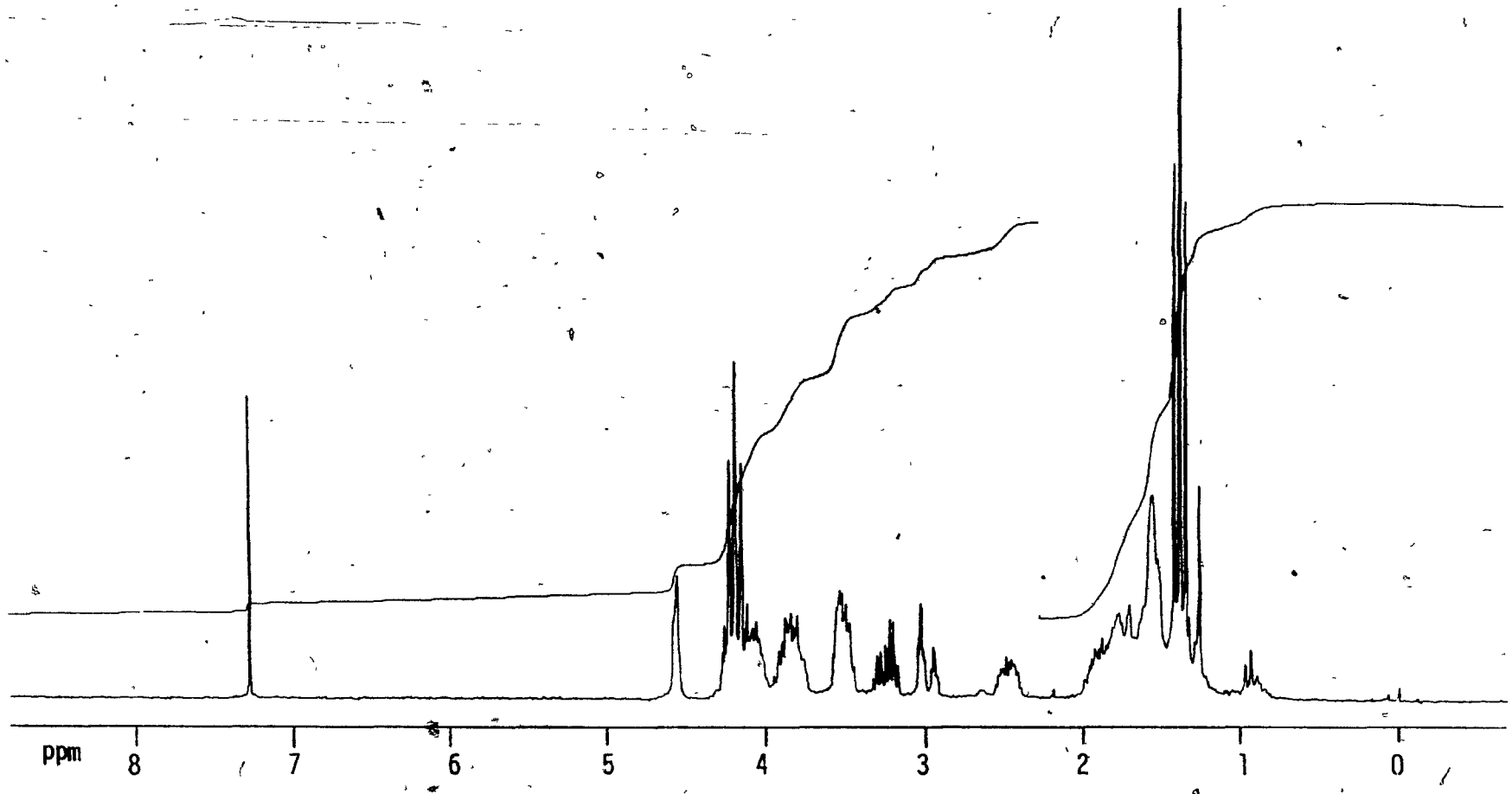
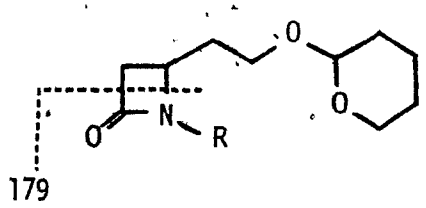


Fig. 14: 200 MHz pmr spectrum of 159, in $CDCl_3$.



158 R = H

159 R = $P(O)(OEt)_2$

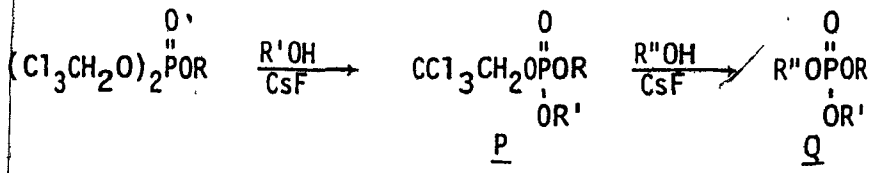
0 R = $P(O)(OR')_2$

presence of two diastereomers, the structures of which were consistent with those depicted. Analysis of an expanded portion of the 159 spectrum allowed us to determine the coupling constants between phosphorus and protons H_1, H_2 and H_3. H_1 was coupled to H_2 (J_{gem} = 16 Hz), to phosphorus (J_{p-H} = 3 Hz) and to H_3 (J_{trans} = 3 Hz). H_2 was coupled to H_1 (J_{gem} = 16 Hz), to phosphorus ($J_{p-H} \simeq 6$ Hz) and to H_3 ($J_{cis} \simeq 6$ Hz).

Having been able to successfully attach the phosphate residue to the nitrogen on the β-lactam ring, we now sought to form the bicyclic system by cyclization of the phosphate and the hydroxy group. There are two possible routes to the desired bicyclic β-lactam J from β-lactam 158: the THP ether 158 could be transformed into an appropriate phosphoroamidate 0, removal of the THP protecting group and would then allow the cyclization by transesterification; the other route involved the conversion of 158 into a phosphoric acid 0 (R' = H), deprotection of THP ether and the cyclization of the resulting compound by a coupling reagent. We attempted the transesterification first, which would eliminate the possible difficulty of handling a very polar phosphoric acid intermediate.

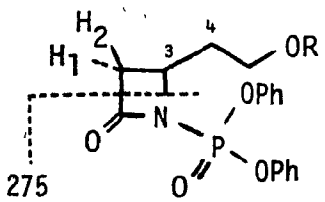
In 1977, Ogilvie *et al.*[100] reported that *bis*(trichloroethyl) alkyl phosphates reacted with alcohols in the presence of cesium fluoride or

tetra-n-butylammonium fluoride affording the adducts P, which could be further transformed into mixed trialkyl phosphates Q. Reactions using



diphenyl alkyl phosphates[100] were found to be even more rapid than the examples mentioned above. In addition, other alkoxy groups were not displaced under those conditions. Based on the above findings, we embarked on the synthesis of the key intermediate 160.

β-Lactam 158 was transformed into the diphenylphosphoramidate 161, obtained in 80% yield after flash chromatography. Deprotection of 161 with PPTS[99] afforded alcohol 160. The mass spectrum of 160 showed a molecular ion at m/e 347 and a peak at m/e 276 (96%) for the fragment shown on structure 160. The 200 MHz pmr spectrum (Fig. 15, p. 78) clearly showed resonances



160 R = H

161 R = THP

at 2.79 ppm for H_1 ($J_{1,2}$ = 16 Hz, $J_{1,3}$ = J_{p,H_1} = 3 Hz), at 3.22 ppm for

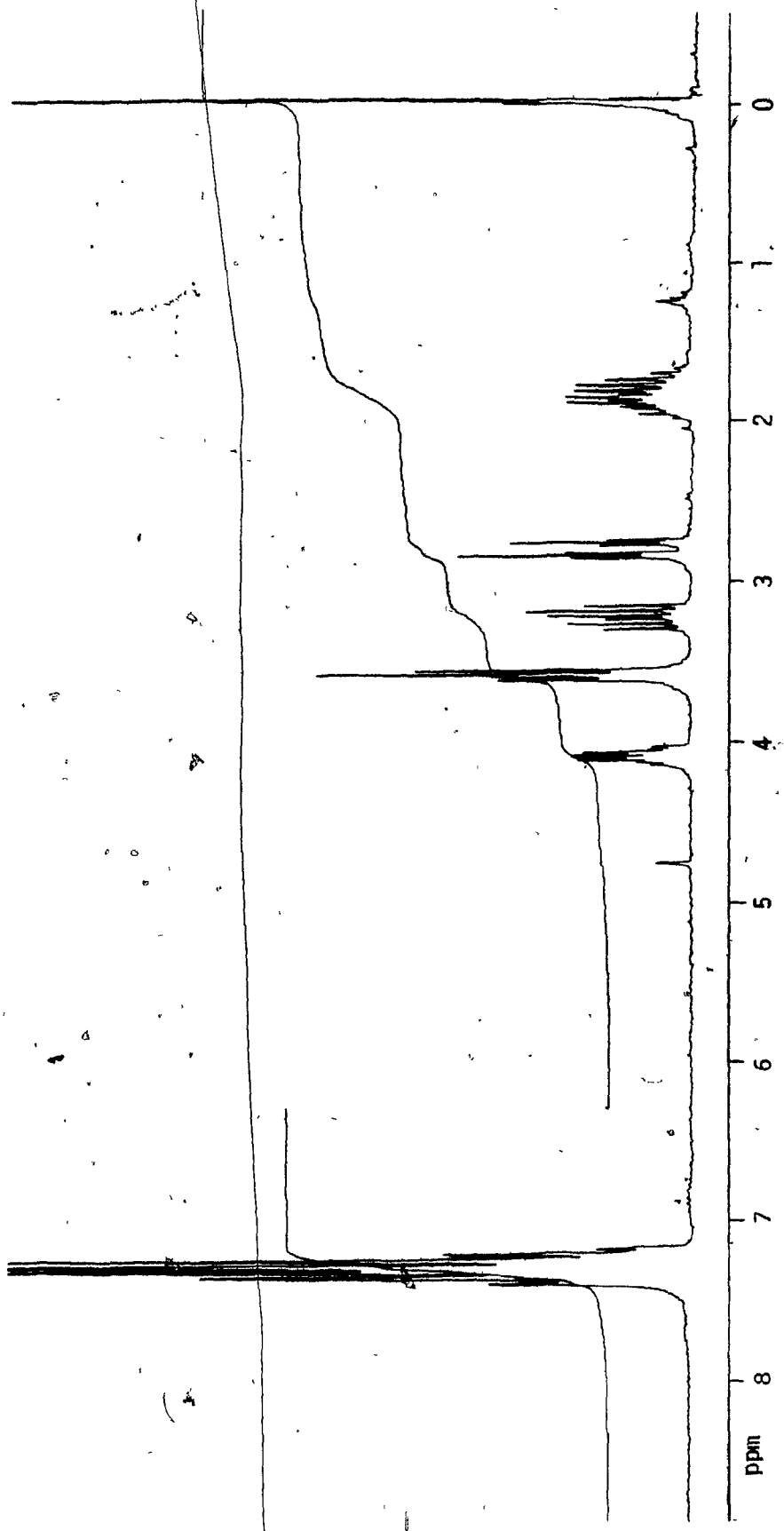
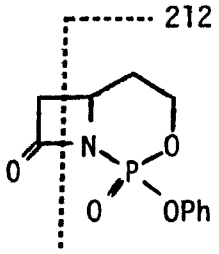


Fig. 15: 200 MHz pmr spectrum of 160, in $CDCl_3$.

H_2 ($J_{1,2}$ = 16 Hz, $J_{2,3}$ = J_{p,H_2} = 6 Hz) and a broad multiplet at 4.10 ppm for H_3. The p-H coupling constant was found by decoupling H_3 from H_1 and H_2.

Reaction of diphenylphosphoramidate 160 with anhydrous cesium fluoride in t-butanol gave the cyclized β-lactam 162, in 20% yield, along with phenol and β-lactam 163, as the sole side product. Attempts to improve the yield of the reaction and to eliminate the side product 163



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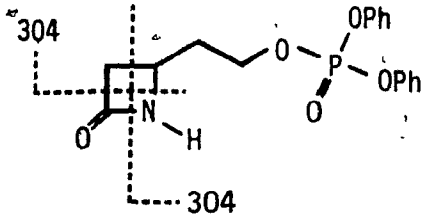


163

were all in vain. This suggested that perhaps the β-lactam nitrogen was in part acting as the leaving group, during treatment with CsF, instead of the phenoxide anion. Due to the strained nature of the bicyclic system, it may also be possible for the phenoxide anion to attack the phosphorus on 162 and displace the β-lactam nitrogen.

The 200 MHz pmr of 162 in acetone d_6 and deuterium oxide (Fig. 16, p. 80) showed multiplets at 1.8-2.2, 2.8-3.0, 3.9-4.1 and 4.3-4.7 ppm for the protons H_3, H_1, H_2 and H_4, respectively. Also, decoupling experiments at 200 MHz affected the above protons in a manner consistent with structure 162. The mass spectrum of 162 showed a molecular ion as

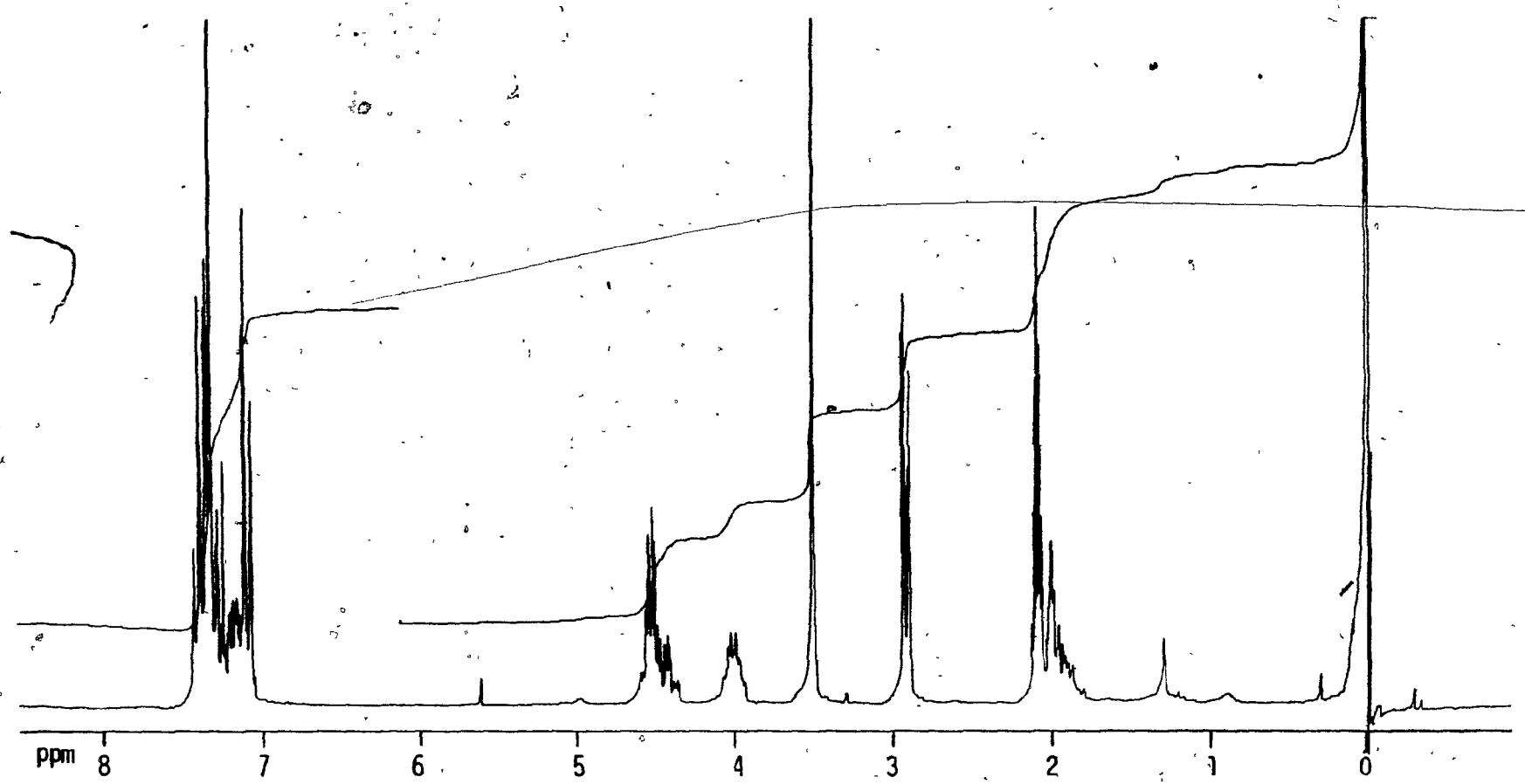


Fig. 16: 200 MHz pmr spectrum of 162, in acetone d_6 and D_2O.

well as a base peak at m/e 212 for the fragment indicated on the structure 162. To our surprise, the infrared absorption frequency for the carbonyl group of β-lactam 162 was 1760 cm^{-1}, which was 30 cm^{-1} lower than the uncyclized β-lactam 160. Biological testing showed 162 was totally devoid of activity toward a variety of bacteria.

