Synthesis of Nocardicin A Analogues

A Thesis

by

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Montreal, Canada

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To those Iranian people who sacrificed their blood for the attainment of freedom, independence and human rights.

Synthesis of Nocardicin A Analogues

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Abstract

The syntheses of D,L-4-hydroxymethylnocardicin A, D,L-4-hydroxymethyl-N-phenylacetylnocardicinic acid, their α-epimers and some aromatic ring-substituted analogues are described, as well as those of homocycloanalogues of nocardicin A.

It is shown that, in contrast to benzylic ketones, benzylic oximes are stable to hydrogenolysis conditions necessary for the removal of benzyl protecting groups of carboxylic acids and phenols.

Synthèse d'Analogues de la Nocardicine A

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Résumé

Sont décrites: la synthèse de la D,L-4-hydroxyméthyle-nocardicine A, de l'acide D,L-4-hydroxyméthyle-N-phenyle-acétylenocardicinique, de leurs épimères α, de quelques analogues avec divers substituents sur le cycle aromatique et d'homocycloanalogues de la nocardicine A.

Il est démontré que, contrairement aux cétones benzyliques, les oximes benzyliques résistent aux conditions d'hydrogénolyse nécessaires au clivage de groupes benzyles protégeant acides carboxyliques et phénols.

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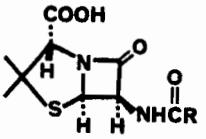
INTRODUCTION

Preface

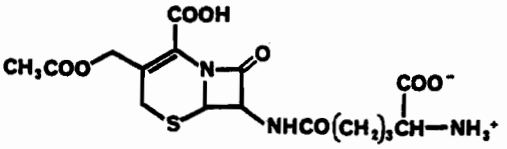
β-Lactams are 4-membered heterocyclic compounds (1), which were first synthesized by Staudinger in 1907[1]. Penicillin (2) was discovered in 1929[2]. It was the first microbial metabolite found to be toxic to the bacterial cell but therapeutic to the mammalian host[3].

(1)



(2)

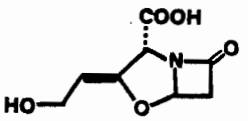
In 1945 Brotzu[4] discovered a Cephalosporium species having antibiotic properties, from which a new β-lactam antibiotic substance cephalosporin C (3) was isolated by Abraham and Newton[5]. The structure was determined in 1961[6]. Hodgkin and Maslen[7] confirmed the structure by means of single crystal X-ray diffraction studies.



(3)

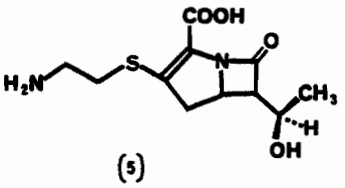
There has been an enormous amount of research on the biological and chemical properties of penicillins and cephalosporins[8-22]. This effort has recently produced 3 new types of β-lactam antibiotics.

The Beecham group[23] has isolated clavulanic acid (4), a potent β-lactamase inhibitor, from a Streptomyces species, and the structure was determined by X-ray analysis of a derivative. The dramatic reduction of MIC (minimal inhibitory concentration) of ampicillin against β-lactamase producing bacteria in the presence of sodium clavulanate was explained by the fact that clavulanic acid is an irreversible β-lactamase inhibitor.



(4)

Thienamycin (5)[24,25] and related compounds PS-5[26], MM4550 (MC 696-SY2-A), MM13902[27,28], were isolated independently by the Merck[25], Beecham[26] and Umezawa[27] groups. They are highly effective against both Gram positive and Gram negative bacteria and show β-lactamase inhibitory activity.



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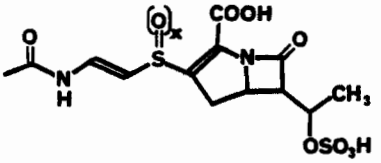
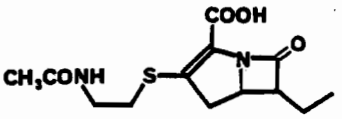


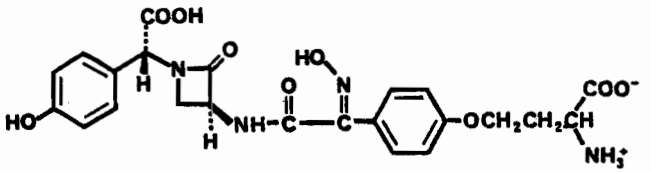
x=1 MM4550 (MC 696-SY$_2$-A)

x=0 MM 13902



PS-5

At almost the same time, work by the Fujisawa group[29-32] led to the discovery of monocyclic β-lactam antibiotics, nocardicin A (6) and B (7), which have an interesting antimicrobial activity in vivo.



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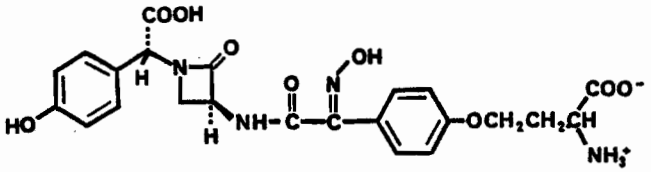


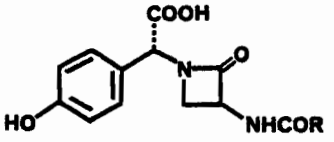
(7)

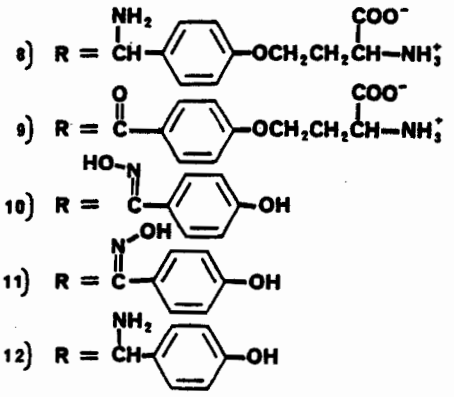
Studies on the new monocyclic β-lactam antibiotics, nocardicins

Bleomycin[33], pachystermines[34], phleomycin[33], wild fire toxin[35] and (S)-alanyl-3-[α-(S)-chloro-3-(S)-hydroxy-2-oxo-3-azetidinyl methyl]-S-alanine[36] have been reported as natural substances having a monocyclic β-lactam ring. These substances, however, are weaker in antimicrobial activity than nocardicin A.

Nocardicin A (6) and B (7)[29-32], novel monocyclic β-lactam antibiotics produced by a strain of Nocardia, were

isolated by the Fujisawa Pharmaceutical Company in Japan[29,30]. Their structures were well established by spectroscopic and chemical means[31]. They possess relatively high antimicrobial activity and are stereochemically and biologically related to penicillins and cephalosporins[31]. Nocardicin B (7) was isolated as a minor component from the same culture, and shows reduced activity. Other nocardicins[37] C (8), D (9), E (10), F (11) and G (12), whose biological activities are less than that of nocardicin A, have been isolated from the fermentation broth of the original strain and its mutants.





Nocardicin A exerts a potent antimicrobial activity against Gram negative bacteria, especially Pseudomonas aeruginosa, Proteus species, Serratia marcescens and the Neisseria.

The in vitro antimicrobial activity of nocardicin A against Ps.aeruginosa was about twice that of carbenicillin[38]. Nocardicin A inhibited 30 strains of S.marcescens, usually resistant to β-lactam antibiotics[38]. However, nocardicin A had no significant in vitro activity against Staphylococci and Escherichia coli. The in vitro activity of nocardicin A against Ps.aeruginosa and Pr.mirabilis was greatly influenced by the assay media used[38]. Sodium chloride was identified as a major inhibitor. Some amino acids, sugars and divalent cations were found to be minor inhibitors[39]. How these factors inhibit the antimicrobial activity of nocardicin A is still unsettled, although data suggest that sodium chloride may modify the structure of the bacterial outer layer to decrease its penetrability to nocardicin A[39]. Generally the in vitro antimicrobial activity of most antibiotics decreases in the presence of serum. Nocardicin A however appeared to act synergistically with serum bactericidal factors against Ps.aeruginosa[39]. When nocardicin A was given to mice the therapeutic effect of the drug was stronger than had been anticipated from in vitro studies[32]. Therefore, the therapeutic efficacy of nocardicin A against infections in mice may not be affected by the inhibitors existing in conventional heart infusion broth (HI) medium. This means that the concentration of inhibitors in the living body are not high enough

to suppress the activity of the drug. Nocardicin A proved to be active against organisms resistant to β-lactam antibiotics[32]. The antimicrobial activity of nocardicin A is characterized by the absence of cross resistance with other β-lactam antibiotics such as cephalosporins and penicillins, and shows a potent activity against organisms resistant to these antibiotics[38]. This may be explained by the fact that nocardicin A is resistant to β-lactamases. Nocardicin A also has a low toxicity, its LD50 being larger than 2g/kg in any route tested[29].

Structural chemistry and antibacterial activity

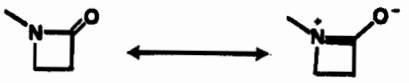
Since the discovery of penicillin and cephalosporin C, thousands of derivatives and analogues have been synthesized[40]. Previous work[41,42] had indicated that at least four requirements were necessary for optimal activity.

1) A reactive bicyclic β-lactam, activated by ring strain and electronic factors.
2) A cis fused β-lactam ring.
3) An amide function α to the β-lactam carbonyl function.
4) An acidic function on the carbon atom adjacent to the azetidinone nitrogen.

However, the newer β-lactams, nocardicin A, thienamycin and clavulanic acid, do not have all of the above features.

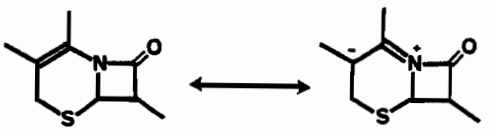
In 1969, Morin[43] obtained a positive correlation between the infrared absorption of the β-lactam carbonyl group and biological activity. The higher frequency indicates higher chemical reactivity and a potential for higher biological activity.

Woodward[44] suggested that in a monocyclic β-lactam normal amide resonance occurs (13), because of coplanarity of the amide function.



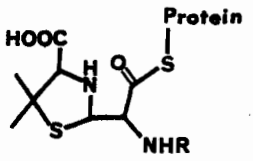
(13)

In the penicillins, however, the nitrogen atom cannot be planar for steric reasons. The absorption band[45] increases in frequency from about 1725 cm^{-1} for the unstrained β-lactams to 1780 cm^{-1} for the penicillins, as the planarity of the nitrogen atom decreases, which indicates an increase in double bond character between the carbon and oxygen atom. In the cephalosporins, another factor in addition to the lack of planarity of the amide nitrogen may add to the lability of the β-lactam amide bond. In these compounds, the possibility exists for enamine type resonance (14).



(14)

The evidence clearly shows that the antibiotic lactam amide bond must be more susceptible to cleavage than a normal amide bond. This situation certainly favors the trans peptidase reaction with the penicillins to open the β-lactam ring and to form a pencilloylenzyme intermediate (15)[46,47].



(15)

The only exception to this rule is provided by nocardicin A (6), which is remarkably active against Gram-negative organisms in vivo[32], although it displays but little activity in vitro[39,48]. It differs from all hitherto described β-lactam antibiotics, (1770-1780 cm^{-1}) in that the β-lactam ring is not fused to a five or six-membered ring, so that its β-lactam frequency is relatively low (1725 cm^{-1}), reflecting an enhanced stability against nucleophilic attack.

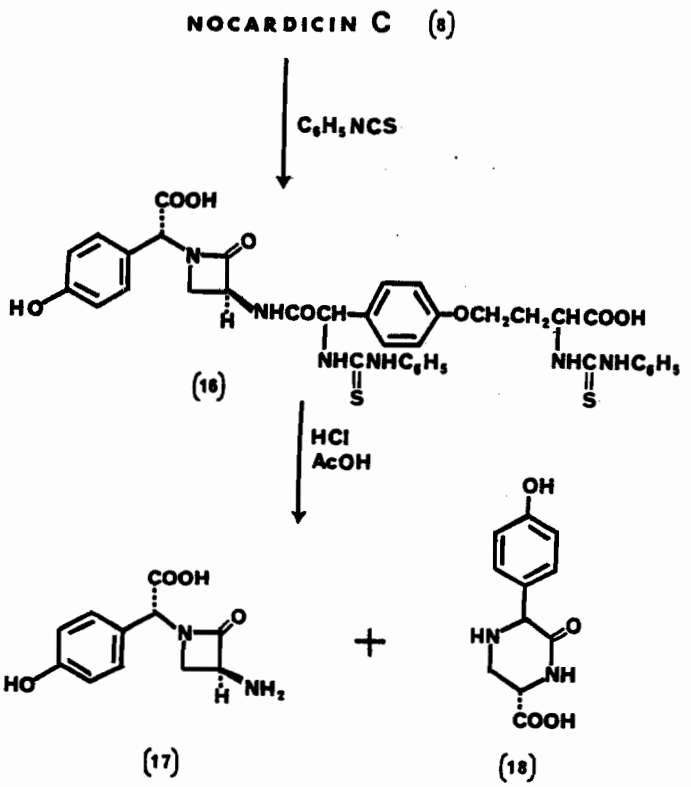
Total syntheses of nocardicin A[3,49]

1) The first successful synthesis of nocardicin A was announced by T. Kamiya[50], and can be broken down into three distinct problems.

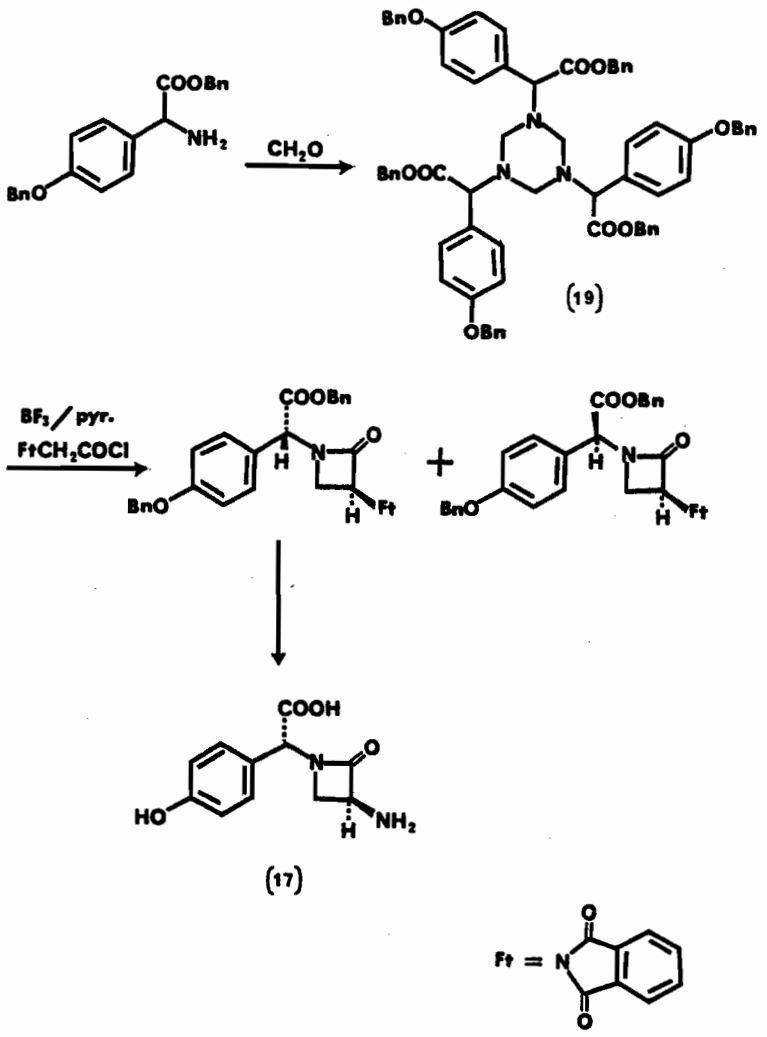
a) Synthesis of 3-aminonocardicinic acid (3-ANA)

3-Aminonocardicinic acid is an important precursor for

preparing new acyl derivatives. Kamiya et al. produced 3-ANA by hydrolysis of a natural nocardicin. Nocardicin C (8) was treated with phenylisothiocyanate to give bisthiourea derivative (16) in quantitative yield[51]. Treatment of (16) with concentrated hydrochloric acid in acetic acid at room temperature gave 3-ANA (17). The yield was only 40% because 3-ANA is relatively unstable under acidic conditions and is converted into piperazinone (18).



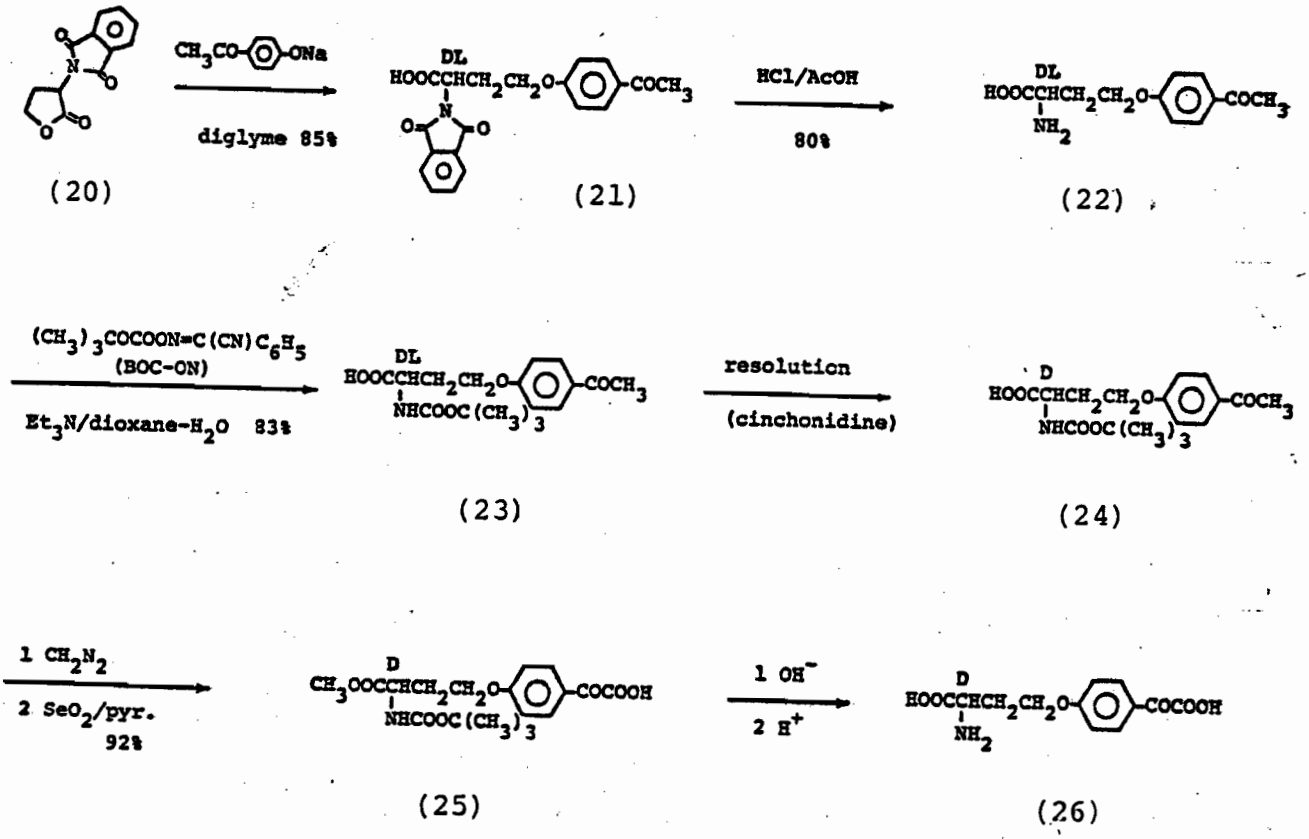
Fujisawa chemists have recently developed a synthesis of 3-ANA, starting from triazine (19)[52,53], which is outlined below.



b) Synthesis of the nocardicin A side chain[3,54]

The starting material for synthesis of the side chain (26) was phthalimidobutyrolactone (20), which was heated with p-hydroxyacetophenone sodium salt in diglyme at reflux for 6 hrs. The phthalimido acid (21) was obtained in 86% yield. Treatment of (21) with concentrated hydrochloric acid gave the amino acid (22) in 80% yield. Treatment of this amino acid with N-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON) and triethylamine in 50% aqueous dioxane at room temperature for 2 hrs gave BOC-protected amino acid (23) in

83% yield. After resolution of (23) with cinchonidine, the desired enantiomer (D-24) was converted into its methyl-ester, which was then oxidized with selenium dioxide in pyridine at 80° for 5 hrs to give the oxo-acid (25)[55] in 92% yield. The oxo-acid (25) was hydrolysed to amino acid (26), which was identical with that obtained from nocardicin A[3].



c) Reaction of 3-ANA (17) with BOC-protected amino acid (25)

The BOC-protected D-amino acid (25) was converted into the mixed anhydride using ethyl chloroformate and triethylamine, and immediately treated with a methylene chloride solution of the trimethylsilyl ester of 3-ANA at -78° for 2 hrs. The protected nocardicin D was obtained in good yield. Removal of the protecting groups gave nocardicin D (9) in overall 43% yield. Then compound (9) was treated with hydroxylamine in water (PH 7) at 50° for 1 hr to give nocardicin A in 60% yield.

