SOME ASPECTS OF THE SYNTHESES OF β-LACTAM ANTIBIOTICS AND D,L-2'-EPI-SHOWDOMYCIN

A Thesis

by

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Department of Chemistry
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Some Aspects of the Syntheses of β-lactam Antibiotics and D,L-2'-Epi-showdomycin

Youla Stratigoula Tsantrizos

Department of Chemistry
McGill Univeristy
Montreal, Quebec, Canada

ABSTRACT

Section I

D,L-cis-N-2'-hydroxy-5'-nitrophenyl-3-D-2"-amino-2"-phenylacetamido-4-hydroxymethyl-2-azetidinone (22) and 7-phenylacetamido-3'-diphenyl-t-butylsilyloxy-6'-nitro-benzo[3,4]-0-2-isocephem (35) were synthesized. They both exhibit high infrared absorption frequencies at 1760 (22) and 1790 cm^{-1} (35). The latter was too unstable to survive for the time required for biological testing, since it decomposed in dimethyl sulfoxide within 30 minutes.

Section II

The synthetic sequence leading to D,L-2'-epishowdomycin(III) was improved and several intermediates were characterized unambiguously for the first time. Enough crystalline III was prepared to allow for biological testing, which showed it to be inactive against a series of viruses.

Quelques aspects de la synthèse d'antibiotiques β-lactames et du D,L-2,épi-showdomycine

Youla Stratigoula Tsantrizos
Département de Chimie
Université McGill
Montréal, Québec, Canada

Résumé

Première Section

Les deux composés suivants ont été synthétisés: le D,L-cis-N-2'-hydroxy-5'-nitrophényle-3-D-2"-amino-2"-phénylacétamido-4-hydroxymethyle-2-azétidinone (22) et le 7-phénylacetamido-3'-diphényl-t-butylsilyloxy-6'-nitro-benzo-[3,4]-O-2-isocépheme (35). Les deux possèdent de hautes fréquences d'absorption dans l'infrarouge, à 1760 cm^{-1} pour 22 et à 1790 cm^{-1} pour 35. Ce dernier est trop instable pour survivre le temps requis pour effectuer les tests biologiques, puisqu'il se décompose en moins de trente minutes, dans le diméthyl sulfoxide.

Deuxième Section

La séquence synthétique menant au D,L-2-épi-showdomycine (III) a été améliorée, et plusieurs intermédiaires ont été caractérisés sans ambiguités pour la première fois. Un échantillon de III, suffisant aux tests biologique, n'a démontré aucune activité contre une série de virus.

To Dr. G. Just and A. Ugolini for their continuous encouragement and advice, but most of all their moral support.

Acknowledgements

I would like to thank my parents, who have always done everything for me.

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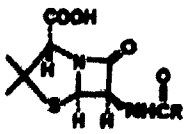
Table of Contents

SECTION I

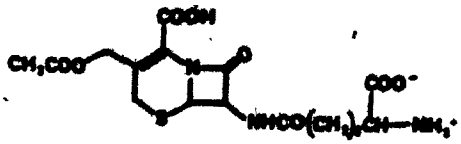
INTRODUCTION

Synthetic Studies of β-lactams

The discovery that the important antibiotics, penicillin[40] (1) and cephalosporin[41,42] (2), contain a β-lactam ring (3) in their structure, has provided a continuing impetus for research on β-lactams in many industrial laboratories and academic institutions.



(1)



(2)

(3)

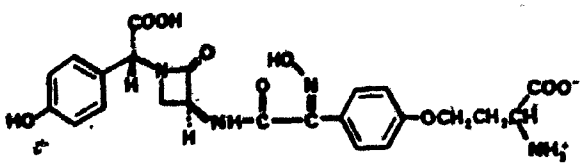
No reduction of activity in this challenging field appears in sight, because penicillins and cephalosporins have been the drugs of choice for treating bacterial infections in man.

The earliest reference to a synthesis of β-lactams appears to have been made by Einhorn[1] in 1883, and he was followed by other scientists making similar claims. The first authentic

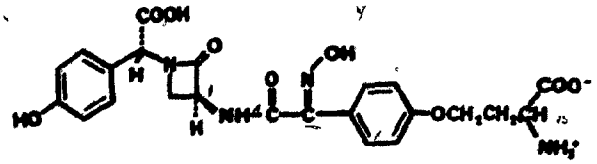
synthesis of β-lactams, however, is considered to have been achieved by Staudinger[2] in 1907. The enormous amount of research that followed the establishment of the structure of cephalosporin[12] in 1961 has recently produced three new types of β-lactam antibiotics:

Nocardicin A and B[3-6,39]; Thienamycin[7] and related compounds PS-5[8], MM4550 (MC696-SY2-A), MM13902[9,10]; and Clavulanic Acid.[11] They all show specific and interesting antimicrobial activity.

Nocardicin

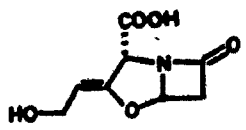


A

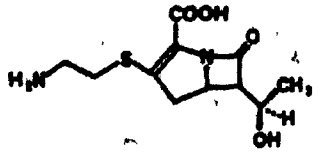


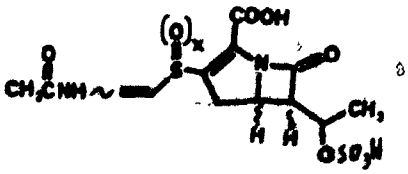
B

Clavulanic acid



Thienamycin





X=1 MM4550(MC696-SY2-A

X=0 MM13902

Mode of action of the β-lactam antibiotics

The bacterial cell-wall, peptidoglycan, is believed to be the site of attack of β-lactam antibiotics. Peptidoglycan, which is a complex polymer, is entirely absent in mammals which can well explain the very low toxicity, to man, of these drugs. The cell wall is synthesized in three major stages which are briefly outlined in Figure 1.

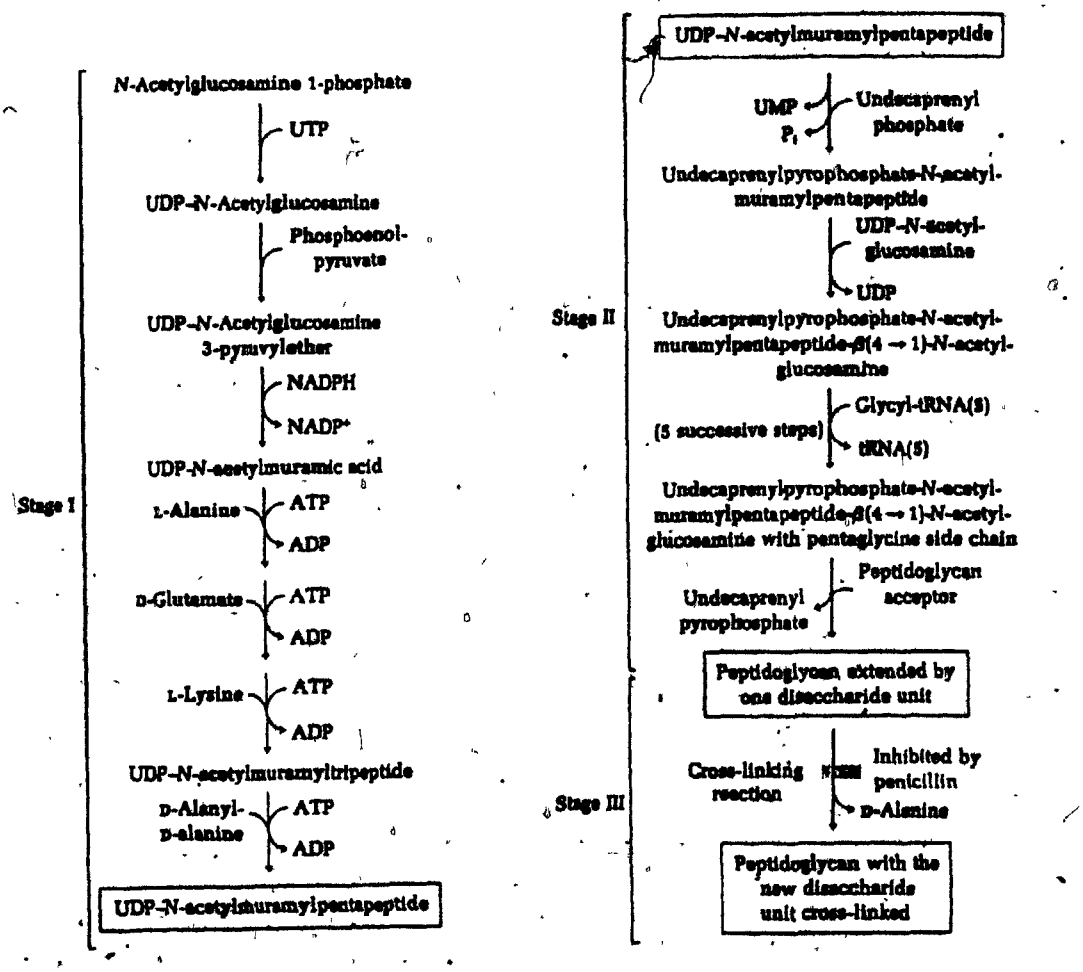


Figure 1: Steps in the biosynthesis of a bacterial cell-wall.

In the last stage of cell-wall synthesis, the cross linkage between parallel peptidoglycan chains is established through a transpeptidation reaction (Fig. 2), in which the amino terminal of a glycine residue, of the cross-linking chain, displaces the carboxyl terminal of D-alanine from the end of the pentapeptide side chain. β-Lactams are thought to inhibit this last step. Incomplete synthesis of the giant macromolecule composing the cell wall, which envelops the bacterial organism and supports its cell membrane, would result in lysis, caused by the difference in osmolarity between cell cytoplasm and culture medium.

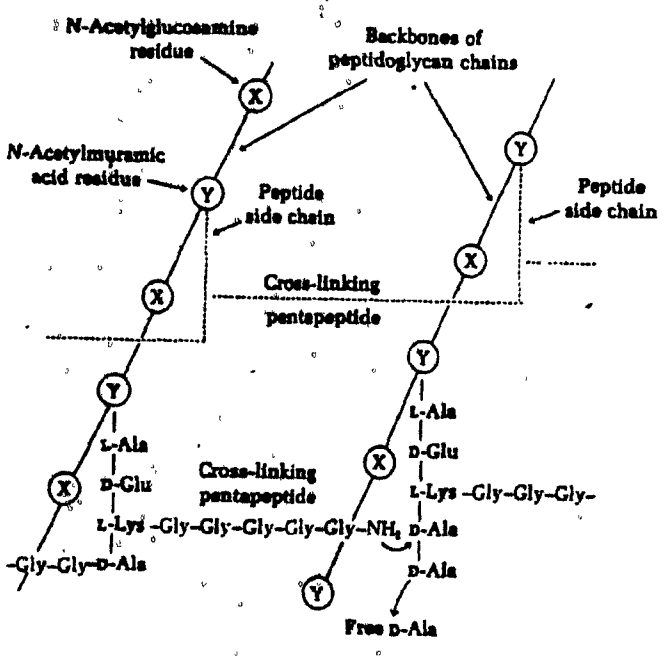


Figure 2: Completion of a cross-link between two adjacent peptidoglycan chains.

Recent studies have shown that the interaction of β-lactam antibiotics with bacterial cells is very complex[13,14]. Bacteria contain a large number of enzymes (transpeptidases, carboxypeptidases, endopeptidases) which are the targets for the β-lactam antibiotics. The affinity of these enzyme for the β-lactam antibiotics varies, and since each enzymes has a distinct function in the biosynthesis of the cell wall, each antibiotic produces characteristic effects on the shape and dimension of the bacteria. Even though inhibition of the above mentioned penicillin binding proteins varies, they all result in cell death. The way in which a particular β-lactam antibiotic kills bacteria depends on the affinity of each of the target enzymes for the antibiotic, and the function of these enzymes in peptidoglycan synthesis. Inhibition may also trigger autolytic enzymes which destroy the cell[15].

Bacterial resistance to pencillins and cephalosporins[16]

Since no species of bacteria is homogeneous, the few mutants which are less sensitive to the particular β-lactam antibiotic used, can survive during the treatment of an infection, to produce a resistant strain. In these types of bacteria three possible biological changes explain their resistance: slight change of the target enzyme, which lowers the affinity of a given β-lactam antibiotic, decrease permeability of the cell wall, or production of certain proteins, β-lactamases, which destroy the antibiotic before it reaches the target enyzmes.

Synthesis of β-lactam antibiotics

(a) Structure requirement

Until recently, it was believed that for the development of an active antibiotic, the following structural features were necessary:

(I) A cis fused β-lactam ring

(II) An acylamino or a formamidine (-N-C=N-) side chain.

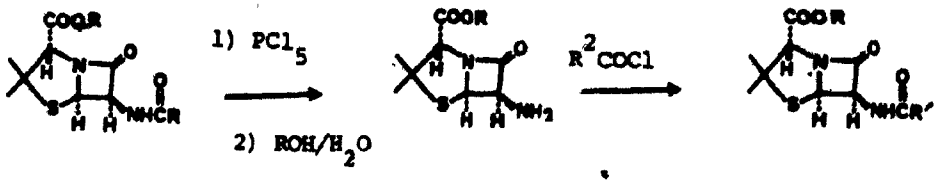
(III) A β-lactam I.R. frequency higher than 1765 cm^{-1}.

(IV) An appropriately placed carboxylic acid function, which however may be replaced by a phosphonic acid group.

Since the isolation of Thienamycin, which has a trans-fused ring and a hydroxyethyl side chain, and Norcardicin A, which has a very low β-lactam frequency (1720 cm^{-1}) the criteria listed above cannot be considered an absolute necessity.

(b) Production of commercial β-lactam antibiotics

Presently all the β-lactam antibiotics used in the treatment of infections, are obtained through fermentation or semi-synthetic procedures. Addition of the appropriately substituted acetic acid to the fermentation broth yields approximately one hundred of the common penicillins. All the others are obtained through acylation of 6-aminopenicillanic acid (6-APA).