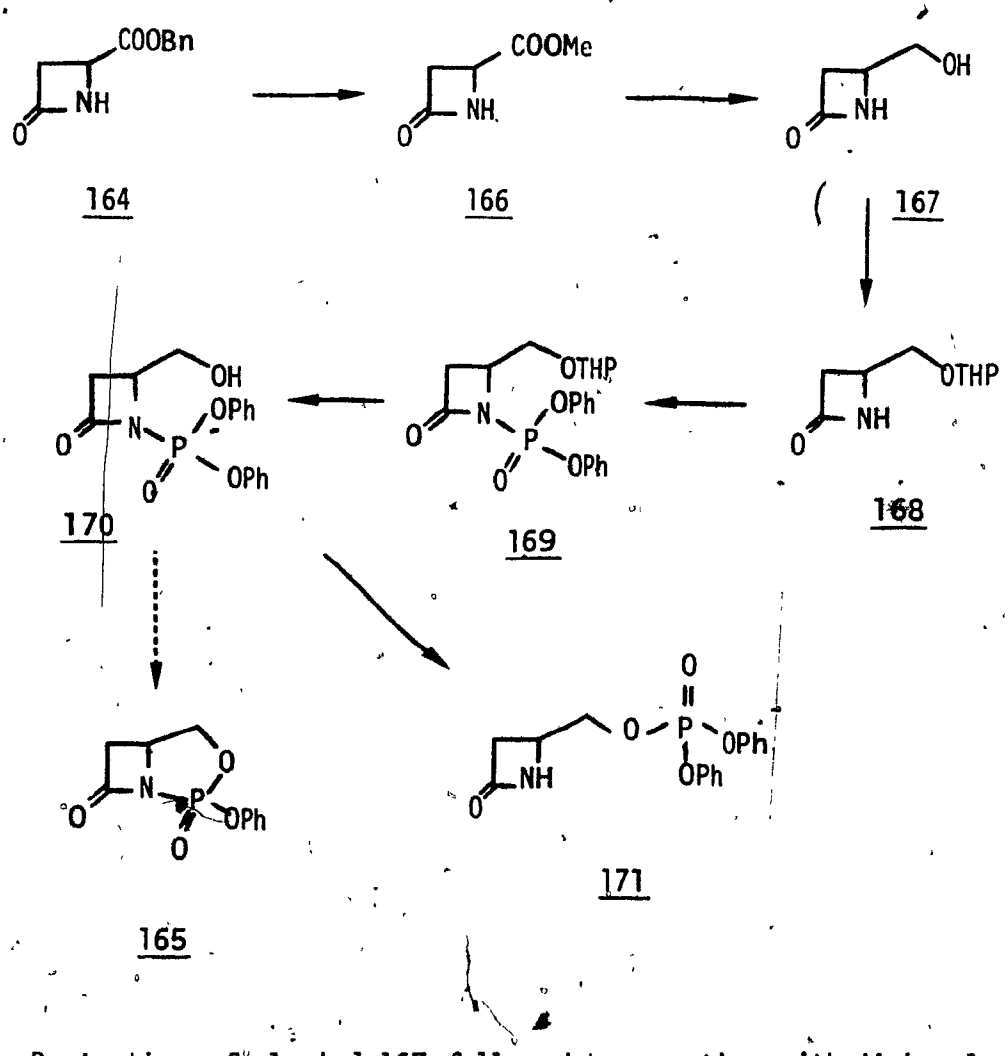
The identity of the side product 163 was proved using infrared, pmr and mass spectral data and by synthesis. Alcohol 138 was reacted with diphenyl chlorophosphate in the presence of pyridine to yield 163. The pmr spectrum (Fig. 17, p. 82) displayed two sets of multiplets at 1.8-2.2 and 4.2-4.5 ppm for the protons of two methylene and a similar pattern for the three of β-lactam protons as that of the alcohol 138. The mass spectrum showed a parent ion at m/e 347 (4.6%) and the fragments at m/e 304 (20.3%), 305 (43.7%) indicated on structure 163.

Unfortunately, preliminary attempts to cleave the phenyl group in 162 with tetra-n-butylammonium fluoride in THF resulted in a very difficult-to-separate reaction mixture.

While this work was in progress, Dr. Christensen at Merck Company generously provided us with β-lactam 164. We were hoping to achieve the synthesis of bicyclic β-lactam 165 *via* the same subsequent transformation (see Scheme 5). Thus, β-lactam 164 was hydrogenated with palladium on charcoal followed by methylation with diazomethane to afford methyl ester 166. Reduction of 166 with sodium borohydride in tetrahydrofuran-water[101] gave the desired alcohol 167. Later, the formation of alcohol 167 was achieved by direct reduction of 164 using sodium borohydride in methanol[36].

Fig. 17: 200 MHz pmr spectrum of 163, in $CDCl_3$.

Scheme 5



Protection of alcohol 167 followed by reaction with diphenyl chlorophosphate gave β-lactam 169. Deprotection of THP ether 169 afforded β-lactam 170. Unfortunately, treatment of 170 with CsF in a usual manner did not yield any of the desired bicyclic β-lactam 165. Instead, β-lactam 171 was found to be the only product. All of the pmr, infrared and mass spectral data were in accordance with the structure 171. β-Lactam 171 was

also synthesized by the reaction of β-lactam 138 with diphenyl chlorophosphate and triethylamine.

At this stage, we decided to attempt the second route (Scheme 6) of the cyclization through the coupling reagent such as DCC. Thus, dibenzyl chlorophosphate 172 was chosen, instead of diphenyl chlorophosphate as the required reagent. It is known that benzyl protecting groups can be removed by hydrogenation. According to the method of Friedman[102], reaction of phosphorus trichloride and benzyl alcohol in the presence of pyridine afforded dibenzyl phosphite 173. After chlorination of 173 with N-chloro-

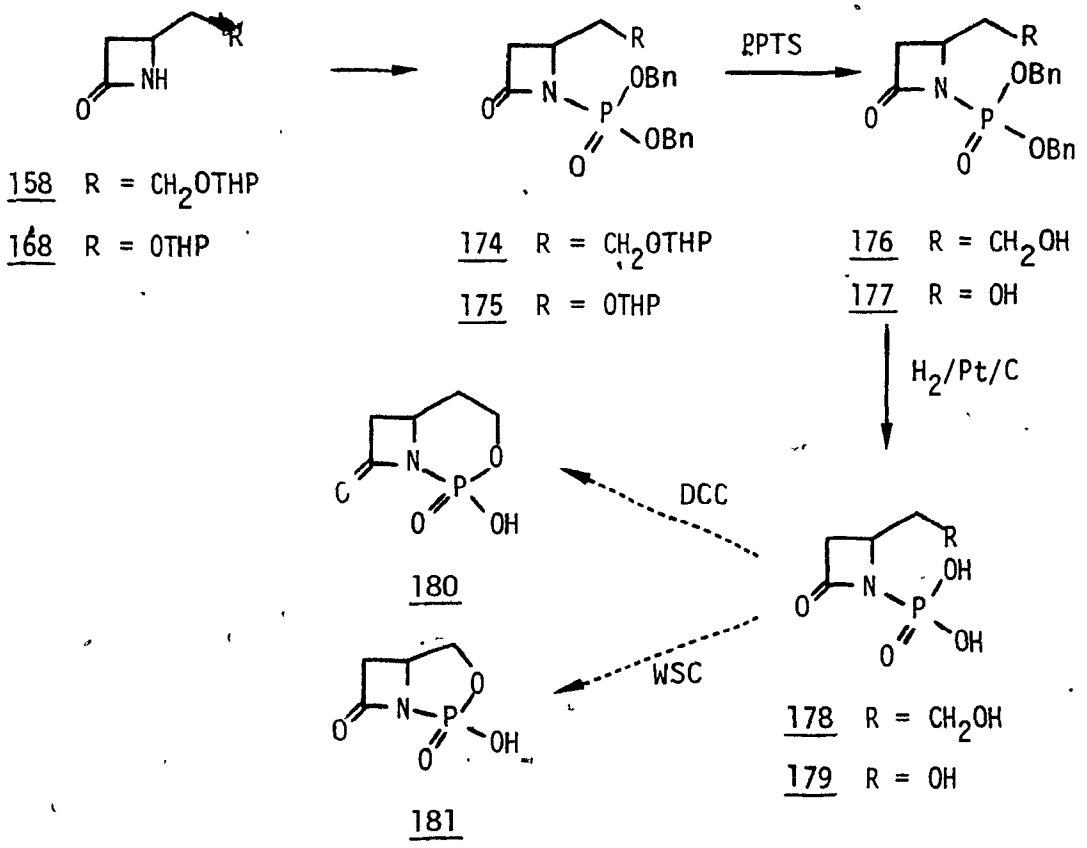
$$PCl_3 + C_6H_5CH_2OH \xrightarrow{py.} HOP(OCH_2C_6H_5)_2 \xrightarrow{NCS} ClP(O)(OBn)_2$$

173 172

succinimide, the corresponding benzyl chlorophosphate 172 was obtained[103].

Treatment of β-lactams 158 and 168 with n-butyllithium at -78°, followed by the addition of dibenzyl chlorophosphate afforded β-lactams 174 and 175. Deprotection of THP ether (174 and 175) and the hydrogenation of 176 and 177 yielded β-lactams 178 and 179. However, attempted cyclization of 178 and 179 with DCC[104] or 1-ethyl-3[3-(dimethylamino)-propyl]carbodiimide (WSC) gave mostly unidentified products.

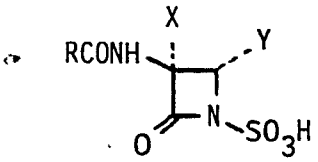
Scheme 6



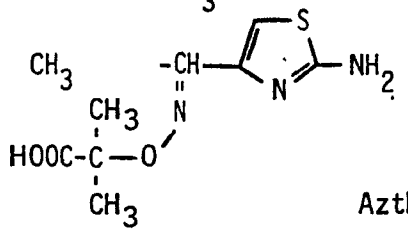
SYNTHESIS TOWARD MONOBACTAM ANALOGUES

In very recent publications[105], two research groups, Takeda Chemical Industries in Japan and The Squibb Institute in USA, described a new family of monocyclic β-lactam antibiotics produced by bacteria. This class of β-lactams containing the novel structure skeleton R have been named as "monobactam". Most of them showed activity against Gram-negative bacteria. Particularly, a highly active β-lactamase-stable

derivative V, azthreonam, synthesized by the Squibb Institute, has been developed for clinical evaluation.

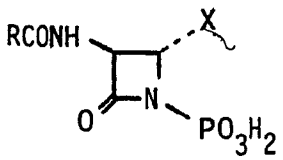


R

	X	Y	R
I	OCH_3	H	$-\overset{*}{C}H(CH_3)-NHCOCH_2CH_2\overset{*}{C}H(COOH)-NH_2$
			*D-alanine:Sulfazecin
			*L-alanine:Isosulfazecin
II	H	H	$-CH(CH_3)-NHCOCH_2CH_2CH(COOH)-NH_2$
III	OCH_3	H	$-CH_3$
IV	H	H	$-CH_3$
V	H	CH_3	

Azthreonam

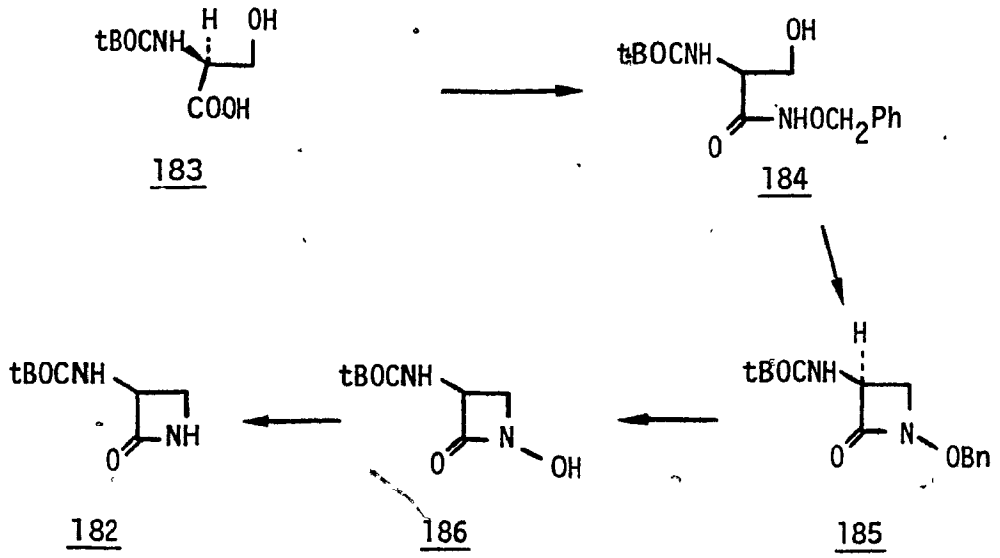
We therefore were interested in the synthesis of some monobactam analogues S by replacing the sulfonate moiety with the phosphate residue. Based on the finding of model studies, it was difficult to react a secondary



S X = H, CH_3

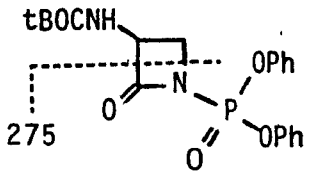
amide with a dialkyl chlorophosphate. We thought it might be possible to apply the established methodology for attaching the phosphate moiety to the nitrogen of a β-lactam ring in the presence of an acylamino side chain.

The simplest β-lactam 182, as the starting material, was prepared according to the method developed by Miller *et al.*[39]. N-t-butoxycarbonyl-L-serine 183 was converted into the corresponding hydroxamic acid 184 using O-benzylhydroxylamine and WSC. Cyclization of 184 with triphenyl-



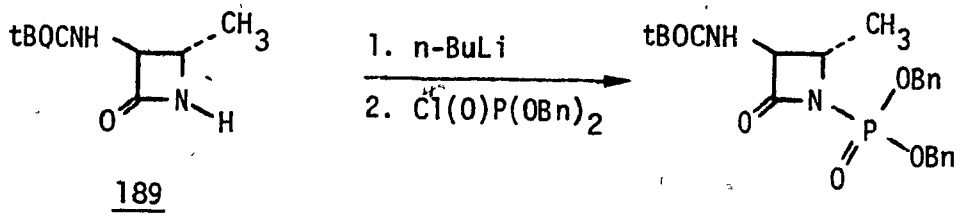
phosphine, triethylamine and carbon tetrachloride afforded β-lactam 185. Hydrogenation of 185 yielded 186 and titanium trichloride reduction gave 182. Treatment of 182 with n-butyllithium at -78°, followed by the addition of diphenyl chlorophosphate, afforded as expected β-lactam 187 in good yield.

In the infrared spectrum, the β-lactam absorption of 187 was found at 1800 cm^{-1}. The mass spectrum showed a parent ion at m/e 418 (1.5%) and the



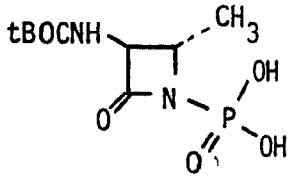
187

fragment at m/e 276 (70.2%). Also, β-lactam 188 was synthesized from β-lactam 189* *via* the same method for the preparation of 182. The infrared



189

188



190

spectrum of β-lactam 188 showed the absorption at 1790 cm^{-1} for the β-lactam carbonyl function. Dr. D. Dugat, in our laboratory, successfully hydrogenated β-lactam 188 to give phosphornic acid 190.

In conclusion, the reactivity and the synthesis of various phosphorus reagents and an amide or an unsubstituted β-lactam on nitrogen

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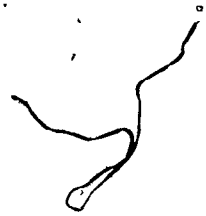
*β-Lactam 189 was prepared by Dr. D. Dugat in our laboratory.

has been explored. The attachment of a dialkyl chlorophosphate to the nitrogen of a β-lactam ring was achieved. The formation of two new ring systems 180 and 181 have been studied and β-lactam 162 was successfully prepared. Different protecting groups of phosphate residue were examined and it was shown that the benzyl protecting group could be cleaved in the case of β-lactams 178 and 179. Preliminary tests* indicated the failure of removing the t-BOC protecting group in phosphorylated β-lactam 188. However, the synthesis of monobactam S was considered beyond the scope of this thesis.



*This work was attempted by Dr. D. Dugat in our laboratory.

CONTRIBUTIONS TO KNOWLEDGE

Nocardicin analogues 31 and 52 have been prepared. Decarbonylation of 64 by tris(triphenylphosphine) rhodium chloride was achieved.

The synthesis of β-lactam 95 was accomplished. The reaction of azidoacetyl chloride with phenylpropargylidene Schiff bases derived from phenylpropargyl aldehyde and various substituted anilines, afforded β-lactams in fair to good yields.

A new bicylic β-lactam 162 was synthesized. The key step in the synthesis of 162 involved the phosphorylation of the nitrogen on the β-lactam ring.

β-Lactam 188, a key intermediate, for the synthesis of monobactam analogue 5 was synthesized.

EXPERIMENTAL

GENERAL EXPERIMENTAL

Melting points (mp) were determined on a Gallenkamp block and are uncorrected, unless otherwise specified. Mass spectra (ms) were obtained on HP 5984 or LKB 9000 mass spectrometers and intensities are reported in parts per thousand ($^{0}/_{00}$). Chemical ionization mass spectra (CI-ms) (using isobutane) and gas chromatographic mass spectra (gc-ms) were recorded on the HP 5984 instrument. Infrared (ir) spectra were obtained on Unicam SP 1000 Perkin Elmer 257 and 297 spectrophotometers. Proton magnetic resonance (^{1}Hmr) spectra were acquired on Varian T-60, T-60A and XL-200 spectrometers, with tetramethylsilane (TMS) as internal standard. Chemical shifts are given in the δ scale, in parts per million (ppm). Doublets (d), triplets (t) and quartets (q) are reported by their center positions, while multiplets (m) are described by a range of absorption. Other abbreviations used are: (s) for singlet, (b) for broad and (app) for apparent.

Analytical thin-layer chromatography (tlc) was carried out on Merck silica gel 60 F_{254} pre-coated aluminum plates, and was visualized by dipping into a solution of 2.5 g ammonium molybdate and 1 g ceric sulfate in 10 mL concentrated H_2SO_4/90 mL H_2O and heating on a hot plate, or by dipping into a solution of 1% of p-dimethylaminobenzaldehyde in 96% ethanol, followed by exposing it to HCl vapors. "Flash chromatography", described by Still *et al.*[106], was performed using 32-63 μ Woelm silica gel (from ICN Nutritional Biochemicals, Cleveland, Ohio). Analytical gas chromatography was performed on a HP 5750 instrument, using a 3% OV-101

(3 ft) column.

Dry solvents were obtained by storing over molecular sieves[107] (DMF, DME, acetonitrile, benzene, methanol, methylene chloride, toluene), or by refluxing in the presence of sodium-benzophenone[108] (THF), or were available commercially (Et_2O, EtOH).

All evaporations were done, unless otherwise mentioned, under reduced pressure (water aspirator) with a bath temperature of 25-40°.

Midwest Microlabs (Indianapolis, Indiana) performed the elemental analyses.

CHAPTER I

2-(2,5-Dimethoxyphenyl)glycinonitrile 33

2,5-Dimethoxybenzaldehyde 32 (3.32 g, 20 mmol) was dissolved in methanol (100 mL). Ammonium chloride (2.67 g, 50 mmol) and sodium cyanide (1.22 g, 25 mmol) was added. The solution was saturated with ammonia gas at 0° for 15 min in a pressure bottle. It was allowed to warm to room temperature and stirred overnight. The solution was evaporated to dryness and water was added. It was extracted with ether (3 x 80 mL), washed with 10% HCl and neutralized the aqueous layer with sodium carbonate. Extraction with ethyl acetate (3 x 70 mL), drying ($MgSO_4$) and evaporation gave 3.34 g (87%) of 33 as a red oil.

^{1}Hmr ($CDCl_3$) δ: 2.16 (bs, 2H, NH_2), 3.74, 3.82 (2s, 6H, $2CH_3$), 5.00 (s, 1H, CH), 6.80-7.12 (m, 3H, C_6H_3); ir ($CHCl_3$) ν_{max}: 3300-3400 (NH_2), 2210 (CN) cm^{-1}.