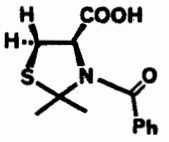
$$(25) + 3\text{ ANA } (17) \longrightarrow (9)$$

$$(9) \xrightarrow{H_2NOH} \text{Nocardicin A } (6)$$

2) Recently R.D.G. Cooper et al.[49,56] have found another stereoselective way to synthesize nocardicin A.

Condensation of L-cysteine with acetone followed by acylation with benzoyl chloride gave thiazolidine (27). The amino group of D-p-hydroxyphenyl glycine was protected as the tert-butoxy carbonyl derivative. Benzylation of the hydroxy groups followed by deprotection of the amino group gave compound (28) in overall 70% yield. Coupling of amine (28) with (27) in the presence of N,N'-dicyclohexylcarbodiimide (DCC) gave

dipeptide (29) in 90% yield. Compound (29) was treated with benzoylperoxide in refluxing benzene to give benzoate (30) in 45-55% yield[57-59]. This was then converted to chloro compound (31) quantitatively using hydrochloric acid in methylene chloride at 0°.



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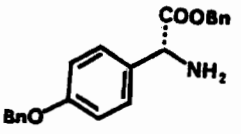

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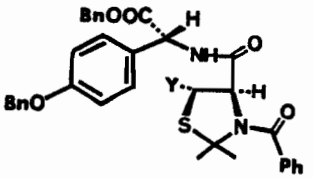
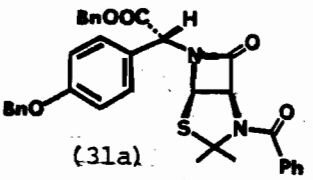


29) Y = H

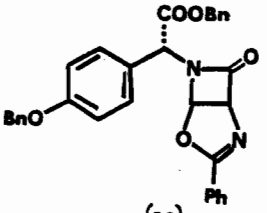
30) Y = OCOBn

31) Y = Cl

Closure of the β-lactam ring was readily accomplished[59], using sodium hydride, in 85% yield. However, an isomeric mixture of two β-lactams was formed, which was treated with pyridine to give exclusively isomer (31a). The stereochemistry was the same as that of the naturally occuring β-lactam. Treatment of this β-lactam with mercuric acetate in aqueous tetrahydrofuran gave a high yield of oxazoline (32), which was readily cleaved to (32a) in the presence of phosphorus pentachloride. Removal of the two chlorine atoms was effected by tributyltin hydride and gave Schiff base (32b).



(31a)



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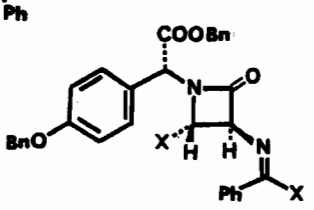
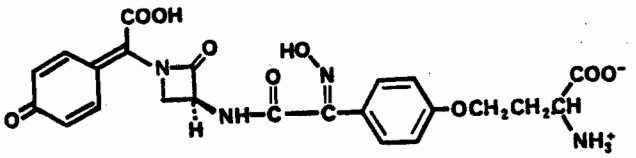


32 a) X = Cl
32 b) X = H

Acid hydrolysis of Schiff base (32b), followed by debenzylation, gave 3-ANA (17) in good yield. The nucleus of 3-ANA was then converted to nocardicin A by the same methodology described by the Fujisawa[3] chemists.

Brief description of the project

Nocardicin A[29-32], the first "monocyclic" β-lactam antibiotic described, possesses relatively high antimicrobial activity *in vivo* rather than *in vitro*. It occurred to us that this *in vivo* activation may well be linked to an oxidation to the corresponding quinone structure (33) in which



(33)

the β-lactam frequency should be considerably enhanced, thus increasing its biological activity. Because of the difficulties in preparing quinone methines[60], it was decided to prepare, in addition to other derivatives (Figure 1), nocardicin analogues bearing two orto-related hydroxy groups in which an *in vivo* and/or *in vitro* oxidation to an o-quinone may be more easily achieved, and which may perhaps exist in part as the p-quinone-methine tautomer (Figure 2). Since several total syntheses of nocardicin have already been reported[3,49], it was decided to prepare 4-hydroxymethyl-nocardicinic acid, which would allow the synthesis of fused nocardicins with a

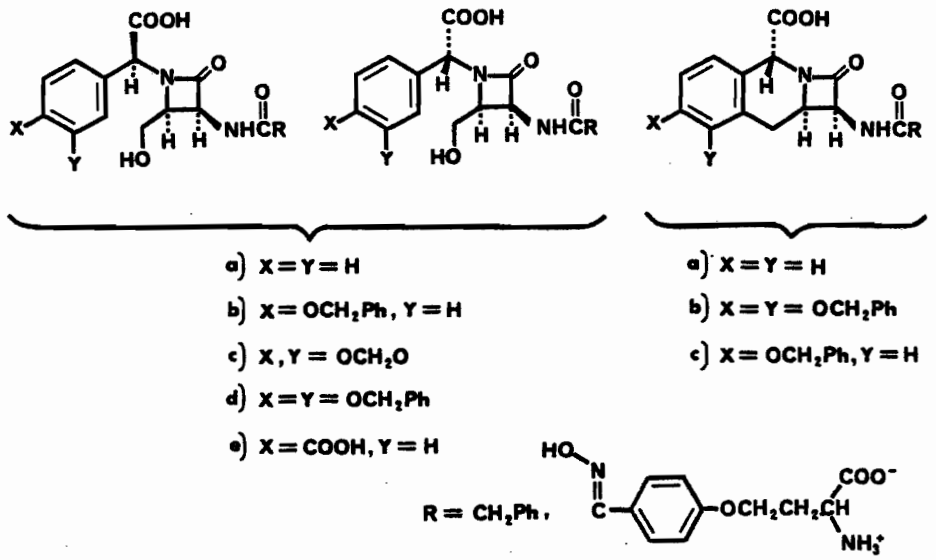


FIGURE 1:

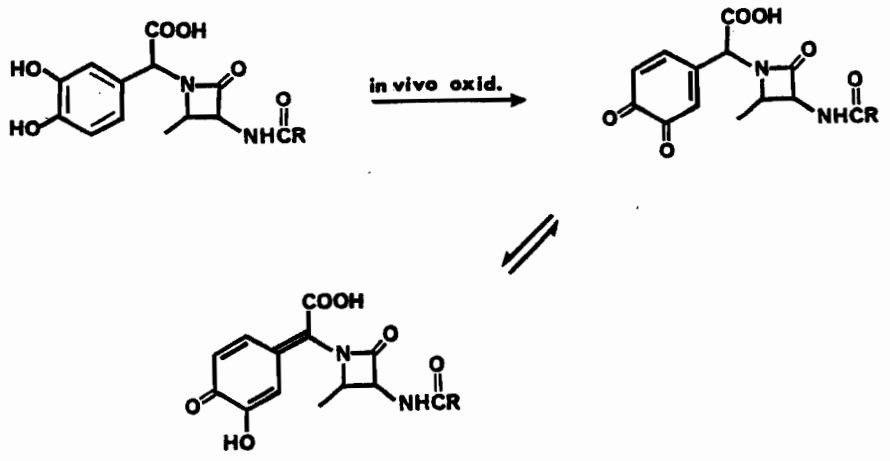


FIGURE 2:

corresponding increase in strain.

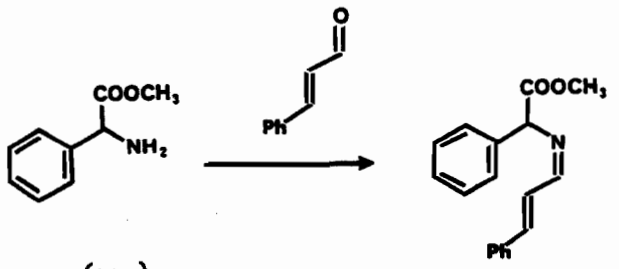
This work consists of three chapters. The first chapter describes the successful synthesis of D,L-4-hydroxymethyl nocardicinic acid derivatives, having a phenylacetylamino side chain, which is readily available, easy to manipulate and known to confer antibiotic activity (e.g. benzylpenicillin). The second chapter describes successful synthesis of homocyclo analogues of nocardicin A having a phenylacetylamino side chain. And the third chapter describes syntheses of nocardicin analogues, having the proper side chain of nocardicin A.

CHAPTER 1

The synthesis of nocardicin analogues carrying a phenylacetyl side-chain

Recently, Doyle et al.[42] were able to obtain exclusively cis-β-lactams in good yield by a method originally discovered by Staudinger[1] and extended by Bose[61-65], in which azidoacetyl chloride was added to the appropriate cinnamylidene Schiff bases. In view of their success, it was decided to use their method for the preparation of our β-lactams.

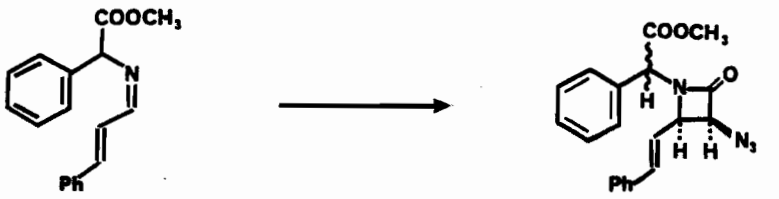
As a model, methyl D,L-phenylglycinate (34a) was treated with cinnamaldehyde to give cinnamylidene Schiff base* (35a) in quantitative yield. The i.r. spectrum showed the presence of the C=N absorption at 1635 cm^{-1} and the lack of absorption above 3100 cm^{-1}. The n.m.r. spectrum showed the characteristic Schiff base proton at 8.0 p.p.m.



(34a) (35a)

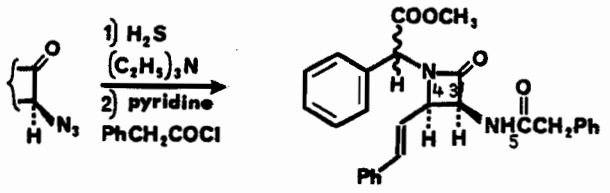
*All Schiff bases are written in the cis form solely to simplify pictorial manipulation.

Schiff base (35a) was treated with azidoacetyl chloride[42] in the presence of triethylamine using methylene chloride as solvent at -20° to obtain β-lactams (36a/37a) as a mixture of epimers at the carboxyl bearing carbon. The i.r. spectrum showed the presence of β-lactam carbonyl absorption at 1760 cm^{-1} and the azido function at 2100 cm^{-1}. The mass spectrum showed M^+ and M^+-N_2. At this stage, it was impossible to assign the stereochemistry of the β-lactam protons, because the β-lactam proton absorption peaks of one diastereomer overlapped with the absorption peaks of the other diastereomer. Fortunately chromatography on silica gel using methylene chloride as eluent separated one of the diastereomers, which was obtained in 30% yield. The coupling constant between the two β-lactam protons was found to be 5 Hz, which proved the cis-stereochemistry for the β-lactam ring according to the Karplus[66] equation, which predicts a larger coupling for cis protons than trans. Indeed Kagan[67,68] found that the coupling constant for a cis β-lactam is equal to about 5-6 Hz while the trans coupling constant is in the vicinity of 2 Hz. All the β-lactams obtained by this method were cis-fused[42] as could be determined by n.m.r. of all derivatives in which the relevant protons did not overlap with other signals. When methyl D-phenyl glycinate (D-34) was transformed to (36a/37a) both the mixture of diastereomers and single compound were optically inactive, indicating that racemization took place as observed previously[69] in a similar reaction in which D-serine had been used as a starting material.



(35a) (36a/37a)

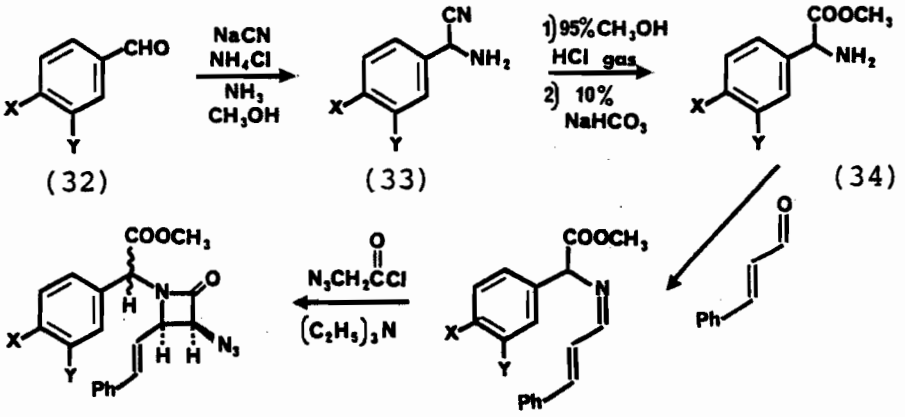
The azide function in (36a/37a) was reduced with hydrogen sulfide-triethyl amine[42], and resulting amine directly acylated with phenylacetyl chloride in the presence of pyridine to give amides (38a/39a). In this stage also, the stereochemistry of the ring junction could not be determined by n.m.r., because of the presence of two diastereomers. However, two recrystallizations from absolute ethanol separated one of the diastereomers. In the n.m.r. spectrum, H_3 appeared as a quartet ($J_{3,4}$ = 5 Hz, $J_{3,5}$ = 8 Hz) characteristic of cis-acylamino β-lactams, at 5.3-5.6 p.p.m.



(36a/37a) (38a/39a)

Having established that the cyclo addition of azidoacetyl chloride proceeded well, oxygenated derivatives of phenylglycine were prepared.

p-Benzyloxybenzaldehyde (32b), piperonal (32c), 3,4-dibenzyloxybenzaldehyde (32d) and 4-carboxybenzaldehyde (32h) were transformed to the corresponding cyanoamine (33b-d) and (33h) by means of sodium cyanide, ammonium chloride and ammonia gas in methanol, or methanol-tetrahydrofuran (THF) in the case of (32b) and (32d). In the case of (32d), cyanoamine formation (32d → 33d) proceeded in 5% only (page 25). The i.r. spectra showed the presence of an amine at 3300-3500 cm^{-1} and a cyano function at 2200 cm^{-1}. The cyanoamines (33) were dissolved in a mixture of methanol and water (95:5) into which was bubbled without cooling hydrogen chloride until saturation. The solution was refluxed gently for 3 hrs and then was treated with aqueous sodium bicarbonate to give the amino esters (34b-e) (page 25). The i.r. spectra showed the presence of an amine at 3300-3400 cm^{-1} and an ester group at 1740 cm^{-1}. N.m.r. spectra and elemental analyses were also in agreement with the structure proposed. Schiff base formation (34b-e → 35b-e) proceeded in general quantitatively, and β-lactam formation (35b-e → 36b-e/37b-e) in approximately 80% as described above for the model compound (page 25). Their spectra were similar to that of model compound (36a/37a) except for variations due to aromatic substituents. All their mass spectra showed $M^{+}-N_2$.



a) X = Y = H

b) X = OCH_2Ph, Y = H

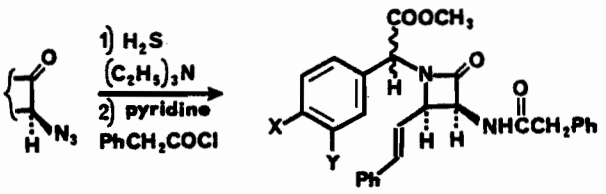
c) X, Y = OCH_2O

d) X = Y = OCH_2Ph

e) X = $COOCH_3$, Y = H

h) X = COOH, Y = H

Reduction of the azide function of (36b-e/37b-e) with hydrogen sulfide in the presence of triethylamine[42] followed by acylation with phenylacetyl chloride afforded amides (38b-e/39b-e). The i.r. spectrum showed the presence of β-lactam carbonyl absorption at 1756 cm^{-1}, the ester function at 1740 cm^{-1} and the amide carbonyl absorption at 1680 cm^{-1}. Their n.m.r. spectra were similar to that of (38a/39a) except for variations due to aromatic substituents. All their mass spectra showed M^+ and fragments[70] characteristic of β-lactams. Their elemental analyses also supported the structures assigned.



(36/37) (38/39)

a) X = Y = H b) X = OCH_2Ph, Y = H c) X, Y = OCH_2O d) X = Y = OCH_2Ph e) X = $COOCH_3$, Y = H

Since, at this point, it was not known whether the methyl ester group could be hydrolysed in the presence of a β-lactam group, β-lactams (38a-e/39a-e) were treated with one equivalent of a 1% aqueous solution of sodium hydroxide

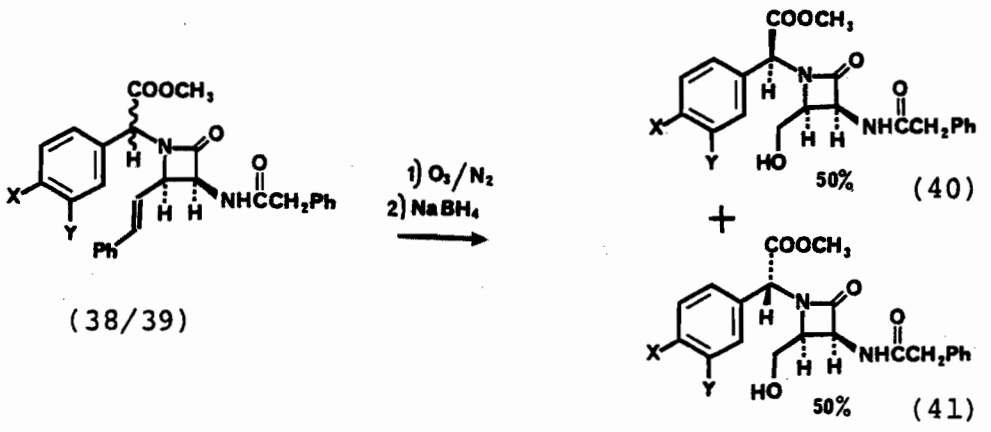
in methanol at room temperature. Esters (38a-d/39a-d) were transformed to the corresponding acids in 60-65% yield, and were characterized by remethylation with diazomethane. In the case of carbomethoxyphenyl derivative (38e/39e), the β-lactam function was hydrolysed more rapidly than either ester group as evidenced by i.r. and n.m.r.

Having prepared the β-lactams in acceptable yield, we next turned our attention to the transformation of the styryl group to a hydroxymethyl group by a sequence of ozonolysis-sodium borohydride reduction, as described by Doyle et al.[42].

Styryl-β-lactam (38c/39c) derived from piperonal was ozonized in methanol at -78°, and the crude product reduced with sodium borohydride at -40°. Although it was possible to isolate (40c/41c) by preparative t.l.c., the yield was very low, and it seemed that the methylene dioxy bridge was attacked during ozonolysis. Moreover, the methylene bridge could not be cleaved in the presence of the β-lactams with boron trichloride-methylene chloride[71] or boron tribromide[72], so that no further work was done in this series.

Ozonolysis of the p-benzyloxy β-lactam (38b/39b), using standard ozonolysis conditions, followed by sodium borohydride reduction at -40°, gave the expected diols (40b/41b) in 20% yield only. However, when ozone and nitrogen were introduced simultaneously[73,74], and the reaction mixture then reduced with sodium borohydride, (40b/41b) were obtained in over 80% yield. Similar results were obtained in the dibenzyloxy series (40d/41d). The diastereomic mixtures (40a,b

and d/41a,b and d) were separated into their constituents by column chromatography using silica gel, with chloroform as eluent.