6-APA

The cephalosporins are also obtained either through fermentation or partial synthesis from penicillin, and similar semi-synthetic procedures.

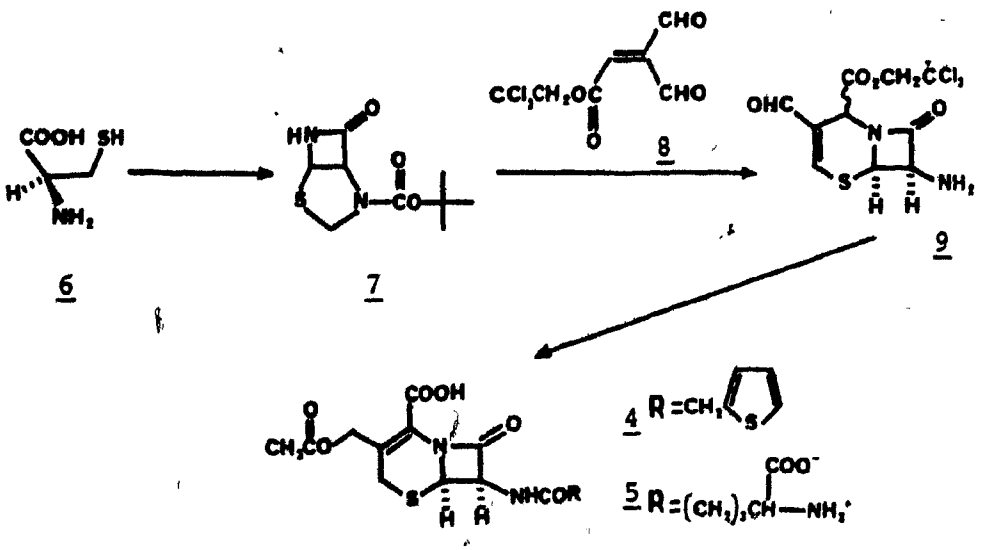
This success in manipulating microorganisms to produce "unnatural" penicillins and cephalosporins is remarkable in view of the fact that the details of their biosynthesis are still not known after decades of biochemical research. The involvement of a tripeptide (L-valine, L-cysteine, L-amino-adipic acid) seems to be very likely, although intermediates between it and penicillin have never been isolated.

(c) Total synthesis

Total synthesis allows for the formation of many novel structures which could be used in the search for better β-lactam antibiotics.

The first synthesis of a penicillin was reported by Sheehan[20], and in 1965 Woodward[21] announced the first succesful

stereospecific synthesis of cephalosporin C (4) and cephalothin (5).

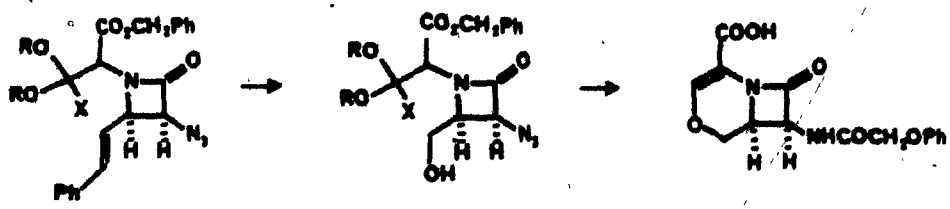


In this ingenious synthesis, L-cysteine 6 was converted to a key intermediate 7. Subsequent reactions with dialdehyde 8 gave 9 and several other manipulations transformed it to 4 and 5.

Since then other approaches to the synthesis of penicillins and cephalosporins have been reported. Bose[22] and co-workers have developed one of the most useful and versatile routes for β-lactam formation, using the reaction of imines with acid chlorides.

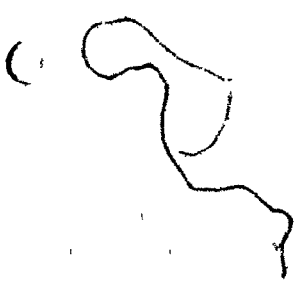
Unfortunately the products from this approach generally give trans-isomers, which must be isomerized[23] to the desired cis-isomer by a low yield isomerization procedure.

Bristol Laboratories[24] later reported one of the most versatile and reliable methods for generating cis-β-lactams such as 10 from the key intermediate E.

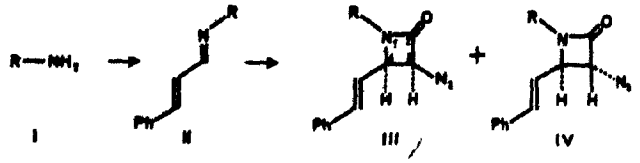


E

10



The formation of the cis-β-lactam ring involves addition of azidoacetyl chloride to a mixture of cinnamylidene Schiff base and triethylamine in methylene chloride. We have used this method successfully to synthesize a number of substituted cis-β-lactams in high yield, but we have also found[38] that even this approach is not free of limitations. As it is shown below the % yield of cis and trans isomers obtained greatly depends on the pk_A of the amine used to prepare the Schiff base, and a Schiff base derived from o-trialkylsilyloxy-p-nitroaniline gives trans-β-lactam only.



R = 	pK_A	yield cis isolated (by NMR)	yield trans isolated (by NMR)
a) X=Y=H	4.6[d]	50%(90%), mp 121-125	
b) X=pCl, Y=H	3.98[d]	40%(90%), mp 121-121.5	
c) X=o-CH_2OTBDMS, Y=H	>1[a]	70%(95%), mp 85.5-86	
d) X=o-OTBDMS, Y=H	>1[a]	60%(90%), mp 84-85	
e) X=o-CH_2OTBDMS, Y=o'-TBDMS	>1[a]	65%(80%)	
f) X=Y=o-OTBDMS	>1[a]	40%(>70%), mp 95-96	
g) X=NO_2, Y=H	2.46[d]	80%	
h) X=o-OMe, Y=p-COOMe	2.4[b]	85%	
i) X=p-Cl, Y=o-Cl	2.05[d]	(60%)	(15%)
j) X=o-COOH, Y=o'-OMe	2.0[c]	(15%)	(15%)
k) X=o-COOH, Y=H	2.2[c]		(30%), (30%, THF)
l) X=o-TBDMS, Y=p-NO_2	<1.5[a]		(2%), in THF: 50%(70%)
m) X=p-NO_2, Y=H	1[d]	(50%)	(25%)
n) X=p-NO_2, Y=o-CH_3	~1[a]	(5%)	(50%)

TBDMS = tert-butyldimethylsilyl

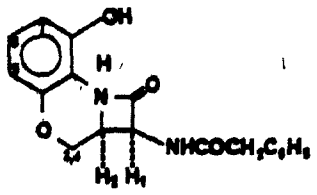
a = estimated, b = measured, c = measured on corresponding methyl esters
d = from literature[3]

Aim of project

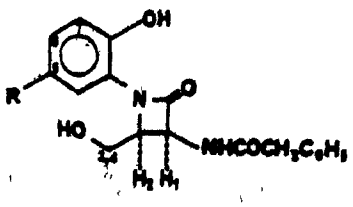
During the last few years we have been interested in the synthesis of penicillin and cephalosporin analogues, having a cis-fused β-lactam ring with a high frequency infrared absorption, an acylamino side chain and a modified acidic function.

Two of the first compounds of this type, synthesized by Mr. A. Ugolini, were analogues I and X, which have a simple acylamino side chain and a phenolic instead of the carboxylic group found in naturally occurring β-lactam antibiotics.

Biological studies on analogue I showed weak antibacterial properties against a number of organisms whereas Xa and Xb showed no biological activity.



I



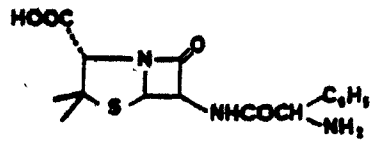
X a) R=H
b) R=NO_2

With the encouragement of these biological results and hoping to synthesize a substantially more active β-lactam antibiotic, it was decided to modify the structure of I in the following way:

a) introduction of an acylamino side-chain which is well known for its biological properties,

and b) increase of the β-lactam ring strain by attaching an electron-withdrawing substituent on the benzene ring.

In their search for penicillin derivatives which would be effective against Gram-negative organisms, when administered orally, Doyle, Fosker, Nayler and Smith[29] synthesized in 1962 Ampicillin III which proved to be active against most bacteria[30].

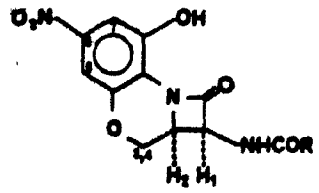


III

Its effectiveness was attributed to its satisfactory absorption, which was thought to be in part due to its unusual stability towards acids. This group prepared numerous other penicillins, which all contained amino-substituted side-chains. They found that the presence of the α-amino-substituent always led to penicillins with marked stability to acids, but this was lost when the substituent was remote from the amide linkage. Because the acylamino side chain in Ampicillin (III) fulfills our requirement (a), it was used to prepare another derivative

of Xb, analogue 22.* This type of compound could, in principle, also serve as a model for cyclization studies. In any event, for reasons which will become apparent in chapter 1, no model studies for cyclization were carried out.

Fulfillment of our second requirement, ideally, could be achieved by introduction of a nitro group para to the β-lactam ring (50).



50

Direct nitration of compound I would theoretically give 50 as one of its products. Preliminary nitration results on I, indicated that three isomers were formed, and that they were unstable and difficult to separate.

We therefore focussed our attention on an alternate route, which involved the formation of a nitroaminoresorcinol, its transformation to a Schiff base, and condensation which azidoacetyl chloride to give β-lactam 51, which is a potential precurser to compound 50 or one of its positional isomers.

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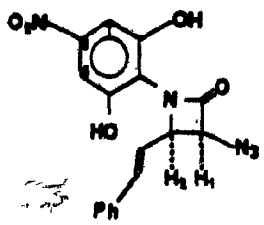
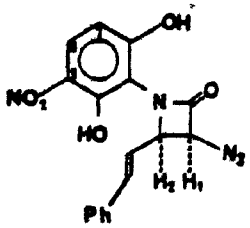
*for analogue 22, see page 17



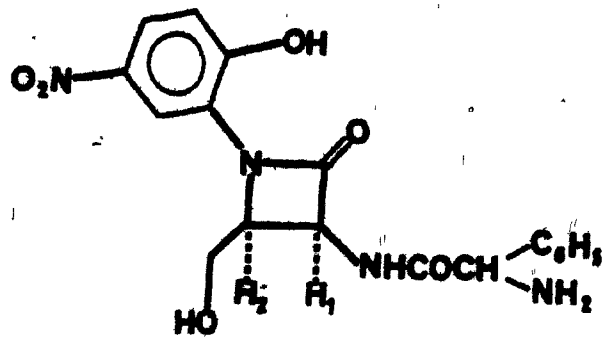
51a



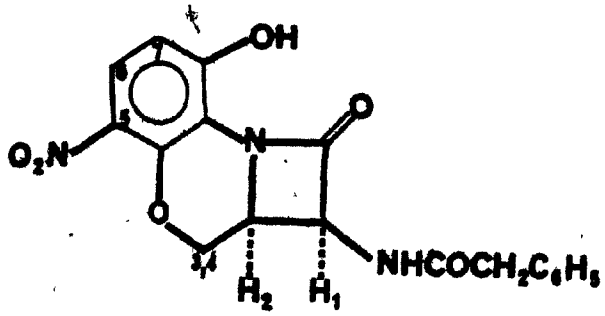
51b

Since the cinnamylidene Schiff base of 4-nitro-2,6-dihydroxyaniline would be expected to give mainly trans-fused β-lactam, we first investigated the reactions of the readily available 5-nitro-2-hydroxyaniline, expecting to extend the procedures developed to the less accessible 3-nitro-2,6-dihydroxy aniline.

As will be discussed in chapters 1 and 2, experimental difficulties prevented us from carrying out our original project, we therefore returned to the first mentioned approach of direct nitration of I.



22



35

The following two chapters describe the successful syntheses of 22 and 35. These analogues along with their precursors were biologically tested, in the hope that introduction of the proper functionalities would improve the antibacterial properties of our initial compound I.

DISCUSSION

CHAPTER 1

Synthesis of β-lactam 22

The synthesis of compounds with the general structure 14 (R = trialkylsilyl), was accomplished using procedures developed in our laboratory[26,27,28,33] as outlined in Figure 4.