Methyl-(2,5-Dimethoxyphenyl)glycinate 35

2-(2,5-Dimethoxyphenyl)glycinonitrile 33 (1.0 g, 5.2 mmol) was dissolved in 60 mL methanol/water (95:5). Hydrochloride gas was bubbled in at room temperature until saturation for 10 min without cooling. The mixture was refluxed gently for 2 h and evaporated to dryness. Saturated sodium carbonate (20 mL) was added. The solution was extracted with ethyl acetate (3 x 20 mL), dried and evaporated to give 0.71 g (61%) of 35.

^{1}Hmr ($CDCl_3$) δ: 2.40 (bs, 2H, NH_2), 3.70, 3.80, 3.82 (3s, 9H, $3CH_3$), 4.79 (s, 1H, CH), 6.85 (s, 3H, C_6H_3); ir ($CHCl_3$) ν_{max}: 3300-3400 (NH_2), 1740 (C=O, ester) cm^{-1}.

Schiff Base 36

To a solution of methyl-(2,5-dimethoxyphenyl)glycinate 35 (1.83 g, 8.13 mmol) in dry CH_2Cl_2 (100 mL) was added cinnamaldehyde (1.07 g, 8.13 mmol). The mixture was refluxed and CH_2Cl_2 distilled out slowly with the constant addition of dry CH_2Cl_2 so as to maintain the same volume in the reaction vessel for 6 h. The solution was cooled, added $MgSO_4$ (1 g) and stirred for 18 h at room temperature. Filtration and evaporation afforded 2.76 g (100%) of Schiff base 36.

^{1}Hmr ($CDCl_3$) δ: 3.40-3.60 (m, 9H, $3CH_3$), 5.10 (s, 1H, CH), 6.30-7.00 (m, 10H, $C_6H_5CH{=}CH$, C_6H_3), 7.40-7.60 (b, 1H, CH=N); ir (CH_2Cl_2) ν_{max}: 1740-1750 (C=O, ester), 1630 (C=C) cm^{-1}.

Azido-β-Lactam 37.

To the freshly prepared Schiff base 36 (2.75 g, 8.12 mmol) in dry CH_2Cl_2 (60 mL) was added triethylamine (0.82 g, 8.12 mmol). A solution of azidoacetyl chloride (0.97 g, 8.12 mmol) in dry CH_2Cl_2 (10 mL) was added at -20 °C, dropwise over 10 min. The mixture was warmed up by itself and stirred an additional hour. The solution was washed with water (2 x 50 mL), dried ($MgSO_4$) and evaporated to obtain the crude product.

Chromatography over silica gel using methylene chloride as eluant gave 1.63 g (50%) of 37 as two diastereomers.

^{1}Hmr ($CDCl_3$) δ: 3.60-3.90 (m, 9H, $3CH_3$), 4.20-4.40 (m, 1H, C<u>H</u>-CHN_3), 4.88-4.95 (2d, 1H, CHN_3, J = 5 Hz), 5.91, 5.93 (2s, 1H, CH), 6.5-7.7 (m, 10H, C_6H_3, C_6H_5CH=CH); ir (CH_2Cl_2) ν_{max}: 2100 (N_3), 1780 (C=O, β-lactam), 1760 (C=O, ester) cm^{-1}.

Acylamino β-Lactam 38

To a diastereomeric mixture of azido β-lactam 37 (1 g, 2.49 mmol) in dry CH_2Cl_2 (50 mL) was added triethylamine (0.25 g, 2.49 mmol). The solution was bubbled into H_2S gas for 15 min at 0° and was warmed to room temperature to stir for an additional hour. It was then bubbled into nitrogen gas for 15 min. Phenylacetyl chloride (0.42 g, 2.72 mmol) and pyridine (0.2 g, 2.53 mmol) were added to the reaction mixture. After stirring at room temperature for 2 h, it was washed with water, 10% HCl and saturated $NaHCO_3$, dried and evaporated to give 1.42 g of crude product. Chromatography over silica gel using CH_2Cl_2 and then $CHCl_3$ as eluant obtained 0.70 g (55%) of β-lactam 38.

^{1}Hmr ($CDCl_3$) δ: 3.42 (bs, 2H, $COCH_2Ph$), 3.40-3.60 (m, 9H, $3CH_3$), 4.00-4.20 (m, 1H, C<u>H</u>-CHNH), 4.60-4.80 (m, 1H, C<u>H</u>NH), 5.70, 5.80 (2s, 1H, CH), 6.2-7.4 (m, 11H, C_6H_3, C_6H_5CH=CH, NH); ir ($CHCl_3$) ν_{max}: 3300-3420 (NH), 1740-1760 (C=O, β-lactam, ester), 1675 (amide) cm^{-1}.

2,5-Dihydroxybenzaldehyde 39

2,5-Dimethoxybenzaldehyde 32 (3.4 g, 20.4 mmol) in dry CH_2Cl_2 (100 mL). Boron tribromide (13.06 g, 51 mmol) in dry CH_2Cl_2 (40 mL) was added dropwise over a period of a half hour at -20°C. The solution was warmed to room temperature and stirred overnight. It was poured onto ice and extracted with ethyl acetate. Drying ($MgSO_4$), filtration and evaporation yielded 2.80 g (99%) of 39 as green-black solid; m.p. 98-99°C.

^{1}Hmr ($CDCl_3$) δ: 6.71-7.34 (m, 3H, C_6H_3), 7.35-8.00 (b, 1H, OH), 9.80 (s, 1H, CHO), 10.40-10.60 (bs, 1H, OH); ir (CH_2Cl_2) ν_{max}: 3500-3000 (OH), 1660 (CHO) cm^{-1}.

2,5-Dibenzyloxybenzaldehyde 40

A solution of 2,5-dihydroxybenzaldehyde 39 (13.68 g, 0.099 mol) in absolute ethanol (450 mL) was refluxed for 18 h, containing benzyl chloride (23.76 g, 0.188 mol) and potassium carbonate (25.94 g, 0.188 mol). After filtration and evaporation, the residue was dissolved in ether and treated with charcoal to afford the crude product, which was chromatographed over silica gel using CH_2Cl_2 as eluant. Crystallization from ether-pentene gave 23.25 g (74%) of 2,5-dibenzyloxybenzaldehyde 40 as yellow cystals; m.p. 89-90°C.

^{1}Hmr ($CDCl_3$) δ: 5.08, 5.18 (2s, 4H, $2CH_2$), 7.0-7.6 (m, 13H, C_6H_3, $2C_6H_5$), 10.60 (s, 1H, CHO), ir (CH_2Cl_2) ν_{max}: 1700 (CHO) cm^{-1}.

2,5-Dibenzoxyphenylglycinonitrile 43

2,5-Dibenzyloxybenzaldehyde 40 (2 g, 6.29 mmol) was dissolved in 100 mL of methanol and 30 mL of tetrahydrofuran. Ammonium chloride (0.674 g, 12.6 mmol) and sodium cyanide (0.318 g, 6.49 mmol) was added. The mixture was saturated with ammonium gas at 0° for 15 min. The solution was allowed to warm up to room temperature and stirred for 18 h in a pressure bottle. It was evaporated to dryness and water was added. Filtration afforded 1.90 g of the crude 41, which was used without purification.

^{1}Hmr ($CDCl_3$) δ: 1.60-2.00 (bs, 2H, NH_2), 5.02, 5.10 (2s, 4H, $2CH_2$), 6.90-7.60 (m, 13H, aromatic); ir (KBr) ν_{max}: 2200 (CN) cm^{-1}.

Methyl-2,5-dibenzoxyphenylglycinate 43 was obtained from the crude 41, *via* the procedures for the preparation of 35, in 45% yield after chromatography on silica gel using chloroform as eluant.

^{1}Hmr ($CDCl_3$) δ: 1.80-2.00 (bs, 2H, NH_2), 3.67 (s, 3H, CH_3), 4.81 (s, 1H, CH), 5.02, 5.05 (2s, 4H, $2CH_2$), 6.80-7.60 (m, 13H, aromatic); ir (CH_2Cl_2) ν_{max}: 3000 (NH_2), 1740 (C=O, ester) cm^{-1}.

Aminoester 44

^{1}Hmr ($CDCl_3$) δ: 3.67 (s, 3H, CH_3), 3.70-4.00 (bs, 3H, OH, NH_2), 4.60 (s, 1H, CH), 5.04 (s, 2H, CH_2), 6.50-7.50 (m, 8H, C_6H_3, C_6H_5); ir (CH_2Cl_2) ν_{max}: 3500-3000 (NH_2, OH), 1740 (C=O, ester) cm^{-1}; ms (70 eV, 54°), m/e (‰): 287 (28, $M^{+\cdot}$), 228 (595, $M^{+\cdot}-COOCH_3^{\cdot}$), 91 (1000).

Schiff Base 45 and Azido-β-Lactam 46

Schiff base 45 was obtained from 43, *via* the procedures for the preparation of 36.

^{1}Hmr ($CDCl_3$) δ: 3.62 (s, 3H, CH_3), 4.90, 4.95 (2s, 4H, $2CH_2$), 5.50 (s, 1H, CH), 6.50-7.60 (m, 20H, C_6H_3, $2C_6H_5$, $C_6H_5CH{=}CH$), 7.90 (d, 1H, CH=N, J = 7Hz); ir (CH_2Cl_2) ν_{max}: 1630 (C=N), 1740 (C=O, ester) cm^{-1}.

β-Lactam 46 was obtained from 45, *via* the procedures for the preparation of 37, in 90% yield, as a 1:1 mixture of diastereomers after chromatography (CH_2Cl_2).

^{1}Hmr ($CDCl_3$) δ: 3.48, 3.52 (2s, 3H, CH_3), 3.90-4.20 (m, 1H, $C\underline{H}$-CHN_3), 4.53 (d, 1H, $C\underline{H}N_3$, J = 5Hz), 4.70, 4.75, 4.81, 4.90 (4s, 4H, $2C\underline{H}_2C_6H_5$), 5.72, 5.78 (2s, 1H, CH), 5.22-7.40 (m, 20H, C_6H_3, $2C_6H_5$, $C_6H_5CH{=}CH$); ir (CH_2Cl_2) ν_{max}: 2100 (N_3), 1770 (C=O, β-lactam), 1740 (C=O, ester) cm^{-1}.

β-Lactam 47

A diastereomeric mixture of β-lactam 46 (1.43 g, 2.49 mmol) in a mixture of CH_2Cl_2 (50 mL) and absolute methanol (100 mL) was saturated with nitrogen gas at -78° for 5 min. A mixture of ozone and nitrogen gas was bubbled in for 35 min, until the KI starch paper showed blue color. The excess ozone was flushed out with nitrogen gas for 15 min. The temperature was allowed to rise to -40°, at which time $NaBH_4$ (0.132 g, 3.48 mmol) was added. The temperature of the solution was risen to room

temperature within 2 h, following which 10% HCl (5 mL) was added. The solution was evaporated to dryness. The residue was added water (30 mL) and extracted with ethyl acetate, washed with water, dried ($MgSO_4$), and evaporated to give the crude product. A wash with a mixture of ether-hexane (1:5) removed benzyl alcohol. Chromatography on silica gel and elution with chloroform afforded 0.75 g (60%) of a 3:1 mixture of diastereomers 47/48. Separation of two diastereomers by chromatography on silica gel using $CHCl_3$ gave 0.5 g of the more polar isomer 47.

^{1}Hmr ($CDCl_3$) δ: 3.10-3.40 (bs, 2H, $C\underline{H}_2OH$), 3.50 (s, 3H, CH_3), 3.40-3.80 (b, 1H, OH), 3.90-4.10 (m, 1H, $C\underline{H}CH_2OH$), 4.60 (d, 1H, $C\underline{H}N_3$, J = 4Hz), 4.80-5.20 (m, 4H, $2C\underline{H}_2C_6H_5$), 5.64 (s, 1H, CH), 6.60-7.40 (m, 13H, C_6H_3, $2C_6H_5$); ir ($CDCl_3$) ν_{max}: 3400-3600 (OH), 2100 (N_3), 1760 (C=O, β-lactam), 1750 (C=O, ester) cm^{-1}.

β-Lactam 49

To a solution of β-lactam 47 (1.25 g, 2.49 mmol) in dry CH_2Cl_2 (50 mL) at 0° was added triethylamine (0.283 g, 2.80 mmol). A stream of H_2S was bubbled in for 15 min. The mixture was stirred at room temperature for 2 h. The excess H_2S gas was removed by passing through nitrogen gas for 30 min. The solution was washed with water, dried ($MgSO_4$) and evaporated to afford 0.65 g (55%) of β-lactam 49, after chromatography on silica gel using chloroform and chloroform:ethyl acetate (1:1).

^{1}Hmr ($CDCl_3$) δ: 2.00-2.60 (bs, 3H, OH, NH_2), 3.20-3.40 (m, 2H, $C\underline{H}_2OH$), 3.54 (s, 3H, CH_3), 3.70-4.00 (m, 1H, $C\underline{H}$-$CHNH_2$), 4.23 (d, 1H, $C\underline{H}NH_2$, J = 5Hz),

4.95, 5.00 (2s, 4H, $2CH_2C_6H_5$), 5.64 (s, 1H, CH), 6.80-7.40 (m, 13H, C_6H_3, $2C_6H_5$); ir ($CHCl_3$) ν_{max}: 3350 (NH_2), 1750 (C=O, β-lactam), 1740 (C=O, ester) cm^{-1}; ms (70 eV, 20-110°), m/e (°/₀₀): 476 (31, $M^{+\cdot}$), 403 (1000, $M^{+\cdot}$-$NH_2CH{=}CHCH_2OH$).

β-Lactam 50

To a solution of β-lactam 49 (0.65 g, 1.36 mmol) in dry CH_2Cl_2 (40 mL) containing triethylamine (0.2 g, 1.98 mmol) was added dropwise a solution of trimethylsilyl chloride (0.2 g, 1.84 mmol) in dry CH_2Cl_2 (10 mL) over 15 min. After stirring another half hour, EEDQ (0.4 g, 1.62 mmol) and phenoxyacetic acid (0.3 g, 1.97 mmol) were added. The solution was allowed to stir at room temperature for 18 h. After evaporation of the solvent, the residue was dissolved in ether, washed with 10% HCl and 10% $NaHCO_3$, dried ($MgSO_4$) and evaporated to afford 0.83 g of the crude product. Chromatography on silica gel using CH_2Cl_2 gave 0.65 g of β-lactam 50 in 78% yield.

^{1}Hmr ($CDCl_3$) δ: 2.60-2.80 (b, 1H, OH), 3.20-3.40 (b, 2H, $C\underline{H}_2OH$), 3.60 (s, 3H, CH_3), 4.0 (m, 1H, $C\underline{H}$-CHNH), 4.42 (s, 2H, $C\underline{H}_2OPh$), 5.00 (s, 4H, $2C\underline{H}_2C_6H_5$), 5.20-5.40 (m, 1H, $C\underline{H}NH$), 5.50 (s, 1H, CH), 6.60-8.00 (m, 19H, C_6H_3, $3C_6H_5$, NH); ir (CH_2Cl_2) ν_{max}: 3400 (NH), 1760 (C=O, β-lactam), 1740 (C=O, ester) cm^{-1}; ms (70 eV, 70-130°), m/e (°/₀₀): 610 (1.15, $M^{+\cdot}$), 551, 63, $M^{+\cdot}$-$COOCH_3\cdot$), 519 (39, $M^{+\cdot}$-$CH_2C_6H_5\cdot$), 403 (427, $M^{+\cdot}$-$OHCH_2CH{=}CHNH$-$COCH_2OC_6H_5$), 344 (1000, $M^{+\cdot}$-$COOCH_3\cdot$-$HOCH_2CH{=}CHNHCOCH_2OC_6H_5$).

β-Lactam 51

To a solution of β-lactam 50 (0.65 g, 1.06 mmol) in methanol (30 mL) was added dropwise 1% NaOH (10 mL) during 10 min. The solution was stirred for 15 min and acidified by 6 M HCl to pH = 3. The methanol was evaporated. The aqueous layer was extracted with ethyl acetate (3 x 10 mL). Drying ($MgSO_4$) and evaporation afforded 0.54 g (86%) of β-lactam 51.

^{1}Hmr ($CDCl_3$-D_2O) δ: 3.10-4.00 (m, 3H, CH_2OH, CH-CHNH), 4.20-4.50 (s, 2H, $CH_2OC_6H_5$), 4.70-5.00 (s, 4H, $2CH_2C_6H_5$), 5.30 (d, 1H, CHNH, J = 5Hz), 5.90 (s, 1H, CH), 6.80-7.40 (m, 18H, C_6H_3, $3C_6H_5$); ir ($CHCl_3$) ν_{max}: 3390 (NH), 1760 (C=O, β-lactam), 1730 (C=O, ester), 1670 (amide) cm^{-1}.

β-Lactam 52

To β-lactam 51 (0.54 g, 0.91 mmol) in absolute ethanol (35 mL) was added 10% Pd/C (0.2 g). The mixture was hydrogenated at 40 psi for 2 h (the pressure dropped to 38 psi). The solution was filtered and evaporated to give 0.30 g (80%) of β-lactam 52.

^{1}Hmr (D_2O-$NaHCO_3$) δ: 3.5-4.0 (m, 5H, CH_2OH, CH_2OPh, CH-CHNH), 5.5-5.6 (d, 1H, CHNH, J = 5Hz), 5.8 (s, 1H, CH), 7-7.8 (m, 8H, C_6H_3, C_6H_5); ir (Nujor) ν_{max}: 2800-3500 (OH, COOH), 1750 (C=O, β-lactam), 1740 (C=O, acid), 1650 (amide) cm^{-1}; uv (EtOH) λ_{max}: 285 nm (ε 2100), a shoulder at 225 nm.

β-Lactam 48

A 3:1 mixture of diastereomers 47/48 (1.50 g, 2.99 mmol) in benzene (50 mL) containing triethylamine (0.758 g, 7.5 mmol) was refluxed for 18 h. The solution was cooled and washed with water, dried ($MgSO_4$) and evaporated to give the crude product. Chromatography on silica gel using CH_2Cl_2 to remove the impurities and $CHCl_3$ to obtain 1.1 g (73%) of a 3:7 mixture of diastereomers 47/48. Separation of two diastereomers by preparative layer chromatography on silica gel using chloroform/ethyl acetate (6:1) afforded 0.65 g (60%) of the less polar isomer 48.