(38/39)

a) X = Y = H

b) X = OCH_2Ph, Y = H

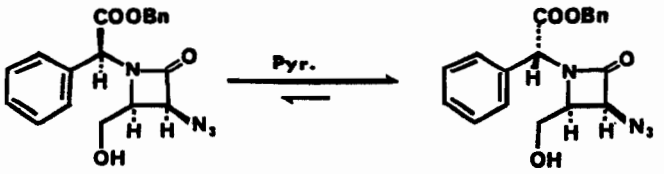
c) X, Y = OCH_2O

d) X = Y = OCH_2Ph

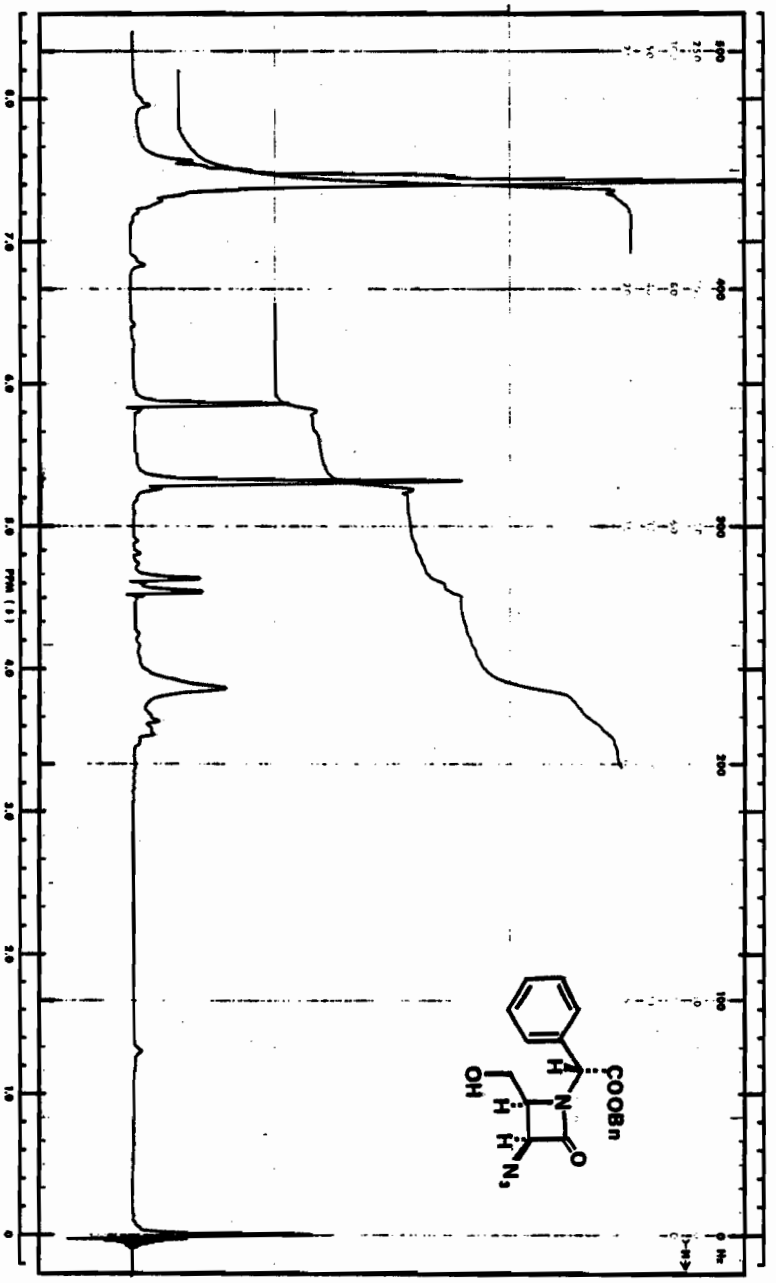
e) X = $COOCH_3$, Y = H

The less polar products (41) were assigned the stereochemistry of the "natural" nocardicin A based on the fact that the ester function absorbed in the infrared at 1740 cm^{-1} (hydrogen bonded carbomethoxy group) and the hydroxyl function at 3300-3500 cm^{-1} (hydrogen-bonded hydroxyl), with a con-

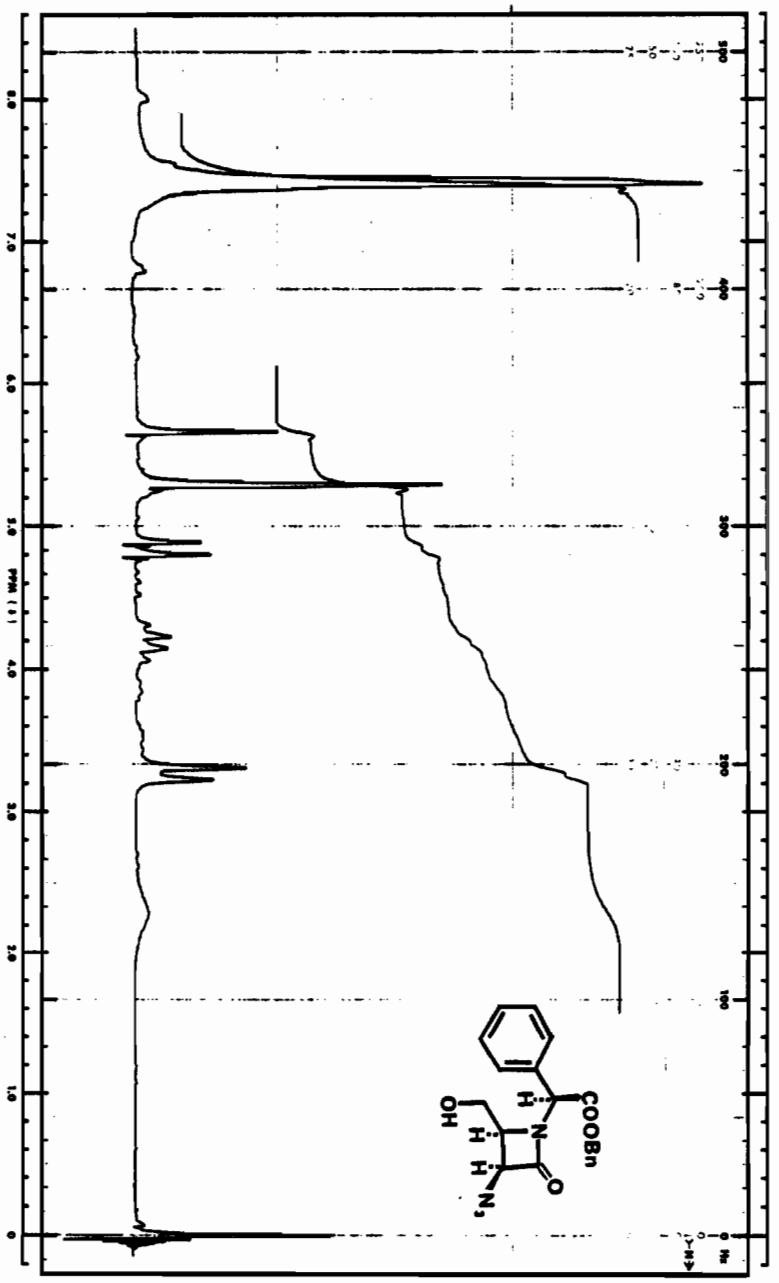
comitant lowering of the chemical shift of the proton α to the carbomethoxy group to 5.8 p.p.m. The corresponding numbers for the more polar isomers (40) were 1750 cm^{-1}, 3400-3600 cm^{-1} and 5.5 p.p.m. Model studies indicated that hydrogen-bonding of the ester and hydroxyl group resulted in the phenyl group being placed away from the β-lactam ring in the case of (41), whereas in isomers (40) hydrogen-bonding would place the phenyl group very close to the β-lactam ring. Equilibration of two related isomers by heating either (52a) or (53a) for a day at 80° in the presence of pyridine using benzene as solvent gave a 30:70 ratio of (52a) and (53a) indicating that the hydrogen bond made (53a) slightly more stable than (52a). (For their synthesis, see Chapter 2).



(52 a) (53 a)



p.m.r. spectrum of β-lactam (53a) in chloroform-d. (60 MHz).

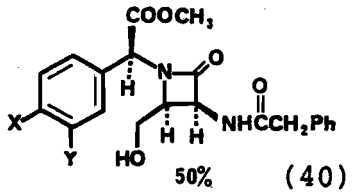


p.m.r. spectrum of β-lactam (52a) in chloroform-d. (60 MHz)

When the hydroxy group in (52a) or (53a) was blocked by mesylation or acetylation, equilibration gave a 50:50 mixture of the corresponding mesylates (56a/57a) or acetates (64a/65a), as established by n.m.r. (For their synthesis, see Chapter 2). The configurational assignment is also somewhat strengthened by the fact that transformation of (41b) to an analogue possessing the proper nocardicin A side chain gave a biologically active compound, whereas a similar compound derived from (40b) was devoid of activity[75] (page 60).

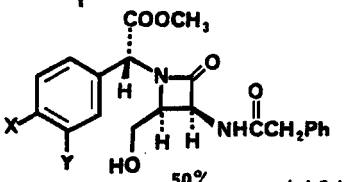
Catalytic hydrogenation of (40b), (40d), (41b) and (41d) over Pd/C, followed by hydrolysis of the ester group with 1% aqueous sodium hydroxide, or hydrolysis followed by catalytic hydrogenation, gave the final products (44f), (44g), (45f) and (45g) in good yield. These compounds were characterized by i.r., n.m.r., mass spectroscopy and also by their remethylation with diazomethane.

None of these compounds display any notable antimicrobial activity either <u>in vivo</u> or <u>in vitro</u>.



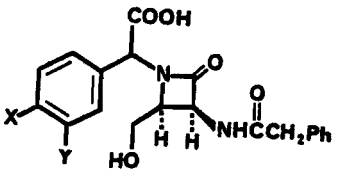
50% (40)

+



50% (41)

1% NaOH →



a) X = Y = H

b) X = OCH_2Ph, Y = H

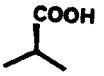
c) X, Y = OCH_2O

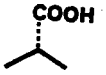
d) X = Y = OCH_2Ph

e) X = $COOCH_3$, Y = H

f) X = OH, Y = H

g) X = Y = OH

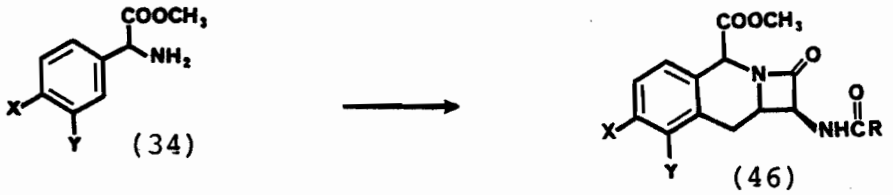
(44) 

(45) 

CHAPTER 2

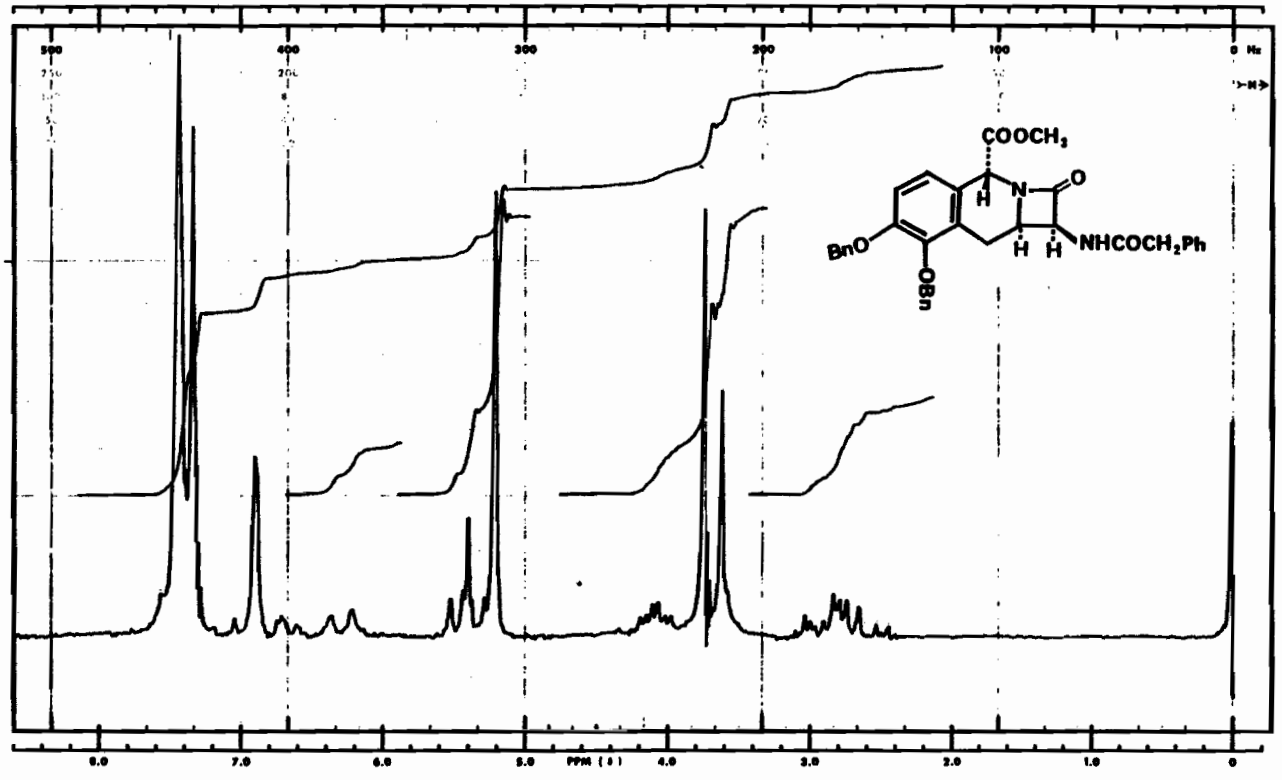
The synthesis of homocycloanalogues of nocardicin A

In the previous chapter we described the synthesis of monocyclic β-lactams in which the β-lactam frequency was 1750-1760 cm^{-1} and which, not surprisingly because of the lack of nocardicin A side chain, showed no significant antimicrobial activity. It was decided to prepare the tricyclic system (46) which we expected to absorb at higher frequency and which should therefore be similar to Δ^2-cephem and nocardicin A. Most reactions described were carried out initially on the known methyl esters corresponding to benzyl esters (48a-c). However, β-lactams (46a-c) could not be hydrolysed without destruction of the β-lactam ring, so that



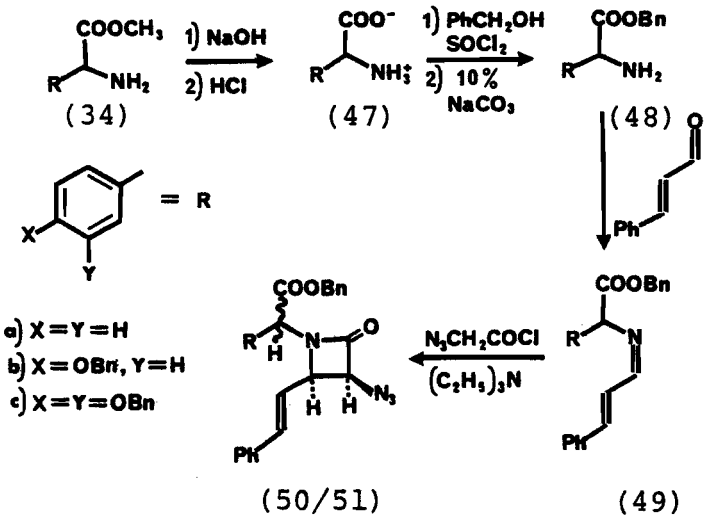
a) X=Y=H, b) X=OBn, Y=H, c) X=Y=OBn

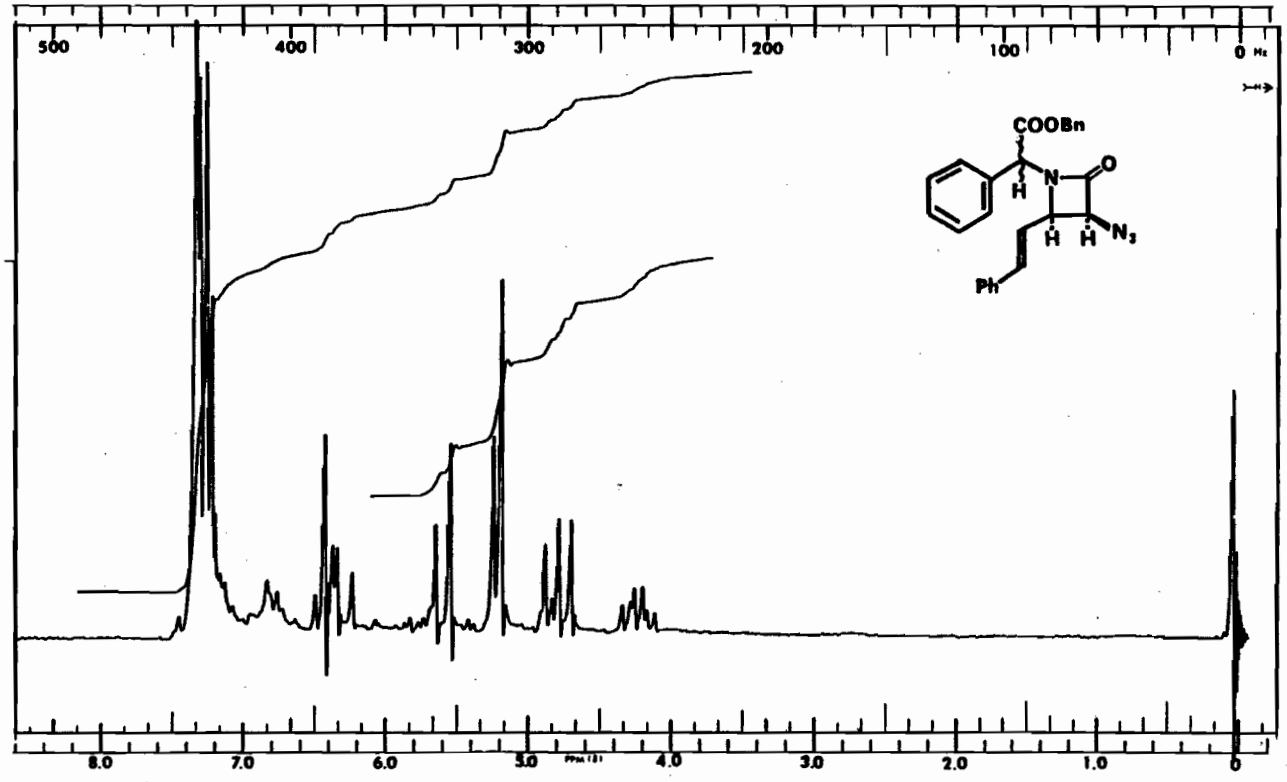
the reaction sequence was carried out on benzyl esters of (48a-c). Their reactions followed those developed for the methyl esters[76], and will be described in detail.



p.m.r. spectrum of β-lactam (46c) in chloroform-d. (60 MHz)

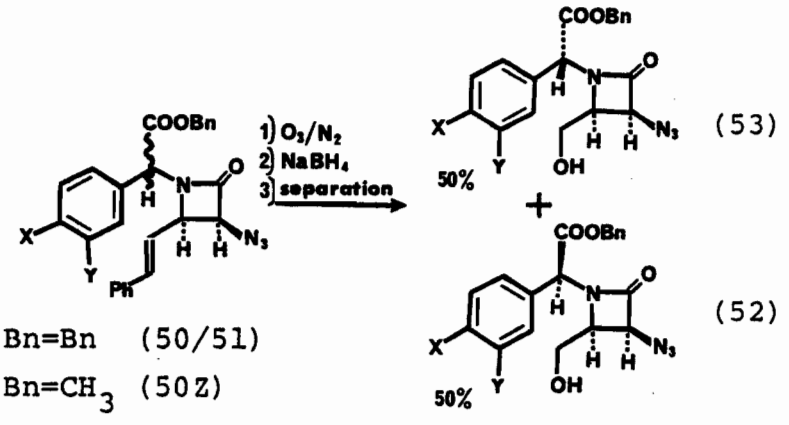
Methyl glycinates (34a-c) were hydrolysed with 4% aqueous sodium hydroxide, and acidified with hydrochloric acid to pH 3 to give the corresponding amino acids (47a-c) quantitatively. The amino acids (47a-c) were benzylated with benzyl alcohol in the presence of thionyl chloride[77] to afford (48a-c) as their hydrochloride salts, which were converted to their parent amine using 10% aqueous sodium carbonate in about 82% yield. Amino esters (48a-c) were condensed with one equivalent of cinnamaldehyde in dry methylene chloride. The mixture was slowly distilled to remove water and dry methylene chloride added throughout the reaction. The formation of the Schiff bases (49a-c) were completed after 10 hrs. The i.r. spectra showed the presence of the C=N bond at 1635 cm^{-1} and no absorption above 3100 cm^{-1}. The n.m.r. spectra showed the characteristic Schiff base proton at 8.0 p.p.m. Treatment of (49a-c) with one equivalent of azidoacetyl chloride and one equivalent of triethylamine in methylene chloride at -20° for 1 hr afforded an inseparable mixture of β-lactams (50a-c/ 51a-c) in approximately 70% yield. The ratio of the two diastereomers (50/51) was 1:1 as shown in the n.m.r. spectra of the crude compounds, and the coupling constant of the β-lactam protons was 5 Hz, which confirmed the cis-stereochemistry of the β-lactams. All their mass spectra showed $M^{+}-N_2$ and fragments characteristic of β-lactams[70].