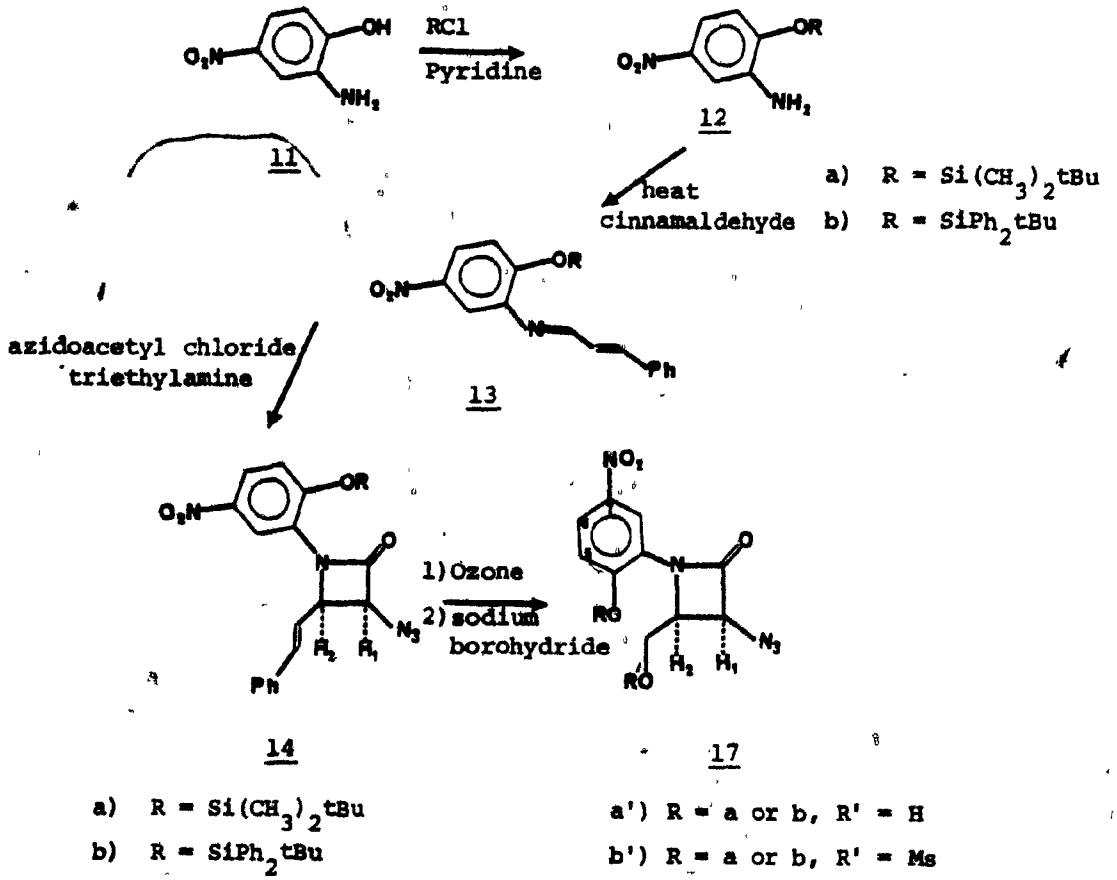


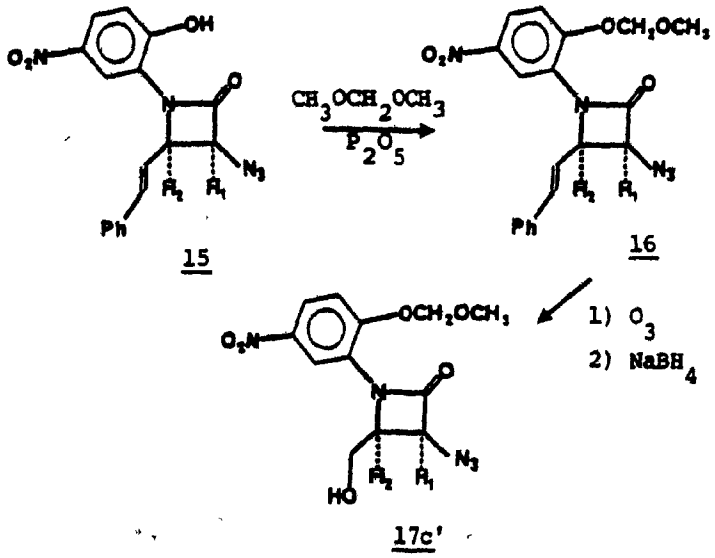
Figure 4: Synthesis of β-lactam 14

We then decided to ozonolyze the cinnamylidene group of 14 and reduce the resulting ozonide with sodium borohydride to the alcohol 17a' in order to proceed with the fluoride induced

cyclization of the mesylate 17b', of alcohol 17a' (Chapter 2). Several attempts, using different experimental conditions, to ozonolyze and reduce 14a were unsuccessful giving very small amounts of 17a' and a mixture of side products, one of them being dimethyl-t-butylsilanol. The different experimental conditions involved such variations as solvent mixture and temperature i.e. (1:3 or 2:1) methylene chloride: isopropanol at -78° and (4:1.5 or 1:1) ethanol: methylene chloride at -78° and -60°.

The dimethyl-t-butylsilyl ether was apparently unstable under all the reaction conditions used, probably due to the p-nitro group which makes the phenolic group very acidic. We therefore repeated the reaction sequence outlined above, using the more stable diphenyl-t-butylsilyl ether 14b. Unfortunately, similar results were obtained and no pure alcohol 17a' (R = $SiPh_3$ t-butyl) could be synthesized.

We then chose to use a methoxymethyl ether as the protecting group. Since this group could not be introduced directly on the phenolic aniline 11 because of alkylation of the amine function, introduction of it was achieved after formation of β-lactam 14.

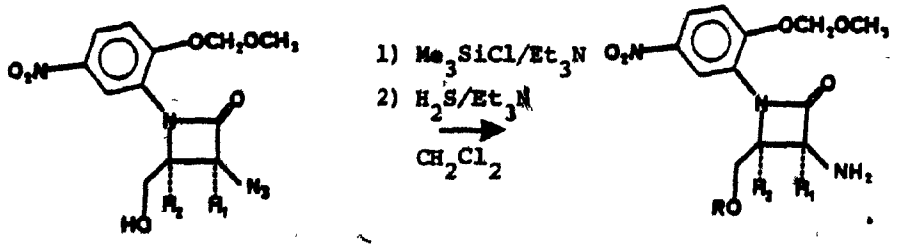


A solution of tetra-n-butylammonium fluoride was added to compound 14 (a or b) in tetrahydrofuran at 0° to give phenol 15, which was then reacted with methoxymethyl methyl ether and phosphorus pentoxide to give 16. The structure of compound 16 was consistent with its I.R. and its p.m.r. spectra, which showed along with all the appropriate signals, seen in compounds 14 and 15, a singlet (3H) at 3.4 p.p.m. due to the $-OCH_3$ group and another singlet (2H) at 5.3 p.p.m ($-OCH_2O-$). Ozonolysis of 16 in ethanol- methylene chloride (8:3) at -78° followed by borohydride reduction gave 17c' in an overall yield of 70%.

Since cyclization of 17c' would now be a cumbersome process, we decided to forego this step and continue the synthesis with the addition of the Ampicillin side chain. The free alcohol of 17c' seemed to be preferable to the large cinnamylidine group, since no naturally occurring β-lactam antibiotic has a large substituent at this position.

Reduction of 17c' with hydrogen sulphide/triethylamine[31] gave amino alcohol 18 in 84% yield.

To couple the side chain to the amine group, the free alcohol of 18 was protected with trimethylsilyl chloride/ triethylamine and compound 19 was obtained after evaporation of the methylene chloride and was further reacted without purification.

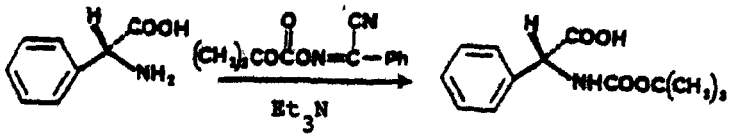


17c'

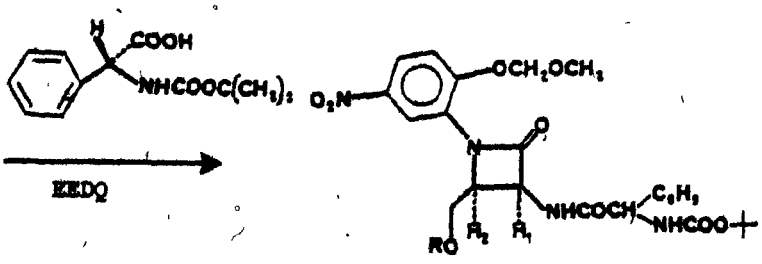
18 R = H

19 R = $Si(CH_3)_3$

The Ampicillin side chain was introduced as tBOC-phenyl glycine, obtained[32] from BOC-ON(V) and optically active D(-)phenyl-glycine as outlined below.



The transformation of the racemic amine 19 to the diastereomeric mixture of amide 20 was achieved through a slow coupling reaction (15hr) with optically active t-BOC-phenyl-glycine/ EEDQ[55] in methylene chloride.



20 R = $Si(CH_3)_3$

21 R = H

Compound 20 was obtained in 95% yield as a mixture of two diastereomers, as evidenced by thin layer chromatography behavior of some later derivatives (21,22), which showed up as elongated spots. Conventional chromatography could not be used to separate the two diastereomers of 20 since, not unexpectedly, they had almost the same R_f value.

Hoping that the diastereomers might be separable on a partially hydrolyzed mixture of diastereomers, we tried removing one protecting group at a time. We were only successful in obtaining desilylated alcohol 21, which can easily be made from 20 upon mild acid treatment.

However, the methoxymethyl and t-BOC protecting groups were found to be of almost equal stability to acid and therefore reaction conditions affecting one always affected the other as well. They were subsequently both cleanly removed with 80% trifluoroacetic acid to give nitrophenol 22.

Unfortunately, neither diastereomers of 21 or of 22 could be separated at either stage due to their similar physical properties.

A 90 MHz p.m.r. spectrum of 22 (figures 5,6) distinctly showed the presence of two diastereomers. The first spectrum (fig. 5) was recorded at 25° and the second (fig. 6) at 60°, where rotational freedom is achieved. The compound was dissolved in D_2O resulting in complete exchange of the amine, amide and hydroxyl protons. In both spectra the -COCH- proton showed as two peaks with a small change in the chemical shift

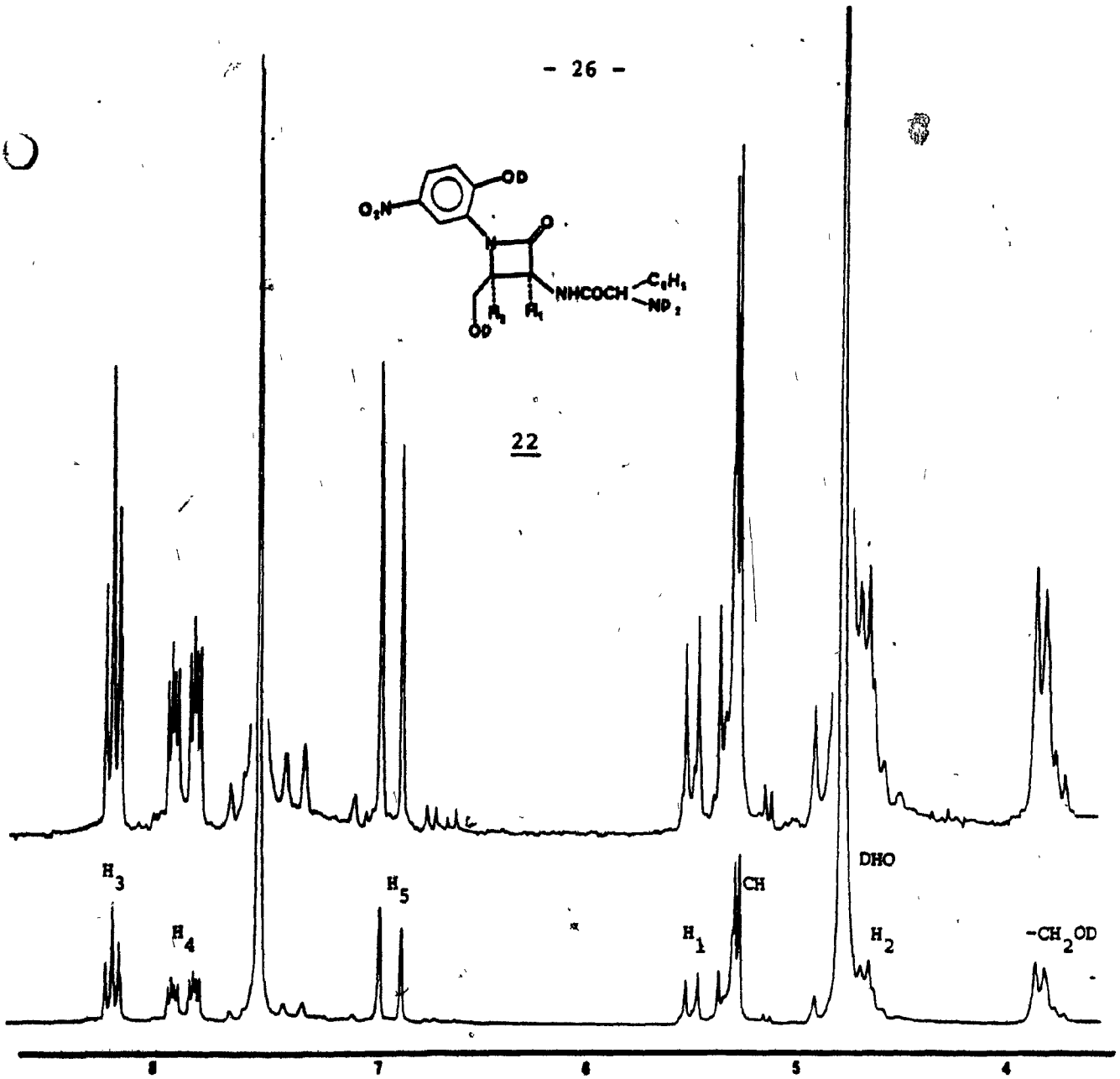


Figure 5: 90 MHz p.m.r. spectra of compound 22 in D_2O at 25°C.