^{1}Hmr ($CDCl_3$) δ: 3.20-3.90 (m, 4H, $CHCH_2OH$), 3.60 (s, 3H, CH_3), 4.30 (d, 1H, CHN_3, J = 5Hz), 5.00 (b, 4H, $2CH_2C_6H_5$), 5.89 (s, 1H, CH), 6.80-7.50 (m, 13H, C_6H_3, $2C_6H_5$); ir ($CHCl_3$) ν_{max}: 3450 (OH), 3400 (NH_2), 1750 (C=O, β-lactam), 1740 (C=O, ester) cm^{-1}.

β-Lactam 53

Obtained from 48, *via* the procedures for the preparation of 49, in 50% yield.

^{1}Hmr ($CDCl_3$) δ: 2.20-3.20 (b, 3H, OH, NH_2), 3.20-3.80 (m, 6H, $CHCH_2OH$, CH_3), 4.00 (d, 1H, $CHNH_2$, J = 5Hz), 5.01, 5.08 (2s, 4H, $2CH_2C_6H_5$), 5.90 (s, 1H, CH), 6.70-7.50 (m, 13H, C_6H_3, $2C_6H_5$); ir ($CHCl_3$) ν_{max}: 3450 (OH), 3400 (NH_2), 1750 (C=O, β-lactam), 1740 (C=O, ester) cm^{-1}.

β-Lactam 55

Obtained from 53, *via* the procedures for the preparation of 50, in 60% yield after chromatography (CH_2Cl_2, then $CHCl_3$).

^{1}Hmr ($CDCl_3$) δ: 2.20-2.40 (b, 2H, $OCH_2\underline{CH_2}CH$), 3.40-3.60 (m, 2H, $\underline{CH_2}OH$), 3.64 (s, 3H, CH_3), 3.80-4.20 (m, 3H, $OCH_2CH_2\underline{CH}$), 4.20-4.70 (m, 1H, $\underline{CH}$-CHNH), 4.95-5.20 (m, 8H, $4C\underline{H_2}C_6H_5$), 5.20-5.50 (m, 1H, $C\underline{H}NH$), 5.95 (s, 1H, CH), 6.50-8.40 (m, 29H, C_6H_3, C_6H_4, $4C_6H_5$, 2NH); ir (CH_2Cl_2) ν_{max}: 3600-3300 (OH, NH), 1760 (C=O, β-lactam), 1730 (C=O, ester), 1660-1680 (amide, ketone) cm^{-1}; uv (EtOH) λ_{max}: 218 nm (ε 20000), 298 nm (ε 17100).

β-Lactam 69

Obtained from 68, *via* the procedures for the preparation of 50, in 72% yield after flash chromatography (petroleum ether:EtOAc, 1:1).

^{1}Hmr ($CDCl_3$) δ: 2.30 (b, 2H, $OCH_2\underline{CH_2}CH$), 3.20-3.80 (m, 2H, $\underline{CH_2}$-CHNH), 3.70 (s, 3H, CH_3), 3.80-4.20 (m, 3H, $OCH_2CH_2\underline{CH}$), 4.40-4.80 (m, 1H, $C\underline{H}NH$), 5.08, 5.20 (2s, $2C\underline{H_2}C_6H_5$), 5.60 - 6.00 (m, 2H, CH, NH), 6.60-6.90 (d, 2H, J = 8Hz, aromatic), 7.32 (s, 15H, $3C_6H_5$), 7.80-8.00 (m, 1H, NH), 8.20 (d, 2H, J = 8Hz, aromatic); ir (film) ν_{max}: 3500-3200 (NH), 1740 (C=O, β-lactam and ester), 1660, 1595 cm^{-1}; uv λ_{max}: (EtOH), 213 nm (ε 18400), 293 nm (ε 16300).

β-Lactam 56

β-Lactam 55 (0.36 g, 0.379 mmol) was dissolved in a mixture of

ethanol (5 mL) and pyridine (5 mL). Hydroxylamine hydrochloride (0.35 g, 5.03 mmol) was added. The mixture was heated at ∿ 70° for 2 h. After adding chloroform (50 mL), the solution was washed with 5% HCl, dried ($MgSO_4$), filtered and evaporated to give crude product, which was chromatographed on silica gel using chloroform/ethyl acetate (1:1) to afford 0.25 g (68%) of β-lactam 56.

^{1}Hmr ($CDCl_3$) δ: 2.0-2.4 (b, 2H, $OCH_2\underline{CH_2}CH$), 3.3-4.0 (m, 9H, CH_2OH, CH_3, $OCH_2CH_2\underline{CH}$), 4.4-4.6 (m, 1H, $\underline{CH}$-CHNH), 4.8-5.2 (m, 8H, $4\underline{CH_2}C_6H_5$). 5.4-5.7 (m, 1H, $\underline{CH}NH$), 6.4-7.6 (m, 30H, C_6H_3, C_6H_4, $4C_6H_5$, 2NH, OH); ir (CH_2Cl_2) ν_{max}: 3600-3000 (NH, OH), 1770-1720 (C=O, β-lactam, ester), 1680 (amide, ketone) cm^{-1}; uv (EtOH) λ_{max}: 274 nm (ε 17000), a shoulder at 230 nm.

β-Lactam 70

Obtained from 69, *via* the procedures for the preparation of 56, in 83% yield after flash chromatography (EtOAc : Petroleum ether, 1:1.5.

^{1}Hmr ($CDCl_3$ + DMSO d_6) δ: 2.10-2.60 (m, 2H, $OCH_2\underline{CH_2}CH$), 3.20-3.80 (bm, 2H, CH_2N), 3.78 (s, 3H, CH_3), 3.80-4.40 (m, 3H, $OCH_2CH_2\underline{CH}$), 4.40-4.80 (m, 1H, $\underline{CH}NH$), 5.18, 5.20 (2s, 4H, $2CH_2Ph$), 5.80 (m, 1H, CH), 6.60-8.40 (m, 21H, aromatic, NH); ir (film) ν_{max}: 1760 (C=O, β-lactam), 1740, 1720 (C=O, ester) cm^{-1}; uv (EtOH) λ_{max}: 208 nm (ε 15400), 268 nm (ε 11300).

β-Lactam 31

To a solution of β-lactam 56 (0.25 g, 0.26 mmol) in methanol (10 mL) was added dropwise 1% NaOH (5 mL) over 10 min. After stirring for 15 min, the solution was acidified carefully by 6 M HCl to pH = 3. The methanol was evaporated and the aqueous solution was extracted with ethyl acetate (3 x 10 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in absolute ethanol (25 mL) and 10% Pd/C (0.15 g) was added. The mixture was hydrogenated at room temperature at 40 psi for an hour. Filtration and evaporation afforded 0.15 g (75%) of β-lactam 31; m.p. 95° (yellow), 171-174° (dec.).

^{1}Hmr (D_2O-$NaHCO_3$) δ: 2.2-2.4 (m, 2H, CH_2), 3.0-3.2 (m, 2H, CH_2OH), 3.8-4.2 (m, 3H, OCH_2CH_2CH), 4.4-4.6 (m, 1H, β-lactam), 5.2-5.4 (b, 1H, β-lactam), 5.9 (b, 1H, CH), 6.8-7.8 (m, 7H, aromatic); ir (nujol) ν_{max}: 3400-3100 and 2500-2700 (OH, COOH, NH), 1750 (β-lactam), 1650, 1610 (amide) cm^{-1}; uv (EtOH): a shoulder at 225 nm, ν_{max} 273 nm (ε 175050); (EtOH, 0.1 N, NaOH): a shoulder at 235 nm, ν_{max} 283 nm (ε 12042).

β-Lactam 71

Obtained from 70 *via* the procedures for the preparation of 31.

^{1}Hmr (D_2O, $NaHCO_3$) δ: 2.5 (m, 2H, OCH_2CH_2CH), 3.2-4.0 (m, 2H, CH_2N), 4.0-4.2 (m, 3H, OCH_2CH_2CH), 4.4 (m, 1H, CH), 5.5, 5.55 (2s, 1H, CH), 6.8-7.6 (m, 9H, aromatic); ir (nujol) ν_{max}: 3400-3000 (COOH, OH), 1750 (C=O, β-lactam), 1730 (acid); uv (EtOH) ν_{max}: 207 nm (ε 10230), 268 nm

(ε 6390), (EtOH, 0.1 N NaOH) ν_{max}: 211 nm (ε 13430), 284 nm (ε 5210).

Aldehyde β-Lactam 60

Azido β-lactam 59 (0.5 g, 1.38 mmol) was ozonized at -30° in methanol (60 mL) for 30 min. The excess ozone was removed by passing in nitrogen gas over 15 min. Dimethyl sulfide (1 ml) was added. The solution was allowed to warm up to room temperature. After evaporation of the methanol, the residue was dissolved in benzene (60 mL), washed with water (2 x 30 mL) and dried over $MgSO_4$. The benzene solution was azeotroped for 1 h followed by evaporation. The oil residue was warmed to 45° under high vacuum for 20 h to remove benzaldehyde. The aldehyde 60 was obtained, in 90% (0.35 g) yield.

^{1}Hmr (acetone-d_6) δ: 3.72 (s, 3H, CH_3), 3.8-5.1 (m, 2H, CH-CH), 5.65, 5.70 (2s, 1H, CH), 7.1-7.6 (bs, 5H, C_6H_5), 8.60, 9.50 (2d, 1H, CHO); ir ($CHCl_3$) ν_{max}: 2100 (N_3), 1770 (C=O, β-lactam), 1740 (C=O, ester) cm^{-1}.

Amino β-Lactam 61

Obtained from 59, *via* the procedure for the preparation of 49, in 90% yield after flash chromatography (ethyl acetate:petroleum ether, 2:1).

^{1}Hmr ($CDCl_3$) δ: 1.73 (s, 2H, NH_2), 3.68, 3.70 (2s, 3H, CH_3), 3.9-4.8 (m, 2H, CH-CH), 5.42, 5.50 (2s, 1H, CH), 5.6-6.4 (m, 2H, CH=CH), 6.90-7.40 (m, 10H, $2C_6H_5$); ir ($CHCl_3$) ν_{max}: 3400-3000 (NH_2), 1750 (C=O, ester) cm^{-1}.

β-Lactam 62

Amine 61 (0.93 g, 2.77 mmol) in dry methylene chloride (10 mL) was added di-tert-butyl dicarbonate (0.60 g, 2.77 mmol). The mixture was stirred at room temperature for a period of 3 h. The solution was washed with water (2 x 10 mL), dried ($MgSO_4$) and the CH_2Cl_2 was removed to give an oil. The product was purified by flash chromatography (petroleum ether:ethyl acetate, 2.5:1) to afford 0.80 g (67%) of 62 as a light yellow foam.

^{1}Hmr ($CDCl_3$) δ: 1.30 (s, 9H, t-Bu), 3.71, 3.75 (s, s, 3H, CH_3), 4.10-4.40 (m, 1H, CH), 4.80-5.40 (m, 1H, CH), 5.51, 5.61 (s, s, 1H, CH), 5.80-6.50 (m, 2H, CH=CH), 6.80-7.60 (m, 11H, NH, $2C_6H_5$); ir (film) ν_{max}: 3500-3200 (NH), 1760 (C=O, β-lactam), 1740 (C=O, ester) cm^{-1}; ms (70 eV, 78°), m/e (°/₀₀): 380 (56, $M^{+\cdot}$-56), 379 (11, $M^{+\cdot}$-t-Bu·).

β-Lactam 63

Amine 61 (250 mg, 0.74 mmol) was stirred with trifluoroacetic anhydride (170 mg, 0.81 mmol) for 3.5 h. The mixture was under high vacuum overnight. The residue was purified by flash chromatography (petroleum ether:ethyl acetate, 2:1) to yield 220 mg (68%) of β-lactam 63.

^{1}Hmr ($CDCl_3$) δ: 3.60, 3.73 (2s, 3H, CH_3), 4.1-4.4, 4.7-5.0 (m, m, 1H, CHCH=CH), 5.2-5.5 (m, 1H, CHNH), 5.58 (s, 1H, CH), 5.6-6.6 (m, 2H, CH=CH), 6.8-7.5 (m, 10H, $2C_6H_5$), 8.50, 8.88 (2d, J = 16 Hz, 1H, NH); ir ($CHCl_3$) ν_{max}: 3400-3000 (NH), 1760 (C=O, β-lactam), 1735 (C=O, ester) cm^{-1}.

β-Lactam 64

β-Lactam 62 (0.80 g, 1.77 mmol) in dry methylene chloride (40 mL) was ozonized at -78 C over 20 min until the solution turned to blue. The excess ozone was removed by passing the solution into nitrogen gas for 15 min. The dimethyl sulfide (1 mL) was added. The solution was allowed to warm to room temperature in a period of 2 h. The reaction mixture was washed with water (3 x 30 mL), dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash chromatography (petroleum ether:ethyl acetate, 1.5:1) to give 0.50 g (82%) of aldehyde 64 as a white solid.

^{1}Hmr ($CDCl_3$) δ: 1.35, 1.40 (2s, 9H, t-Bu), 3.70 (s, 3H, CH_3), 4.1-5.3 (m, 2H, CH-CH), 5.70, 5.75 (2s, 1H, CH), 5.8-6.1 (m, 1H, NH), 7.40 (s, 5H, C_6H_5), 8.58, 9.55 (2d, 1H, CHO); ir (film) ν_{max}: 3500-3200 (NH), 1770 (C=O, β-lactam), 1740 (C=O, ester), 1720 cm^{-1}; ms (70 eV, 64°), m/e (%): 362 (10, $M^{+\cdot}$), 307 (18, $M^{+\cdot}$-t-Bu·).

Aldehyde β-Lactam 65

Obtained from 63, *via* the procedures for the preparation of 64, in 74% yield.

^{1}Hmr ($CDCl_3$) δ: 3.80-3.82 (2s, 3H, CH_3), 4.10-5.50 (m, 2H, CH-CH), 5.66-5.72 (2s, 1H, CH), 7.0-7.60 (m, 5H, C_6H_5), 8.3-8.7 (b, 1H, NH), 9.0, 10.0 (2d, 1H, CHO); ir ($CHCl_3$) ν_{max}: 3500-3000 (NH), 1770 (C=O, β-lactam), 1730 (C=O, ester) cm^{-1}.

β-Lactam 66

Aldehyde 64 (0.500 g, 1.38 mmol) was dissolved under a nitrogen atmosphere at 60-70°C in 15 mL of d-gas benzene. With strong stirring, $(PPh_3)_3RhCl$ (1.29 g, 1.38 mmol) was added. After refluxing for 3 h, the solution was cooled in an ice-bath to room temperature and then passed CO through it for 10 min. After evaporation, to the residue was added CH_2Cl_2 (20 mL). Lemmon-yellow crystals of $(Ph_3P)_2COCl$ was filtered off and washed with CH_2Cl_2. Evaporation of the filtrate in vacuum gave the crude product which was purified by flash chromatography (petroleum ether:ethyl acetate, 1.5:1) to afford 0.32 g (70%) of 66 as a foam.

^{1}Hmr ($CDCl_3$) δ: 1.35, 1.41 (s, s, 9H, t-Bu), 2.9-4.0 (m, 2H, CH_2), 3.75 (s, 3H, CH_3), 4.6-5.0 (b, 1H, $\underline{CH}$-CH_2), 5.60 (s, 1H, CH), 5.70-5.90 (m, 1H, NH), 7.38 (s, 5H, C_6H_5); ir (film) ν_{max}: 3500-3200 (NH), 1765 (C=O, β-lactam), 1740 (C=O, ester), 1710 (t-BOCCO) cm^{-1}; ms (70 eV, 85°), m/e (%): 279, 278 (5, $M^{+\cdot}$-56), 277 (10, $M^{+\cdot}$-t-Bu$^{\cdot}$), 57 (1000).

β-Lactam 67[3]

Obtained from 65, *via* the procedures for the preparation of 66, in 42% yield after flash chromatography (petroleum ether:ethyl acetate, 5:3).

^{1}Hmr ($CDCl_3$) δ: 3.30-3.60 (m, 2H, $\underline{CH_2}$-CH), 3.70 (s, 3H, CH_3), 5.0 (m, 1H, CH_2-$\underline{CH}$), 5.54 (s, 1H, CH_3), 7.10-7.40 (m, 5H, C_6H_5), 8.10 (bd, 1H, NH);

ir ($CHCl_3$) ν_{max}: 3400 (NH), 1765 (C=O, β-lactam), 1730 (C=O, ester) cm^{-1}; ms (70 eV, 73°), m/e (°/₀₀): 271 (81, $M^{+\cdot}-COOCH_3\cdot$), 191 (238, $M^{+\cdot}-CH_2=CHNHCOCF_3$), 132 (1000, $M^{+\cdot}-COOCH_3\cdot-CH_2=CHNHCOCF_3$).

Note: It should contain two isomers based on the crude pmr and tlc. Only one isomer was isolated out.

β-Lactam 68

β-Lactam 66 (0.32 g, 0.96 mmol) was dissolved in methylene chloride (7 mL) and trifluoroacetic acid (3 mL). After stirring at room temperature for 1 h, the solution was evaporated in vacuum to dryness. The residue was purified by flash chromatography (ethyl acetate:methanol, 7:1) to give 0.14 g (62%) of β-lactam 68 as a yellow oil.

^{1}Hmr ($CDCl_3$) δ: 1.90 (bs, 2H, NH_2 exchanged with D_2O), 2.60-3.40 (m, 2H, CH_2), 3.67 (s, 3H, CH_3), 3.8-4.3 (m, 1H, $\underline{CH}-NH_2$), 5.38 (s, 1H, CH), 7.23 (s, 5H, C_6H_5); ir (film) ν_{max}: 3400-3100 (NH_2), 1735 (C=O, β-lactam and ester) cm^{-1}; ms (70 eV, 50°), m/e (°/₀₀): 234 (1, $M^{+\cdot}$), 177 (243, $M^{+\cdot}-NH_2CH=C=O$), 150 (27), 149 (78); Anal. Calcd. for $C_{12}H_{14}N_2O_3$: C, 61.54; H, 5.98; N, 11.96; Found: C, 61.27; H, 5.71; N, 11.69.

CHAPTER II

3-Hydroxy-4-Aminobenzoic Acid 75

A solution of 3-hydroxy-4-nitrobenzoic acid 74 (2 g, 10.9 mmol) in absolute ethanol (150 mL) containing PtO_2 (100 mg) was hydrogenated at 40 psi for 2 h. Filtration and evaporation gave amine 75 (1.67 g) in quantitative yield.

^{1}Hmr (acetone d_6) δ: 4.60 (b, 3H, NH_2, OH), 6.14-7.09 (m, 3H, C_6H_3); ir (KBr) ν_{max}: 3400, 3300, 3000-2500 (NH_2, OH, COOH), 1660, 1600 (COOH) cm^{-1}; ms (70 eV, 47°). m/e (°/₀₀): 153 (1000, $M^{+\cdot}$).