COOCH3
1) NaOH
2) HCl
R
NH2
(34)
COO⁻
NH3⁺
(47)
1) PhCH2OH
SOCl2
2) 10%
NaCO3
COOBn
(48)
= R
X
Y
a) X=Y=H
b) X=OBn, Y=H
c) X=Y=OBn
N3CH2COCl
(C2H5)3N
Ph
(50/51)
(49)



p.m.r. spectrum of β-lactams (50a/51a) in chloroform-d. (60 MHz)

Ozonolysis of the diastereomeric mixtures of azido-β-lactam (50a-c/51a-c) with ozone in the presence of a nitrogen stream[74] in a mixture of methylene chloride and absolute ethanol at -78° and reduction with sodium borohydride at -40° afforded a mixture of diastereomeric alcohols (52a-c/53a-c) contaminated with some ethyl ester, presumably arising from transesterification. The diastereomeric mixtures (52/53) were separated by column chromatography using silica gel. Less polar diastereomers (53a-c) were eluted with methylene chloride (i.r. absorption for esters at 1740 cm^{-1}). More polar diastereomers (52a-c) were eluted with chloroform (i.r. absorption for esters at 1750 cm^{-1}). The relative configuration at the carbon next to the ester group was assigned as described previously (page 29).

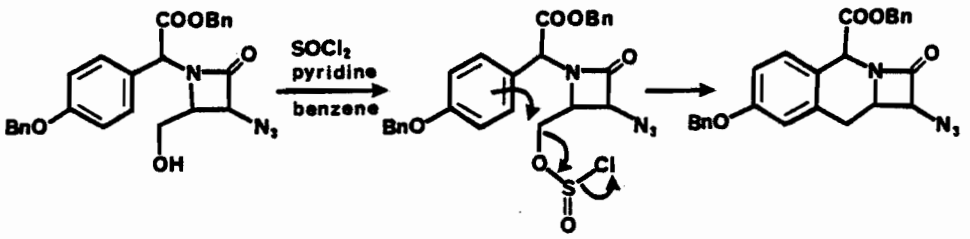


Alcohols (53a) and (52a) were transformed to their respective mesylates (56a) and (57a) (page 48). Attempts to cyclize these mesylates in benzene containing triethylamine failed. Attempted conversion of the mesylates to their respective iodide using sodium iodide in acetone or tetra-n-butylammonium iodide in refluxing benzene, failed, presumably because of steric hindrance[78]. Successful cyclization could be achieved when alcohol (52a) was treated with two equivalents of thionyl chloride and three equivalents of pyridine in dry benzene at 70-74° for 7 hrs, in which case 70% cyclization product (66a) and 20% chloro compounds (60a/61a) were obtained (page 46).

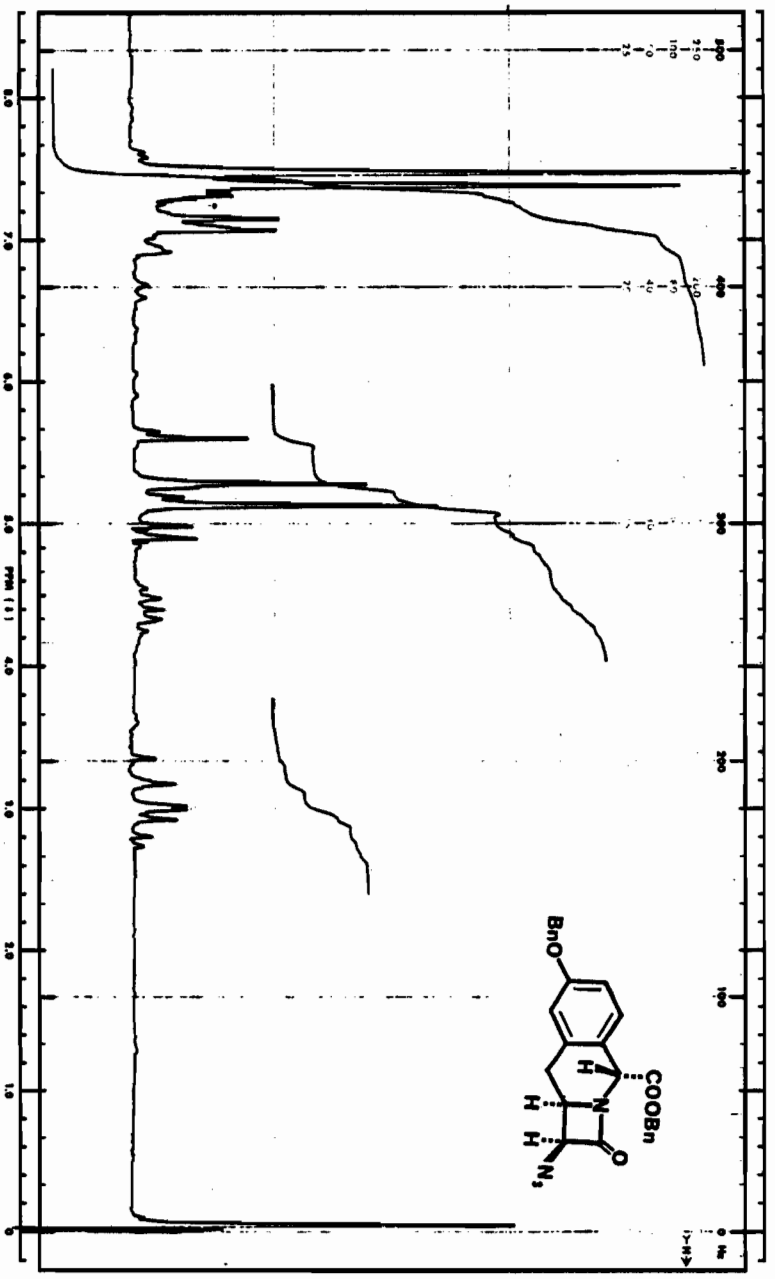
Various experiments showed that cyclization and chlorination were competitive reactions, and that the ratio of products formed depended on the reaction temperature. The following table summarizes our results, and indicates that an optimum yield of cyclized compound was obtained at 70-74° (table 1). The fact that the chloro compounds (60/61) were not an intermediate in the formation of the cyclized compound (66) was proved by recovering of starting material when chloro compounds (60/61) were submitted to the same reaction condition.

TABLE 1:

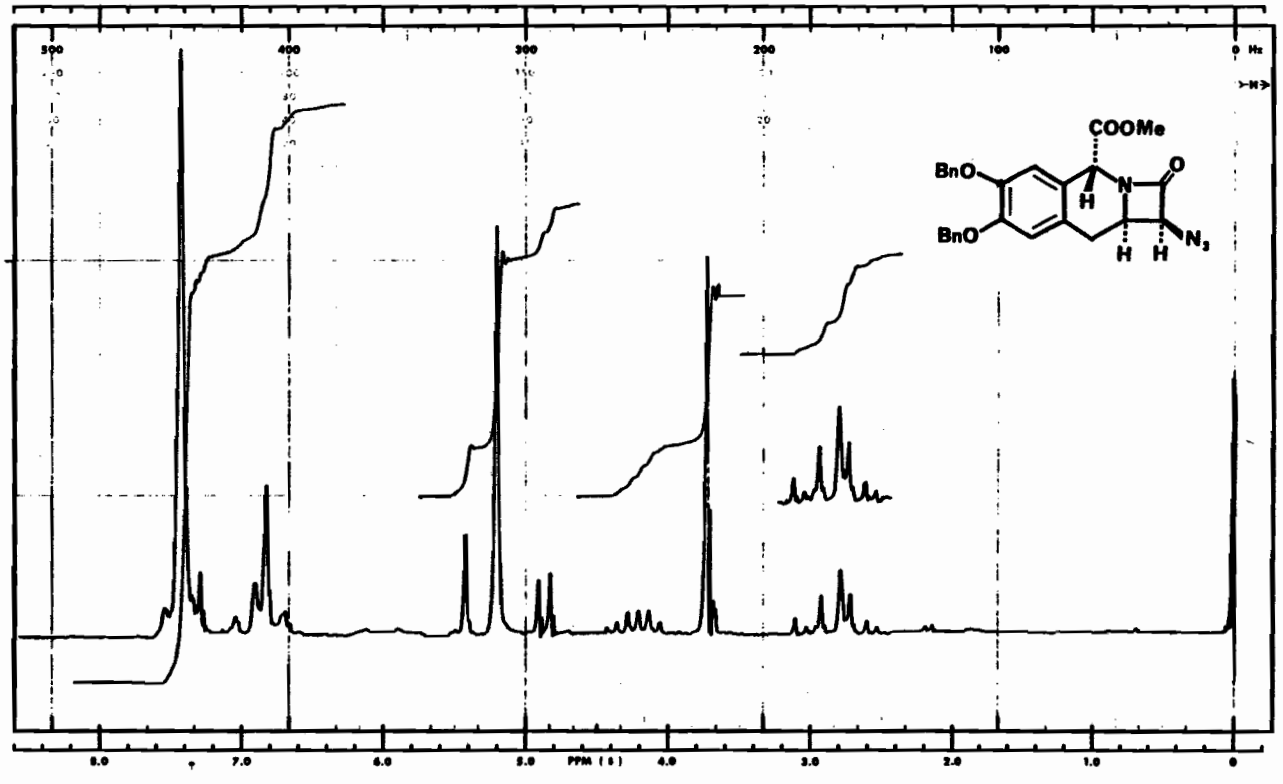
CYCLIZATION



T °C	% CYCL.	% CHLORIN.	% START. M.	TIME (hr)
30	trace	trace	≃90	24
60	≃50	trace	≃50	8
70	≃60	≃15	≃25	2
72–74	≃80	≃20	0	7
80	≃60	≃40	0	7
85	≃30	≃70	0	2



p.m.r. spectrum of β-lactam (66b) in chloroform-d. (60 MHz)

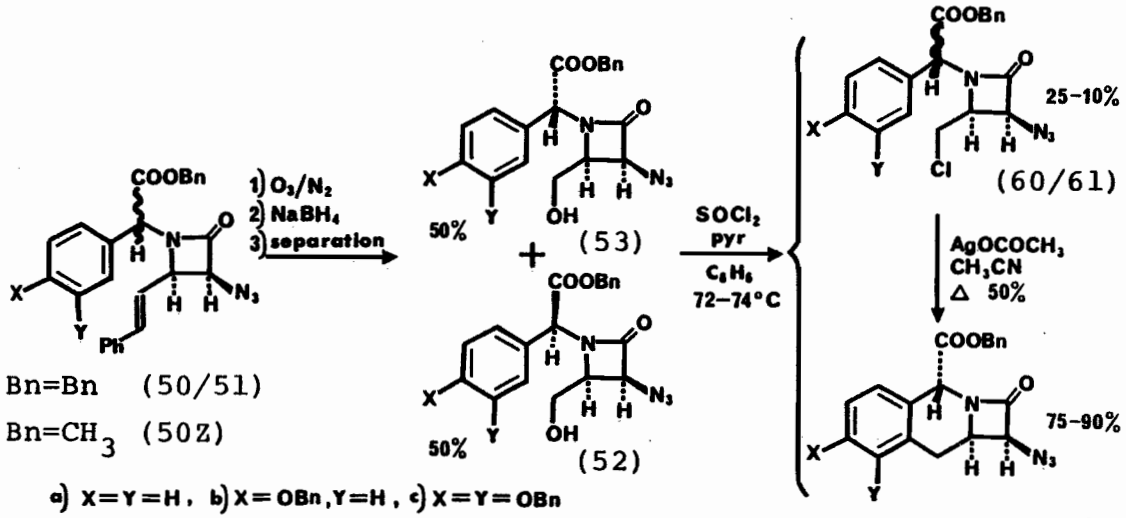


p.m.r. spectrum of β-lactam (66zc) in chloroform-d. (60 MHz)

As expected, the cyclization increased the β-lactam i.r. absorption frequency from 1760 cm^{-1} to 1770-1780 cm^{-1} by decreasing the planarity of the β-lactam nitrogen. This decrease in amide resonance reduced the double bond character of the β-lactam bond and therefore increased the i.r. absorption frequency of the β-lactam[43]. The n.m.r. spectra showed J = 5 Hz for the proton next to the azide function, characteristic of cis-fused β-lactam ring. The mass spectra showed M^+- (N_3-CH=C=O) and other appropriate fragments.

In the case of (52b-c), an 80-85% yield of cyclization products was obtained using the same conditions, whereas isomers (53a-c) gave an approximately 1:1 mixture of cyclization products (66a-c) and chlorination products (60a-c/61a-c). It should be noted that both diastereomers gave one cyclization product only, so that epimerization must have occurred during or after the cyclization reaction.

The chloro compounds (60a-c/61a-c) could be converted to the cyclized compounds (66a-c), by means of silver acetate in refluxing acetonitrile.



Bn=Bn (50/51)

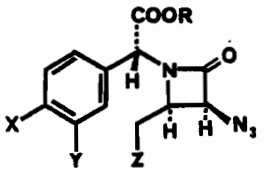
Bn=CH_3 (50Z)

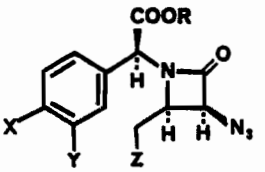
a) X=Y=H, b) X=OBn, Y=H, c) X=Y=OBn

Bn=Bn (66)

Bn=CH_3 (66Z)

Since both diastereoisomers (52a-c) and (53a-c) gave one cyclization product only, and no firm stereochemical assignment could be made at this point, we next attempted to effect cyclization without isomerization of the benzylic proton. Alcohol (52a) was treated with three equivalents of thionyl chloride in boiling benzene for 90 minutes. The resulting chlorosulfite (63a), the structure of which was established by n.m.r. and hydrolysis to starting alcohol (52a), was treated with three equivalents of silver acetate in boiling benzene for 2 hrs. Chromatographic separation gave acetate (65a) and cyclization product (67) in 75 and 4% yield respectively. The structure of acetate (65a) was deduced by comparison with acetate (65a), obtained from treatment of alcohol (52a) with boiling acetic anhydride. The n.m.r. spectrum of the cyclized compound (67) was very similar to that of the cyclized compound (66a), except for the position of the benzylic CH_2-group adjacent to the β-lactam ring, which appeared at approximately 0.5 p.p.m. downfield from the corresponding proton in (66a). Upon treatment of (67) with pyridine in refluxing benzene for 7 hrs, it was converted quantitatively to (66a).





54 R=CH_3, Z=OH

55 R=CH_3, Z=OH

56 R=Bn, Z=OMS

57 R=Bn, Z=OMS

58 R=CH_3, Z=Cl

59 R=CH_3, Z=Cl

60 R=Bn, Z=Cl

61 R=Bn, Z=Cl

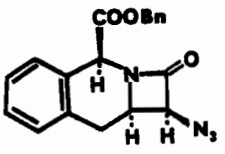
62 R=Bn, Z=OSOCl

63 R=Bn, Z=OSOCl

64 R=Bn, Z=OAC

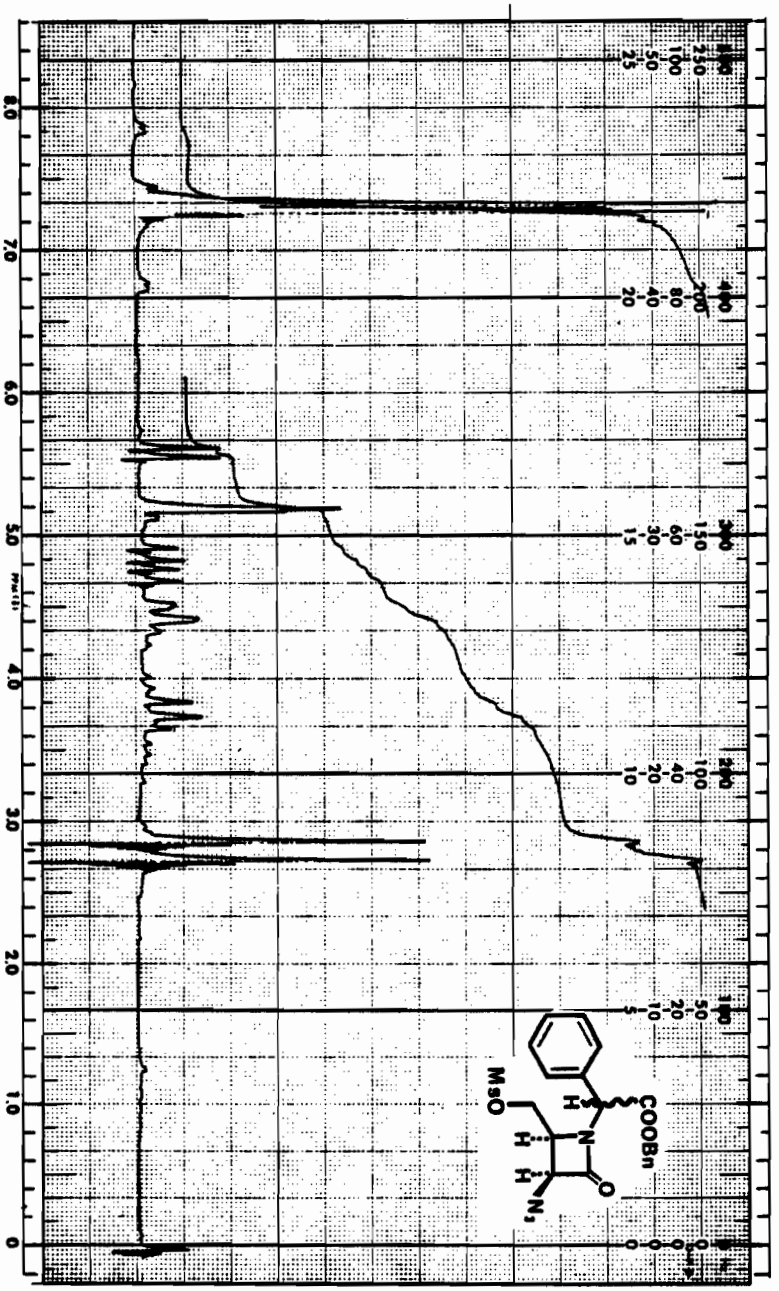
65 R=Bn, Z=OAC

a) X=Y=H, b) X=OBn, Y=H, c) X=Y=OBn



(67)

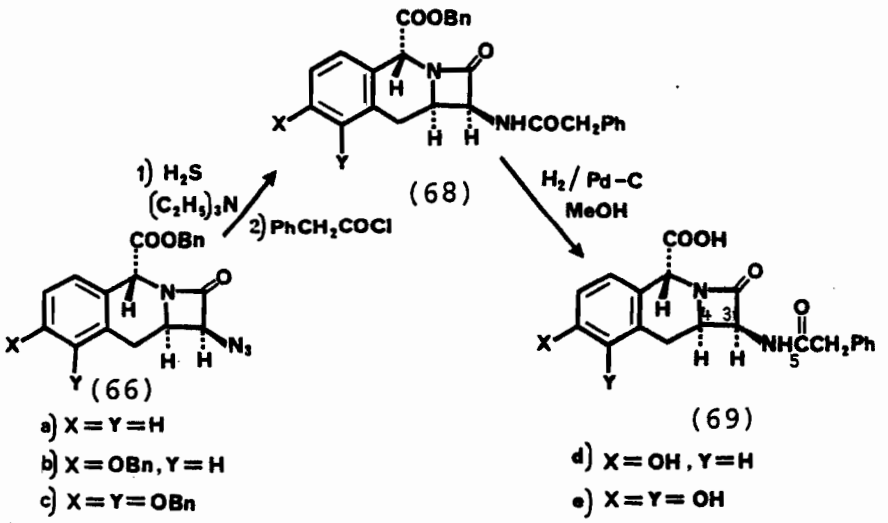
The proton α- to the carbobenzyloxy group and the protons of the mesylate group were clearly different in (56a), δ 5.6 (CHCOOBn) and 2.85 p.p.m. (SO_2CH_3) and (57a) δ 5.51 and 2.65 p.p.m. There was equally no major problem in distinguishing acetates (64a), δ 5.65 (CHCOOBn) and 2.1 p.p.m. (OAc), and (65a), δ 5.60 and 1.98 p.p.m. In chlorosulphites (62a) and (63a) the benzylic methine protons appeared much closer at 5.60 and 5.59 p.p.m. respectively. For chloro compounds (58a/59a) and (60a/61a), all n.m.r. signals were identical in $CDCl_3$ or C_6D_6. In order to prove that a mixture of chloro compounds (58a/59a) and (60a/61a) was obtained during the cyclization reactions using thionyl chloride-pyridine in benzene, (60a/61a) was treated with three equivalents of silver nitrate in boiling acetonitrile for 2 hrs. A 30% yield of a 1:1 mixture of products of cyclization (66a) and (67) was obtained as established by n.m.r. only. The two isomers could not be separated by t.l.c. Epimerization using pyridine in boiling benzene converted the mixture of cyclization products to the more stable (66a) having the "natural" nocardicin stereochemistry. In related bicyclic nocardicin precursors, Cooper et al.[49], have observed a similar epimerization.