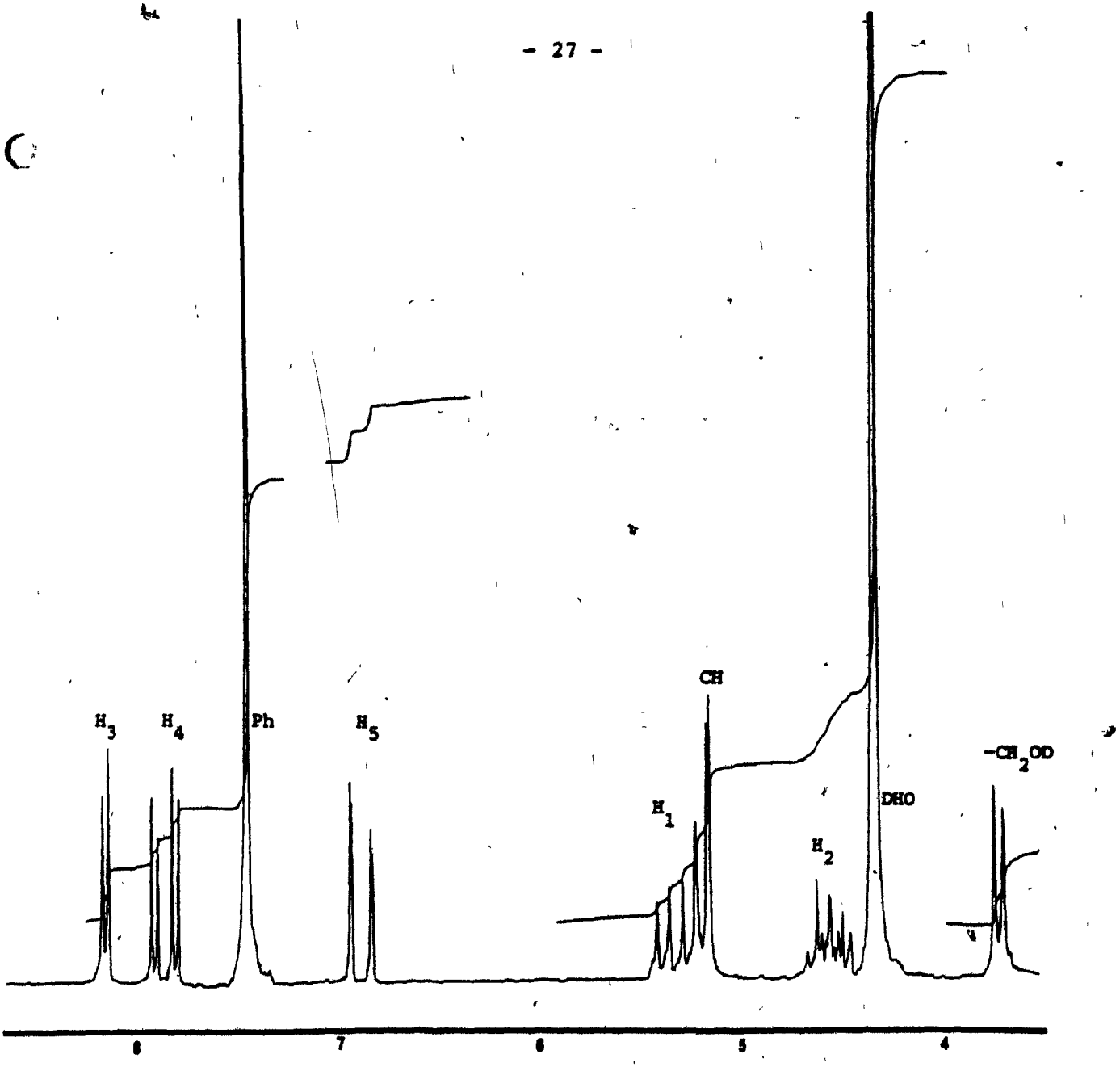
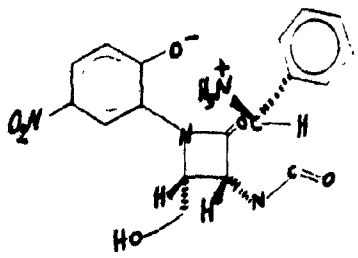


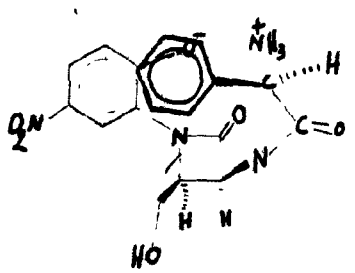
Figure 6: 90 MHz p.m.r. spectra of compound 22 in D_2O at 60°C.

difference between them, upon going from 25° to 60°, revealing the presence of two diastereomers. The largest effect is seen in the absorption of the aromatic protons H_3, H_4 and H_5, which appear as two distinct sets at 25°, merging to one at 60°. The rest of the protons show only broader and slightly more complex peaks at 25° compared to 60°.

Compound 22 is expected to exist as a zwitterion. Therefore strong ionic interactions are expected to take place between the phenoxide and the ammonium groups.



diastereomer I

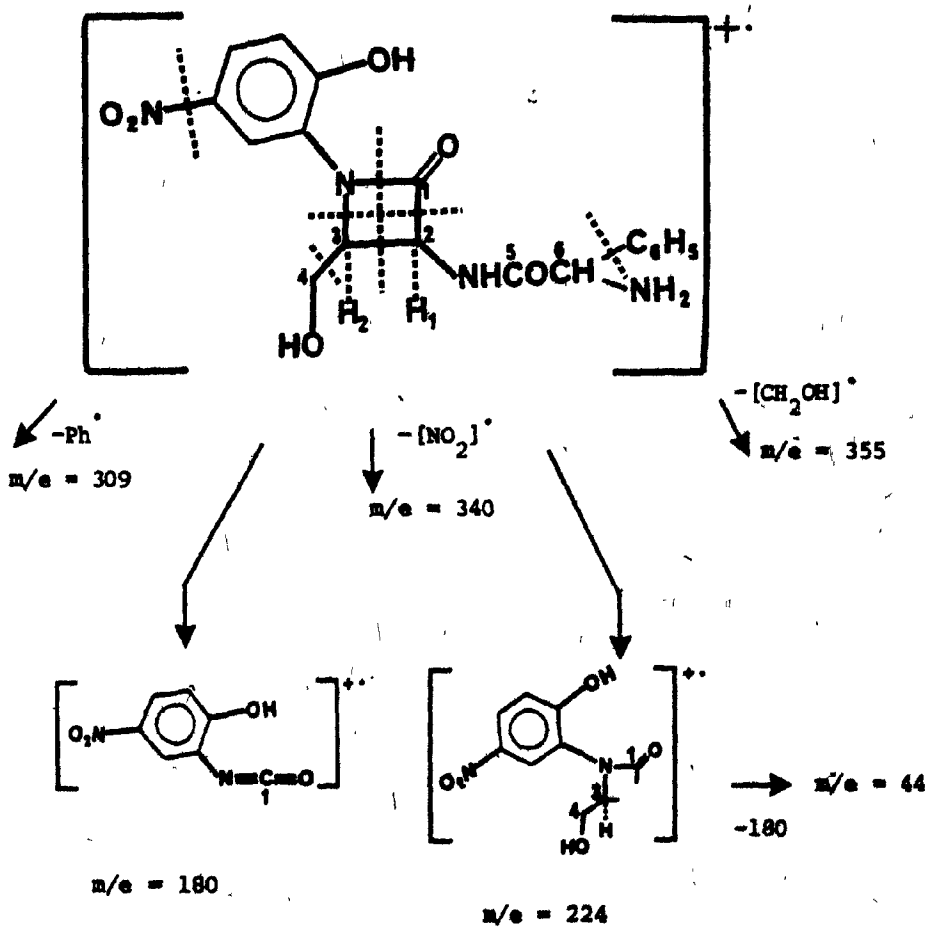


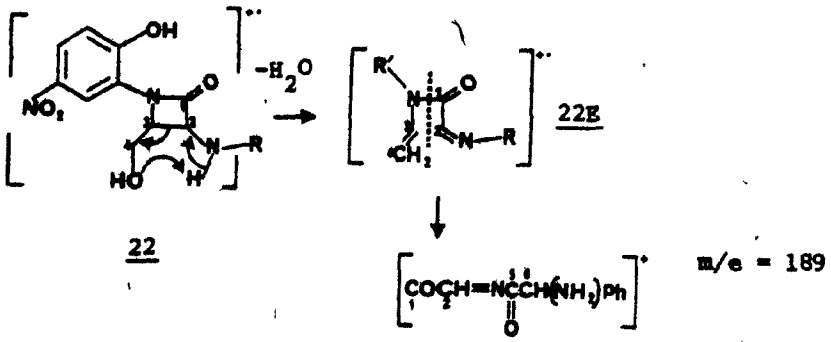
diastereomer II

Molecular models indicate that if these electrostatic interactions are operative in both diastereomers, in one case the two benzene rings are lined on top of one another whereas in the other isomer they are pointing away from each other. This may explain why the major differences between the two spectra lie in the aromatic protons. Of course, the possibility

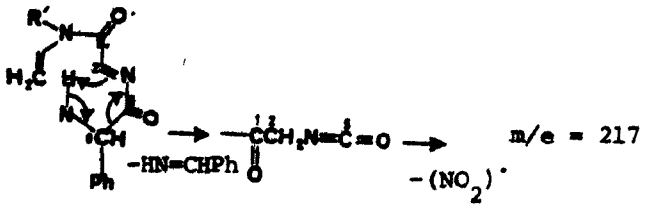
of a more complex explanation cannot be eliminated. It is important though to note that the effect seen at 60° is completely reversible upon cooling of the sample, and that free rotation seems to have been established in both diastereomers at 60°.

The mass spectrum of compound 22 shows simple fragmentation patterns as indicated below and is consistent with the structure assigned.





22 22E m/e = 189



22E 22E'

The ion of mass 189 may be explained if one postulates the existence of intermediate ion 22E, which undergoes cleavage of the amide bond. Ion 22E is likely to arise through loss of water, via a McLafferty-type rearrangement. Similarly, the presence of the intense ion of mass 217 could be explained by invoking a second rearrangement intermediate 22E', which may arise through loss of benzaldehyde imine followed by loss of a nitro radical. Neither 22E nor 22E' are detected, therefore other mechanismscannot be excluded.

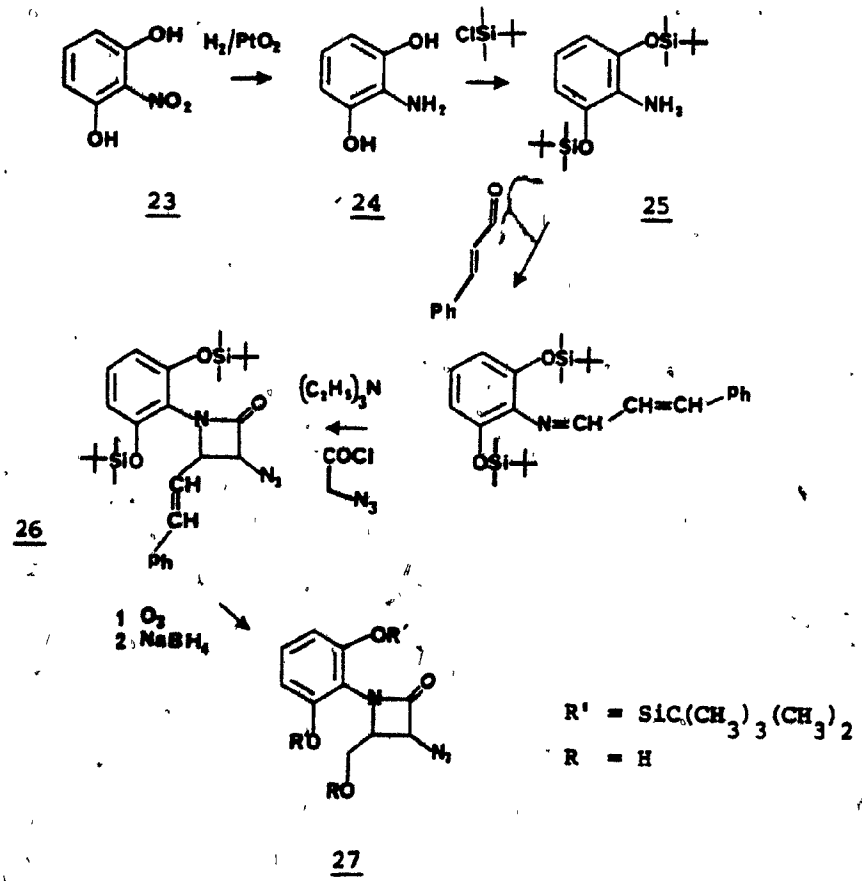
However, a rigorous mass spectral analysis is not crucial to the structure determination of compound 22.

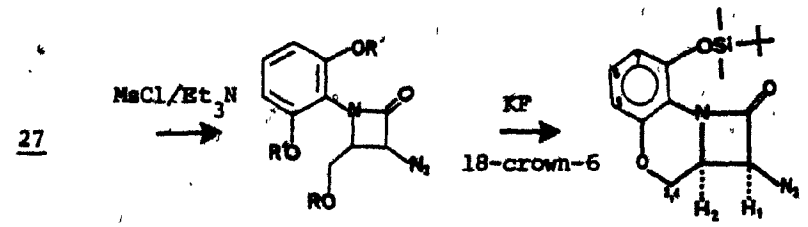
CHAPTER 2

Total synthesis of β-lactam 35

Because of the instability of nitrophenylsilyl ethers to ozonolysis (chapter 1), the synthetic scheme starting from a 3-nitro-2,6-dihydroxyaniline was abandoned, and attention was focused on direct nitration of analogues 28 and II.

The scheme[26,27,28,33] outlined in figure 8, was used to obtain the intermediate (II) for the synthesis described in this chapter.





28

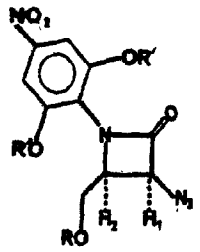
R' = $-SiC(CH_3)_3(CH_3)_2$

R = $-SO_2CH_3$

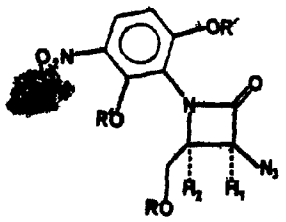
II

Figure 8: Synthesis of analogue II from nitroresorcinol

Since nitration of II would have given three compounds which were known (introduction) to be difficult to separate, we thought of nitrating compound 28 instead, and carrying out the cyclization on the separated nitro compounds 29-A and 29-B. Compound 28 was treated with nitronium tetrafluoro borate at -20° and, as expected, two compounds (29A, 29B) were isolated from the reaction mixture. Attempts to purify the mixture of compounds 29, which were well separated on thin layer chromatography, proved unsuccessful, resulting in complete decomposition of 29A and extensive desilylation of 29B.