Methyl-3-Hydroxy-4-Amino-Benzoate 77

Obtained from 75, by treatment with diazomethane[79] in Et_2O; m.p. 93-95°.

^{1}Hmr (acetone d_6) δ: 3.74 (s, 3H, CH_3), 4.80-5.60 (b, 3H, NH_2, OH), 6.55-7.60 (m, 3H, C_6H_3); ir (CH_2Cl_2) ν_{max}: 3580, 3500, 3400 (NH_2, OH), 1710 (C=O) cm^{-1}; ms (70 eV, 30°): 167 (645, $M^{+\cdot}$), 136 (1000, $M^{+\cdot}$-$OCH_3^{\cdot}$).

Methyl-3-t-Butyldimethylsilyloxy-4-Amino-Benzoate 78

To methyl-3-hydroxy-4-amino-benzoate 77 (2 g, 11.9 mmol) in dry DMF (15 mL) was added imidazole (2.04 g, 29.9 mmol) and t-butyldimethylsilyl chloride (1.98 g, 13.2 mmol). After stirring at room temperature for 18 h,

the solution was partitioned between water and ether. The ether layer was washed with water (4 x 50 mL), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether:ethyl acetate, 9:1) to give 2.71 (81%) of amine 78, as a pale yellow solid; m.p. 59-61°.

^{1}Hmr ($CDCl_3$) δ: 0.38 (s, 6H, $Si(CH_3)_2$), 1.10 (s, 9H, tBuSi), 3.94 (s, 3H, CH_3), 4.30 (b, 2H, NH_2), 6.62-7.64 (m, 3H, aromatic); ir (KBr) ν_{max}: 3500, 3400 (NH_2), 3000, 1630, 1710 (ester, C=O) cm^{-1}; ms (70 eV, 30°), m/e (°/₀₀): 281 (145, $M^{+\cdot}$), 224 (1000, $M^{+\cdot}$-t-Bu·); Anal. Calcd. for $C_{14}H_{23}NO_3Si$: C 59.74, H 8.06, N 4.98; Found: C 59.51, H 8.06, N 4.79.

t-Butyldimethylsilyl-3-t-Butyldimethylsilyloxy-4-Amino-Benzoate 85

To amine 75 (0.2 g, 1.31 mmol) in dry DMF (5 mL) was added imidazole (0.445 g, 6.53 mmol) and t-butyldimethylsilyl chloride (0.433 g, 2.88 mmol). The solution was stirred at room temperature overnight and then partitioned between H_2O (30 mL) and ether (50 mL). The ether layer was washed with water (4 x 50 mL), dried and evaporated to obtain 0.44 g (88%) of red oil 85.

^{1}Hmr ($CDCl_3$) δ: 0.24 (s, 6H, $Si(CH_3)_2$), 0.34 (s, 6H, $Si(CH_3)_2$), 0.98 (s, 18H, t-BuSi), 4.24 (b, 2H, NH_2), 6.48-7.56 (m, 3H, C_6H_3); ir ($CHCl_3$) ν_{max}: 3500, 3400 (NH_2), 1665 (C=O) cm^{-1}; ms (70 eV, 30°), m/e (°/₀₀): 381 (101, $M^{+\cdot}$), 324 (1000, $M^{+\cdot}$-t-Bu·).

3-t-Butyldimethylsilyloxy-4-Amino-Benzoic Acid 84

To amine 85 (2.00 g, 5.24 mmol) in methanol (30 mL) was added 5 drops of concentrated HCl. The solution was stirred at 50° for half an hour. Evaporation gave acid 84 (1.40 g) as pale yellow solid in quantitative yield, m.p. 160-162°.

^{1}Hmr ($CDCl_3$, DMSO d_6) δ: 0.20 (s, 6H, $Si(CH_3)_2$), 0.98 (s, 9H, t-BuSi), 5.60-6.00 (b, 3H, OH, NH_2), 6.60-7.54 (m, 3H, C_6H_3); ir ($CHCl_3$) ν_{max}: 3500, 3400 (NH_2), 3200-2500 (COOH), 1675 (C=O), 1610 cm^{-1}; ms (70 eV, 30°), m/e (%): 267 (66, $M^{+\cdot}$), 210 (1000, $M^{+\cdot}$-t-Bu·).

Azido β-Lactams 80 and 88

Amine 84 (0.5 g, 1.87 mmol) and cinnamaldehyde (0.25 g, 1.87 mmol) in benzene (50 mL) were refluxed for 4 h, using a Dean Stark trap. The solvent was evaporated to obtain the Schiff base as a yellow solid. To this Schiff base was added triethylamine (0.227 g, 2.25 mmol) in CH_2Cl_2 (20 mL) and then dropwise trimethylsilyl chloride (0.264 g, 2.43 mmol) in CH_2Cl_2 (5 mL) at 0°. After stirring for 10 min, triethylamine (0.2 g, 1.98 mmol) was added, and a solution of azidoacetyl chloride (0.247 g, 0.206 mmol) in CH_2Cl_2 (5 mL) was added at -20°. After stirring an additional hour, the solution was washed with water (2 x 40 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (ethyl acetate:petroleum ether, 9:1) afforded 0.45 g (51.8%) of β-lactam 88 as a pale yellow solid, m.p. 150-152°d.

Schiff base 86; ^{1}Hmr ($CDCl_3$) δ: 0.25 (s, 6H, $Si(CH_3)_2$), 1.05 (s, 9H, t-BuSi), 6.98-8.40 (m, 11H, C_6H_3, C_6H_5-CH=CH-CH), 10.2-10.4 (b, 1H, COOH); ir (CH_2Cl_2) ν_{max}: 3000-2500 (COOH), 1680 (COOH), 1630 (C=N) cm^{-1}.

β-Lactam 88; ^{1}Hmr ($CDCl_3$) δ: 0.35 (s, 6H, $Si(CH_3)_2$), 1.05 (s, 9H, t-BuSi), 5.0-5.2 (m, 1H, CHCH=CH), 5.35-5.42 (d, 1H, CHN_3, J = 5 Hz), 6.00-6.44 (dd, 1H, GH=CHPh, J = 16,5 Hz), 6.62-6.88 (d, 1H, PhCH, J = 16 Hz), 7.33-7.78 (m, 9H, C_6H_3, C_6H_5), 10.33 (b, 1H, COOH); ir (CH_2Cl_2) ν_{max}: 3200-2500 (COOH), 2100 (N_3), 1765 (β-lactam, C=O), 1680 (ester) cm^{-1}; Anal. Calcd. for $C_{24}H_{28}O_4N_4Si$: C 62.07, H 6.03, N 12.07; Found: C 62.04, H 6.24, N 11.92.

β-Lactam 88 was treated with CH_2N_2 in ether to give ester 80 as a pale yellow solid; m.p. 90-91°.

^{1}Hmr ($CDCl_3$) δ: 0.34 (s, 6H, $Si(GH_3)_2$), 1.03 (s, 9H, t-BuSi), 3.95 (s, 3H, CH_3), 4.93-5.28 (m, 2H, N_3CHCH), 5.90-6.28 (dd, 1H, PhCH=CH, J = 5,16 Hz), 6.50-6.77 (d, 1H, PhCH, J = 16 Hz), 7.20-7.75 (m, 8H, C_6H_3, C_6H_5); ir (CH_2Cl_2) ν_{max}: 2100 (N_3), 1765 (β-lactam, C=O), 1720 (ester) cm^{-1}; ms (70 eV, 62°), m/e (°/₀₀): 450 (13, $M^{+\cdot}-N_2$), 393 (1000, $M^{+\cdot}-N_2\cdot$-t-Bu·); Anal. Calcd. for $C_{25}H_{30}N_4O_4Si$: C 62.76, H 6.28, N 11.72; Found: C 62.29, H 6.51, N 11.46.

3-Trimethylsilyloxy-4-Aminobenzoic Acid 90

To 3-hydroxy-4-aminobenzoic acid 75 (1.38 g, 9.02 mmol) in dry CH_2Cl_2 (40 mL) was added triethylamine (2 g, 19.8 mmol) and added dropwise

a solution of trimethylsilyl chloride (2.15 g, 19.8 mmol) in dry CH_2Cl_2 (20 mL) over 15 min. After stirring for an additional 30 min, the solution was washed with H_2O (2 x 40 mL), dried ($MgSO_4$) and evaporated to afford trimethylsilyl-3-trimethylsilyloxyl-4-amino-benzoate.

^{1}Hmr ($CDCl_3$) δ: 0.38, 0.45 (2s, 18H, 2 $Si(CH_3)_3$), 4.70 (b, 2H, NH_2), 6.64-7.71 (m, 3H, C_6H_3).

The disilylated amine in methanol (30 mL) was stirred at room temperature for 30 min. Evaporation gave 2.03 g of 3-trimethylsilyloxy-4-aminobenzoic acid 90 as an oil.

^{1}Hmr ($CDCl_3$-DMSO d_6) δ: 0.20 (s, 9H, $Si(CH_3)_3$), 6.32 (b, 2H, NH_2), 6.37-7.38 (m, 3H, C_6H_3); ir (CH_2Cl_2) ν_{max}: 3500, 3400 (NH_2), 3300-2400 (COOH), 1670, 1620 cm^{-1}.

β-Lactam 87

A solution of 3-trimethylsilyloxy-4-aminobenzoic acid 90 (0.73 g, 3.24 mmol) and cinnamaldehyde (0.43 g, 3.25 mmol) in benzene (20 mL) was refluxed 1.5 h using a Dean-Stark trap. Evaporation of the benzene afforded the Schiff base as a yellow solid.

^{1}Hmr ($CDCl_3$+DMSO d_6) δ: 0.25, 0.45 (2s, 9H, $Si(CH_3)_3$), 6.80-7.70 (m, 11H, C_6H_3, $C_6H_5CH=CH$), 8.2-8.5 (b, 1H, COOH); ir (CH_2Cl_2) ν_{max}: 1730 (C=O, acid), 1670 (C=N) cm^{-1}.

To the Schiff base was added triethylamine (0.36 g, 3.58 mmol) in

dry CH_2Cl_2 (20 mL) and added dropwise trimethylsilyl chloride (0.389 g, 3.58 mmol) in dry CH_2Cl_2 (10 mL) at 0°. After stirring another 20 min at room temperature, triethylamine (0.36 g, 3.58 mmol) was added, a solution of azidoacetyl chloride (0.43 g, 3.58 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise at -20°. The reaction mixture was warmed up to room temperature and stirred for an additional hour. The solution was washed with H_2O (2 x 20 mL), dried ($MgSO_4$) and evaporated to dryness. The resulting oil was dissolved in methanol (15 mL). After adding one drop of concentrated HCl, the solution was stirred at room temperature for 10 min. The methanol was evaporated. The residue was treated with CH_2N_2 in ether to yield the ester. The crude product was purified by flash chromatography (petroleum ether:ethyl acetate, 3:1) to afford 0.71 g (60%) of β-lactam 87; m.p. 129-131°.

^{1}Hmr ($CDCl_3$) δ: 3.82 (s, 3H, CH_3), 4.95-5.02 (m, 2H, CH-CHN_3), 5.90-7.60 (m, 10H, C_6H_3, C_6H_5CH=CH), 9.42 (s, 1H, OH); ir (CH_2Cl_2) ν_{max}: 3500-3000 (OH), 2100 (N_3), 1730-1720 (C=O, β-lactam, ester) cm^{-1}; ms (70 eV, 56°), m/e (°/₀₀): 364 (8, $M^{+\cdot}$), 349 (11, $M^{+\cdot}-CH_3^{\cdot}$), 336 (153, $M^{+\cdot}-N_2^{\cdot}$), 280 (485), 143 (1000); Anal. Calcd. for $C_{19}H_{16}N_4O_4$: C 62.64, H 4.40, N 15.38; Found: C 62.55, H 4.39, N 15.27.

Amino β-Lactam 94

To a solution of β-lactam 80 (1.0 g, 2.09 mmol) in CH_2Cl_2 (25 mL) was added triethylamine (0.232 g, 2.30 mmol), and a stream of H_2S gas

was bubbled in for 15 min at 0°. The mixture was allowed to warm and was stirred at room temperature. After 2 h, nitrogen gas was bubbled in for 30 min. The solution was washed with water (3 x 30 mL), dried ($MgSO_4$) and evaporated to give 0.76 g (80%) of amine 94, after flash chromatography (ethyl acetate:petroleum ether, 3:2).

^{1}Hmr ($CDCl_3$) δ: 0.34 (s, 6H, $Si(CH_3)_2$), 1.01 (s, 9H, t-BuSi), 3.86 (s, 3H, CH_3), 4.51-4.81 (b, 1H, C<u>H</u>=CHPh), 5.28 (m, 1H, C<u>H</u>NH_2), 6.20-8.00 (m, 12H, C_6H_3, C_6H_5C<u>H</u>=C<u>H</u>, NH_2); ir ($CHCl_3$) ν_{max}: 3500-3400 (NH_2), 1740 (β-lactam, C=O) cm^{-1}; ms (70 eV, 53°), m/e (°/₀₀): 452 (166, $M^{+\cdot}$), 437 (10, $M^{+\cdot}-CH_3^{\cdot}$), 421 (17, $M^{+\cdot}-OCH_3^{\cdot}$), 396 (1000, $M^{+\cdot}-OCH_3^{\cdot}-CH_3^{\cdot}$), 395 (167, $M^{+\cdot}$-t-Bu$^{\cdot}$, $M^{+\cdot}-NH_2CH{=}CO$).

Amide β-Lactam 95

To amine 94 (0.72 g, 1.59 mmol) in CH_2Cl_2 (20 mL) was added triethylamine (0.177 g, 1.75 mmol) and phenylacetyl chloride (0.27 g, 1.75 mmol). After stirring at room temperature for 30 min, the solution was washed with water (2 x 30 mL), dried ($MgSO_4$) and evaporated. Purification of the residue by flash chromatography (petroleum ether: ethyl acetate, 2:1) gave 0.56 g (62%) of amide 95 as a white solid; m.p. 168-170 .

^{1}Hmr ($CDCl_3$) δ: 0.32, 0.35 (2s, 6H, $Si(CH_3)_2$), 0.98 (s, 9H, t-BuSi), 3.50 (s, 2H, $PhCH_2CO$), 3.80 (s, 3H, CH_3), 5.10-5.40 (dd, 1H, C<u>H</u>CH=CHPh, J = 5,6 Hz), 5.54-5.60 (d, 1H, C<u>H</u>NH, J = 5 Hz), 5.74-6.11 (dd, 1H, C<u>H</u>=CHPh,

J = 6,16 Hz), 6.29-6.57 (d, CH=CHPh, J = 16 Hz), 7.00-7.73 (m, 14H, C_6H_3, $2C_6H_5$, NH); ir ($CHCl_3$) ν_{max}: 3400 (NH), 1760 (β-lactam, C=O), 1720 (ester) cm^{-1}; ms (70 eV, 82°), m/e (°/₀₀): 570 (64, $M^{+\cdot}$), 513 (199, $M^{+\cdot}$-t-Bu·), 378 (906).

β-Lactam 96

β-Lactam 95 (360 mg, 0.63 mmol) in absolute ethanol (20 mL) containing PtO_2 (100 mg) was hydrogenated at 40 psi for 2 h. Filtration and evaporation gave 50 mg (14%) of β-lactam 96, after flash chromatography (petroleum ether:ethyl acetate, 2.8:1).

^{1}Hmr ($CDCl_3$) δ: 0.200, 0.260 (2s, 6H, $Si(CH_3)_2$), 0.97 (s, 9H, t-BuSi), 1.60-2.70 (m, 4H, $PhCH_2CH_2$), 3.67 (s, 2H, $PhCH_2CO$), 3.95 (s, 3H, CH_3), 4.52-4.82 (m, 1H, $PhCH_2CH_2CH$), 5.45-5.71 (dd, 1H, CHNH, J = 5,8 Hz), 6.90-7.80 (m, 14H, aromatics, NH); ir ($CHCl_3$) ν_{max}: 3400 (NH), 1750 (β-lactam, C=O), 1720 (ester, C=O) cm^{-1}, ms (70 eV, 87°), m/e (°/₀₀): 572 (19, $M^{+\cdot}$), 541 (18, $M^{+\cdot}$-OCH_3·), 515 (295, $M^{+\cdot}$-t-Bu·), 398 (808).

Azido β-Lactam 98

Aniline (62 mg, 0.67 mmol) and phenylpropargyl aldehyde (86 mg, 0.67 mmol) in benzene (20 mL) were refluxed for 2.5 h, using a Dean-Stark trap. After evaporation of the benzene, the residue was dissolved in CH_2Cl_2 (10 mL) containing triethylamine (73 mg, 0.73 mmol), to this was added dropwise azidoacetyl chloride (79 mg, 0.67 mmol) in CH_2Cl_2 (5 mL)

at -20°. After stirring for an additional hour at room temperature, the mixture was washed with water (2 x 10 mL), dried ($MgSO_4$) and evaporated to give 110 mg (58%) of β-lactam 98, after flash chromatography (1:8, ethyl acetate:petroleum ether); m.p. 85-86.5°.

^{1}Hmr ($CDCl_3$) δ : 4.80 (d, 1H, CHN_3, J = 5 Hz), 5.01 (d, 1H, CH-CHN_3, J = 5 Hz), 6.73-7.88 (m, 10H, $2C_6H_5$); ir ($CHCl_3$) ν_{max}: 2220 (C≡C), 2100 (N_3), 1765 (β-lactam, C=O) cm^{-1}; ms (70 eV, 25-30°), m/e (°/₀₀): 288 (13, $M^{+\cdot}$), 260 (227, $M^{+\cdot}-N_2$).

Phenol β-Lactam 100

A solution of 2-hydroxyaniline (0.109 g, 1 mmol) and phenylpropargyl aldehyde (0.13 g, 1 mmol) in benzene (20 mL) were refluxed for 2.5 h using a Dean Stark trap. Removal of the benzene afforded the Schiff base as a red solid. To this Schiff base in CH_2Cl_2 (10 mL) was added triethylamine (0.22 g, 2.2 mmol) and then dropwise trimethylsilyl chloride (0.119 g, 1.1 mmol) in CH_2Cl_2 (5 mL) at 0°. After stirring for 15 min at room temperature, a solution of azidoacetyl chloride (0.12 g, 1 mmol) was added at -20°. The mixture was allowed to warm and was stirred at room temperature for an hour. Methanol (5 mL) and a drop of concentrated HCl were added. The solution was washed with water (2 x 15 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (4.5:1, petroleum ether:ethyl acetate) afforded 150 mg (50%) of β-lactam 100 as pale yellow crystals; m.p. 110-111°.