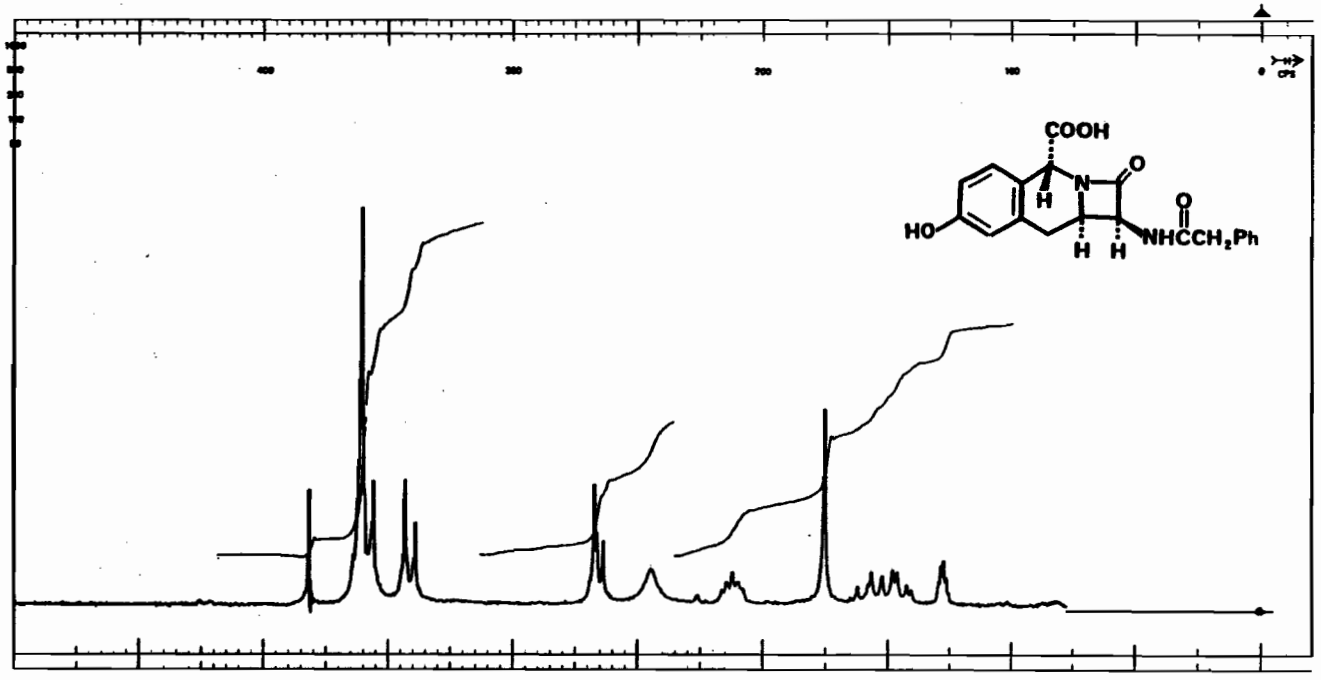


p.m.r. spectrum of β-lactams (56a/57a) in chloroform-d. (60 MHz)

Reduction of azides (66b,c) with hydrogen sulfide in the presence of triethylamine, followed by acylation with phenylacetyl chloride in the presence of pyridine, resulted in the formation of the corresponding amides (68b,c). In the n.m.r. spectra, H_3 appeared as a quartet ($J_{3,4} = 5$ Hz, $J_{3,5} = 10$ Hz), characteristic of cis fused acylamino β-lactams, at 5.3 p.p.m. The elemental analyses supported the structure assigned.

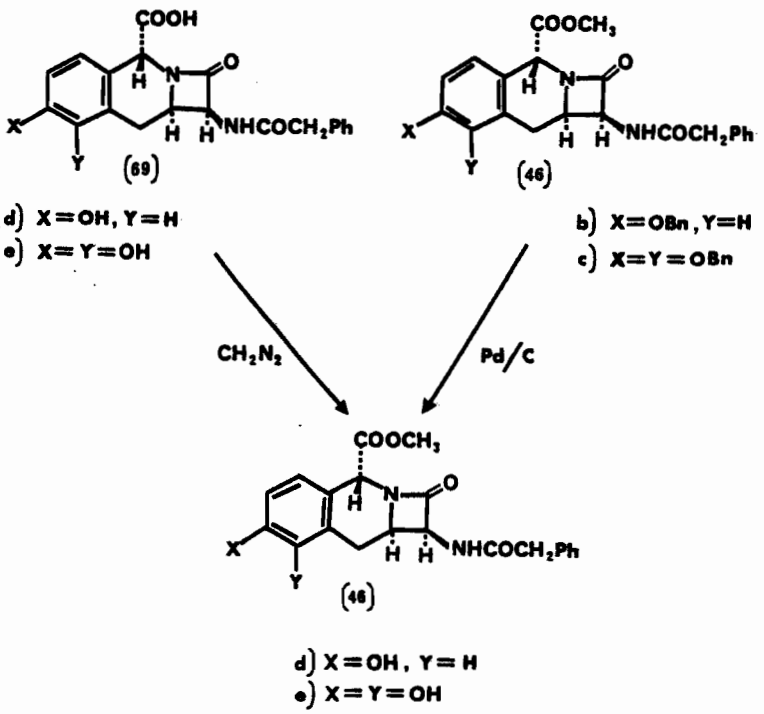
Catalytic hydrogenation of (68b,c) using 10% Pd/C in methanol at 40 psi gave (69d,e) in good yield. The structures of (69d,e) were established by i.r., n.m.r. and also mass spectra of their methyl esters (46d,e).





p.m.r. spectrum of β-lactam (69d) in $CDCl_3$:DMSO:D_2O. (100 MHz)

These were obtained by treatment of compounds (69d,e) with diazomethane, and were identical to those obtained from catalytic hydrogenation of (46b,c).



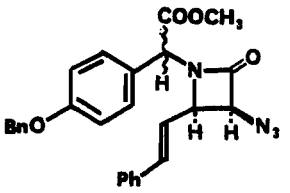
CHAPTER 3

The synthesis of nocardicin A analogues carrying the nocardicin A side chain

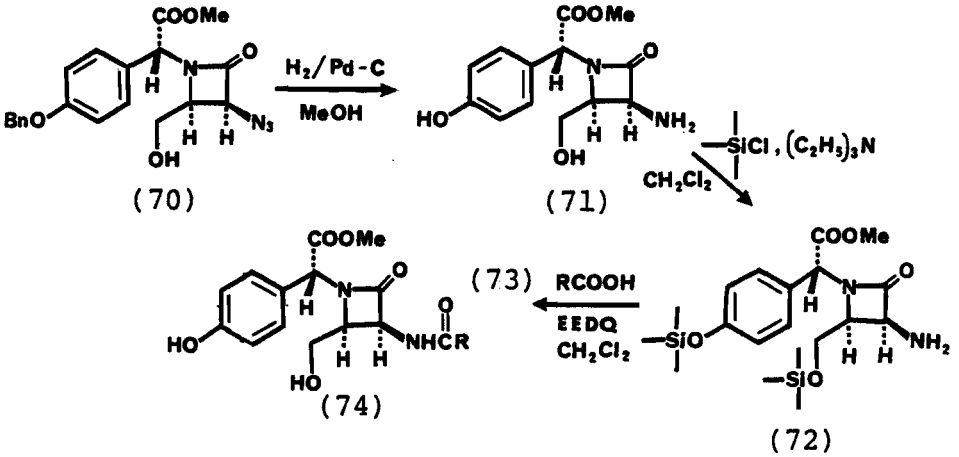
The formation of the amide bond in nocardicin A[3,56], and the removal of benzyl protecting groups in the presence of a phenylglyoxyl amide (page 62) or phenyloximinoglyoxyl amide (page 65) function can cause considerable difficulties[56]. The use of benzyl protecting groups is virtually mandatory for the efficient synthesis of strained nocardicin A analogues, since other groups may not be removed cleanly, and the purification of the final product can be somewhat tedious. This chapter will deal with a fairly general solution of that problem, and the synthesis of DL-4-hydroxymethyl nocardicin A (mixture of diastereomeric racemates) where the use of benzyl protecting groups are not necessary.

A mixture of diastereomeric styryl azides 5b were ozonised and reduced to a mixture of (70/70A) which could be separated into their constituents 70 and 70A as described for (40) and (41) (page 28).

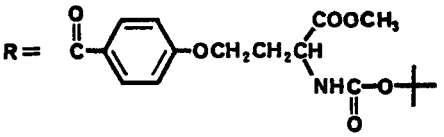
DL-Azido-β-lactam (70) was reduced catalytically with Pd/C in methanol, and the resulting phenolic amino alcohol (71), obtained in nearly quantitative yield, was silylated in situ to give (72). Coupling of (72) with glyoxylic acid (DL-73) using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[79-81] in methylene chloride for 16 hrs, gave, after washing with 10% hydrochloric acid and 10% sodium bicarbonate, followed by chromatography on silica gel, 80% of amide (74) as a mixture of unseparable diastereomeric racemates. Its β-lactam carbonyl absorption appeared at 1760 cm^{-1}. After D_2O exchange, the n.m.r. spectrum of the β-lactam proton

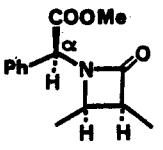


(5 b)



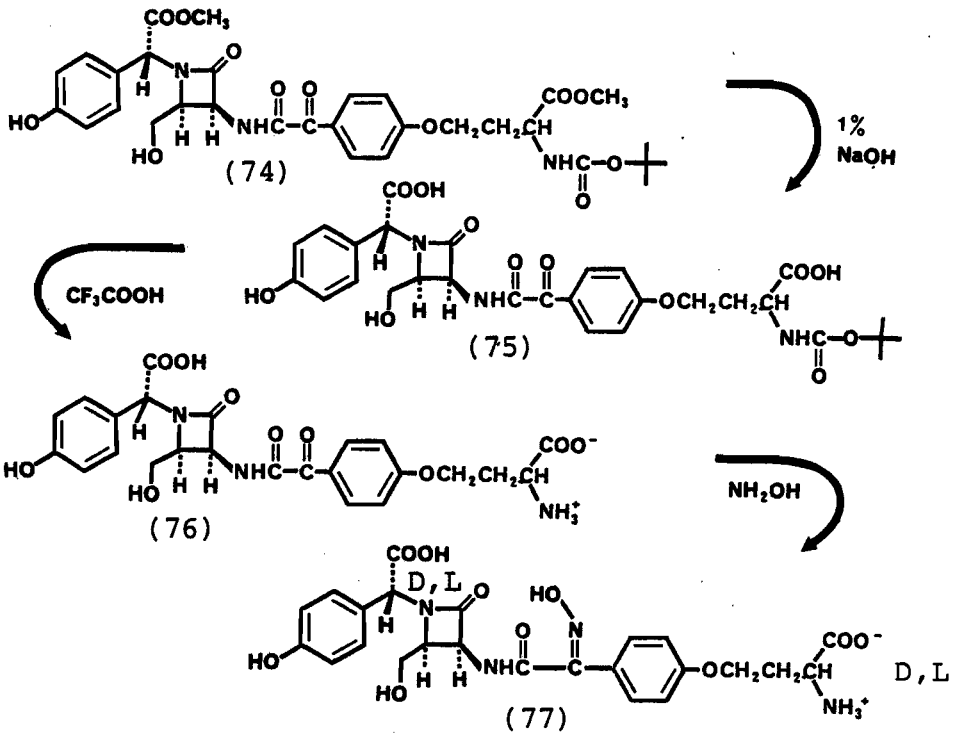
(70) (71) (72) (73) (74)





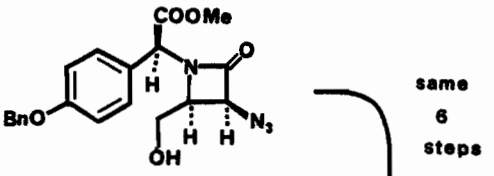
next to the amide function appeared at 5.5 p.p.m. as a doublet (J = 5 Hz). One part of the two sets of aromatic AB systems which did not overlap with the other signals appeared at 8.3 p.p.m. (J = 8 Hz).

Hydrolysis of diester (74) with two equivalents of sodium hydroxide in aqueous methanol afforded diacid (75) in about 70% yield. Its infrared spectrum showed absorption at 1740 cm^{-1} indicative of the β-lactam function. The n.m.r. spectrum also showed the presence of β-lactam hydrogens, and after deuterium exchange, the hydrogen next to the amide function appeared as a doublet at 5.4 p.p.m. (J = 5 Hz). Compound (75) was treated with trifluoroacetic acid[82-84] to give amino acid (76) in about 90% yield. This compound decomposed at 220-4°. The infrared spectrum indicated the presence of the β-lactam function at 1750 cm^{-1} and the amide function at 1660 cm^{-1}. U.v. spectral data [λ_{max} (EtOH, H_2O), 226 nm (ε, 18500), 229 (Σ, 15800)] were consistent with the presence of a para-alkylated phenol and a conjugated alkoxyphenyl derivative. Oximation was carried out using hydroxylamine hydrochloride in water at pH 7[85.89]. Purification by ion exchange using resin XAD4[86] gave (77) in 60% yield. This compound decomposed at 217-20°. Its spectral characteristics were very similar to those of nocardicin A (6), except for the presence of an extra CH_2OH group in the n.m.r. (see experimental part).

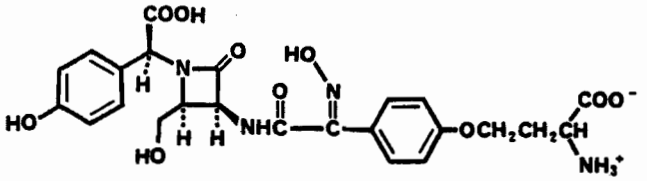

(74)
1%
NaOH
(75)
CF3COOH
(76)
NH2OH
D,L
(77)
D,L

Compound (77) has an in vitro activity of 30 mcg/ml vs. S. lutea PCl-1001, which is the only organism of Fujisawa's test panel available for the time being. Nocardicin A has an M.I.C. of 6.25 mcg/ml vs. S. lutea PCl-1001. Thus (77) is approximately equivalent in activity to natural Nocardicin A[87], taking into consideration that it consists of four stereoisomers, three of which can be presumed not to be active.

The same sequence could also be applied to the epimer (70A) giving (77A); (77A) did not show any biological activity.



(70A)

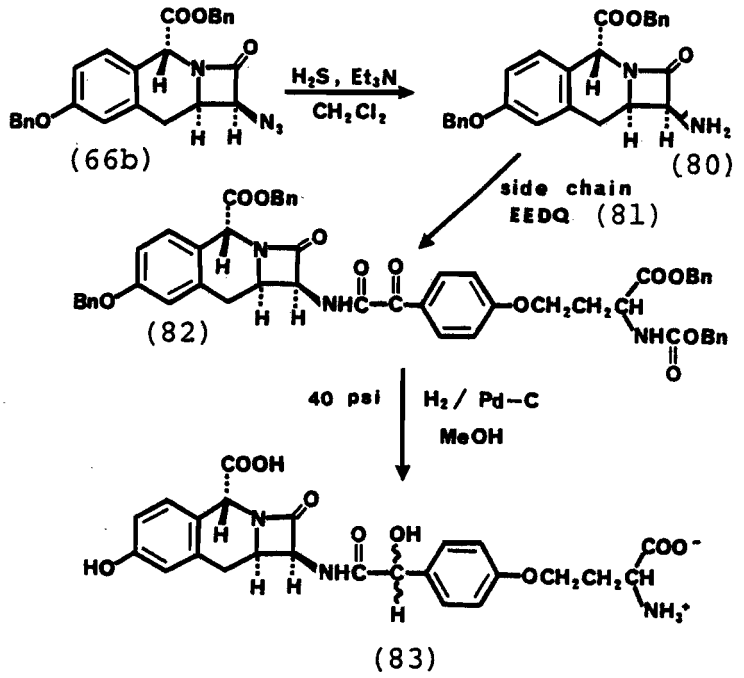


(77A)

In the case of the cyclized nocardicinic acid analogue (66b), a methyl ester could not be used because the β-lactam ring did not survive the alkaline conditions necessary for its hydrolysis, and a benzyl derivative was used.

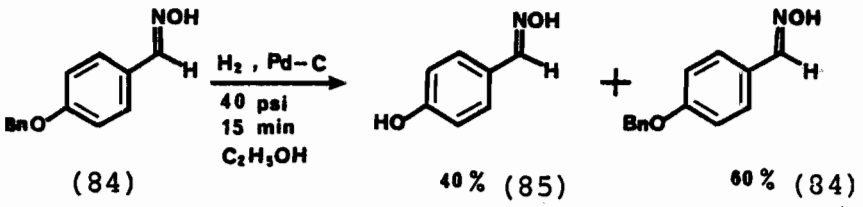
DL-Azido-β-lactam (66b) was converted to amine (80) by means of hydrogen sulfide-triethylamine. Column chromatography gave pure amine, which was coupled with DL-glyoxylic acid (81), using EEDQ in methylene chloride. After purification, an 80% yield of amide (82) was obtained. The i.r. spectrum indicated the presence of the β-lactam group at 1770 cm^{-1}, ester groups at 1745 cm^{-1} and the amide group at 1665 cm^{-1}. The n.m.r. spectrum showed the β-lactam proton next to the amide function as a quartet at 5.5 p.p.m. ($J_1 = 5$ Hz, $J_2 = 10$ Hz) and other appropriate signals.

Catalytic hydrogenation of (82) in methanol, using Pd/C as catalyst, resulted in complete debenzylation and reduction of the keto function[88] to alcohol (83), as evidenced by n.m.r. and u.v. spectra, and the inability to form an oxime (see experimental part).


COOBn
BnO
H_2S, Et_3N
CH_2Cl_2
N_3
NH_2
side chain
EEDQ
(81)
NHC
OCH$_2$CH$_2$CH
NHCOBn
40 psi
H_2 / Pd–C
MeOH
COOH
HO
OH
COO$^-$
NH_3^+

(66b) (80) (82) (83)

We next investigated if debenzylation by catalytic hydrogenation could be achieved in the presence of an oxime function. We chose as a model p-benzyloxybenzaldehyde oxime (84). We found that catalytic reduction in ethanol using 10% Pd/C at 35 psi for 15-30 minutes removed selectively the benzyl group, and that only a mixture of (84) and (85) was isolated.



(84) 40% (85) 60% (84)

Therefore, it was decided to carry out the catalytic debenzylation of the oxime of the keto-amide (82).

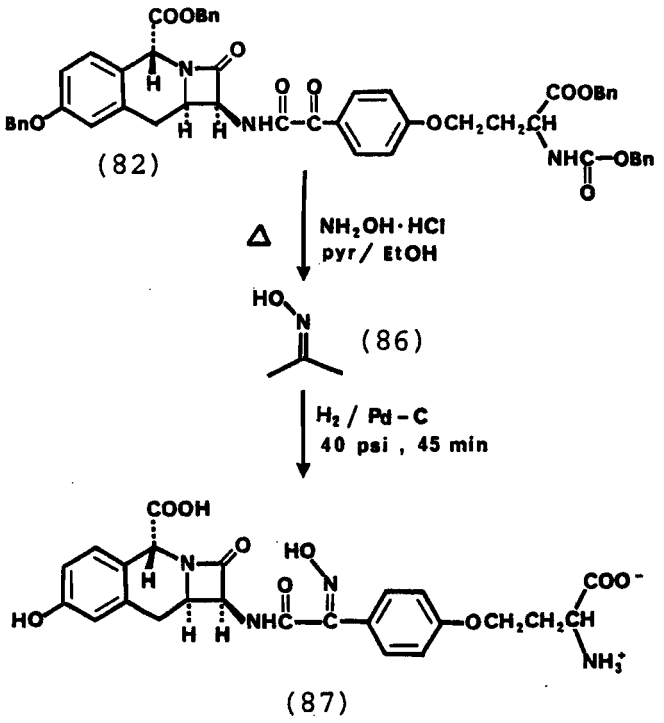
As mentioned before, the monocyclic β-lactam ring is relatively stable, especially in comparison with the fused β-lactam. Treatment of monocyclic β-lactam antibiotics with hydroxylamine gave the corresponding oxime without any problem. Fortunately, we found that the fused β-lactam

ring (82) also could be converted to syn-oxime (86) using hydroxylamine hydrochloride in pyridine-ethanol[90]. Its i.r. spectrum indicated the presence of a β-lactam function at 1775 cm^{-1} and of an oxime function at 1520 cm^{-1}. U.v. studies suggested the presence of a conjugated alkoxy phenyl derivative, λ_{max} (EtOH) 273 nm (ε, 15000).

Catalytic hydrogenation in absolute ethanol for 40 minutes at 35-40 psi, using 10% Pd/C as catalyst, converted oxime (86) to the deblocked oxime (87) in 81% yield. Its spectral characteristics were very similar to those of nocardicin A, except for the presence of a CH_2 group in the n.m.r. (see experimental part).