29-A



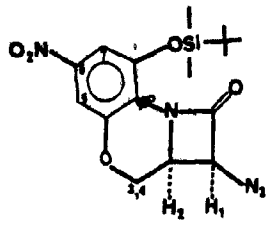
29-B

R' = $SiC(CH_3)_3(CH_3)_2$

R = $-SO_2CH_3$

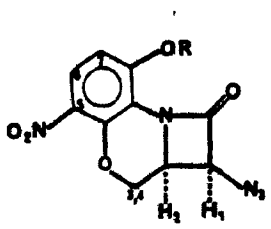
It is known that p-nitrophenols (pK_A = 7.2) behave almost like carboxylic acids. The presence of the nitro group in 29B well explains the extreme instability of the silylether. In compound 29A the nitro substituent para to the β-lactam ring, introduces so much chemical reactivity that the amide linkage is easily hydrolyzed on contact with silica gel. Because of this instability of 29-A and 29-B, it was thought that the most convenient approach would be introduction of the nitro group as late in the synthesis as possible.

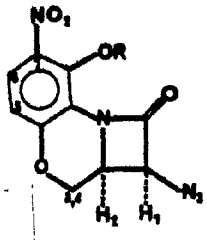
Nitration of II showed very similar complications. The p.m.r. spectrum of the crude reaction mixture clearly showed the presence of three compounds (30-M, 30-P, 30-O)*, characterized by three sets of doublets, two of large coupling constant (J = 9 Hz), as would be expected for ortho protons (30-P, 30-O), and one set at about 7.4 p.p.m. with the two doublets overlapping and a small coupling constant of 3 Hz indicating meta protons (30-M). Attempts to purify and separate the three compounds lead to complete decomposition of 30-M and some desilylation of 30-O and 30-P upon chromatography.



30-M

*M.o.p refers to the nitro group being ortho, meta, para to the silyloxy group.





30-P R = $-SiC(CH_3)_3(CH_3)_2$

31-P R = H

30-O R = $-SiC(CH_3)_3(CH_3)_2$

31-O R = H

Since nitration had to be carried out at this stage, to avoid nitration of the phenyl ring on the acylamino side chain to be introduced in the next step, we concentrated on optimization of yields for compounds 30. Several work-up conditions were tried and it was found that neutralization of fluoboric acid, formed during the reaction, immediately after completion of the nitration and while still cold (-20°) would give minimum decomposition. Compounds 30-P and 30-O were found, through p.m.r. and I.R. studies, to be sensitive to moisture and traces of acid found in solvents, even after they had been separated and crystallized. The p.m.r. spectra (in C_6D_6) of the two compounds, shown in figure 9*, cannot fully prove the structure of each compound. Their p.m.r. spectra were also recorded in deuterated chloroform (figure 10) and a