^{1}Hmr ($CDCl_3$) δ : 4.80 (d, 1H, CHN_3, J = 5 Hz), 5.11 (d, 1H, CH-CHN_3,

J = 5 Hz), 6.61-7.67 (m, 9H, C_6H_5, C_6H_4), 9.22 (s, 1H, OH); ir ($CHCl_3$) ν_{max}: 3300-2900 (OH), 2210 (C≡C), 2100 (N_3), 1730 (β-lactam, C=O); ms (15 eV, 52°), m/e (°/₀₀): 304 (146, $M^{+\cdot}$), 276 (211, $M^{+\cdot}-N_2\cdot$).

β-Lactam 101

Obtained from amine 84 *via* the same procedure for the preparation of 80, in 50% yield after flash chromatography (EtOAc:petroleum ether, 1:7.5).

Schiff base; ^{1}Hmr ($CDCl_3$) δ: 0.25 (s, 6H, $Si(CH_3)_2$), 1.04 (s, 9H, t-BuSi), 6.60-8.00 (m, 8H, aromatic), 9.20 (s, 1H, N=CH), 10.6-10.9 (b, 1H, COOH); ir ($CHCl_3$) ν_{max}: 3500-2500 (COOH), 2200 (C≡C), 1680 (C=O), 1660 (C=N) cm^{-1}; β-lactam 101; ^{1}Hmr ($CDCl_3$) δ: 0.35, 0.40 (2s, 6H, $Si(CH_3)_2$), 1.02 (s, 9H, t-BuSi), 3.85 (s, 3H, OCH_3), 4.80 (d, 1H, HCH, J = 5 Hz), 5.40 (d, 1H, CHN_3; J = 5 Hz), 6.80-7.65 (m, 8H, aromatic); ir ($CHCl_3$) ν_{max}: 2100 (N_3), 1780 (β-lactam, C=O), 1720 (ester) cm^{-1}; CI-ms (68°): 477 (608, MH^+), 449 (1000, $MH^+-N_2\cdot$).

β-Lactams 104 and 105

Amine 102 (1.96 g, 5 mmol) and phenylpropargyl aldehyde (0.65 g, 5 mmol) in benzene (50 mL) containing a trace of p-TsOH, were refluxed overnight using a Dean Stark trap. Evaporation of the solvent afforded the Schiff base 103 as a brown oil. To this Schiff base in CH_2Cl_2 (50 mL) and triethylamine (0.56 g, 5.5 mmol) was added dropwise azidoacetyl

chloride (0.66 g, 5.5 mmol) in CH_2Cl_2 (10 mL) at -20° over 10 min. After stirring for an additional hour at room temperature, the mixture was washed with water (2 x 50 mL), dried ($MgSO_4$) and evaporated to give 0.88 g (30%) of *trans* 105 and 0.87 g (30%) of *cis* β-lactam 104, after flash chromatography (1:7, ethyl acetate:petroleum ether).

Schiff base 103; ^{1}Hmr ($CDCl_3$) δ: 1.20 (2s, 9H, t-BuSi), 6.38-7.77 (m, 18H, aromatic), 7.69 (s, 1H, CH=N); ir ($CHCl_3$) ν_{max}: 2200 (C≡C), 1650 (C=N), 1580 (NO_2) cm^{-1}.

trans β-Lactam 105; ^{1}Hmr ($CDCl_3$) δ: 1.17 (2s, 9H, t-BuSi), 4.97 (d, 1H, CHN_3, J = 2 Hz), 5.17 (d, 1H, C<u>H</u>-CHN_3, J = 2 Hz), 6.31-8.44 (m, 18H, aromatic); ir (film) ν_{max}: 2210 (C≡C), 2100 (N_3), 1780 (β-lactam, C=O), 1520, 1340 (NO_2) cm^{-1}; ms (70 eV, 140°), m/e (‰): 559 (8, $M^{+\cdot}-N_2^{\cdot}$), 502 (8, $M^{+\cdot}-N_2^{\cdot}$ - t-Bu$^{\cdot}$), 361 (1000).

cis β-Lactam 104; ^{1}Hmr ($CDCl_3$) δ: 1.19 (s, 9H, t-BuSi), 4.98 (d, 1H, CHN_3, J = 5 Hz), 5.54 (d, 1H, C<u>H</u>-CHN_3, J = 5 Hz), 6.34-8.47 (m, 18H, aromatic); ir ($CHCl_3$) ν_{max}: 2210 (C≡C), 2100 (N_3), 1770 (β-lactam, C=O), 1580, 1340 (NO_2) cm^{-1}.

β-Lactam 106

Aniline (0.3 g, 3.22 mmol) and β-phenylcinnamaldehyde (0.672 g, 3.23 mmol) in benzene (30 mL) was refluxed for 3 h using a Dean-Stark trap. Evaporation of the benzene gave the Schiff base as yellow oil.

^{1}Hmr ($CDCl_3$) δ: 6.80-7.45 (m, 16H, $3C_6H_5$, CH), 8.02 (d, 1H, NCH, J = 10 Hz); ir ($CHCl_3$) ν_{max}: 1610 (C=N) cm^{-1}.

To the freshly prepared Schiff base in dry CH_2Cl_2 (50 mL) containing triethylamine (0.36 g, 3.56 mmol) was added dropwise a solution of azidoacetyl chloride (0.42 g, 3.50 mmol) in dry CH_2Cl_2 (10 mL) at -20° for 10 min. After stirring for another hour, the solution was washed with H_2O (2 x 20 mL), dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography (petroleum ether:ethyl acetate, 9:1) to afford 0.758 g (64%) of β-lactam 106; m.p. 97-98°.

^{1}Hmr ($CDCl_3$) δ: 4.75-4.95 (m, 2H, $CHCHN_3$), 6.05-6.37 (m, 1H, CH=C), 7.0-7.6 (m, 15H, $3C_6H_5$); ^{1}Hmr (C_6D_6) δ: 3.75 (d, 1H, CHN_3, J = 5 Hz), 4.00 (dd, 1H, CH-CHN_3, J = 5, 9 Hz), 5.60-5.75 (d, 1H, CH=C, J = 9 Hz), 6.50-7.20 (m, 15H, $3C_6H_5$); ir ($CHCl_3$) ν_{max}: 2100 (N_3), 1760 (C=O, β-lactam), 1600 (C=C) cm^{-1}; ms (70 eV, 67°), m/e (°/₀₀): 338 (126, $M^{+\cdot}-N_2$), 282 (1000).

β-Lactam 107

Obtained from 102 *via* the procedure for the preparation of 106; in 43% yield, after flash chromatography (EtOAc:petroleum ether, 1:8); m.p. 123-125°.

^{1}Hmr (C_6D_6) δ: 1.20 (s, 9H, t-BuSi), 4.60 (d, 1H, CHN_3, J = 5 Hz), 4.70-5.00 (dd, 1H, CH-CHN_3, J = 5, 9 Hz), 6.50-8.30 (m, 24H, C_6H_3, $4C_6H_5$); ir (CH_2Cl_2) ν_{max}: 2100 (C≡N), 1770 (C=O, β-lactam), 1520, 1340 (NO_2) cm^{-1};

ms (70 eV, 75°), m/e (°/₀₀): 637 (22, $M^{+\cdot}-N_2\cdot$), 580 (69, $M^{+\cdot}-N_2\cdot$ - t-Bu·), 343 (1000).

β-Lactam 108

Obtained from aniline and o-nitrocinnamaldehyde, *via* the procedure for the preparation of β-lactam 106, in 47% yield after flash chromatography (EtOAc:petroleum ether, 1:5); m.p. 115-117°d.

^{1}Hmr ($CDCl_3$) δ: 4.91 (d, 1H, CHN_3, J = 5 Hz), 4.93 (m, 1H, CHN), 6.00-6.44 (m, 1H, $\underline{CH}=CHC_6H_4$), 7.00-8.20 (m, 10H, $CH=\underline{CH}C_6\underline{H}_4$, C_6H_5); ir (CH_2Cl_2) ν_{max}: 2100 (C≡N), 1760 (C=O, β-lactam), 1520, 1340, 1370 (NO_2) cm^{-1}; ms (70 eV, 53°), m/e (°/₀₀): 335 (54, $M^{+\cdot}$), 307 (41, $M^{+\cdot}-N_2\cdot$), 77 (1000).

β-Lactam 109

Obtained from amine 102 *via* the procedure for the preparation of β-lactam 108, in 50% yield after flash chromatography (EtOAc:petroleum ether, 1:4).

^{1}Hmr ($CDCl_3$) δ: 1.12 (s, 9H, t-BuSi), 5.10 (d, 1H, CHN_3, J = 5 Hz), 5.12-5.40 (m, 1H, CHN), 6.00-8.40 (m, 18H, $2C_6H_3$, CH=CH, $2C_6H_5$); ir (CH_2Cl_2) ν_{max}: 1780 (C=O, β-lactam), 1525, 1340 (NO_2) cm^{-1}.

Epoxide β-Lactam 111

To β-lactam 110 (0.5 g, 0.909 mmol) in hexane (40 mL) was added

m-chloroperbenzoic acid (85%) (0.20 g, 1.1 mmol). The mixture was heated to 60° for 18 h. The solution was washed successively with 10% Na_2SO_3 (30 mL), aqueous $NaHCO_3$ (30 mL), water (40 mL), dried ($MgSO_4$) and evaporated. Flash chromatography (petroleum ether:ethyl acetate, 10:1.2) afforded in 90% yield two isomers of 111, R_f = 0.35 and 0.25, which were not completely separated.

Less polar isomer; ^{1}Hmr ($CDCl_3$) δ: 0.10 (m, 12H, $4CH_3$), 0.50-1.00 (m, 18H, t-BuSi), 3.00, 3.15 (dd, 1H, $C_6H_5CHOC\underline{H}$, J = 2, 7 Hz), 3.31 (d, 1H, $C_6H_5C\underline{H}O$, J = 2 Hz), 3.65, 3.80 (dd, 1H, $C\underline{H}$-CHN_3, J = 4, 7 Hz), 4.82 (d, 1H, CHN_3, J = 4 Hz), 6.20-7.20 (m, 8H, C_6H_5, C_6H_3). More polar isomer; ^{1}Hmr ($CDCl_3$) δ: 0.10 (2s, 12H, $4CH_3$), 0.90 (s, 18H, t-BuSi), 3.21, 3.31 (dd, 1H, $C_6H_5CHOC\underline{H}$, J = 2, 6 Hz), 3.60 (d, 1H, $C_6H_5C\underline{H}O$, J = 2 Hz), 4.00 (m, 1H, $C\underline{H}$-CHN_3), 4.90 (d, 1H, $C\underline{H}N_3$, J = 5 Hz), 6.40-7.35 (m, 8H, C_6H_5, C_6H_3); ir (film) ν_{max}: 2100 (N_3), 1775 (β-lactam, C=O) cm^{-1}; ms (70 eV, 100°), m/e (°/₀₀): 509 (203, $M^{+\cdot}$-t-Bu·), 483 (40, $M^{+\cdot}$-N_3CH=C=O), 481 (233, $M^{+\cdot}$-t-Bu·-N_2·), 426 (32, $M^{+\cdot}$-t-Bu·-N_3CH=C=O), 322 (478, $M^{+\cdot}$-t-Bu·-C_6H_5CHOCH CH=CHN_3).

β-Lactam 112

To silylated epoxide 111 (1.50 g, 2.65 mmol) in dry THF (30 mL) was added glacial acetic acid (0.477 g, 7.95 mmol), followed by dropwise addition of a solution of tetra-n-butylammonium fluoride in THF (0.5 M, 10.6 mL), at 0°. After 15 min the solution was added to pH 4.4 buffer (25 mL) and extracted with ether (3 x 25 mL). The organic layer was washed

with water (2 x 30 mL), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether:ethyl acetate, 2:1) affording 0.54 g (60%) of β-lactam 112.

^{1}Hmr ($CDCl_3$) δ: 3.10-3.40 (m, 1H, CH-CHOCHPh), 3.60, 3.84 (dd, 1H, CHOCHPh, J = 2 Hz), 4.40-4.80 (m, 1H, $CHCHN_3$), 5.00 (d, 1H, $CHCHN_3$, J = 5 Hz), 6.20-7.40 (m, 9H, OH, aromatic), 7.60-8.40 (b, 1H, OH); ir (CH_2Cl_2) ν_{max}: 3500-2900 (OH), 2100 (C≡N), 1725 (β-lactam, C=O) cm^{-1}; ms (70 eV, 100°), m/e (°/₀₀): 310 (41, $M^{+\cdot}-N_2$), 151 (215, $M^{+\cdot}$-PhCHOCHCH=CHN_3).

Tricyclic β-Lactam 113

β-Lactam 112 (195 mg, 0.577 mmol) in CH_2Cl_2 (10 mL) and camphorsulfonic acid (147 mg, 0.634 mmol) were stirred for an hour at room temperature. The solution was washed with water (2 x 10 mL), dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography (petroleum ether:ethyl acetate, 1:1.3) to afford β-lactam 113, 10.8 mg (5.5%).

^{1}Hmr 200 MHz ($CDCl_3$) δ: 3.90 (dd, 1H, NCH, J = 5, 8 Hz), 4.21 (dd, 1H, OHCH, J = 8, 8 Hz), 4.44 (d, 1H, C_6H_5CH, J = 8 Hz), 5.14 (d, 1H, N_3CH, J = 5 Hz), 6.40-7.76 (m, 10H, aromatic, OH); ir (CH_2Cl_2) ν_{max}: 3500-3000 (OH), 2100 (N_3), 1730 (β-lactam, C=O) cm^{-1}; ms (70 eV, 92°), m/e (°/₀₀): 338 (60, $M^{+\cdot}$), 310 (285, $M^{+\cdot}-N_2$), 255 (67, $M^{+\cdot}-N_3CH$=C=O).

β-Lactam 114

To β-lactam 113 (10.8 mg, 0.032 mmol) in acetic anhydride (0.5 mL) was added a catalytic amount of 4-dimethylaminopyridine. After stirring overnight at room temperature, ethyl acetate (20 mL) was added. The mixture was washed with water (2 x 15 mL), dried ($MgSO_4$) and evaporated. The residue was purified by HPLC (petroleum ether:ethyl acetate:methanol, 8.5:1.5:0.05) to give β-lactam 114, 13 mg (96%).

^{1}Hmr 200 MHz ($CDCl_3$) δ: 1.68, 2.30 (2s, 6H, $2CH_3$), 4.04 (dd, 1H, HCH, J = 5, 8 Hz), 4.55 (d, 1H, $C\underline{H}C_6H_5$, J = 8 Hz), 4.98 (d, 1H, CHN_3, J = 5 Hz), 5.50 (dd, 1H, $C\underline{H}OCOCH_3$, J = 8, 8 Hz), 6.85-7.44 (m, 8H, aromatic); ir (CH_2Cl_2) ν_{max}: 2100 (N_3), 1780 (β-lactam, C=O) cm^{-1}; CI-ms (210°): 423 (280, MH^+), 395 (226, $MH^+-N_2\cdot$), 381 (1000).

CHAPTER III

Phenyl Cyclophosphoramidate 122

To 3-amino-1-propanol 123 (1 g, 13.3 mmol) in dry methylene chloride (20 mL) was added pyridine (3.16 g, 40 mmol) and phenyldichlorophosphate (2.8 g, 13.3 mmol) at 0°. The mixture was stirred at room temperature for 18 h. The solution was washed with water (2 x 15 mL), dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography (ethyl acetate) to afford 2.24 g (79%) of 122 as a colorless oil.

^{1}Hmr ($CDCl_3$) δ: 1.2-2.4 (m, 2H, CH_2), 2.8-3.7 (m, 2H, CH_2N), 4.1-4.7 (m, 3H, NH, CH_2O), 7.32 (s, 5H, C_6H_5); ir (film) ν_{max}: 3250 (NH, amide), 1590, 1485, 1260, 1210 cm^{-1}; ms (70 eV, 69°), m/e (°/₀₀): 213 (643, $M^{+\cdot}$), 120 (382, $M^{+\cdot}$-$C_6H_5O^{\cdot}$).

Amide 125

To the cyclophosphoramidate 122 (125 mg, 0.59 mmol) in dry tetrahydrofuran (10 mL) was added 1.5 M n-butyllithium (0.39 mL, 0.59 mmol) at -78°. The mixture was stirred for 15 min and then a solution of benzoyl chloride (82.3 mg, 0.59 mmol) in dry tetrahydrofuran (2 mL) was added dropwise. The solution was stirred one more hour. After the addition of methylene chloride (20 mL), the solution was washed with H_2O (2 x 30 mL), dried $MgSO_4$) and evaporated. The resulting oil was purified by flash chromatography (petroleum ether:ethyl acetate, 1:1) to afford 167 mg

(90%) of amide 125.

^{1}Hmr ($CDCl_3$) δ: 1.7-2.6 (m, 2H, CH_2), 3.2-3.9 (m, 1H, CHN), 4.0-4.8 (m, 3H, NCH, CH_2O), 6.8-7.7 (m, 10H, $2C_6H_5$); ir (film) ν_{max}: 1670 (C=O, amide), 1590, 1490 (aromatic C=C), 1320 cm^{-1}; ms (70 eV, 43°), m/e (°/₀₀): 317 (46, $M^{+\cdot}$), 214 (199, $M^{+\cdot}-C_6H_5O^{\cdot}$), 105 (1000, $C_6H_5C{\equiv}C^{+\cdot}$).

Amide 127

To a solution of 122 (1.44 g, 6.76 mmol) in THF (10 mL), ethyl malonyl chloride (1.02 g, 6.78 mmol) in THF (5 mL) was added. The reaction was followed by means of tlc. When starting material disappeared (∿ 15 h), the solvent was evaporated. The residue was introduced into a flash chromatography column to afford 1.85 g (84%) of 127 as a colorless oil.

^{1}Hmr ($CDCl_3$) δ: 1.24 (t, 3H, CH_3), 1.6-2.5 (m, 2H, $NCH_2\underline{CH_2}CH_2O$), 3.0-3.6 (m, 1H, CHN), 3.83, 3.90 (2s, 2H, CH_2CO), 4.11 (q, 2H, $CH_3\underline{CH_2}$), 4.1-4.9 (m, 3H, CH_2O, CHN), 7.30 (s, 5H, C_6H_5); ir (film) ν_{max}: 1740 (C=O, ester), 1690 (C=O, amide) cm^{-1}; ms (20 eV, 20°), m/e (°/₀₀): 327 (20, $M^{+\cdot}$), 234 (1000, $M^{+\cdot}-C_6H_5O^{\cdot}$).