Further reduction of (87) by the method described by Hashimoto et al.[31], using Pd/C in water on the sodium salt of (87), resulted in the reduction of the oxime function, as evidenced by a change in u.v. spectrum similar to the one described[31], and presumably giving the corresponding amine. However, no attempt was made to characterize this compound because of the lack of material. It should be noted that (87) too was obtained as a mixture of two diastereomeric racemates.

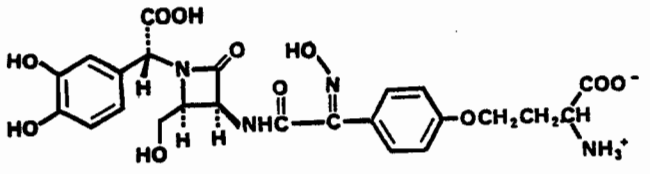
This compound did not show any noticeable antimicrobial activity either in vivo or in vitro.


COOBn
BnO
NHC
OCH2CH2CH
NHC—OBn
(82)
Δ
NH2OH·HCl
pyr / EtOH
HO
N
(86)
H2 / Pd-C
40 psi , 45 min
COOH
HO
COO⁻
NH3⁺
(87)

Since neither hydroxymethylnocardicin A nor homocyclonocardicin A showed any *in vivo* antibacterial activity, no attempt was made to synthesize hydroxynocardicin A (88), especially since the yield of the starting dibenzyloxyphenyl cyanoamine was only 5% (page 24).

The activity of 4-hydroxymethylnocardicin A *in vitro* was about the same as that of nocardicin A against S.lutea, but the lack of activity of this compound *in vivo* may mean that the 4-hydroxymethyl function somehow interferes with the *in vivo* oxidation of this compound. Unfortunately model studies on (69d) indicated that the oxidation did not proceed cleanly and that no pure product could be isolated.

In view of this fact no attempt was made to transform 4-hydroxymethylnocardicinic acid into its quinone methine oxidation product.



(88)

Contributions to knowledge

(1) Homocyclo analogues of nocardicin A (69d-e) and (87) were synthesised. A key step in the synthesis of these compounds involved the novel cyclization of (52) and (53) with thionyl chloride.

(2) The relative stereochemistry of the carbobenzyloxy group with respect to the cis-β-lactam protons was precisely defined for the key intermediates.

(3) EEDQ was shown to be an excellent condensing agent for the formation of the amide bond in nocardicin A analogues.

(4) A simple catalytic procedure for deblocking four benzyl protecting groups in the presence of an oximino function is described.

General Experimental

Solvents were of reagent grade unless otherwise specified. I.r. spectra were run on Unicam SP1000 and P.E. 257 spectrophotometers. N.m.r. spectra were run on Varian T-60A, HA-100 and Brucker Ft-90 MHz spectrometers. Mass spectra were taken on an AEI-MS-902 mass spectrometer using the direct sample inlet system with a 70 eV ionization energy or on a LKB-9000 mass spectrometer. U.v. spectra were run on a Cary 17 spectrophotometer.

Melting points were determined on a Gallenkamp block in open capillary tubes and are uncorrected.

Merck S160 silica gel was used for column chromatography. Merck silica gel HF 254 was used for thin layer chromatography (1 mm) on glass plates (20 x 20 cm). Elemental analyses were performed by Heterocyclic Chemical Corporation or Midwest Microlab Ltd.

EXPERIMENTAL

CHAPTER 1

Aminonitriles 33b,c,d,h

Benzaldehydes 32 (0.02 mole) were dissolved in solvent A. Ammonium chloride (2.6 g, 0.05 mole) and sodium cyanide (1.25 g, 0.025 mole) were added, and the solution was saturated with ammonia at 0° (10 mins), and stirred in a pressure bottle at 25-30° for 15 hrs. After evaporation, solvent B was added, and the product either filtered and recrystallized from solvent C, or extracted with ethyl acetate. The n.m.r. (DMSO-d_6) showed a 2H broad singlet at D pp., exchangeable with D_2O (NH_2), a 1H-singlet at δE pp, (CH), aromatic proton at F pp, and other appropriate signals. All compounds absorbed in the infrared at 3300-3500 cm^{-1} (NH_2) and 2200 cm^{-1} (CN). Yield G%.

33b: A 100 ml methanol, 30 ml THF; B water; C methanol; m.p. 93-5°; D 2.8-3.4; E 4.83; F 6.8-7.5 (9H); 5.1 p.p.m., 2H, s, CH_2Ph; m/e 238 (M^+); G = 90%.

33c: A 100 ml methanol; B water, extracted with ethyl acetate; D 2.0; E 4.80; F 6.8-7.2 (3H); 6.0 p.p.m., 2H, s, O-CH_2-O; G = 80%.

33d: A 100 ml methanol, 30 ml THF; B water; the precipitate consisted of methanol insoluble 33d, and large amounts of soluble cyanohydrin; m.p. 49-50°; D 1.63-1.82; E 4.90; F 7.0-7.7 (13H); 5.2 p.p.m., 4H, s, $(CH_2Ph)_2$; m/e 344 (M^+); G = 5%.

33h: A 100 ml methanol; B acetic acid; filtration; water; filtration. m.p. > 250° (dec.); D 4.8 (3H); E 5.4; F 7.6-8.2 (4H); G = 80%.

Amino acid 34b,c,d,e

Cyanoamines 33b,c,d,h (0.018 mole) were dissolved in 250 ml methanol-water (95:5), and HCl-gas was bubbled in at room temperature without cooling for 10 mins. The solution was gently refluxed for 3 hrs, the solvent then evaporated and a mixture of 20 ml acetone - 60 ml ether was added. The colourless precipitate was washed with ether, and 34b,c,e,·HCl obtained in approximately 80% yield, except for 34d·HCl which was obtained in 50% yield. All compounds obtained had n.m.r. spectra similar to those described for the cyanoamines except for additional methyl ester peaks.

34b·HCl: Calcd. for $C_{16}H_{18}O_3NCl$: C 62.44, H 5.85, N 4.55; found: C 62.22, H 5.95, N 4.53.

34d·HCl: Calcd. for $C_{23}H_{24}O_4NCl$: C 66.75, H 5.80, N 3.38; found: C 66.35, H 5.50, N 3.30.

All hydrochlorides were converted to the parent amine 34 according to the following procedure:

34b·HCl: (4.6 g, 0.015 mole) was mixed with 50 ml of 10% aqueous $NaHCO_3$, and the resulting amine 34b extracted into 50 ml of ether. After evaporation, 3.25 g of 34b (80% yield) was obtained as an oil. N.m.r. ($CDCl_3$) δ 1.8 (s, 2H, NH_2, exchanged with D_2O); 3.5 (s, 3H, OCH_3), 4.4 (s, 1H, CH), 4.9 (s, 2H, CH_2O), 6.65-7.4 p.p.m. (m, 9H, aromatic); i.r. (CH_2Cl_2) 3300-3400 (NH_2), 1740 cm^{-1} (ester).

Schiff base 35a,b,c,d,e

All the Schiff bases were prepared in a similar manner and used without purification. The following is a representative procedure:

To a solution of 34b (2.71 g, 0.01 mole) in 100 ml dry CH_2Cl_2 was added cinnamaldehyde (1.32 g, 0.01 mole). The solution was brought to reflux and the CH_2Cl_2 distilled slowly with the constant addition of dry CH_2Cl_2 so as to maintain the same volume of liquid in the reaction vessel. After the water of reaction was all removed ($\sim$ 7 hrs), the solution was cooled and $MgSO_4$ was added. After 2.5 hrs, it was filtered and evaporated to yield 3.85 g (100%) Schiff base 35b as an oil; n.m.r. ($CDCl_3$) δ 3.8 (s, 3H, CH_3), 5.1 (s, 2H, CH_2), 5.3 (s, 1H, CH), 6.8 (m, 2H, CH=CH), 7-7.6 (m, 14H, Ph), 8.0 (m, 1H, N=CH); i.r. (CH_2Cl_2) 1735 (ester), 1635 (HC=N) cm^{-1}. In the case of 35d, the yield seemed to be somewhat lower (85%) as ascertained by n.m.r.

Azido-β-lactams 36/37a-e

All β-lactams were prepared in an identical manner and obtained in approximately 80% yield. Their spectra were similar except for variations due to aromatic substituents. All their mass spectra showed M^+-N_2 or, in the case of 36/37a, M^+.

The following is a representative procedure:

β-Lactam 36/37b: To the freshly prepared Schiff base (3.85 g, 0.01 mole) in 100 ml dry CH_2Cl_2 was added at -20° (dry ice - CCl_4) triethyl amine (1.01 g, 0.01 mole). A solution of azidoacetyl chloride (1.2 g, 0.01 mole) in 30 ml dry CH_2Cl_2 was added dropwise over 10 mins. The solution was stirred for 1 hr and evaporated to dryness. The residue was dissolved in ether, treated with charcoal, filtered and evaporated to give 3.7 g (80%) 36/37b as an oily mixture of diastereomers; n.m.r. ($CDCl_3$) δ 3.8 (d, 3H, CH_3) 4.2-4.4 (m, 1H, β-lactam) 4.7-4.9 (m, 1H, β-lactam), 5.1 (s, 2H, CH_2), 5.5-5.6 (d, 1H, CH), 6.5 (m, 2H, CH=CH), 6.8-7.6 (m, 14H, Ph). Chromatography over silica gel using CH_2Cl_2 as eluent separated one of the diastereomers, which was obtained in 30% yield as an oil. N.m.r. ($CDCl_3$) δ 3.75 (s, 3H, CH_3), 4.2-4.4 (m, 1H, β-lactam), 4.8 (d, 1H, J = 5 Hz, β-lactam), 5.1 (s, 2H, CH_2), 5.5 (s, 1H, CH), 6.5 (m, 2H, CH=CH), 6.8-7.5 (m, 14H, Ph); i.r. (CH_2Cl_2) 2100 (N_3), 1760 (β-lactam), 1740 (ester) cm^{-1}, identical to that of the diastereomeric mixture; m/e 440 (M^+-N_2).

Phenylacetamido-β-lactams 38/39a-e

All transformations were performed in an identical manner, and the mixture of diastereomers 38/39 obtained in 70% yield after purification. Their spectra were similar except for variations due to aromatic substituents. All their mass spectra showed M^+, M^+-(Ph-CH_2CONHCH=C=O), M^+-(Ph-CH=CH-CH=CH-NHCOCH_2Ph) except for the dibenzyloxy derivative which showed no molecular ion but M^+-(Ph-CH_2CONHCH=C=O), M^+-(Ph-CH=CH-CH=CH-NHCOCH_2Ph).

The following is a representative procedure:

To a solution of a diastereomeric mixture of azido β-lactam 36/37b (4.68 g, 0.01 mole) in 100 ml dry CH_2Cl_2 at 0° was added triethyl amine (1.2 g, 0.012 mole). A stream of H_2S gas was bubbled in for 45 mins. The solution was allowed to stand for 2 hrs at room temperature. Evolution of nitrogen gas was observed. A stream of nitrogen gas was bubbled in for 30 mins, then was added 2.3 g (0.03 mole) pyridine, followed by dropwise addition of 1.8 g (0.012 mole) phenylacetyl chloride in 20 ml CH_2Cl_2. The solution was stirred for 2 hrs at 25°, then was washed with 10% HCl solution, 10% $NaHCO_3$ solution and brine. It was then dried over Na_2SO_4, and evaporated to give 5 g (89%) of impure amide 38/39b, which was chromatographed on silica gel. Methylene chloride eluted impurities, and chloroform gave 4 g (70%) of β-lactam 38/39b, m.p. 100-6°, as a mixture of diastereomers. N.m.r. ($CDCl_3$) δ 3.4 (s, 2H, amide CH_2), 3.6-3.8 (2s, 3H, CH_3), 4.2-4.4 (m, 1H, β-lactam), 4.8-5.1 (2s, 2H, CH_2), 5.4-5.7

(m, 2H, CH and β-lactam), 6.2-6.5 (m, 2H, CH=CH), 7-7.6 (m, 20H, Ph and NH). Two recrystallizations from absolute ethanol separated one of the diastereomers, m.p. 168-9°. N.m.r. ($CDCl_3$) δ 3.42 (s, 2H, CH_2), 3.7 (s, 3H, CH_3), 4.2-4.4 (m, 1H, β-lactam), 5.1 (s, 2H, CH_2), 5.3-5.6 (s, 1H, CHCOOMe and m, 1H, β-lactam, $J_1 = 5$ Hz and $J_2 = 8$ Hz), 6.2-6.6 (m, 2H, CH=CH), 6.2-7.6 (m, 20H, Ph and NH); i.r. (CH_2Cl_2) 3390 (NH), 1756 (β-lactam), 1740 (ester), 1680 (amide) cm^{-1}, identical to that of the mixture of diastereomers; m/e 560 (M^+),

38b or 39b: Calcd for $C_{35}H_{32}O_5N_2$: C 74.98, H 5.75, N 5.00; found: C 74.63, H 5.98, N 4.88.

38/39c: Calcd for $C_{29}H_{26}O_6N_2$: C 69.87, H 5.22, N 5.62; found: C 69.69, H 5.27, N 5.37; m.p. 69-70° (ethanol).

38/39d: Calcd for $C_{42}H_{38}O_6N_2$: C 75.67, H 5.70, N 4.20; found: C 75.33, H 5.79, N 3.88; m.p. 130-1° (ethanol).

4-Hydroxymethyl-β-lactams 40b,40d,41b and 41d

Both mixtures of diastereomeric monobenzyloxylactam 38/39b and dibenzyloxylactam 38/39d were submitted to identical reaction conditions and separation procedures, which will be described for 38/39b only.

β-Lactam 38/39b (2 g, 0.0035 mole) in a mixture of 50 ml CH_2Cl_2 and 100 ml dry methanol was saturated with nitrogen gas at -78° (3 mins). Then a mixture of O_3-N_2 gas was bubbled in until the KI starch paper showed excess ozone (30 mins). The excess ozone was removed by passing a stream of N_2 for 10 mins. The temperature was allowed to rise to -40°, at which time was added $NaBH_4$ (0.2 g, 0.005 mole). The temperature of the solution was permitted to rise to 25° over 2 hrs, following which 5 ml 10% HCl was added. The solution was evaporated to 50 ml, diluted with H_2O and was extracted with ethyl acetate, washed with water, dried over Na_2SO_4, and evaporated to give crude product in quantitative yield.

A wash with a mixture of ether-hexane (1:14) removed benzyl alcohol, and a 1:1 mixture of diastereomers (1.5 g, 85%) of 40/41b, m.p. 50-4°, was obtained, as evidenced by n.m.r.

Chromatography on silica gel and elution with $CHCl_3$ separated completely the diastereomers 41b and 40b. The less polar diastereomer 41b was obtained in 40% yield, and melted at 84-85° after crystallization from diethyl ether. N.m.r. ($CDCl_3$) δ 3.4 (b, 2H, CH_2O), 3.6 (s, 2H, CH_2CO), 3.8 (s, 3H, CH_3), 4.2-4.6 (m, 1H, C<u>H</u>-CH_2OH), 5.18 (s, 2H, CH_2Ph), 5.4-5.7 (<u>q</u>, 1H, C<u>H</u>-NHCO, $J_1 = 5$ Hz and $J_2 = 10$ Hz after D_2O exchange: d, $J = 5$ Hz), 5.8

(s, 1H, CH), 7-7.6 (m, 15H, Ph and NH); i.r. (CH_2Cl_2) 3380-3440 (NH and OH), 1760 (β-lactam), 1740 (ester), 1670 (amide) cm^{-1}.

40b was obtained in 40% yield and was crystallized from diethyl ether, m.p. 59-60°. N.m.r. ($CDCl_3$) δ 2.8-3.2 (b, 2H, CH_2O), 3.5 (s, 2H, CH_2CO), 3.7 (s, 3H, CH_3), 4-4.1 (m, 1H, C<u>H</u>-CH_2OH), 5.1 (s, 2H, CH_2Ph), 5.4-5.7 (m, 1H, C<u>H</u>-NHCO, J_1 = 5 Hz and J_2 = 10 Hz, and 1H, s, CH), 6.8-7.8 (m, 15H, Ph and NH); i.r. (CH_2Cl_2) 3380-3440 (NH and OH), 1760 (β-lactam), 1750 (ester), 1670 (amide) cm^{-1}; m/e 429 (M^+-COOMe), 297 (M^+-$HOCH_2CH$=$CHNHCOCH_2Ph$), identical to that of the other diastereomer. Calcd for $C_{28}H_{28}O_6N_2$: C 68.84, H 5.87, N, 5.73; found: C 68.62, H 6.05, N 5.47.

41d was obtained by the same purification method in 40% yield and melted at 85-7° (Et_2O) n.m.r. ($CDCl_3$) δ 3-3.3 (b, 2H, CH_2O), 3.6 (s, 2H, CH_2CO), 3.8 (s, 3H, CH_3), 4.2-4.4 (m, 1H, C<u>H</u>-CH_2OH), 5.2 (s, 4H, $2CH_2Ph$), 5.3-5.6 (<u>q</u>, 1H, C<u>H</u>-NHCO, J_1 = 5 Hz and J_2 = 10 Hz), 5.7 (s, 1H, CH), 6.7-7.6 (m, 19H, Ph, NH). Its i.r. was identical to that of 41b.

40d was obtained by the same purification method in 40% yield, m.p. 70-2°. N.m.r. ($CDCl_3$) δ 2.6-2.7 (b, 1H, OH exchanged with D_2O), 3.0-3.2 (b, 2H, CH_2O), 3.6 (s, 2H, CH_2CO), 3.7 (s, 3H, CH_3), 3.9-4.0 (m, 1H, C<u>H</u>-CH_2OH), 5.2 (s, 4H, $2CH_2Ph$), 5.4-5.72 (m, 1H, C<u>H</u>-NHCO, J_1 = 5 Hz and J_2 = 10 Hz and 1H, s, CH), 6.8-7.6 (m, 19H, Ph, NH); i.r. was identical to that of 40b; m/e 594 (M^+) identical to that of the other diastereomer. Calcd for $C_{35}H_{34}O_7N_2$: C 70.71, H 5.72, N 4.71; found: C 70.69, H 5.70, N 4.69.

β-Lactam 40f and 41f

To β-lactam 41b (1.5 g, 0.003 mole) in 60 ml methanol was added 10% Pd/C (0.2 g), and the mixture was hydrogenated at room temperature and 40 psi for 1 hr. (The pressure dropped to 37 psi). The solution was then filtered and evaporated to give 1 g (83%) of 41f, m.p. 76-8° (Et_2O); n.m.r. ($CDCl_3$) δ 3.4-3.6 (b, 2H, CH_2O), 3.7 (s, 2H, CH_2CO), 3.8 (s, 3H, CH_3) 4.2-4.6 (b, 2H, OH and C<u>H</u>-CH_2OH), 5.4-5.67 (<u>q</u>, 1H, C<u>H</u>-NHCOR, $J_1 = 5$ Hz and $J_2 = 10$ Hz), 5.7 (s, 1H, CH), 6.8-7.5 (m, 10H, Ph, and NH), 8.3 (b, 1H, phenolic OH); i.r. (CH_2Cl_2) 3350 (OH and NH), 1760 (β-lactam), 1725 (ester), 1660 (amide) cm^{-1}; 40f was obtained by the same method in 80% yield, m.p. 68-9° (Et_2O); n.m.r. ($CDCl_3$) δ 3.2 (b, 2H, CH_2O), 3.6 (s, 2H, CH_2CO), 3.79 (s, 3H, CH_3), 4.2 (b, 2H, OH and C<u>H</u>-CH_2OH), 5.4-5.7 (m, 2H, CH and C<u>H</u>NHCOR, $J_1 = 5$ Hz and $J_2 = 10$ Hz), 6.8-7.6 (m, 11H, Ph, NH and phenolic OH); i.r. (CH_2Cl_2) 3350 (OH and NH), 1760 (β-lactam), 1735 (ester), 1660 (amide) cm^{-1}; m/e 339 (M^+-COOMe), 148 [M^+-COOMe + $HOCH_2CH$=$CHNHCOCH_2Ph$)] identical to that of the diastereomer 41f.

β-Lactam 44f and 45f

β-Lactam 41f (1 g, 0.0025 mole) was dissolved in 30 ml methanol. 1% aqueous NaOH (10 ml) was added dropwise over a period of 10 mins. It was then stirred for 15 mins and acidified with HCl to pH 3. The methanol was evaporated and the aqueous solution was extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gave 0.8 g (84%) 45f, m.p. 160-2°, after dissolving in 5% aqueous $NaHCO_3$, and acidifying to pH 3. N.m.r. ($CDCl_3$-DMSO-d_6) δ 3.4-3.7 (b, 5H, CH_2O, CH_2CO, C<u>H</u>-CH_2OH), δ 5.2-5.4 (q, 1H, C<u>H</u>-NHCOR, $J_1 = 5$ Hz and $J_2 = 10$ Hz), 5.6 (s, 1H, CH), 6.8-7.4 (m, 11H, Ph, COOH, OH), 8.0 (b, 1H, NH). Addition of D_2O resulted in exchange of 4 protons and considerable sharpening of spectrum. I.r. (KBr) 3250-3350 (OH, NH, COOH), 1735 (β-lactam), 1710 (acid), 1625 (amide) cm^{-1}. Treatment of 45f with diazomethane resulted in quantitative formation of 41f. 44f Was obtained from 40f by the same method in 80% yield; m.p. 117-120°. N.m.r. was identical to that of the other diastereomer; i.r. (KBr) 3300-3400 (NH, OH, COOH), 1740-1730 (β-lactam and acid), 1660 (amide) cm^{-1}. Treatment of 44f with diazomethane gave 40f quantitatively.