*Figures 9-O and 9-P

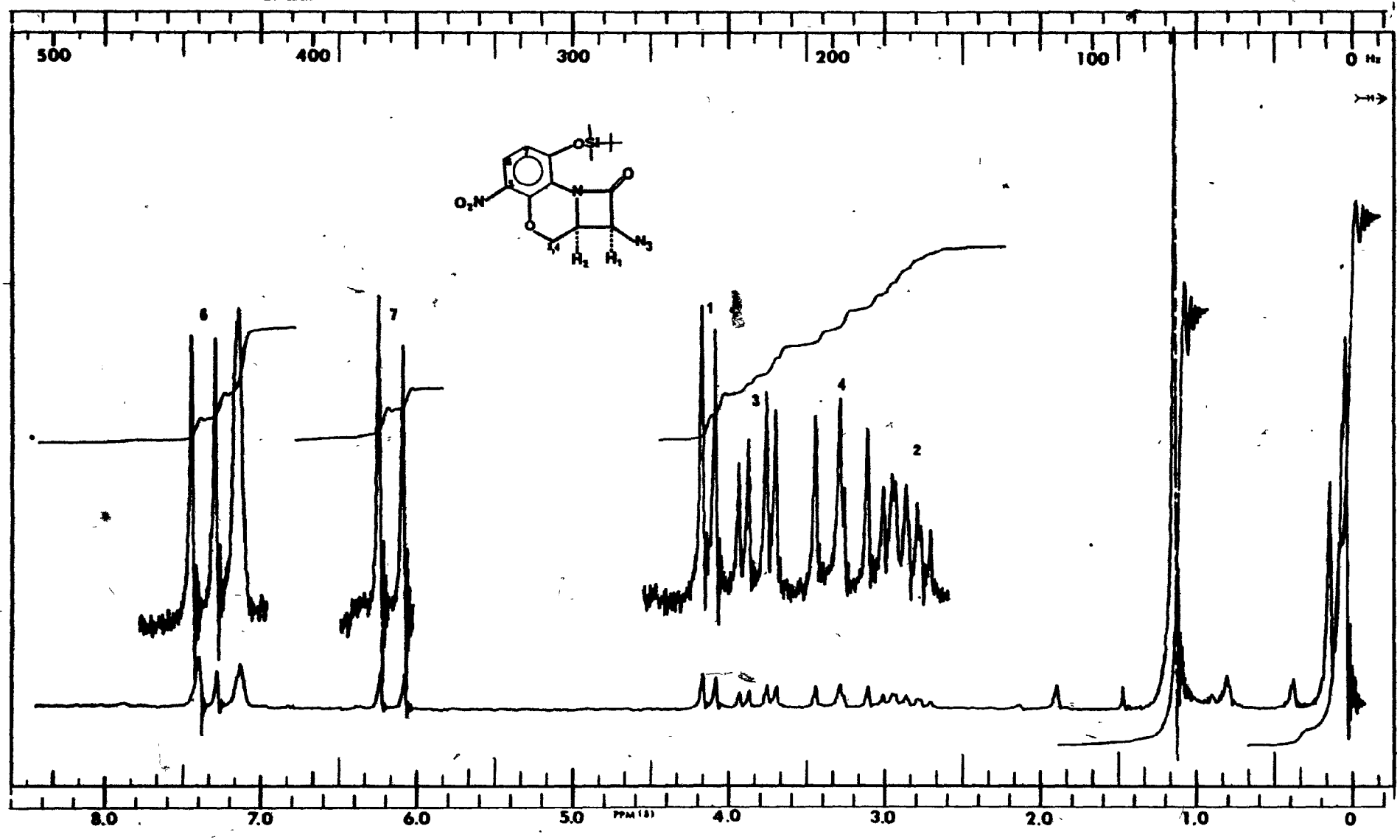


Figure 9-P: P.m.r. spectrum of compound 30-P in C_6D_6

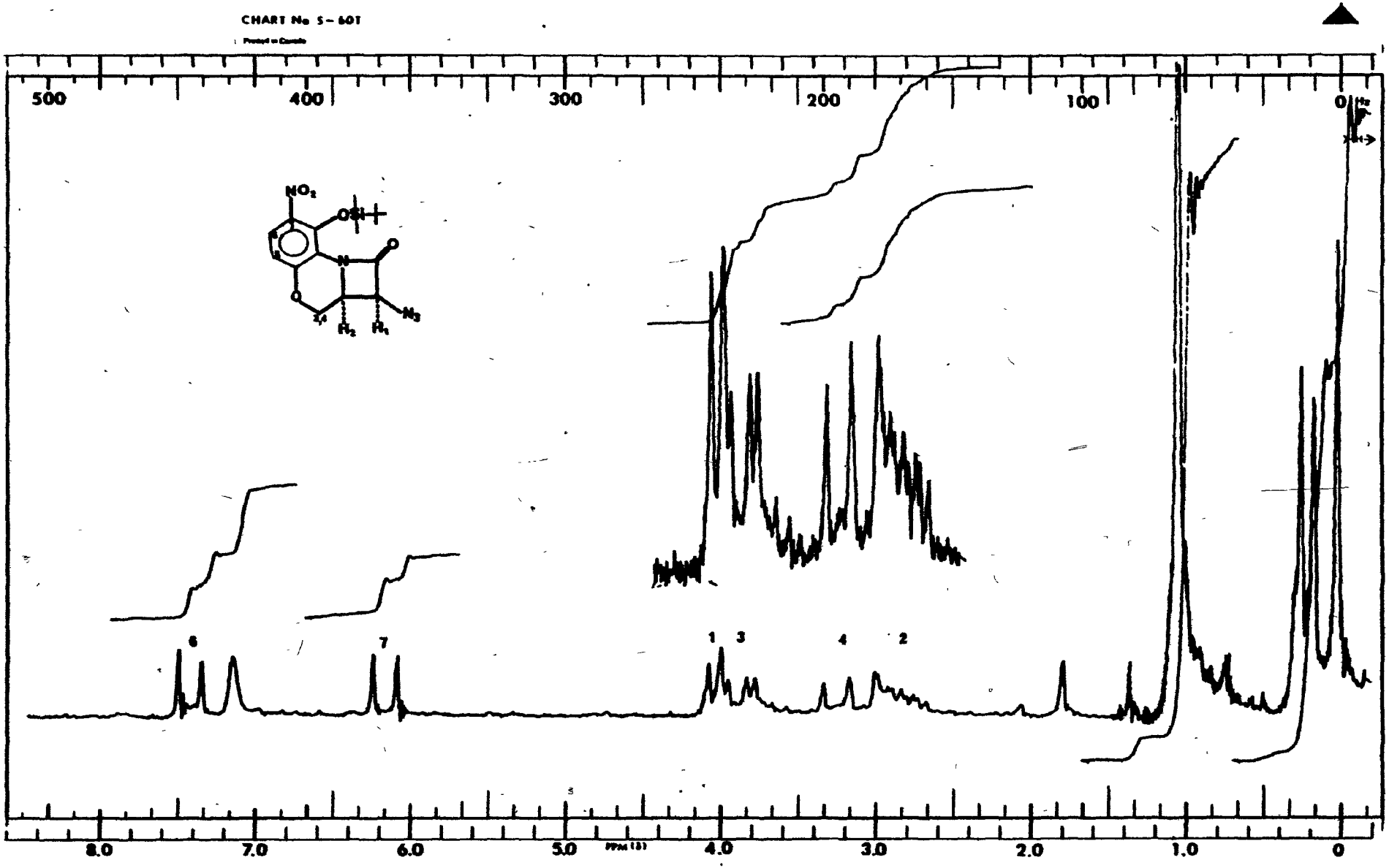


Figure 9-O: - P.m.r. spectrum of 30-O in C_6D_6

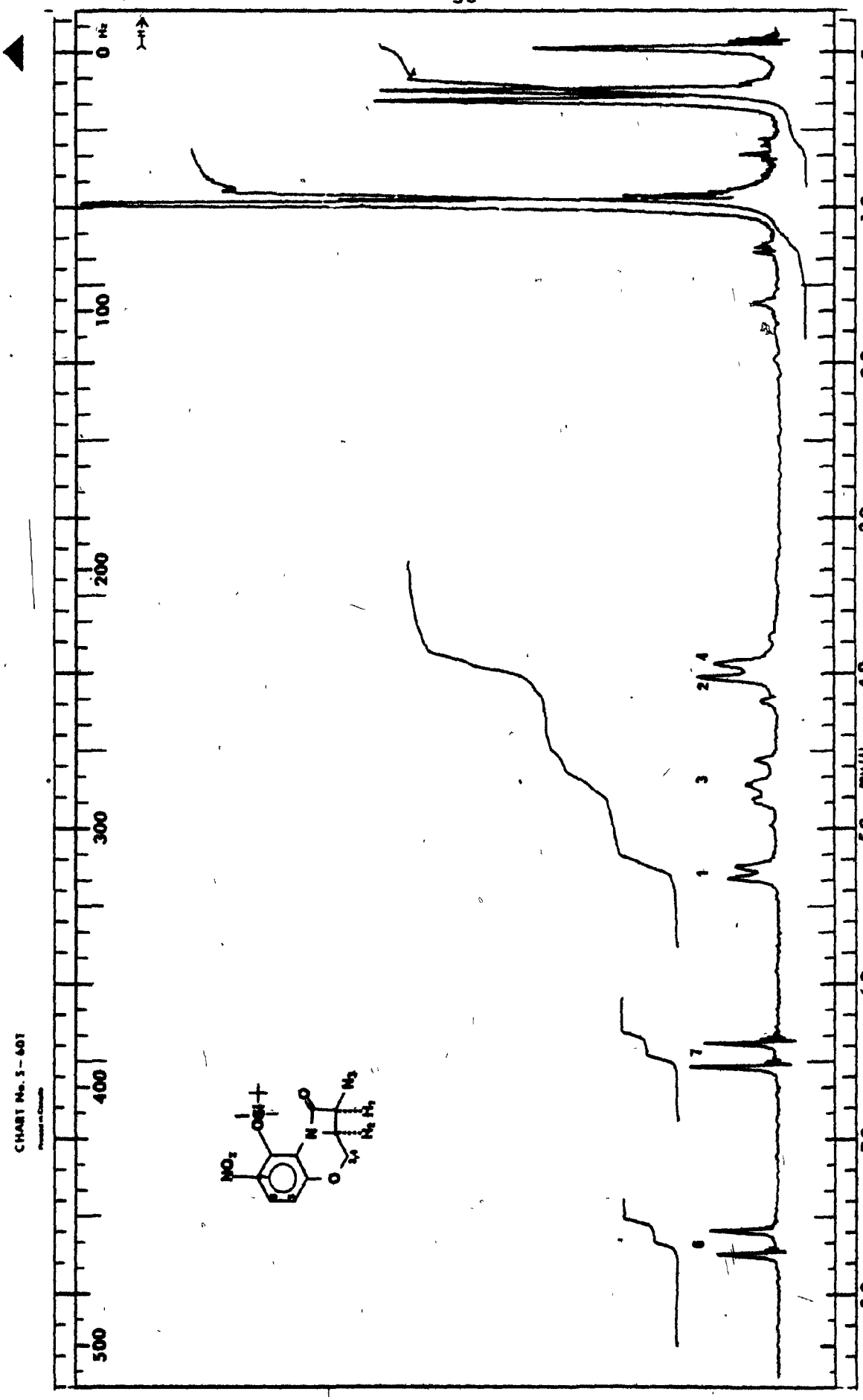


Figure 10-O: P.m.r. spectrum of 30-O in $CDCl_3$

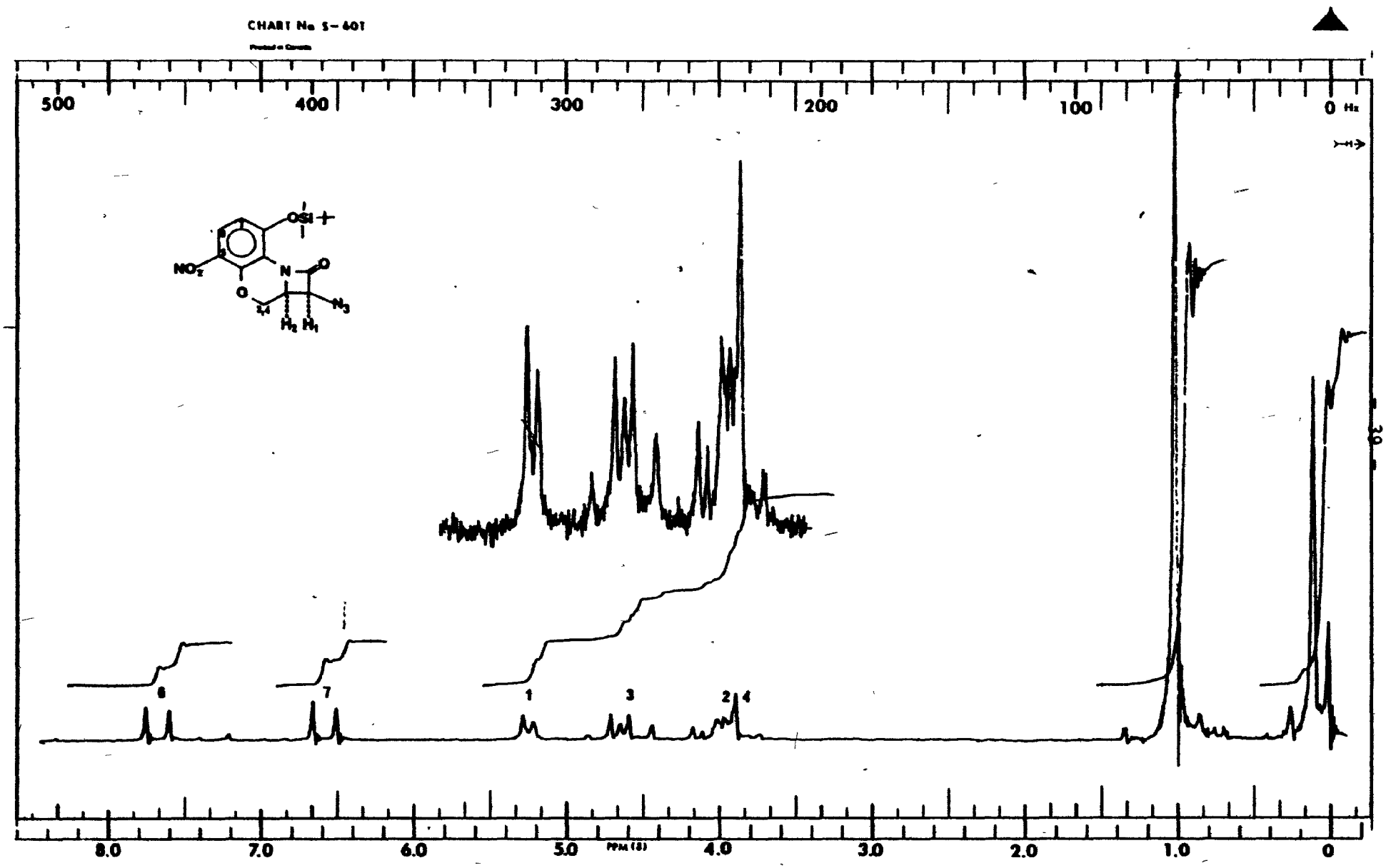
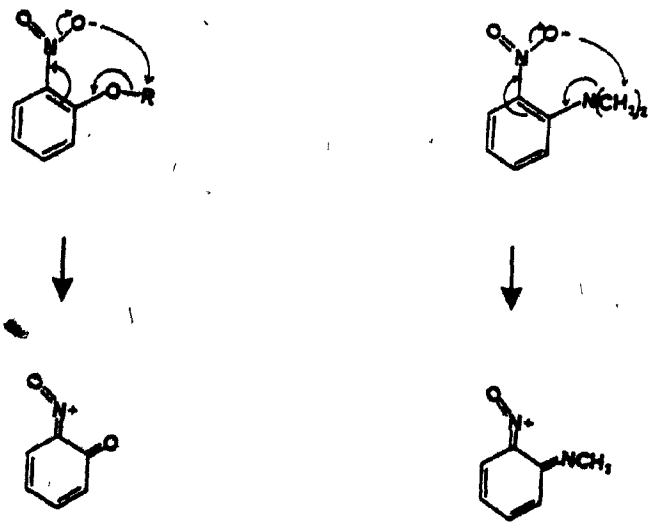


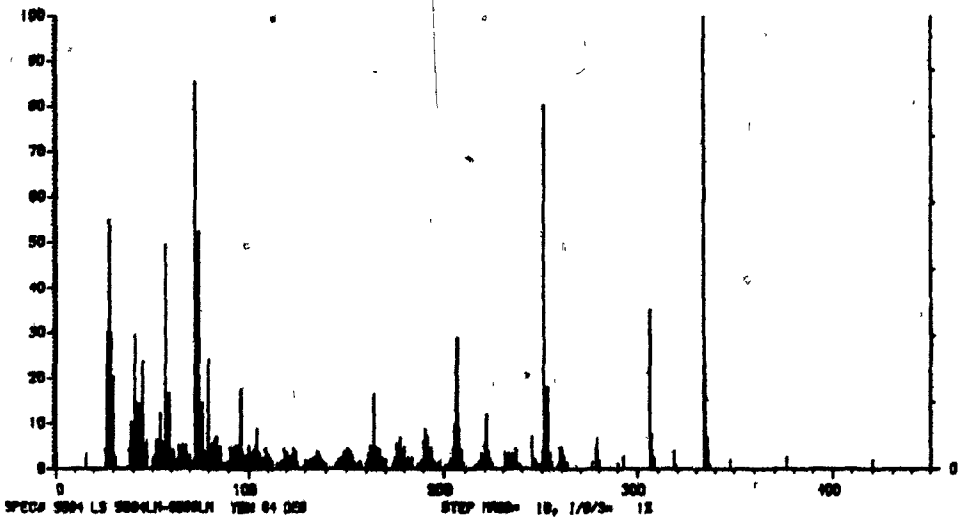
Figure 10-P: P.m.r. spectrum of 30-P in $CDCl_3$

significant difference between the two was now observed. The resonance peak, due to the methyl protons on the silyl ether, was recorded as a single peak for compound 30-P and as two peaks for compound 30-O. The presence of two non-equivalent methyl groups and their chemical shift at about 0.2 p.p.m. downfield in O compared to P, indicates steric hindrance and deshielding, most probably due to the presence of a nitro group ortho to the silyl ether. Ultraviolet spectra did not help to differentiate between the two structures

The structures of the two compounds was assigned as shown (30-P, 30-O) using the very convincing results obtained through mass spectrometry (Fig. 11). It is known that aromatic nitro compounds which have ortho -CR, -NR or -OR groups are characterized by their intense ions of mass (M^+-OR), obtained from the rearrangement shown in the examples below[34].



30-P



30-O

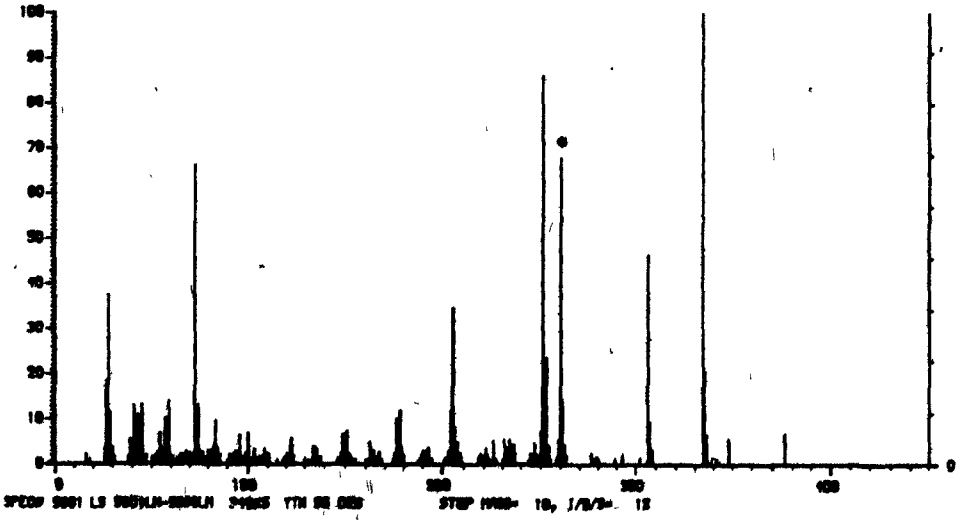
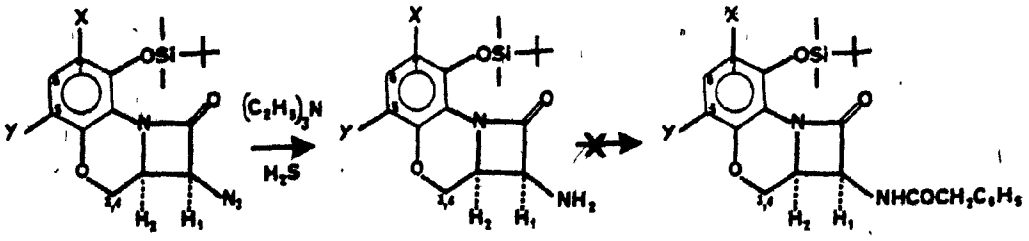


Figure 11: Mass spectra of analogues **30-P** and **30-O**

The very intense peak at m/e 260 (M^+-OSiMe$_2$t-butyl) seen in the mass spectrum of compound 30-O is almost completely absent from the spectrum of 30-P strongly suggesting an ortho-nitro silyl ether for analogue 30-P, in agreement with the p.m.r. data.

Both compounds show intense ions at m/e: 334 (base peak, M^+-t-butyl), 306 (334-N_2, from the azide group), 251 (334-83, 83 = -COCH-N_3), 205 (251-NO_2) as indicated in figure 11.

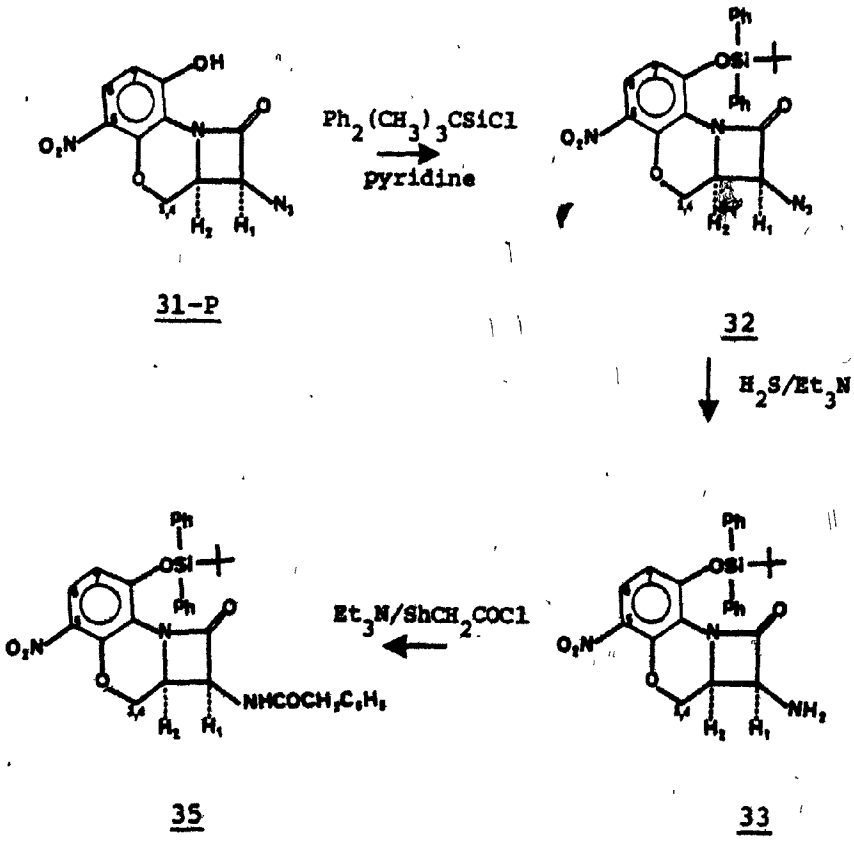
Subsequently a mixture of 30-O and 30-P was reacted with hydrogen sulfide/triethylamine to obtain the unstable amines 34(a,b) in good yield. Further reaction of crude compound 34 in an attempt to attach the acylamino side chain was unsuccessful, initially leading to hydrolysis of the silyl ether, followed by hydrolysis of the β-lactam ring, as evidenced by NMR which shows the presence of silanol and I.R., which shows a decrease in intensity of the β-lactam absorption with time.



30 34

a) X = H, Y = NO_2
b) X = NO_2, Y = H

We then thought that protection of the phenol group, with diphenyl-t-butylsilyl chloride, would give a more stable β-lactam. It was found that treatment of phenol 31-P with diphenyl-t-butylsilyl chloride/triethylamine gave compound 32 which was well characterized by its p.m.r. spectrum (Fig. 12). However, phenol 31-O was unreactive under the same reaction conditions, further confirming that the phenolic group was sterically hindered by the presence of an ortho nitro substituent.