Diazo-Amide 130

To a solution of 127 (1.85 g, 5.66 mmol) in acetonitrile (20 mL) containing triethylamine (0.63 g, 6.24 mmol) was added p-toluenesulfonyl azide (1.18 g, 5.98 mmol) at 0°. The mixture was stirred for 40 h at room temperature. The solvent was evaporated below 30° and replaced by

methylene chloride (40 mL). After being washed with 0.4 N potassium hydroxide, water, dried ($MgSO_4$) and evaporated, the residue was purified by flash chromatography (petroleum ether:ethyl acetate, 1:1) to afford 1.70 g (85%) of 130 as a yellow oil.

^{1}Hmr ($CDCl_3$) δ: 1.30 (t, 3H, CH_3), 1.6-2.5 (m, 2H, CH_2), 3.2-3.8 (m, 1H, NCH), 4.30 (q, 2H, $CH_3\underline{CH_2}$), 4.0-4.7 (m, 3H, NCH, $NCH_2\underline{CH_2}CH_2O$), 7.23 (s, 5H, C_6H_5); ir (film) ν_{max}: 2135 (N_2), 1730 (C=O, ester), 1690, 1650, 1590, 1490 cm^{-1}; ms (20 eV, 60°), m/e (‰): 353 (32, $M^{+\cdot}$), 325 (245, $M^{+\cdot}-N_2$), 260 (77, $M^{+\cdot}-C_6H_5O^{\cdot}$), 253 (341), 94 (1000).

Trichloroethyl Cyclophosphoramidate 132

A solution of 2,2,2-trichloroethyl alcohol (0.49 g, 3.28 mmol) and triethylamine (0.33 g, 3.27 mmol) in anhydrous tetrahydrofuran (3 mL) was added dropwise at -78° into phosphorus oxychloride (0.5 g, 3.27 mmol) in THF (5 mL) under nitrogen atmosphere. After stirring 0.5 h, the temperature slowly increased to room temperature within 1 h and the mixture was cooled again to -78°. To the cooled mixture, a solution of 3-amino-1-propanol (0.245 g, 3.26 mmol) and triethylamine (0.693 g, 6.86 mmol) in THF (3 mL) was added. The mixture was allowed to warm to room temperature and stirred for another 4 h. The tetrahydrofuran was evaporated. The residue was added to methylene chloride (50 mL) and water (40 mL). The organic layer was washed with 1% HCl (30 mL), dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography (ethyl acetate) to obtain 0.81 g (93%) of 132 as a light yellow oil.

^{1}Hmr ($CDCl_3$) δ: 1.5-2.5 (m, 2H, $NCH_2\underline{CH_2}CH_2O$), 3.0-3.7 (m, 2H, NCH_2), 4.1-4.8 (m, 3H, NH, OCH_2). 4.50 (d, 2H, CH_2CCl_3, J = 7 Hz); ir (film) ν_{max}: 3250 (NH), 1420, 1335, 1260, 1100 cm^{-1}; ms (70 eV, 20°), m/e (°/₀₀): 273 (4, $M^{+\cdot}$+6, Cl_3^{37}), 271 (18, $M^{+\cdot}$+4, $Cl_1^{35}Cl_2^{37}$), 269 (55, $M^{+\cdot}$+2, $Cl_2^{35}Cl_1^{37}$), 267 (57, $M^{+\cdot}$, Cl_3^{35}), 120 (1000, $M^{+\cdot}$-$OCH_2CCl_3\cdot$).

Amide 133

Obtained from 132 *via* the procedure for the preparation of 127, in 90% yield after flash chromatography (petroleum ether:EtOAc, 1.5:1).

^{1}Hmr ($CDCl_3$) δ: 1.30 (t, 3H, CH_3), 1.7-2.3 (m, 2H, $NCH_2\underline{CH_2}CH_2O$), 3.1-3.7 (m, 1H, NCH), 3.8-4.8 (mb, 3H, OCH_2, NCH), 3.90, 4.04 (2s, 2H, CH_2), 4.20 (q, 2H, $CH_3\underline{CH_2}$), 4.70 (d, 2H, OCH_2CCl_3, J = 7 Hz); ir (film) ν_{max}: 1735 (C=O, ester), 1690 (C=O, amide), 1330, 1300 cm^{-1}; ms (70 eV, 37°), m/e (°/₀₀): 385 (15, $M^{+\cdot}$+4, $Cl_1^{35}Cl_2^{37}$), 383 (36, $M^{+\cdot}$+2, $Cl_2^{35}Cl_1^{37}$), 381 (38, $M^{+\cdot}$, Cl_3^{35}), 234 (194, $M^{+\cdot}$-$OCH_2CCl_3\cdot$).

Diazo-Amide 134

Obtained from 133 *via* the procedure for the preparation of 130, in 80% yield after flash chromatography (petroleum ether:EtOAc, 1.5:1).

^{1}Hmr ($CDCl_3$) δ: 1.30 (t, 3H, CH_3), 1.6-2.3 (m, 2H, $NCH_2\underline{CH_2}CH_2O$), 3.1-3.8 (m, 1H, NCH), 3.8-4.8 (m, 5H, NCH, CH_2O, OCH_2CH_3), 4.80 (d, 2H, OCH_2CCl_3, J = 7 Hz); ir (film) ν_{max}: 2115 (N_2), 1720 (C=O, ester), 1650 (C=O, amide), 1330 cm^{-1}; ms (70 eV, 51°), m/e (°/₀₀): 272 (14, $M^{+\cdot}$-$EtOOCCN_2CO\cdot$+6,

Cl_3^{37}), 270 (44, $M^{+\cdot}$-EtOOCCN$_2$CO$^{\cdot}$+4, $Cl_1^{35}Cl_2^{37}$), 268 (81, $M^{+\cdot}$-EtOOCCN$_2$CO$^{\cdot}$+2, $Cl_2^{35}Cl_1^{37}$), 266 (14, $M^{+\cdot}$-EtOOCCN$_2$CO$^{\cdot}$, Cl_3^{35}).

Phosphonate β-Lactam 142

To a solution of alcohol 138 (347 mg, 3.02 mmol) and triphenylphosphine (790 mg, 3.02 mmol) in dry dimethylformamide (3 mL) was added, in portion, N-bromosuccinamide (538 mg, 3.02 mmol). After the addition, the reaction mixture was stirred at room temperature for 30 min. The solution was added water (15 mL) and extracted with ether (4 x 10 mL). The ether layer was washed with water (2 x 10 mL), dried ($MgSO_4$) and concentrated to afford the crude bromide 141 (contaminated with triphenylphosphine oxide).

^{1}Hmr ($CDCl_3$) δ: 2.00 (dt, 2H, $C\underline{H}_2CH_2Br$), 2.46 (bd, 1H, OCC$\underline{H}$H, J_{gem} = 16 Hz), 3.00 (ddd, 1H, OCC$\underline{H}$H, J_{gem} = 16 Hz, J_{cis} = 5 Hz, J_{NH} = 2 Hz), 3.34 (t, 2H, CH_2Br), 3.5-3.8 (m, 1H, NCH).

To the crude bromide was added triethyl phosphite (2 mL). The mixture was heated in an oil bath at 120° for 18 h. Flash chromatography of the residue using ethyl acetate first and then acetone afforded 150 mg (21%) of phosphonate 142.

^{1}Hmr ($CDCl_3$) δ: 1.15 (t, 6H, $2CH_3$), 1.4-2.0 (m, 4H, CH_2CH_2P), 2.27 (bd, 1H, COC$\underline{H}$H, J_{gem} = 15 Hz), 2.80 (ddd, 1H, COC$\underline{H}$H, J_{gem} = 15 Hz, J_{cis} = 5 Hz, J_{NH} = 2 Hz), 3.0-3.5 (m, 1H, NCH), 3.7-4.3 (m, 4H, $C\underline{H}_2CH_3$), 7.5-8.0 (bs, 1H, NH); ir (film) ν_{max}: 3600-3100 (NH), 1745 (C=O, β-lactam) cm^{-1};

ms (70 eV, 85°), m/e ($^0/_{00}$): 235 (7, $M^{+\cdot}$), 193 (887, $M^{+\cdot}-CH_2=C=O$), 137 (1000, $OP(OEt)_2^+$).

Mesylate 147

To alcohol 138 (266 mg, 2.31 mmol) in dry methylene chloride (10 mL) and triethylamine (0.35 mL, 2.52 mmol), under a nitrogen atmosphere, was added methanesulfonyl chloride (0.19 mL, 2.45 mmol) in methylene chloride (2 mL) at 0°. The solution was stirred for 30 min at 0° and allowed to warm up to room temperature. The reaction mixture, after washing with water (2 x 10 mL), drying ($MgSO_4$) and evaporation *in vacuo*, afforded 410 mg (92%) of mesylate 147.

^{1}Hmr ($CDCl_3$) δ: 1.9-2.4 (m, 2H, CH_2CH_2O), 2.6 (bd, 1H, $COCHH$, J_{gem} = 15 Hz), 3.0-3.4 (m, 1H, $COCHH$), 3.0 (s, 3H, CH_3), 3.5-4.0 (m, 1H, CHN), 4.30 (t, 2H, CH_2O), 6.80 (bs, 1H, NH); ir (film) ν_{max}: 3400-3100 (NH), 1740 (C=O, β-lactam) cm^{-1}; CI-ms (70°), m/e ($^0/_{00}$): 194 (1000, MH^+), 152 (588, $MH^+-CH_2=C=O$).

Iodide 146

To mesylate 147 (169 mg, 0.87 mmol) in acetone (15 mL) was added sodium iodide (132 mg, 0.88 mmol). The mixture was refluxed in an oil bath at 60 for 7 h. After evaporation of the acetone, ethyl acetate (20 mL) was added to this residue. The resulting solution was washed with water (2 x 15 mL) dried ($MgSO_4$) and evaporated to afford 180 mg (92%)

of iodide 146.

^{1}Hmr ($CDCl_3$) δ: 2.0-2.3 (m, 2H, $C\underline{H}_2CH_2I$), 2.63 (bd, 1H, OCC$\underline{H}$H, J_{gem} = 15 Hz), 3.0-3.4 (m, 1H, OCC$\underline{H}$H), 3.20 (t, 2H, $C\underline{H}_2I$), 3.52-3.94 (m, 1H, NCH). 6.70 (bs, 1H, NH); ir (CH_2Cl_2) ν_{max}: 3500-3400 (NH), 1760 (C=O, β-lactam) cm^{-1}.

Silylated β-Lactam 149

To mesylate 147 (274 mg, 1.42 mmol) in dimethylformamide (4 mL) was added triethylamine (376 mg, 3.72 mmol) and t-butyldimethylsilyl chloride (236 mg, 1.57 mmol). The mixture was stirred at room temperature for 18 h. To this was added water (15 mL) and then extracted with ether (4 x 10 mL). The ether solution, after washing with water (2 x 15 mL), drying ($MgSO_4$) and evaporation, afforded silylate product 149 (400 mg, 91%).

^{1}Hmr ($CDCl_3$) δ: 0.22 (s, 6H, $2CH_3$), 1.98 (s, 9H, t-BuSi), 1.6-2.4 (m, 2H, $C\underline{H}_2CH_2O$), 2.55-3.20 (m, 2H, $COCH_2$), 3.02 (s, 3H, CH_3), 3.4-4.9 (m, 1H, CHN), 4.34 (t, 2H, CH_2O); ir (film) ν_{max}: 1740 (C=O, β-lactam); ms (70 eV, 42°), m/e ($^0/_{00}$): 250 (107, $M^{+\cdot}$-t-Bu·), 208 (26, $M^{+\cdot}$-t-Bu·-CH_2=C=O), 153 (1000).

N-Benzoyl-3-Amino-1-Propanol 150

To a solution of 3-amino-1-propanol (1.5 g, 20 mmol) in 5 N NaOH (4 mL) was added, in the meantime, benzoyl chloride (2.81 g, 20 mmol) and

5 N NaOH (4 mL) at 0°. The solution was warmed up to room temperature and stirred for 18 h. The solution was neutralized by concentrated HCl and extracted with ethyl acetate (4 x 10 mL). Drying ($MgSO_4$) and evaporation afforded 3.1 g (87%) of 150 as a colorless oil.

^{1}Hmr ($CDCl_3$) δ: 1.4-1.9 (m, 2H, CH_2), 3.2-3.8 (m, 4H, CH_2N, CH_2O), 4.80 (s, 1H, OH), 7.0-8.1 (m, 6H, NH, C_6H_5); ir (film) ν_{max}: 3400-3200 (OH), 1650 (C=O, amide) cm^{-1}; ms (70 eV, 72°), m/e (°/₀₀): 179 (15, $M^{+\cdot}$), 105 (1000, $C_6H_5C{\equiv}O^{+\cdot}$).

Imine 152

To a solution of alcohol 150 (0.5 g, 2.79 mmol) in dry tetrahydrofuran (15 mL) contaminated triethylamine (0.78 mL, 5.61 mmol) was added dropwise a solution of phosphorus oxychloride (0.26 mL, 2.80 mmol) in tetrahydrofuran at -78°. The resulting solution was then allowed to warm up to room temperature. After stirring for 3 h, toluene (20 mL) was added to the solution and filtered through a celite pad. The filtrate was concentrated to afford the crude product. This was purified by flash chromatography (petroleum ether:ethyl acetate, 1:4) to afford 0.36 g (80%) of 152.

^{1}Hmr ($CDCl_3$) δ: 2.00 (tt, 2H, CH_2), 3.50 (t, 2H, NCH_2), 4.25 (t, 2H, CH_2O), 7.2-8.0 (m, 5H, C_6H_5); ir (film) ν_{max}: 1655 (C=N), 1350, 1270 cm^{-1}; ms (70 eV, 19°), m/e (°/₀₀): 161 (858, $M^{+\cdot}$), 103 (447, $C_6H_5CN^{+\cdot}$), 77 (1000, $C_6H_5^{+\cdot}$); Anal. Calcd. for $C_{10}H_{11}NO$: C, 74.53; H, 6.83; N, 8.70;

Found: C, 74.30; H, 6.76; N, 8.93.

Silylated Alcohol 153

To alcohol 150 (2.5 g, 14 mmol) in dry DMF (10 mL) was added t-butyldimethylsilyl chloride (2.31 g, 15.3 mmol) and imidazole (2.38 g, 34.9 mmol). After stirring 1 h at room temperature, the solution was partitioned between ether and water. The ether layer was washed, dried and evaporated to afford 3.8 g (93%) of silylated alcohol 153.

^{1}Hmr ($CDCl_3$) δ: 0.20 (s, 6H, $Si(CH_3)_2$), 1.02 (s, 9H, t-BuSi), 1.7-2.0 (m, 2H, CH_2), 3.84 (t, 2H, CH_2N), 4.15 (t, 2H, CH_2O), 7.0-7.5 (b, 1H, NH), 7.4-8.0 (m, 5H, C_6H_5); ir (film) ν_{max}: 3300 (NH), 1640 (C=O, amide), 1550, 1250 cm^{-1}; ms (70 eV, 71°), m/e (°/₀₀): 278 (22, $M^{+\cdot}-CH_3\cdot$), 236 (773, $M^{+\cdot}$-t-Bu·), 105 (1000, $C_6H_5C{\equiv}O^{+\cdot}$).

Phosphite 154

To the silyl ether 153 (365 mg, 1.24 mmol) in dry tetrahydrofuran (15 mL), under a nitrogen atmosphere, was added dropwise 1.6 M n-butyllithium (0.77 mL, 1.23 mmol) at -78°. This was stirred for 20 min, and added to a solution of diethyl chlorophosphite (0.18 mL, 1.26 mmol) in dry tetrahydrofuran (3 mL). The resulting mixture was stirred for 1 h at -78°, and then warmed up to room temperature. Evaporation and purification using flash chromatography (petroleum ether:ethyl acetate, 9:1) afforded 180 mg (35%) of 154.

^{1}Hmr ($CDCl_3$) δ: 0.22 (s, 6H, $(CH_3)_2Si$), 1.08 (s, 9H, t-BuSi), 1.43 (t, 6H, $2CH_3$). 1.7-2.3 (m, 2H, CH_2), 3.6-4.2 (m, 8H, OCH_2, NCH_2, $2CH_2CH_3$), 7.48 (s, 5H, C_6H_5); ir (film) ν_{max}: 1650 (C=O, amide), 1540, 1250 cm^{-1}; ms (70 eV, 20°). m/e (°/₀₀): 236 (132, $M^{+\cdot}$-t-Bu·-$P(OEt)_2$·+1), 105 (1000, $C_6H_5C{\equiv}O^{+\cdot}$), 77 (412).

β-Lactam 158

A solution of β-lactam 138 (4.39 mg, 3.82 mmol) and dihydropyran (482 mg, 5.73 mmol) in dry methylene chloride (10 mL) containing pyridinium p-toluenesulfonate (PPTS) (96 mg, 0.382 mmol) was stirred for 8 h at room temperature. The solution was diluted with ether and washed with half-saturated brine to remove the catalyst. After drying and evaporation of the solvent, flash chromatography (ethyl acetate) gave 410 mg (54%) THP ether 158.

^{1}Hmr ($CDCl_3$) δ: 1.4-2.1 (m, 8H, CH_2CH_2O, $CHCH_2CH_2CH_2$). 2.49 (ddd, 1H, CHHCO, J_{gem} = 13 Hz, J_{trans} = 2.2 Hz, J_{NH} = 1.1 Hz), 3.10 (ddd, 1H, CHHCO, J_{gem} = 13 Hz, J_{cis} = 4.2 Hz, J_{NH} = 1.1 Hz), 3.3-4.1 (m, 5H, CHNH, $2CH_2O$), 4.6 (bm, 1H, $CH_2OCHOCH_2$), 6.3-6.7 (bs, 1H, NH); ir (film) ν_{max}: 3300 (NH), 1760 (C=O) cm^{-1}; ms (70 eV, 20°), m/e (°/₀₀): 199 (99 , $M^{+\cdot}$), 99, (103, $M^{+\cdot}$-$HOCH(CH_2)_4O$), 85 (1000, THP^+).

β-Lactam 159

Obtained from β-lactam 158 and diethyl chlorophosphate, *via* the

procedure for the preparation of 161, in 75% yield after flash chromatography (EtOAc).