β-Lactam 44d and 45d

40d and 41d were hydrolysed to 44d and 45d in about 80% yield using the method described for the formation of 45f.

45d: m.p. 122-5°; n.m.r. ($CDCl_3$, DMSO-d_6, D_2O) δ 3.3-3.7 (m, 5H, CH_2O, CH_2CO, C<u>H</u>-CH_2OD), 5.2 (s, 4H, $2CH_2O$), 5.3-5.5 (d, 1H, C<u>H</u>-NDCOR), 5.63 (s, 1H, CH), 6.8-7.4 (m, 18H, Ph); i.r. (KBr) 3200-3400 (NH, OH, COOH), 1750-1730 (β-lactam and acid), 1640 (amide) cm^{-1}; treatment of 45d with CH_2N_2 gave 41d quantitatively.

44d: m.p. 95-8°; n.m.r. was identical to that of the 45d; i.r. (KBr) 3200-3400 (NH, OH, COOH), 1750-1740 (β-lactam and acid), 1680 (amide) cm^{-1}; treatment of 44d with CH_2N_2 gave 40d.

β-Lactams, 41g, 44g and 45g

44d and 45d were debenzylated to 44g and 45g in 80% yield by catalytic hydrogenation as described for the preparation of 41f.

45g: m.p. 116-9° (dec.) n.m.r. (acetone-d_6, D_2O) δ 3.5-3.8 (b, 5H, CH_2O, CH_2CO, C<u>H</u>-CH_2O), 5.3-5.5 (d, 1H, CH-ND-COR, J = 5 Hz), 5.6 (s, 1H, CH), 6.8-7.4 (m, 8H, Ph); i.r. (KBr) 2800-3500 (OH, COOH), 1750 (β-lactam), 1730 (acid), 1640 (amide) cm^{-1}. Treatment of 45g with CH_2N_2 gave 41g quantitatively.

41g: m.p. 84-7°; n.m.r. ($CDCl_3$-D_2O) δ 3.4-3.8 (m, 8H, CH_2O, CH_2CO, CH_3, C<u>H</u>-CH_2O), 5.2-5.4 (d, 1H, C<u>H</u>-NDCOCH_2Ph, J = 5 Hz), 5.7 (s, 1H, CH), 6.5-7.4 (m, 8H, Ph); i.r. ($CHCl_3$) 3100-3500 (OH, NH), 1770 (β-lactam), 1740 (ester), 1680 (amide) cm^{-1}; m/e 414 (M^+).

44g: m.p. 95-8°; its n.m.r. was identical to that of 45g; i.r. (KBr) 2800-3500 (OH, COOH), 1750-1740 (β-lactam, acid), 1670 (amide) cm^{-1}. It was treated with CH_2N_2 to give the corresponding methyl ester 40g; m/e 414 (M^+).

EXPERIMENTAL

CHAPTER 2

Amino acids 47b,c

Amino esters 34b,c (0.036 mole) were stirred in 4% aqueous NaOH (40 ml) for 10 mins and acidified with HCl to pH 3. The colourless precipitate was filtered, washed with water and dried; 47b,c were obtained in about 90% yield. 47b, m.p. 270-3° (dec.). N.m.r. (DMSO-d_6) δ 5.05 (s, 1H, CH), 5.1 (s, 2H, CH_2), 7.05-7.61 (m, 9H, Ph), 11.4-11.6 (b, 3H, NH_3^+). 47c, m.p. 259-61° (dec.). Its n.m.r. spectra was similar to that of 47b except for variations due to aromatic substituents.

Amino esters 48a,b,c

Amino acids 47a,b,c (0.027 mole) were suspended in benzyl alcohol (200 ml) at 0-5°. Thionyl chloride (30 ml) was added slowly, over a period of 20 mins. Then the reaction mixture was heated at 90° while stirring for 5 hrs. Then it was cooled and dry diethyl ether was added. The white precipitate was filtered and washed three times with ether. 48a,b,c HCl were obtained in approximately 65% yield, except for 48c HCl which was obtained in 40% yield. 48a, m.p. 235-6°; 48b, 225-6°; 48c, 210-2°. All compounds obtained had n.m.r. spectra similar to those described for the amino acids except for additional benzyl ester peaks. All hydrochlorides were converted to the parent amine 48 according to the following procedure:

48b HCl (6.8 g, 0.017 mole) was mixed with 50 ml of saturated aqueous $NaHCO_3$ and the resulting amine 48b extracted into 50 ml of ether. After evaporation 5 g of 48b (82% yield) was obtained as an oil. N.m.r. ($CDCl_3$) δ 2.0-2.1 (b, 2H, NH_2 exchanged with D_2O), 4.6 (s, 1H, CH), 5.0-5.02 (d, 4H, $2CH_2$), 6.8-7.6 (m, 14H, Ph); i.r. (CH_2Cl_2) 3300-3400 (NH_2), 1740 (ester) cm^{-1}.

Schiff base 49a,b,c

All the Schiff bases were prepared in a similar manner and used without purification. The following is a representative procedure:

To a solution of 48b (5 g, 0.014 mole) in 100 ml dry CH_2Cl_2 was added cinnamaldehyde (1.9 g, 0.014 mole). The solution was brought to reflux and the CH_2Cl_2 distilled slowly with the constant addition of dry CH_2Cl_2. After $\sim$ 10 hrs the solution was cooled and $MgSO_4$ was added. After 3 hrs, it was filtered and evaporated to yield 6.9 g Schiff base 49b as an oil. N.m.r. ($CDCl_3$) δ 5.15-5.27 (m, 5H, $2CH_2$ and CH), 7.0-7.6 (m, 21H, Ph and CH=CH), 8.0-8.2 (m, 1H, CH=N); i.r. (CH_2Cl_2) 1735 (ester), 1635 (HC=N) cm^{-1}. In the case of 49c, the yield seemed to be somewhat lower (80%) as ascertained by n.m.r.

Azido-β-lactams 50/51a-c

For preparation of these β-lactams, see 36/37a-e (page 73). Their spectra were similar to that of those described, except for ester variations and variations due to aromatic substituents. All their mass spectra showed M^+-N_2.

4-Hydroxymethyl-β-lactams 54a,55a,54b,55b,54c,55c,53a,52a, 53b,52b,53c and 52c

The mixtures of diastereomeric β-lactams 50za, 50zb, 50zc, 50/51a, 50/51b and 50/51c were submitted to identical reaction conditions and separation procedures which will be described for 50/51b only. All their mass spectra showed M^+, M^+-COOR, M^+-($N_3CH=CH-CH_2OH$) and M^+-(N_3-CH=C=O) except for 53b, 52b, 53c and 52c which showed no molecular ion but M^+-($N_3CH=CHCH_2OH$) and M^+-(N_3-CH=C=O).

β-Lactam 50/51b (5.44 g, 0.01 mole) in a mixture of 60 ml CH_2Cl_2 and 100 ml dry methanol was saturated with nitrogen gas at -78° (3 mins). Then a mixture of O_3 and N_2 gas was bubbled in until the KI starch paper showed excess ozone (60 mins). The excess ozone was removed by passing a stream of N_2 for 10 mins. The temperature was allowed to rise to -40°, at which time was added $NaBH_4$(0.38 g, 0.01 mole). The temperature of the solution was permitted to rise to 25° over 2 hrs, following which 10 ml 10% aqueous HCl was added. The solution was evaporated to 50 ml, diluted with water and was extracted with ethyl acetate, washed with water, dried over Na_2SO_4, and evaporated to give crude product in quantitative yield.

A wash with a mixture of ether-hexane (1:14) removed benzyl alcohol, and an oily 1:1 mixture of diastereomers (3g, 63%) of 53/52b contaminated with approximately 5% 54/55b was obtained as evidenced by n.m.r.

Chromatography on silica gel and elution with CH_2Cl_2

separated one of the diastereomers 53b in 30% yield as a foam. 52b was eluted with $CHCl_3$ in 30% yield as a foam.

Less polar diastereomer 53b: n.m.r. ($CDCl_3$) δ 3.4-3.8 (b, 4H, CH-CH_2OH), 4.4-4.6 (d, 1H, CH-N_3, J = 5 Hz), 5.0-5.2 (d, 4H, $2CH_2$), 5.78 (s, 1H, CH), 6.9-7.55 (m, 14H, Ph); i.r. (CH_2Cl_2) 3300-3500 (OH), 2100 (N_3), 1770 (β-lactam), 1740 (ester) cm^{-1}. More polar diastereomer 52b: n.m.r. ($CDCl_3$) δ 2.2-2.4 (b, 1H, OH exchanged with D_2O), 3.2-3.4 (b, 2H, CH_2O), 4.0-4.3 (m, 1H, CH-CH_2O), 4.75-4.9 (d, 1H, CH-N_3, J = 5 Hz), 5.01-5.3 (d, 4H, $2CH_2$), 5.5 (s, 1H, CH), 6.9-7.5 (m, 14H, Ph); i.r. (CH_2Cl_2) 3400-3600 (OH), 2100 (N_3), 1770 (β-lactam), 1750 (ester) cm^{-1}.

53c was obtained by the same purification method as 53b in 53% yield. N.m.r. ($CDCl_3$) δ 3.4-3.8 (b, 4H, CH-CH_2OH, exchanged with D_2O), 4.3-4.5 (d, 1H, CH-N_3, J = 5 Hz), 5.0-5.2 (m, 6H, $3CH_2$), 5.71 (s, 1H, CH), 6.8-7.6 (m, 18H, Ph); i.r. was identical to that of 53b.

52c was obtained by the same purification method as 52b in 35% yield. N.m.r. ($CDCl_3$) δ 2.2-2.4 (b, 1H, OH exchanged with D_2O), 3.1-3.25 (d, 2H, CH_2O, J = 6 Hz), 3.8-4.2 (m, 1H, CH-CH_2O), 4.8-4.98 (d, 1H, CH-N_3, J = 5 Hz), 5.1-5.3 (m, 6H, $3CH_2$), 5.7 (s, 1H, CH), 7.0-7.7 (m, 18H, Ph); i.r. was identical to that of 52b.

53a was obtained by the same purification method as 53b in 40% yield. N.m.r. ($CDCl_3$) δ 3.4-3.8 (b, 4H, CH-CH_2OH exchanged with D_2O), 4.4-4.6 (d, 1H, CH-N_3, J = 5 Hz), 5.8 (s, 1H, CH), 7.4 (d, 10H, Ph); i.r. was identical to that

of 53b.

52a was obtained by the same purification method as 52b in 40% yield. N.m.r. ($CDCl_3$) δ 2.2-2.4 (b, 1H, OH exchanged with D_2O), 3.2-3.37 (d, 2H, CH_2O, J = 6 Hz), 4.0-4.3 (m, 1H, $C\underline{H}-CH_2O$), 4.75-4.9 (d, 1H, $C\underline{H}-N_3$, J = 5 Hz), 5.5 (s, 1H, CH), 7.4 (d, 10H, Ph); i.r. was identical to that of 52b.

54a, 54b and 54c were obtained by the same purification method as 53b in 50% yield. Their n.m.r. were similar to that of 53b except for their ester variation and variations due to aromatic substituents. Their i.r. spectra were identical to that of 53b.

55a, 55b and 55c were obtained by the same purification method as 52b in 40% yield. Their n.m.r. were similar to that of the 52b except for their ester variation and variations due to aromatic substituents. Their i.r. spectra were identical to that of 52b.

Mesylates 56a and 57a

To β-lactam 52a (0.366 g, 1 mmole) and NEt_3 (0.101 g, 1 mmole) in 50 ml dry CH_2Cl_2 was added dropwise at -20° (dry ice-CCl_4) a solution of mesyl chloride (0.115 g, 1 mmole) in 10 ml dry CH_2Cl_2 over a period of 5 mins. The solution was stirred for 30 mins and evaporated to dryness. The residue was dissolved in ether, filtered and evaporated to give 0.4 g (90%) of crude product. Chromatography on silica gel using $CHCl_3$ as eluent gave 57a as an oil in 80% yield. N.m.r. ($CDCl_3$) δ 2.65 (s, 3H, CH_3), 3.62-3.82 (t, 2H, CH_2O, $J_1 = 6$ Hz, $J_2 = 11$ Hz), 4.2-4.5 (m, 1H, C<u>H</u>-CH_2O), 4.8-4.96 (d, 1H, C<u>H</u>-N_3, J = 5 Hz), 5.2 (s, 2H, CH_2), 5.51 (s, 1H, CH), 7.38 (d, 10H, Ph); i.r. (CH_2Cl_2) 2100 (N_3), 1780 (β-lactam), 1750 (ester) cm^{-1}.

56a was obtained by the same method in 80% yield. N.m.r. ($CDCl_3$) δ 2.85 (s, 3H, CH_3), 3.62-3.82 (t, 2H, CH_2O, $J_1 = 6$ Hz, $J_2 = 11$ Hz), 4.25-4.5 (m, 1H, CH-CH_2O), 4.6-4.78 (d, 1H, <u>CH</u>-N_3, J = 5 Hz), 5.19 (s, 2H, CH_2), 5.6 (s, 1H, CH), 7.4 (d, 10H, Ph); i.r. was identical to that of 57a.

Isomerization of mesylates 56a and 57a

56a or 57a was refluxed with 3 equivalents pyridine in dry benzene and gave 56a/57a (1:1) quantitatively, as determined by n.m.r.

Chlorosulfites 62a/63a

To a mixture of β-lactams 53a/52a (0.183 g, 0.5 mmole) in 40 ml dry benzene was added thionyl chloride (0.36 g, 3 mmole). The solution was refluxed for 90 mins. and evaporated to dryness to give 62a/63a quantitatively. N.m.r. ($CDCl_3$) δ 3.8 (m, 2H, CH_2O), 4.4-4.8 (m, 2H, $C\underline{H}-C\underline{H}-N_3$), 5.2 (s, 2H, CH_2), 5.59-5.60 (d, 1H, CH), 7.38 (d, 10H, Ph).

62a/63a was treated with water and extracted with ether to give quantitatively 53a/52a.

Chloroesters 58b,c/59b,c and 60a-c/61a-c

All β-lactams were obtained in an identical manner in approximately 70% yield. Their spectra were similar except for the ester variations and variations due to aromatic substituents. All their mass spectra showed M^+.

The following is a representative procedure:

To β-lactams 53b/52b (1 g, 0.002 mole) in 60 ml dry benzene was added pyridine (0.474 g, 0.006 mole). The solution was brought to reflux and thionyl chloride (0.6 g, 0.004 mole) was added. After 2 hrs it was cooled and 50 ml of ether was added. The solution was washed with water, dried over $MgSO_4$, and evaporated to give 0.8 g (85%) of 60/61b as an oil. N.m.r. ($CDCl_3$) δ 3.8-4.1 (b, 3H, $C\underline{H}$-$C\underline{H_2}Cl$), 4.7 (d, 1H, $C\underline{H}N_3$, J = 5 Hz), 5.13-5.23 (d, 4H, $2CH_2$), 5.6 (s, 1H, CH), 7.0-7.6 (m, 14H, Ph); i.r. (CH_2Cl_2) 2100 (N_3), 1760 (β-lactam), 1750 (ester) cm^{-1}; m/e 490-492 (M^+). By this method about 10% 66b was obtained as ascertained by n.m.r.

β-Lactams 66z(a-c) and 66a-c

All β-lactams were obtained in an identical manner in approximately 70-80% yield using method I and 50% yield using method II. Their spectra were similar except for the ester variations and variations due to aromatic substituents. All their mass spectra showed $M^+-(N_3CH=C=O)$, except for 66c in which case no good mass spectrum was obtained.

The following is a representative procedure:

Method I:

To β-lactam 52b (1 g, 0.002 mole) in 60 ml dry benzene was added pyridine (0.474 g, 0.006 mole). Thionyl chloride (0.6 g, 0.004 mole) was added. The solution was kept at 70-3° (bath temperature) for 7 hrs. Then it was cooled, diluted with 50 ml of ether and washed with water, 5% aqueous HCl, dried over $MgSO_4$ and evaporated to give 90% of crude product. Chromatography on silica gel and elution with CH_2Cl_2 gave first 15% of 60b/61b, as ascertained by n.m.r. and then 0.75 g (80%) 66b as an oil. N.m.r. ($CDCl_3$) δ 2.7-3.4 (m, 2H, CH-$\underline{CH_2}$Ph), 4.3-4.6 (m, 1H, $\underline{CH}$-CH_2Ph), 4.81-5.0 (d, 1H, $\underline{CH}N_3$, J = 5 Hz), 5.1-5.3 (d, 4H, $2CH_2$), 6.8-7.5 (m, 13H, Ph); i.r. (CH_2Cl_2) 2100 (N_3), 1775 (β-lactam), 1750 (ester) cm^{-1}; m/e 371 (M^+-$N_3CH=C=O$).

Method II:

Chloro compounds 60b/61b (0.8 g, 0.0016 mole) and AgOAc (4 eq.) in 60 ml dry CH_3CN were refluxed for 2 hrs. Then it was filtered and evaporated to dryness. The residue was

dissolved in 50 ml ether and after filtration was evaporated to give 0.7 g product containing ∿ 50% 66b and ∿ 50% starting material as ascertained by n.m.r. These compounds have about the same polarity and could be separated with difficulty only by column chromatography using silica gel and eluted with CH_2Cl_2.

β-Lactams 65a 67, 64a and 66a

β-Lactam 52a (0.366 g, 0.001 mole) in 60 ml dry benzene containing thionyl chloride (0.36 g, 0.003 mole) was refluxed for 90 mins. Silver acetate (4 eq.) was then added and refluxing continued for 2 hrs. Then it was filtered and evaporated to dryness. The residue was dissolved in 40 ml ether and after filtration was evaporated to give 0.3 g product containing ∿ 80% 65a and ∿ 10% 67 as ascertained by n.m.r. purification by column chromatography using silica gel and elution with CH_2Cl_2 gave ∿ 4% of 67 contaminated with a trace of 65a as an oil, and ∿ 75% of 65a contaminated with a trace of 67 as an oil.

65a, n.m.r. ($CDCl_3$) δ 1.98 (s, 3H, CH_3), 3.78 (d, 2H, CH_2O), 4.4 (m, 1H, $\underline{CH}-CH_2O$), 4.8-4.91 (d, 1H, $\underline{CH}-N_3$, J = 5 Hz), 5.23 (s, 2H, CH_2), 5.6 (s, 1H, CH), 7.4 (s, 10H, Ph); i.r. (CH_2Cl), 2100 (N_3), 1760 (β-lactam), 1740-1750 (ester) cm^{-1}.

67, n.m.r. ($CDCl_3$) δ 3.2-3.9 (m, 2H, $CH-\underline{CH_2}Ph$), 4.4 (m, 1H, $\underline{CH}CH_2Ph$), 4.83-4.97 (d, 1H, $\underline{CH}-N_3$, J = 5 Hz), 5.23 (s, 2H, CH_2), 5.58 (s, 1H, CH), 7.4 (s, 9H, Ph); i.r. (CH_2Cl_2) 2100 (N_3), 1770 (β-lactam), 1750 (ester) cm^{-1}; m/e 348 (M^+).

Boiling 67, which contained approximately 5% 65a, and 2 equivalents pyridine in benzene for 7 hrs gave quantitatively 66a and a trace of an isomeric mixture of 65a/64a as ascertained by n.m.r.

β-Lactams 46b,c and 68b,c

All transformations were performed in an identical manner in approximately 75% yield. Their spectra were similar except for the ester variations and variations due to aromatic substituents. All their mass spectra showed M^+-($PhCH_2CONHCH$=C=O), except for 68c which gave a poor mass spectrum but satisfactory microanalysis results.