31-P

32

35

33

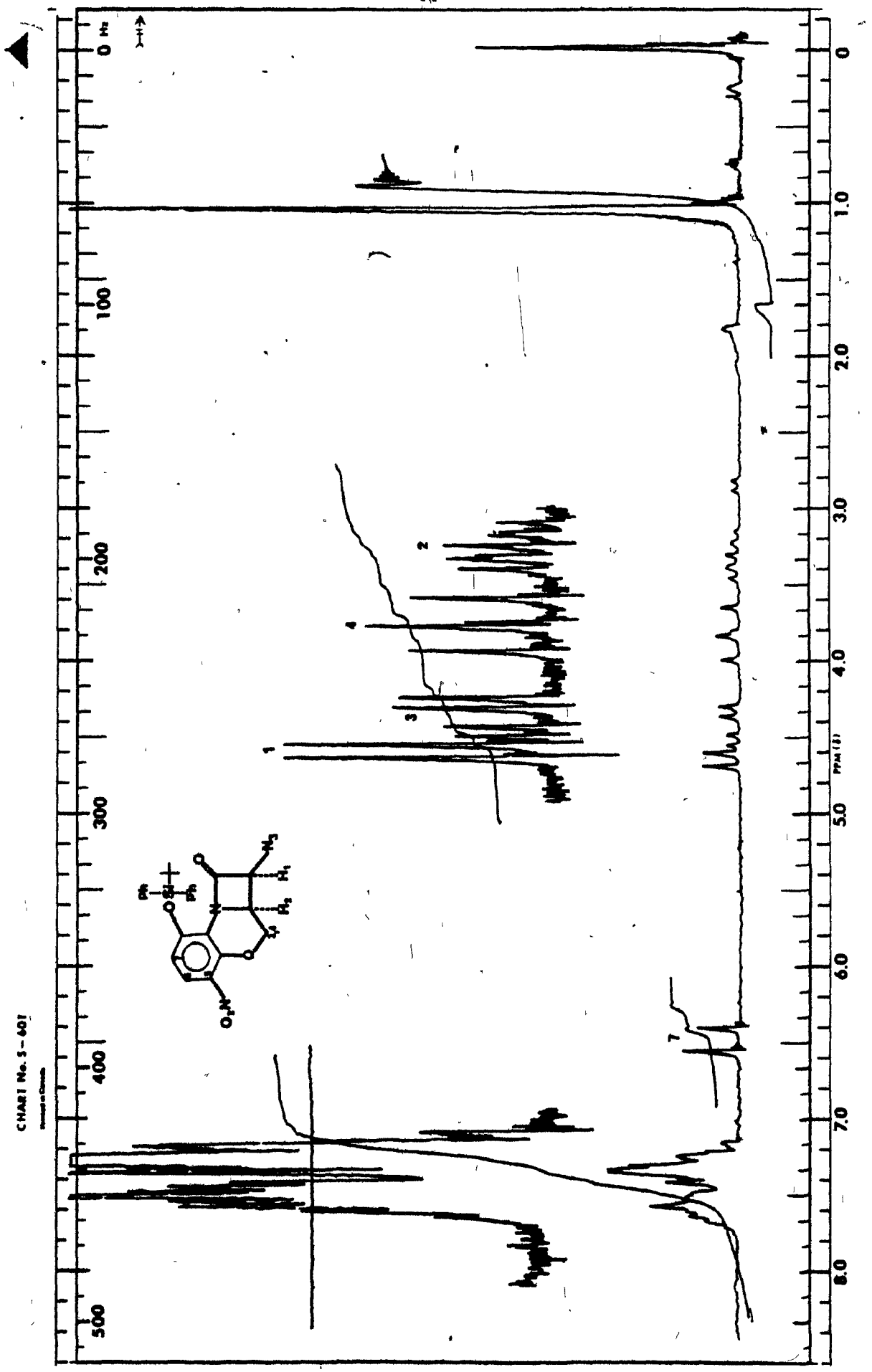


Figure 12: P.m.r. spectrum of analogue 32 in C_6D_6

The azide function of diphenyl-t-butylsilyl ether 32 was then reacted with hydrogen sulphide/triethylamine and the progress of the reaction was followed by the disappearance of the infrared absorption of the azide. Upon complete formation of amine 33, an equivalent of triethylamine was added along with an equivalent of phenylacetyl chloride to form final product 35.

The p.m.r. spectrum of 35 clearly showed the presence of all expected proton resonances: a singlet at 3.5 p.p.m. ($-CO\underline{CH_2}Ph$), a multiplet at 3.3-3.6 p.p.m. due to H_2, two sets of doublets at 3.8 to 4.6 p.p.m., coupled to each other and each integrating to one proton (H_3 and H_4). H_1 was found as a doublet of doublets at 5.1 to 5.3 p.p.m. with one of its coupling constants equal to that of the broad doublet (due to NH) at 8.0 p.p.m. Finally, two doublets (6.6 and 8.7 p.p.m.) were seen coupled to each other by a large coupling constant of 9 Hz, characteristic of ortho protons (H_6, H_7).

The infrared spectrum of 35 was also consistent with the structure given, with an absorption of 1790 cm^{-1} for the carbonyl group of the β-lactam.

Unfortunately, mass spectrometry and elemental analysis could not be used to further support the structure of 35 due to the extreme instability of this compound. It was observed through p.m.r. and I.R. studies that even the traces of moisture or acid found in DMSO-d_6 would cause rapid decomposition of the silyl ether, followed by hydrolysis

of the β-lactam ring, within half an hour.

Due to this extreme instability, reactions to substitute the simple acylamino side-chain ($-NHCOCH_2Ph$) with the Ampicillin side-chain, were not attempted. Biological studies of compounds 22, 30 and 35 were inconclusive due to complete decomposition of these compounds during travel. We therefore showed that a nitro substituent introduces greater reactivity than desired and perhaps replacement of this group with a less electron withdrawing substituent such as an ester might lead to more stable and perhaps biologically active products.

General Experimental

Analytical thin layer chromatography (t.l.c.) was performed on silica gel-coated plates (Machery Nagel Polygram G or Merck Silical Gel 60) and on a preparative scale on silica gel (Merck HF 254) coated glass plates (20 cm x 20 cm x 1 mm). Merck silica gel 60, Woelm alumina (neutral) and Camag alumina (supplied by Ventron) were used for normal chromatography. Silica Woelm 32-63 was used for flash chromatography.

Solvents were reagent grade unless otherwise specified. All evaporations were done under reduced pressure (water aspirator) with a bath temperature of 25-40° unless otherwise specified.

Elemental analyses were performed by Heterocyclic Chemical Corporation of Midwest Microlab Ltd.

Melting points were determined on a Gallenkamp block and are uncorrected. Mass spectra (m.s.) were obtained on an AEI-MS-902 mass spectrometer or on an LKB-900 mass spectrometer at 70 eV using a direct insertion probe. Infrared (i.r.) spectra were obtained on Unicam SP1000, Perkin Elmer 257 and 297 spectrophotometers. Proton magnetic resonance (p.m.r.) spectra were recorded on Varian T-60, T-60A, HA-100 and on Brucker FT 90 spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in the δ scale in parts per million. Doublets ('d'), triplets ('t') and quartets ('q') were recorded at the center of the peaks, and multiplets ('m') as their range of

absorption; other abbreviations used: singlet ('s') and broad ('br'), doublet of doublets ('dd').

U.v. spectra were recorded using a Cary 17 spectrometer.

Experimental

Chapter 1

Methoxy-methyl ether 16

The alcohol 15 (100 mg, 0.3 mmole) was dissolved in 25 ml of dry methylal and stirred vigorously at room temperature. Phosphorus pentoxide (500 mg) was added in small portions, every 10 minutes, and the end of the reaction was determined by T.L.C. Upon completion of the reaction (2 hrs), 25 ml of methylene chloride were added, the phosphorus pentoxide was filtered off, and the filtrate was washed with 5% bicarbonate solution, water and saturated salt solution. Drying of the methylene chloride solution over magnesium sulfate and evaporation gave 60 mg (53% yield) of product (16). P.m.r. ($CDCl_3$): δ 3.4 (s, 3H, $-OCH_3$), 5.0-5.4 (m, 2H, β-lactam) 5.3 (s, 2H, $-OCH_2O-$), 6.0-6.4 (dd, 1H, -CH=CHPh), 6.6-6.9 (d, 1H, -CH=CH-Ph), 7.1-7.4 (m, 6H, Ar), 7.8-8.1 (dd, 1H, H_4), 8.75 (d, 1H, H_3) p.p.m., I.R. ($CHCl_3$): ν_{max} 3450 (-OH), 2100 ($-N_3$), 1770 (β-lactam) cm^{-1}.

β-lactam alcohol 17

Ozone was bubbled through a solution of 16 (396 mg, 1 mmole) in ethanol-methylene chloride (4:1.5 ml) at -70° until a faintly blue color was observed. Excess ozone was flushed out with dry nitrogen while the system was kept at -78°. Then, sodium borohydride (76 mg, 2 mmole) was added. The temperature was allowed to rise to 20° over a period of 5 hrs. The solution was then washed with potassium phosphate buffer solution (pH 4.4), water and brine. Drying of the solution over magnesium sulfate and evaporation gave a light orange oil. It was purified on a flash silica gel column[44] using EtOAc/pet. ether (65:35) as the eluent to give product 17 (227 mg, 70%). P.m.r. (acetone-d_6): δ 3.55 (s, 3H, $-OCH_3$), 4.1 (d, 2H, $-CH_2OH$, J = 3 Hz), 4.7-4.9 (dd, 1H, H_2, J_1=3 Hz, J_2=5 Hz), 5.2 (d, 1H, H_1, J=5 Hz), 5.4 (s, 2H, $-OCH_2O$), 7.35 (d, 1H, H_5, J=9 Hz), 7.9-8.1 (dd, 1H, H_4, J_1= 9 Hz, J_2=3 Hz), 8.8 (d, 1H, H_3, J = 3 Hz) p.p.m., I.R. ($CHCl_3$): ν_{max} 3500, (OH), 2110, ($-N_3$) 1775 (β-lactam C=O) cm^{-1}.

Amine 18

Hydrogen sulfide was bubbled into a solution of 17 (100 mg, 0.3 mmole) in 10 ml of methylene chloride and triethyl amine (35 mg, 0.35 mmole) at 0° for 3 min. After stirring for 1 hr at 20°, the solution was purged with nitrogen (5 min). The reaction mixture was washed with water (20 x 20 ml), dried and evaporated to give an orange oily product, which was crystallized from methylene chloride: petroleum-ether to give 45 mg (84%) of light yellow crystals, m.p. 119-120°C. P.m.r. (acetone-d_6) δ: 3.7 (s, 3H, OCH_3), 4.1 (d, 2H, $\underline{CH_2}OH$), 4.4-4.9 (m, 2H, β-lactam plus OH), 5.2-5.3 (m, 3H, β-lactam plus $-\underline{OCH_2}OCH_3$), 7.2-7.4 (2d, 1H, H_5), 7.9-8.1 (2t, 1H, H_4), 9.1-9.4 (2d, 1H, H_3) p.p.m., I.R. ($CHCl_3$): ν_{max} 3350 (NH_2), 1735 (β-lactam) cm^{-1}.

Synthesis of analogue 21 from alcohol 18 through the silylated compound 19

Alcohol 18 (149 mg, 0.5 mmole) and triethylamine (56 mg, 0.5 mmole) were dissolved in 10 ml of methylene chloride to which trimethylsilyl chloride (60 mg, 0.5 mmole) was added. After stirring for 30 min, the solvent was evaporated, and the residue dissolved in ethyl-ether and filtered to remove triethylammonium hydrochloride. After evaporation, the crude silylated product 19 was redissolved in methylene chloride (5 ml). To this, EEDQ (136 mg, 0.5 mmole) and t-BOC phenyl glycine (138 mg, 0.5 mmole) were added, and the mixture was stirred overnight. The reaction mixture was washed with 5% hydrochloric-acid, 2% bicarbonate and brine solutions, dried, ($MgSO_4$) and evaporated to give 250 mg (95%) of a light yellow oily product, as a mixture of diastereomers. P.m.r. ($CDCl_3$): δ 0 (2s, 9H, $Si(CH_3)_3$), 1.6 (s, 9H, $-C(CH_3)_3$), 3.6 (2s, 3H, $-OCH_3$), 4.1 (bs, 2H, $-\underline{CH_2}-OSi(CH_3)_3$), 4.8 (bm, 1H, H_2), 5.3 (bd, 1H, NH), 5.4 (d, 2H, $-O\underline{CH_2}-OCH_3$), 5.6-6.2 (m, 2H, H_1, COCH), 7.2-7.5 (m, 2H, H_5, NH), 7.4 (s, 5H, Ph), 7.9-8.2 (dd, 1H, H_4), 9.0 (2d, 1H, H_3) p.p.m.

Alcohol 21

A solution of the silylated compound 20 (120 mg, 0.2 Mmole) in 5 ml of 5% hydrochloric acid and 5 ml of a 2:1 tetrahydrofuran water mixture was stirred at 20°C for 30 min. Ethyl acetate extraction gave 100 mg (95% yield) of 21. P.m.r. (acetone-d_6): δ 1.5 (s, 9H, $-100C(CH_3)_3$), 3.6 (s, 3H, $-OCH_3$), 3.9-4.3 (bm, 3H, $-(H_2OH)$, 4.8 (m, 1H, H_2), 5.4 (bd, 1H, COCHN), 5.5 (bs, 2H, $-O\underline{CH_2}OCH_3$), 5.8 (dd, 1H, H_1), 6.7 (bd, 1H, NH), 7.3-7.7 (m, 6H, pH, H_5), 8.0-8.3 (bdd, 2H, H_4, NH), 8.9 (dd, 1H, H_3) p.p.m.; m.s.: m/e 500 (M^+-2CH_3), 205, 206, 209, 235; I.R. (film): ν_{max} 3350 (-OH), 1760 (β-lactam), 1700 (NHCO), 1690 (NHCOO-t-butyl) cm^{-1}.