^{1}Hmr 200 MHz ($CDCl_3$) δ: 1.38 (t, 6H, $2CH_3$), 1.4-2.0, 2.4-2.6 (m, m, 8H, $C\underline{H}_2C\underline{H}_2O$, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.97 (2ddd, 1H, C<u>H</u>HCO, J_{gem} = 16 Hz, $J_{trans} = J_{pH}$ = 3 Hz), 3.22 (2ddd, 1H, C<u>H</u>HCO, J_{gem} = 16 Hz, $J_{cis} = J_{pH}$ = 6 Hz), 3.4-3.6, 3.7-4.0 (m, m, 4H, OCH_2, OCH_2), 4.09 (m, 1H, HCH), 4.19 (m, 4H, $OC\underline{H}_2$, CH_3), 4.56 (bs, 1H, OCHO); ir ($CHCCl_3$) ν_{max}: 1785 (C=O, β-lactam) cm^{-1}; ms (70 eV, 39°), m/e (°/₀₀): 251 (57, $M^{+\cdot}$-DHP), 250 (47, $M^{+\cdot}$-THP·), 234 (100, $M^{+\cdot}$-OTHP·), 180 (1000, $M^{+\cdot}-CH_2CHCH_2CH_2OTHP\cdot+1$), 152 (345, $M^{+\cdot}-COCH_2CH(CH_2)_2O\dot{T}HP\cdot+1$).

β-Lactam 161

Under a nitrogen atmosphere, a solution of β-lactam 158 (253 mg, 1.27 mmol) in dry THF (5 mL) was cooled to -78° and treated with n-BuLi (1 eq.) in THF. After being stirred for 10 min, diphenyl chlorophosphate (342 mg, 1.27 mmol) was added dropwise, and stirring, at -78°, was continued for a period of 30 min. The reaction mixture was warmed to room temperature by itself. After adding methylene chloride (20 mL), the organic layer was washed with water (2 x 15 mL), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (petroleum ether:ethyl acetate; 1:1.5) to afford 438 mg of β-lactam 161 in 80% yield.

^{1}Hmr ($CDCl_3$) δ: 1.2-2.1 (m, 8H, $C\underline{H}_2C\underline{H}_2O$, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.6-3.0 (m, 2H, $C\underline{H}_2CO$), 3.1-4.0 (m, 5H, C<u>H</u>N, $C\underline{H}_2O$, $C\underline{H}_2O$), 4.2 (b, 1H, CH), 7.00 (s, 10H,

$2C_6H_5$); ir (film) ν_{max}: 1790 (C=O, β-lactam) cm^{-1}.

β-Lactam 160

A solution of THP ether 161 (180 mg, 0.42 mmol) and pyridinium p-toluenesulfonate (PPTS) (20 mg, 0.08 mmol) in absolute ethanol (5 mL) was stirred at 55° (oil bath temperature) for 5 h. The solvent was evaporated *in vacuo*, and the residue was chromatographed (EtOAc:petroleum ether, 2:1) to afford 124 mg (85%) of β-lactam 160.

^{1}Hmr 200 MHz ($CDCl_3$ and D_2O) δ: 1.6-2.0 (m, 2H, CH_2CH_2OH), 2.79 (ddd, 1H, COCHH, J_{gem} = 16 Hz, $J_{cis} = J_{pH} \simeq 3$ Hz), 3.22 (ddd, 1H, COCHH, J_{gem} = 16 Hz, $J_{trans} = J_{pH} \simeq 6$ Hz), 3.4-3.8 (m, 2H, CH_2OH), 4.0-4.2 (m, 1H, NCH), 7.38 (m, 10H, $2C_6H_5$); ir (film) ν_{max}: 3450 (OH), 1790 (C=O, β-lactam) cm^{-1}; ms (70 eV, 56°), m/e (°/₀₀): 347 (101, $M^{+\cdot}$), 276 (961, $M^{+\cdot}$-CH_2=CH-$(CH_2)_2OH$+1), 254 (117, $M^{+\cdot}$-$OC_6H_5^{\cdot}$), 77 (1000).

Bicyclic β-Lactam 162

To a solution of 160 (150 mg, 0.43 mmol) in t-BuOH (2 mL) was added CsF (66 mg, 0.43 mmol). After stirring at room temperature for 2 h, the solvent was evaporated. Ethyl acetate was added. The mixture was washed with brine, dried and evaporated to afford 22 mg (20% of β-lactam 162, after flash chromatography (petroleum ether:EtOAc, 1:2); m.p. 103°; ^{1}Hmr (acetone d_6) δ: 1.8-2.2 (m, 2H, CH_2), 2.90 (app.dd, 2H, CH_2CO), 3.8-5.0 (m, 3H, CH, CH_2O), 6.9-7.5 (m, 5H, C_6H_5); ^{1}Hmr 200 MHz (acetone d_6

and D_2O) δ: 1.8-2.2 (m, 2H, CH_2), 2.8-3.0 (m, 2H, CH_2O), 3.9-4.1 (m, 1H, CH), 4.3-4.7 (m, 2H, CH_2O), 6.9-7.5 (m, 5H, C_6H_5); ir (CH_2Cl_2) ν_{max}: 1760 (C=O, β-lactam), 1590, 1490, 1190 cm^{-1}; ms (70 eV, 82°), m/e (°/₀₀): 254 (372, $M^{+\cdot}$+1), 212 (1000, $M^{+\cdot}$-CH_2=C=O).

β-Lactam 163

To a solution of alcohol 138 (150 mg, 1.3 mmol) and pyridine (162 mg, 1.43 mmol) in CH_2Cl_2 (10 mL) was added dropwise diphenyl chlorophosphate (349 g, 1.3 mmol). After stirring for 2 h the solution was washed with water (3 x 5 mL) and brine (8 mL), dried ($MgSO_4$) and evaporated to give 392 mg (87%) of β-lactam 163, after flash chromatography.

^{1}Hmr 200 MHz ($CDCl_3$) δ: 1.8-2.2 (m, 2H, CH_2), 2.60 (ddd, 1H, COCHH), J_{gem} = 15 Hz, J_{trans} = 2 Hz), 3.05 (ddd, 1H, COCHH, J_{gem} = 15 Hz, J_{cis} = 5 Hz, J_{NH} = 2 Hz), 3.6-3.8 (m, 1H, CH), 4.2-4.6 (m, 2H, CH_2O), 6.3 (bs, 1H, NH), 7.1-7.5 (m, 10H, $2C_6H_5$); ir (film) ν_{max}: 1765 (C=O, β-lactam), 1590, 1490 (C=C, aromatic) cm^{-1}; ms (70 eV, 55°), m/e (°/₀₀): 347 (46, $M^{+\cdot}$), 305 (437, $M^{+\cdot}$-CH_2=C=O), 304 (203, $M^{+\cdot}$-OCNH).

β-Lactam 166

A mixture containing β-lactam 164 (8.00 g, 0.039 mol), 5% palladium on charcoal and ethanol (300 mL) was hydrogenated at 40 psi for 2 h. The solution was filtered and evaporated to dryness. The residue was taken up in methylene chloride (100 mL) and was methylated with etheral diazomethane

at 0°. After removing the solvent *in vacuo*, the crude methyl ester was purified by flash chromatography (petroleum ether:ethyl acetate, 1:5) to afford 4.5 g (90%) of β-lactam 166.

^{1}Hmr ($CDCl_3$) δ: 2.90 (ddd, 1H, J_{gem} = 15 Hz, J_{trans} = 2.5 Hz, J_{NH} = 1.5 Hz), 3.34 (ddd, 1H, J_{gem} = 15 Hz, J_{cis} = 5 Hz, J_{NH} = 1.5 Hz), 3.75 (s, 3H, CH_3), 4.20 (dd, 1H, CH, J_{cis} = 5 Hz, J_{trans} = 2.5 Hz), 6.8-7.4 (bs, 1H, NH); ir (film) ν_{max}: 1775 (C=O, β-lactam), 1740 (C=O, ester) cm^{-1}; ms (70 eV, 16°), m/e (°/₀₀): 129 (115, $M^{+\cdot}$), 101 (463), 86 (188), 28 (1000).

Alcohol 167

To a solution of β-lactam 164 (0.205 g, 1 mmol) in methanol (10 mL) was added sodium borohydride (114 g, 3 mmol) in portion. After stirring at room temperature for 18 h, concentrated HCl was added dropwise to neutralize to pH = 5. The precipitate was filtered off and the filtrate was evaporated to dryness. The residue was purified by flash chromatography (EtOAc:methanol, 10:1) to afford 0.05 g (50%) of alcohol 167.

^{1}Hmr (acetone d_6) δ: 2.6 (dd, 1H, COCHH, J_{gem} = 15 Hz, J_{trans} = 2 Hz), 2.9 (m, 1H, COCHH, J_{gem} = 15 Hz, J_{cis} = 5 Hz, J_{NH} = 1.5 Hz), 3.5-3.9 (m, 3H, CH_2O, CH), 4.2 (bs, 1H, OH), 7.3 (bs, 1H, NH); ir (film) ν_{max}: 3400-3000 (NH, OH), 1730 (C=O, β-lactam) cm^{-1}; gc-ms (TMS derivative), m/e (°/₀₀): 245 (24, $M^{+\cdot}$, di-TMS derivative).

β-Lactam 168

Obtained from β-lactam 167, *via* the procedure for the preparation of 158, in 80% yield after flash chromatography (EtOAc).

^{1}Hmr ($CDCl_3$) δ: 1.2-2.0 (m, 6H, $OCH(CH_2)_3$), 2.51 (app.bd, 1H, $COCHH$, J_{gem} = 14 Hz), 2.96 (2ddd, 1H, $COCHH$, J_{gem} = 14, J_{cis} = 4 Hz, J_{NH} = 1.5 Hz), 3.2-4.0 (m, 4H, OCH_2, OCH_2), 4.48 (bs, 1H, OCH), 6.7-7.0 (bs, 1H, NH); ir (film) ν_{max}: 3280 (NH), 1750 (C=O, β-lactam) cm^{-1}; ms (70 eV, 19°), m/e (°/₀₀): 185 (21, $M^{+\cdot}$), 85 (1000, THP^+).

β-Lactam 169

Obtained from 168, *via* the same procedure for the preparation of 161, in 85% yield used for the next reaction without purification.

^{1}Hmr ($CDCl_3$) δ: 1.1-2.0 (m, 6H, $CH_2CH_2CH_2$), 2.9-3.2 (m, 2H, CH_2CO), 3.2-4.0 (m, 5H, $2OCH_2$, NCH), 4.2-4.5 (m, 1H, OCH), 7.12 (s, 10H, $2C_6H_5$); ir (film) ν_{max}: 1795 (β-lactam), 1595, 1490 (aromatic C=C), 1290 (P=O) cm^{-1}; ms (70 eV, 76°), m/e (°/₀₀): 417 (54, $M^{+\cdot}$), 333 (47, $M^{+\cdot}$-DHP), 275 (287, $M^{+\cdot}$-$CH_2CHCH_2OTHP^{\cdot}$), 94 (936, $C_6H_5OH^+$), 77 (1000).

β-Lactam 170

Obtained from 169, *via* the procedure for the preparation of 160, in 60% yield after flash chromatography (petroleum ether:EtOAc, 1:2.5).

^{1}Hmr ($CDCl_3$) δ: 2.5-3.4 (m, 2H, CH_2CO), 3.2-3.5 (b, 1H, OH, exchangeable with D_2O), 3.5-3.7 (m, 2H, $\underline{CH_2}OH$), 4.0-4.1 (m, 1H, CHN), 7.27 (s, 10H, $2C_6H_5$); ir (film) ν_{max}: 3600-3200 (OH), 1790 (C=O, β-lactam), 1590, 1485 (aromatic C=C), 1280 (P=O) cm^{-1}; ms (70 eV, 26°), m/e ($^0/_{00}$): 333 (165, $M^{+\cdot}$), 275 (820, $M^{+\cdot}$-CH_2CHCH_2OH), 233 (280 (O)$P(OC_6H_5)_2^+$), 94 (664, $C_6H_5OH^{+\cdot}$), 77 (1000).

β-Lactam 171

Diphenyl chlorophosphate (0.31 mL, 1.48 mmol) was added dropwise to a solution of alcohol 167 (149 mg, 1.48 mmol) and triethylamine (0.21 mL) in anhydrous tetrahydrofuran (5 mL). After stirring for 0.5 h, to the reaction mixture was added methylene chloride (20 mL). The organic layer was washed with water (2 x 15 mL), dried ($MgSO_4$) and evaporated to dryness. The residue was purified by flash chromatography (EtOAc) to afford 438 mg (89%) of β-lactam 171.

^{1}Hmr ($CDCl_3$) δ: 2.51 (ddd, 1H, $\underline{CH}HCO$, J_{gem} = 15 Hz, J_{trans} = 3 Hz, J_{NH} = 1.5 Hz), 2.83 (ddd, 1H, $\underline{CH}HCO$, J_{gem} = 15 Hz, J_{cis} = 4 Hz, J_{NH} = 1.5 Hz), 3.5-3.8 (m, 1H, CHN), 3.9-4.3 (m, 2H, CH_2O), 6.6 (bs, 1N, NH), 7.00 (s, 10H, $2C_6H_5$); ir (film) ν_{max}: 3260 (NH), 1780 (C=O, β-lactam), 1590, 1490 (aromatic C=C), 1290 (P=O) cm^{-1}; ms (70 eV, 55°), m/e ($^0/_{00}$): 333 (34, $M^{+\cdot}$), 291 (1000, $M^{+\cdot}$-CH_2=C=O), 94 (861, $C_6H_5OH^{+\cdot}$), 77 (820, $C_6H_5^{+\cdot}$).

β-Lactam 174

Obtained from 158 and dibenzyl chlorophosphate, *via* the procedure

for the preparation of 161, used for the next reaction without purification.

^{1}Hmr ($CDCl_3$) δ: 1.3-2.0 (m, 8H, CH_2, $CH_2CH_2CH_2$), 2.4-3.1 (m, 2H, CH_2CO), 3.3-4.0 (m, 5H, NCH, $2CH_2O$), 4.5 (bm, 1H, CH), 5.0, 5.2 (2s, 4H, $2CH_2C_6H_5$), 7.3 (s, 10H, $2C_6H_5$).

β-Lactam 176

Obtained from 174, *via* the same procedure for the preparation of 160, in 74% yield after flash chromatography (EtOAc).

^{1}Hmr ($CDCl_3$) δ: 1.5-2.1 (m, 2H, CH_2), 2.4-3.1 (m, 2H, CH_2CO), 3.2-4.0 (m, 4H, CHN, CH_2O, OH), 5.0, 5.2 (2s, 4H, $2CH_2C_6H_5$), 7.3 (s, 10H, $2C_6H_5$); ir (film) ν_{max}: 3400-3100 (OH), 1785 (C=O, β-lactam) cm^{-1}; ms (20 eV, 20°), m/e (°/₀₀): 375 (3, $M^{+\cdot}$), 284 (170, $M^{+\cdot}-CH_2C_6H_5^{\cdot}$), 178 (1000, $M^{+\cdot}-C_6H_5CHO-C_6H_5CH_2^{\cdot}$).

β-Lactam 178

β-Lactam 176 (0.6 g, 1.6 mmol) in absolute ethanol (20 mL) containing 5% Pd/C (0.1 g) was hydrogenated at 40 psi for 1 h. The mixture was filtered. The filtrate was evaporated to afford 0.29 g (94%) of 178.

^{1}Hmr (D_2O) δ: 1.6-2.5 (m, 2H, CH_2), 2.6-3.4 (m, 2H, CH_2CO), 3.7-4.0 (m, 1H, CH), 3.78 (t, 2H, OCH_2); ir (film) ν_{max}: 3300-300 (OH), 1750 (C=O,

β-lactam) cm^{-1}.

β-Lactam 175

Obtained from 168, *via* the procedure for the preparation of 174, used for the next reaction without purification.

^{1}Hmr ($CDCl_3$) δ: 1.3-2.1 (m, 6H, $CH_2CH_2CH_2$), 2.8-3.1 (m, 2H, CH_2CO), 3.5-4.1 (m, 5H, $2CH_2O$, CH), 4.55 (s, 1H, OCH), 5.05, 5.20 (2s, 4H, $2CH_2$), 7.4 (bs, 10H, $2C_6H_5$).

β-Lactam 177

Obtained from 175, *via* the same procedure for the preparation of 160, in 70% yield after flash chromatography (EtOAc).

^{1}Hmr ($CDCl_3$) δ: 2.7-3.0 (m, 2H, CH_2CO), 3.3-4.0 (m, 4H, CH_2O, CH, OH), 5.05, 5.19 (2d, 4H, $2CH_2$), 7.30 (s, 10H, $2C_6H_5$); ir (film) ν_{max}: 3400-3200 (OH), 1790 (C=O, β-lactam) cm^{-1}; ms (70 eV, 60°), m/e (‰): 277 (61, $(O)P(OBn)_2NH_2^+$), 107 (140, $C_6H_5CH_2CO^+$·), 91 (1000, $C_6H_5CH_2^+$·).

β-Lactam 179

Obtained from 177, *via* the procedure for the preparation of 178, in quantitative yield.

^{1}Hmr (D_2O) δ: 2.7-3.1 (m, 2H, CH_2CO), 3.2-4.1 (m, 3H, CH_2O, CH); ir (film) ν_{max}: 3400-2600 (OH), 1750 (C=O, β-lactam) cm^{-1}.

β-Lactam 187

Obtained from 182, *via* the procedure for the preparation of 161, at -78° for 3 h, in 74% yield after flash chromatography (petroleum ether: EtOAc, 1.5:1).

^{1}Hmr ($CDCl_3$) δ: 1.43 (s, 9H, t-Bu), 3.4-4.0 (m, 2H, CH_2), 4.1-4.5 (m, 1H, CH), 4.8-5.2 (mb, 1H, NH), 7.1-7.4 (m, 10H, $2C_6H_5$); ir (film) ν_{max}: 3400 (NH), 1800 (C=O, β-lactam), 1720, 1590, 1490, 1290 cm^{-1}; gc-ms (70 eV, 66°), m/e (°/₀₀): 418 (15, $M^{+\cdot}$), 276 (702, $(O)P(OPh)_2NHCO^+$), 57 (1000).

β-Lactam 188

Obtained from 189, *via* the procedure for the preparation of 187, in 70% yield after flash chromatography (petroleum ether:EtOAc, 1:1.5).

^{1}Hmr ($CDCl_3$) δ: 0.8-1.4 (m, 12H, CH_3, t-Bu), 3.4-4.4 (m, 2H, CHCH), 4.8-5.2 (m, 4H, $2CH_2$), 5.3-5.6 (m, 1H, NH), 7.3 (s, 10H, $2C_6H_5$); ir (film) ν_{max}: 3320 (NH), 1790 (C=O, β-lactam), 1710 (C=O) cm^{-1}; ms (70 eV, 80°), m/e (°/₀₀): 304 (110, $M^{+\cdot}$ - O=C=CH-NHt-BOC+1, $M^{+\cdot}$ - $CH_3CHCHNHt$-BOC+1), 157 (70, $CH_3CHCHNHt$-BOC^+, $COCHNHt$-BOC^+), 91 (880).

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