The following is a representative procedure:

To a solution of β-lactam 66c (1.5 g, 2.4 mmole) in 60 ml dry CH_2Cl_2 at 0° was added triethylamine (0.24 g, 2.4 mmole). A stream of H_2S gas was bubbled in for 20 mins. The solution was stirred for 2 hrs at room temperature. Evolution of nitrogen gas was observed. A stream of nitrogen gas was bubbled in for 20 mins, then was added 0.46 g (0.006 mole) pyridine, followed by dropwise addition of 0.36 g (0.0024 mole) phenyl acetyl chloride in 15 ml CH_2Cl_2. The solution was stirred for 2 hrs at 0-10°, washed with 5% aqueous, HCl, 5% aqueous $NaHCO_3$ and brine. It was then dried over $MgSO_4$, and evaporated to give quantitatively impure amide 68c, which was chromatographed on silica gel. Methylene chloride eluted impurities, and chloroform gave 1 g (90%) of β-lactam 68c as a foam. Recrystallization from absolute ethanol gave 0.88 g (80%) of 68c, m.p. 140-2°. N.m.r. ($CDCl_3$) δ 2.9-3.1 (m, 2H, CH-$\underline{CH_2}$Ph), 3.6 (s, 2H, CH_2CO), 4.2-4.41 (m, 1H, $\underline{CH}$-CH_2Ph), 5.1-5.2 (d, 6H, $3CH_2$), 5.21-5.59 (q, 1H, $\underline{CH}$-$NHCOCH_2Ph$, $J_1 = 5$ Hz, $J_2 = 10$ Hz), 5.6 (s, 1H, CH), 6.4-6.6 (d, 1H, NH), 6.9-7.6 (m, 22H, Ph); i.r. (CH_2Cl_2) 3350-3400 (NH), 1770 (β-lactam), 1750 (ester), 1670 (amide) cm^{-1}.

Calcd. for $C_{41}H_{36}N_2O_6$: %C 75.44, %H 5.56, %N 4.29; found: %C 75.45, %H 5.56, %N 4.17.

β-Lactams 69d and 69e

Both β-lactams 68b and 68c were submitted to identical hydrogenolysis procedures, and gave 69d and 69e in about 70% yield. Their spectra were similar except for variations due to aromatic substituents. Their mass spectra showed no M^+ but M^+-($PhCH_2CONHCH=C=O$) while their corresponding methyl esters 46d and 46e showed M^+.

The following is a representative procedure:

To β-lactam 68b (1 g, 0.0018 mole), in 50 ml methanol was added 10% Pd/c (0.5 g), and the mixture was hydrogenated at room temperature and 40 psi for 1.5 hrs. The solution was filtered and evaporated to give 0.5 g (72%) of 69d, m.p. 147-50° (dec.). N.m.r. ($CDCl_3$: DMSO-d_6: D_2O) 1:1:3 drops δ 2.7-3.4 (m, 2H, CH-$\underline{CH_2}$Ph), 3.61 (s, 2H, CH_2CO), 4.3 (m, 1H, $\underline{CH}$-CH_2Ph), 5.3-5.38 (d, 1H, $C\underline{H}$-$NDCOCH_2Ph$, J = 5 Hz in the absence of D_2O, q, 1H, J_1 = 5 Hz and J_2 = 10 Hz), 5.45 (s, 1H, CH), 6.9-7.4 (m, 8H, Ph); i.r. (KBr) 3600 ∿ 2700 (NH, OH, COOH), 1760 (β-lactam), 1720 (acid), 1660 (amide) cm^{-1}; m/e 191 (M^+-$PhCH_2CONHCH=C=O$). Treatment of 69d with CH_2N_2 gave 46d quantitatively as a foam; m/e 380 (M^+).

EXPERIMENTAL

CHAPTER 3

Azido alcohol 70 and 70A

β-Lactam 5b (4.68 g, 0.01 mole) in a mixture of 61 ml CH_2Cl_2 and 100 ml dry methanol was saturated with nitrogen gas at -78° (3 mins). Then a mixture of O_3 and N_2 gas was bubbled in until the KI starch paper showed excess ozone (60 mins). The excess ozone was removed by passing a stream of N_2 for 10 mins. The temperature was allowed to rise to -40°, at which time was added $NaBH_4$ (0.38 g, 0.01 mole). The temperature of the solution was permitted to rise to 25° over 2 hrs, following which 10 ml 10% aqueous HCl was added. The solution was evaporated to 50 ml, diluted with water and was extracted with ethyl acetate, washed with water, dried over Na_2SO_4, and evaporated to give crude product in quantitative yield.

A wash with a mixture of ether-hexane (1:14) removed benzyl alcohol, and an oily 1:1 mixture of diastereomers (3 g, 70%) of 70/70A was obtained as evidenced by n.m.r.

Chromatography on silica gel and elution with CH_2Cl_2 separated one of the diastereomers 70 in 30% yield as a foam. 70A was eluted with $CHCl_3$ in 30% yield as a foam.

Less polar diastereomer 70: n.m.r. ($CDCl_3$) δ 3.4-3:8 (b, 4H, C<u>H</u>-C<u>H</u>$_2$O<u>H</u>), 3.7 (s, 1H, CH_3), 4.4-4.6 (d, 1H, C<u>H</u>-N_3, J = 5 Hz), 5.1 (s, 2H, CH_2), 5.78 (s, 1H, CH), 6.9-7.55 (m, 9H, Ph); i.r. (CH_2Cl_2) 3300-3500 (OH), 2100 (N_3), 1770 (β-lactam), 1740 (ester) cm^{-1}.

More polar diastereomer 70A: n.m.r. ($CDCl_3$) δ 2.2-2.4 (b, 1H, OH exchanged with D_2O), 3.2-3.4 (b, 2H, CH_2O), 3.6 (s, 3H,

CH_3), 4.0-4.3 (m, 1H, C<u>H</u>-CH_2O), 4.75-4.9 (d, 1H, <u>CH</u>-N_3, J = 5 Hz), 5.3 (s, 2H, CH_2), 5.5 (s, 1H, CH), 6.9-7.5 (m, 9H, Ph); i.r. (CH_2Cl_2) 3400-3600 (OH), 2100 (N_3), 1770 (β-lactam), 1750 (ester) cm^{-1}. Mass spectra showed M^+, M^+-COOMe, M^+-($N_3CH=CH-CH_2OH$) and M^+-(N_3-CH=C=O) identical to that of the <u>70</u>.

β-Lactam 71

β-Lactam 70 (3.8 g, 0.01 mole) was dissolved in 60 ml methanol. Pd/C (0.4 g) was added, and the mixture was hydrogenated at room temperature and 40 psi for 1.5 hrs. The solution was then filtered and evaporated to give 2.6 g (90%) β-lactam 71, m.p. 110-3°; n.m.r. (DMSO-d_6) δ 3.2 (b, 2H, NH_2), 3.6 (s, 3H, CH_3), 3.8-4.1 (m, 4H, CH_2O and $C\underline{H}$-$CH_2O\underline{H}$), 4.6 (m, 1H, $C\underline{H}$-NH_2), 5.40 (s, 1H, CH), 6.6-7.4 (q, 5H, Ph and phenolic OH); i.r. (Nujol) 3000-3400 (OH, NH_2), 1760-1750 (β-lactam, ester).

71A was obtained by the same method in 90% yield, m.p. 100-3°; n.m.r. (DMSO-d_6) δ 3.2 (b, 2H, NH_2), 3.6 (s, 3H, CH_3), 3.8-4.1 (m, 4H, CH_2O and $C\underline{H}$-$CH_2O\underline{H}$), 4.4-4.6 (m, 1H, $C\underline{H}$-NH_2) 5.38 (s, 1H, CH), 6.6-7.4 (q, 5H, Ph and phenolic OH); i.r. was identical to that of 71.

β-Lactam 72

To a suspension of β-lactam 71 (2.6 g, 0.009 mole) in 50 ml dry CH_2Cl_2 containing triethylamine (1.01 g, 0.01 mole) was added dropwise within 5 mins trimethyl silyl chloride (1.09 g, 0.01 mole) dissolved in 15 ml dry CH_2Cl_2. The solution was stirred for 30 mins at room temperature, evaporated to dryness and diethyl ether was added. Filtration, and evaporation of the filtrate gave 3.4 g (88%) of 72 as an oil. N.m.r. ($CDCl_3$) δ 0.1-0.3 (d, 18H, 2[OSi(Me)$_3$]), 2.2-2.6, (b, 2H, NH_2), 3.8 (s, 3H, CH_3), 4.0-4.6 (m, 4H, CH-CH-CH_2-OSi), 5.4 (s, 1H, CH), 6.8-7.4 (q, 4H, Ph); i.r. (CH_2Cl_2) 3300-3400 (NH_2), 1750 (ester), 1770 (β-lactam).

72A was obtained by the same method in 85% yield. Its i.r. and n.m.r. spectra were identical to those of 72.

β-Lactam 74

β-Lactam 72 (0.21 g, 0.49 mmole) and acid 73 (0.2 g, 0.52 mmole) were dissolved in 40 ml dry CH_2Cl_2. EEDQ (0.13 g, 0.52 mmole) was added and the reaction mixture was stirred for 16 hrs at room temperature. The solution was washed with 10% aqueous HCl and 10% aqueous $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated to give 0.3 g (94%) crude product, which was chromatographed on silica gel using CH_2Cl_2 to remove all impurities. Elution with $CHCl_3$ gave 0.25 g (77.5%) of 74 as a foam. N.m.r. ($CDCl_3$) δ 1.4 (s, 9H, $-CMe_3$), 2.1-2.4 (m, 2H, CH_2), 3.7 (d, 6H, $2CH_3$), 4.0 (m, 5H, C<u>H</u>-C<u>H</u>$_2$O<u>H</u> and MeOOCC<u>H</u> NHR), 4.5 (t, 2H, CH_2OPh), 5.4-5.6 (m, 1H, C<u>H</u>-NHCOR, after D_2O exchange, J = 5 Hz), 5.65 (s, 1H, CH), 6.8-7.1 (m, 9H, Ph, NH, phenolic OH), 8.2-8.4 (d, 2H, aromatic, J = 8 Hz); i.r. ($CHCl_3$) 3200-3450 (OH, NH), 1750-1760 (ester, β-lactam), 1715 (t-BOC), 1670-1680 (amide, ketone) cm^{-1}.

74A was obtained by the same method in 80% yield. I.r. was identical to that of diastereomer 74. N.m.r. was identical to that of 74 except for benzylic methine proton which appeared at δ 5.5 p.p.m.

β-Lactam 75

β-Lactam 74 (0.25 g, 0.38 mmole) was dissolved in 20 ml methanol. 1% NaOH (10 ml) was added dropwise over a period of 10 mins. It was then stirred for 13 mins and acidified with HCl to pH 3. The methanol was evaporated and the aqueous solution was extracted with ethyl acetate. Drying ($MgSO_4$) and evaporation gave 0.2 g (80%) crude product as a foam, which was dissolved in 5% aqueous $NaHCO_3$ and after filtration was acidified with HCl to pH 3 to give 0.15 g (63%) 75, m.p. 165-7° (dec.). N.m.r. (acetone d_6-D_2O) δ 1.4 (s, 9H, $-CMe_3$), 2.18-2.4 (m, 2H, CH_2), 3.6-3.9 (m, 3H, $-C\underline{H}_2$-OD and DOOCC$\underline{H}$ NDR), 4.2-4.5 (m, 3H, C$\underline{H}$-CH_2OD and $C\underline{H}_2$OPh), 5.4 (d, 1H, C$\underline{H}$-NDCOR, J = 5 Hz), 5.6 (s, 1H, CH), 6.9-7.3 (m, 6H, Ph), 8.2-8.4 (d, 2H, aromatic, J = 8 Hz); i.r. (Nujol) 3100-3300 (OH, NH), 1740-1720 (β-lactam, acid, t-BOC), 1660 (amide, ketone).

75A was obtained by the same method in 67% yield, m.p. 158-60° (dec.); i.r. and n.m.r. were identical to those of 75.

β-Lactam 76

A solution of β-lactam 75 (0.2 g, 0.32 mmole) in 5 ml trifluoroacetic acid was stirred at room temperature for 2 hrs, then diethylether was added to give a white precipitate, which was filtered and washed three times with ether to give 0.15 g (90%) of 76, becoming brown at 185°, m.p. 226-20° (dec.). N.m.r. (D_2O-$NaHCO_3$) 2.0-2.4 (m, 2H, CH_2), 3.6-3.9 (b, 3H, CH_2OD and $^{-}OOCCHND_3$), 4.0-4.2 (m, 1H, CH-CH_2OD), 4.3-4.5 (t, 2H, $-CH_2OPh$), 5.4-5.5 (d, 1H, $CHND$-COR), 5.6 (s, 1H, CH), 6.9-7.4 (m, 6H, Ph), 8.2-8.4 (d, 2H, aromatic, J = 8 Hz); i.r. (Nujol) 3450 ∿ 3100 and 2500 ∿ 2700 (OH, NH_2, COOH), 1750-1730 (β-lactam, acid), 1660 (amide and ketone) cm^{-1}. λ_{max} (EtOH-H_2O), 226 nm (ε, 18500), 299 (15800).

76A was obtained by the same method in 92% yield, m.p. 185° (brown) 215-18° (dec.); i.r., n.m.r. and u.v. were identical to those of 76.

β-Lactam 77

β-Lactam 76 (0.15 g, 0.29 mmole) was suspended in 10 ml water. Hydroxylamine hydrochloride (0.1 g, 1.4 mmole) was added, then saturated aqueous solution of $NaHCO_3$ was added dropwise until pH 7 was reached. The solution was heated at 50° for 2 hrs. Then it was cooled and acidified to pH 3 with HCl, (the volume of solution was about 20 ml). The solution was poured into a column containing Resin XAD4. All inorganic salts were removed by water, and the compound was eluted with methanol to give 0.1 g (62%) 77, m.p. 184° (brown) 217-20° (dec.). N.m.r. (D_2O-$NaHCO_3$) δ 2.2-2.42 (2H, CH_2, m), 3.5-3.77 (b, 3H, C<u>H</u>-C<u>H</u>$_2$OD), 3.8-4.0 (t, 1H, DOOCC<u>H</u>ND_2, J = 6 Hz), 4.2-4.5 (t, 2H, CH_2O, J = 6 Hz), 5.3-5.5 (d, 1H, C<u>H</u>-NDCOR, J = 5 Hz), 5.6 (s, 1H, CH), 6.95-7.5 (m, 8H, Ph); i.r. (Nujol) 3400-3100 and 2500 ∿ 2700 (OH, NH, COOH), 1740-1730 (β-lactam, acid), 1660 (amide), 1610 cm^{-1}. λ_{max} (EtOH-H_2O) 220 nm (ε, 20500), 273 (14000), λ_{max} (EtOH-0.1 N NaOH), 245 nm (ε, 23000), 286 (11500).

77A was obtained by the same method in 65% yield, m.p. 180° (brown), 200-4° (dec.); i.r., n.m.r. and u.v. were identical to those of 77.

β-Lactam 80

β-Lactam 66b (0.5 g, 1 mmole) was dissolved in 50 ml dry CH_2Cl_2. Triethylamine (0.12 g, 1.2 mmole) was added at 0°. A stream of hydrogen sulfide gas was bubbled in for 15 mins. The solution was stirred at room temperature for 2 hrs. Evolution of nitrogen gas was observed. A stream of nitrogen gas was bubbled in for 15 mins. The mixture was washed with water (twice), dried and evaporated. The oily product was purified by column chromatography using silica gel, all impurities were eluted with CH_2Cl_2 and compound 80 (0.4 g, 90%) was eluted with $CHCl_3$ as an oil. N.m.r. ($CDCl_3$) δ 1.8-2 (b, 2H, NH_2, exchangeable with D_2O), 3.1 (d, 2H, CH_2 J = 6 Hz), 4.0-4.5 (m, 2H, -CH-CH-NH_2) 5.1-5.3 (d, 4H, $2CH_2Ph$), 5.6 (s, 1H, CH), 6.83-7.6 (m, 13H, Ph); i.r. (CH_2Cl_2) 3300-3350 (NH_2), 1750 (ester), 1770 (β-lactam).

β-Lactam 82

β-Lactam 80 (0.4 g, 0.9 mmole) and acid 81 (0.491 g, 1 mmole) were dissolved in 50 ml CH_2Cl_2. EEDQ (0.247 g, 1 mmole) was added and stirred for 16 hrs at room temperature. The solution was washed with 5% aqueous HCl and 5% aqueous $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated to give 0.9 g (99%) of crude 82 which was chromatographed on silica gel using CH_2Cl_2 to remove impurities. Elution with $CHCl_3$ gave 0.7 g (85%) of 82 as a foam. N.m.r. ($CDCl_3$) δ 2.39 (m, 2H, CH_2), 3.1 (m, 2H, $PhC\underline{H}_2$-CH), 3.9-4.15 (t, 2H, $C\underline{H}$-CH_2 and BnOOC-$C\underline{H}$-NHR, J = 6 Hz), 4.4-4.6 (t, 2H, CH_2OPh, J = 6 Hz), 5.1-5.3 (q, 8H, $4CH_2Ph$), 5.38-5.6 (q, 1H, $C\underline{H}$-NHCOR, J = 5 Hz), 5.62 (s, 1H, CH), 6.8-8.4 (q, 4H, Ph, J = 8 Hz), 7.0-7.4 (m, 25H, Ph, NH); i.r. (CH_2Cl_2) 3400 (NH), 1770 (β-lactam), 1745 (ester), 1725 (BnOCO), 1665 (amide, ketone) cm^{-1}.

β-Lactam 83

β-Lactam 82 (0.2 g, 0.22 mmole) was dissolved in 30 ml methanol. 10% Pd/C (0.1 g) was added, and the mixture was hydrogenated at room temperature and 40 psi for 40 mins. The solution was then filtered and evaporated to give 0.09 g (81%) of 83, m.p. 181 (brown), 200-5° (dec.). N.m.r. (D_2O-$NaHCO_3$) 2.38 (m, 2H, CH_2), 3.2 (m, 2H, Ph-C<u>H</u>$_2$CH), 3.6 (m, 1H, C<u>H</u>-CH_2), 3.8 (t, 1H, $^-OOCCHND_3^+$, J = 6 Hz), 4.2 (m, 2H, CH_2OPh), 5.3 (d, 1H, CH-NDCOR, J = 5 Hz), 5.4 (s, 1H, CH), 5.5-5.8 (d, 1H, CH-OD), 6.9-7.5 (m, 7H, Ph); i.r. (Nujol) 3400-3100 and 2500 ∿ 2700 (OH, NH, COOH), 1760 (β-lactam), 1720 (acid), 1660 (amide), 1610 cm^{-1}. λ_{max} (EtOH-H_2O), 225 nm (ε, 18000), 272 (2000).

β-Lactam 86

β-Lactam 82 (0.2 g, 0.22 mmole) was dissolved in a mixture of 5 ml ethanol and 5 ml pyridine. Hydroxylamine hydrochloride (0.2 g, 2.8 mmole) was added. The solution was warmed at 70° for 2 hrs. Then 50 ml chloroform was added and solution washed with 5% aqueous HCl. Then it was dried over Na_2SO_4, filtered and evaporated to give 0.2 g (90%) crude 86 which was purified by column chromatography using silica gel. Elution with $CHCl_3$ EtOAC (1:1) gave 0.15 g (71%) 86 as a foam. N.m.r. ($CDCl_3$) 2.2 (b, 2H, CH_2), 3.1 (m, 2H, Ph$\underline{CH}_2$CH) 3.8-4.0 (b, 3H, $\underline{CH}$-CH_2, BnOOC$\underline{CH}$NHCOR, OH), 4.3-4.6 (m, 2H, CH_2OPh) 5.0-5.2 (q, 8H, 4CH_2Ph), 5.3-5.5 (q, 1H, $\underline{CH}$-NHR, J = 5 Hz), 5.6 (s, 1H, CH), 6.6-7.4 (m, 29H, Ph and 2NH); i.r. (CH_2Cl_2) 3300-3450 (OH, NH), 1775 (β-lactam), 1750-1730 (ester and BnOCO), 1680 (amide), 1520 (oxime) cm^{-1}. λ_{max} (EtOH), 273 nm (λ, 15000).

β-Lactam 87

β-Lactam 86 (0.15 g, 0.16 mmole) was dissolved in 30 ml absolute ethanol. 10% Pd/C (0.075 g) was added, and the mixture was hydrogenated at room temperature and 35 psi for 40 mins. The solution was then filtered and evaporated to give 0.07 g (81%) of 87, m.p. 160° (brown), 186-9° (dec.). N.m.r. (D_2O-$NaHCO_3$) δ 2.4 (m, 2H, CH_2), 3.1 (m, 2H, $PhC\underline{H}_2CH$), 3.6 (m, 1H, $C\underline{H}$-CH_2), 4.0 (t, 1H, $^{-}OOCCHND_3^{+}$), 4.4 (t, 2H, CH_2OPh, J = 6 Hz), 5.3 (d, CH-NDR, J = 5 Hz), 5.5 (s, 1H, CH), 6.9-7.5 (m, 7H, Ph); i.r. (Nujol) 3400-3100 and 2500 ∿ 2700 (OH, COOH, NH), 1755 (β-lactam) 1720 (acid), 1660 (amide), 1610 cm^{-1}. λ_{max} (EtOH-H_2O), 221 nm (ε, 20800), 272 (14500), λ_{max} (EtOH-0.1N NaOH), 246 nm (ε, 23500), 286 (12000).

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