Amine-diol 22

A solution of alcohol 11 (250 mg, 0.5 mmole) in a trifluoroacetic acid-tetrahydrofuran-water mixture (4 ml : 2 ml : 2 ml) was stirred at room temperature for 3.5 hrs. The reaction mixture was then evaporated to dryness, redissolved in ethyl acetate, and upon addition of methylene chloride, 150 mg (0.4 mmole) of 22 precipitated as light yellow crystals; m.p. (161-162°). P.m.r. (D_2O), 90 MHz, 60°C): δ 3.9 (d, 2H, $-CH_2OD$), 4.7 (m, 1H, H_2), 5.3 (2d, 1H, -COCH-), 5.4 (q, 1H, H_1), 7 (2d, 1H, H_5), 7.5 (s, 5H, Ph) 7.8-7.9 (dd, 1H, H_4), p.p.m. 8.2 (d, 1H, H_3); m.s.: m/e 180, 309, 355, 340, 224, 44, 189, 217.

Experimental

Chapter 2

Cyclized compound II

To a solution of the mesylate 28 (900 mg, 1.6 mmole) in acetonitrile (10 ml) was added potassium fluoride (113 mg, 1.1 mmole) and 18-crown-6 (130 mg, 0.5 mmole). After stirring for 4 hrs at room temperature, the reaction mixture was poured into water (20 ml) and extracted with methylene chloride (2 x 30 ml). Drying ($MgSO_4$) and evaporation afforded the crude product which after flash chromatography[44] crystalized on standing (510 mg, 94%) m.p. 69-70°C. P.m.r. ($CDCl_3$): δ 0.2 (s, 3H, $SiCH_3$), 0.3 (s, 3H, $SiCH_3$), 1.1 (s, 9H, $C(CH_3)_3$), 3.8-4.7 (m, 3H, H_2, H_3, H_4), 5.3 (d, 1H, H_1), 6.5-7.2 (m, 3H, H_5, H_6, H_7) p.p.m., I.R. ($CDCl_3$): ν_{max} 2100 (azide), 1775 (β-lactam) cm^{-1}.

Nitration of analogue II to obtain compounds 30-P and 30-O

To a solution of II (574 mg, 1.6 mmole) in dry acetonitrile (20 ml) under a nitrogen atmosphere at -20°C, nitronium tetrafluoroborate, (3.2 ml, 0.5 M solution in sulfolane) was added dropwise. After 1 hr, dry benzene (10 ml) was added and the mixture was stirred for 10 more min. The reaction mixture was then poured (while still very cold) into 30 ml of pH 4.4 buffer and extracted twice, dried ($MgSO_4$) and evaporated. The ortho-nitro- and para-nitro-silyl ether compounds were isolated and crystallized after flash chromatography. The remaining mixture of nitrophenols was separated from sulfolane via Kugelrohr distillation, resilylated, and purified by flash chromatography to afford more of the desirable products.

Ortho-silyl ether:

m.p. 109° - 110°C. P.m.r. (C_6D_6): δ 0.2 (s, 3H, $SiCH_3$), 0.3 (s, 3H, $SiCH_3$), 1.1 (s, 9H, t-Butyl), 2.6-2.9 (dq, 1H, H_2), 2.9-3.3 (d, 1H, H_3, $J_1=J_2=10$ Hz), 3.8-4.0 (dd, 1H, H_4, $J_1=$ 10 Hz, $J_2=3$Hz), 4.05 (d, 1H, H_1, J=4 Hz), 6.15 (d, 1H, H_3, J=9 Hz), 7.43 (d, 1H, H_6, J=9 Hz) p.p.m.; m.s. m/e: 334 (M^+-t-Butyl), 306 (M^+-t-Butyl-N_2), 260 (M^+-$OSi(CH_3)_2C(CH_3)_3$), 251 (M^+-t-Butyl-N_3CCO); I.R. (film): ν_{max} 2100 ($-N_3$), 1795 (β-lactam) cm^{-1}.

Para-silyl ether:

m.p. 122° - 123°C. P.m.r. (C_6D_6): δ 0.03 (s, 3H, $SiCH_3$), 0.15 (s, 3H, $SiCH_3$), 1.7 (s, 9H, t-Butyl), 2.66 -3.0 (dq, 1H, H_2, J_1=4 Hz, J_2=9 Hz, J_3=5 Hz), 3.15-3.45 (dd, 1H, H_3, J_1=9 Hz, J_2=11 Hz), 3.7-3.9 (dd, 1H, H_4, J_1=11 Hz, J_2=4 Hz), 4.1 (d, 1H, H_1, J=5 Hz), 6.15 (d, 1H, H_7, J=9 Hz), 7.35 (d, 1H, H_6, J=9 Hz) p.p.m.; m.s. m/e: 334 (M^+-t-Butyl), 306 (M^+-t-Butyl-N_2), 251 (M^+-t-Butyl-N_3(CO); I.R. (film): ν_{max} 2100 (azide), 1785 (β-lactam) cm^{-1}. Anal. Calcd for $C_{16}H_{21}N_5O_5Si$: C, 49.10; H, 5.37; N, 17.90. Found: C, 48.91; H, 5.50; N, 17.72.

Reduction of azide 30 to the amine 34

Hydrogen sulphide was bubbled into a solution of the azide 30 (mixture of ortho and para-nitrosilylethers) (170 mg, 0.45 mmole) and triethylamine (49 mg, 0.5 mmole) in dry methylene chloride (10 ml) at 0°. After 1 hr of stirring at room temperature the solution was purged with nitrogen, washed with water (2 x 10 ml), dried and evaporated to give a crude yellow oily product (140 mg). P.m.r. (acetone-d_6): δ 0.3 (2s, 6H, $Si(CH_3)_2$), 1.0 (s, 9H, $C(CH_3)_3$), 2.9 (bs, 2H, NH_2), 4.0-4.3 (m, 2H,), 4.7-4.9 (m, 1H), 5.5-5.6 (dd, 1H, H_1), 6.5-6.7 (2d, 1H, H_7), 7.6 (d, 1H, H_6) p.p.m; I.R. (film): ν_{max} 3350 (amine), 1780 (β-lactam) cm^{-1}.

Formation of diphenyl-t-butylsilylether 32

A mixture of ortho- and para-nitrophenols 31 (138 mg, 0.5 mmole), tert-butyldiphenylsilyl chloride (165 mg, 0.6 mmole) and imidazole (82 mg, 1.2 mmole), in dry dimethylformamide (5 ml) was stirred at room temperature for 15-20 hrs. The reaction mixture was then added to ethyl ether (100 ml), washed with water and brine, dried ($MgSO_4$) and evaporated to dryness to give crude product 32 which was crystallized from CH_2Cl_2-petroleum ether (100 mg, 45%), m.p. 153-154°C. The water-brine layer was further extracted with ethyl acetate in order to isolate ortho-nitrophenol and decomposition products; p.m.r. (C_6D_6): δ 1.1 (s, 9H, $C(CH_3)_3$), 3.15-3.5 (dq, 1H, H_2, J_1=5 Hz, J_2=9 Hz, J_3=4 Hz), 3.65-4.0 (dd, 1H, H_3, J_1=11 Hz, J_2=9 Hz), 4.3-4.55 (dd, 1H, H_4, J_1=4 Hz, J_2=11 Hz). 4.65 (d, 1H, H_1, J=5 Hz), 6.47 (d, 1H, H_7, J=9 Hz), 7.2-7.7 (m, 11H, H_6, 2Ph) p.p.m.

Final product 35

Hydrogen sulphide was bubbled into a solution of the azide 32 (140 mg, 0.3 mmole) and triethylamine (0.05 ml) in methylene chloride (10 ml) at 0° for 3-5 min. After 1 hr, the solution was purged with nitrogen. To the crude product 33 in methylene chloride (10 ml) and triethyl amine (0.05 ml) at 0°, was added dropwise phenylacetyl chloride (0.06 g, 0.4 mmole). After stirring for 1 hr the solution was washed with pH 4.4 buffer (10 ml), water (10 ml), dried ($MgSO_4$) and concentrated. The crude product 35 was crystallized from ethyl acetate-petroleum ether several times to obtain 100 mg (60%) of pale yellow crystalline product, m.p. 190°-191°. P.m.r. (DMSO-d_6): δ (s, 9H, $C(CH_3)_3$), 3.5 (s, 2H, $COCH_2Ph$), 3.3-3.7 (m, 1H, H_2), 3.8-4.2 (dd, 1H, H_3, J_1=11 Hz, J_2=9 Hz), 4.3-4.6 (dd, 1H, H_4, J_1=4 Hz, J=10 Hz), 5.1-5.3 (dd, 1H, H_1, J_1=8 Hz, J_2=5 Hz), 6.6 (d, 1H, H_7, J=9 Hz), 7.2 (s, 10H, $SiPh_2$), 7.2-7.7 (m, 5H, $-CH_2\underline{Ph}$), 8.0 (bd, 1H, NH, J=8 Hz), 8.7 (d, 1H, H_6, J=9 Hz) p.p.m. I.R. (film): ν_{max} 1790 (β-lactam), 1710 (amide) cm^{-1}.

SECTION II

INTRODUCTION

Chapter 3

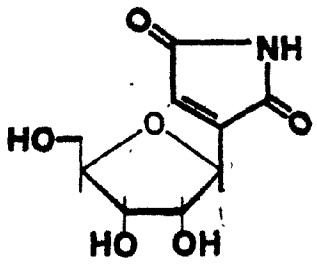
An improved synthesis of D,L-2'-epishowdomycin.

In recent years, the term "nucleoside" has come to designate natural and synthetic N-glycosides and C-nucleosides. Natural nucleosides are compounds of the glycosylamine type in which the aglycone is a purine or pyrimidine base, and the sugar component is D-ribofuranose or 2-deoxy-D-ribofuranose. These are the important constituents of the nucleic acids DNA and RNA. Analogues, in which the C-1 of the sugar residue is bonded to a carbon atom of the heterocyclic base, are referred to as "C-nucleosides", and many naturally occurring C-glycosyl nucleosides have been isolated[45].

The fact that many nucleosides and C-nucleosides exhibit biological activity towards a wide range of organisms is stimulating the synthesis of these compounds.

Showdomycin (60) is a broad-spectrum antibiotic. It was first isolated from streptomyces showdoensis[46], and its structure has been shown to be 2-(β-D-ribofuranosyl) maleimide[47].

The numerous biochemical studies carried out with showdomycin have been reviewed by Suhadolnik[48]. Its biological activity includes the inhibition of protein and DNA synthesis, and of the transport of sugar and amino acids in Escherichia coli[49]. It was also found[46] that showdomycin is active against Ehrlich ascites tumor cells. It appears that the maleimide aglycone moiety of showdomycin is an active alkylating agent which is especially active towards the



60

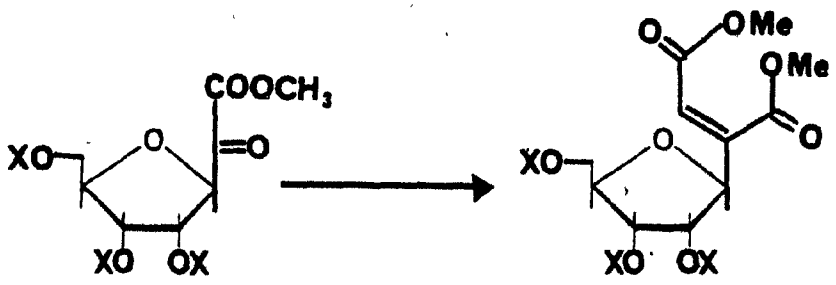
sulfhydryl group of enzymes[50].

The biological activity of showdomycin and of all C-nucleosides has been extensively studied, and attributed[51,52] to the length and the stability of the C-C glycosydic bond. The longer bond of the C-C linkage in C-nucleosides as compared to the C-N linkage in N-nucleosides, allows for a lower rotational energy barrier in the C-nucleosides, permitting them to assume a suitable conformation for interaction with the active site of the inhibited enzyme. As well, a C-C bond is much more stable towards chemical and enzymatic attack than a C-N bond. The latter can be more easily protonated and hydrolyzed to give free base and free sugar.

Therefore the prolonged biological activity that C-nucleosides exhibit, as compared to that of N-nucleosides, may be attributed to the increased chemical stability and the increased conformational flexibility, presumably assisting the binding to enzymes, of C-nucleosides.

Synthesis of epishowdomycin and related compounds

Kalvoda et al.[53] have reported the first synthesis of showdomycin. It involves condensation of the key intermediate 61 with carboethoxymethylenetriphenylphosphorane to give the maleate ester 62. From 62 showdomycin is obtained in a few more steps.

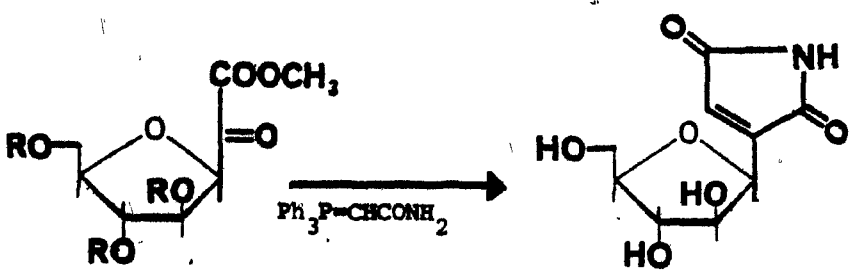


61 62

X = Ac

The intermediate 61 was adopted by Trumnlitz et al.[54] in their synthesis of showdomycin, which involved the condensation of carbamoyl methylenetriphenylphosphorane 63 with keto-ester 61. This approach was also used by Dr. Mu-Ill Lim to prepare D,L-2'-epishowdomycin(III).

The improvement* of his synthetic scheme will be discussed in chapter 3.



63 III

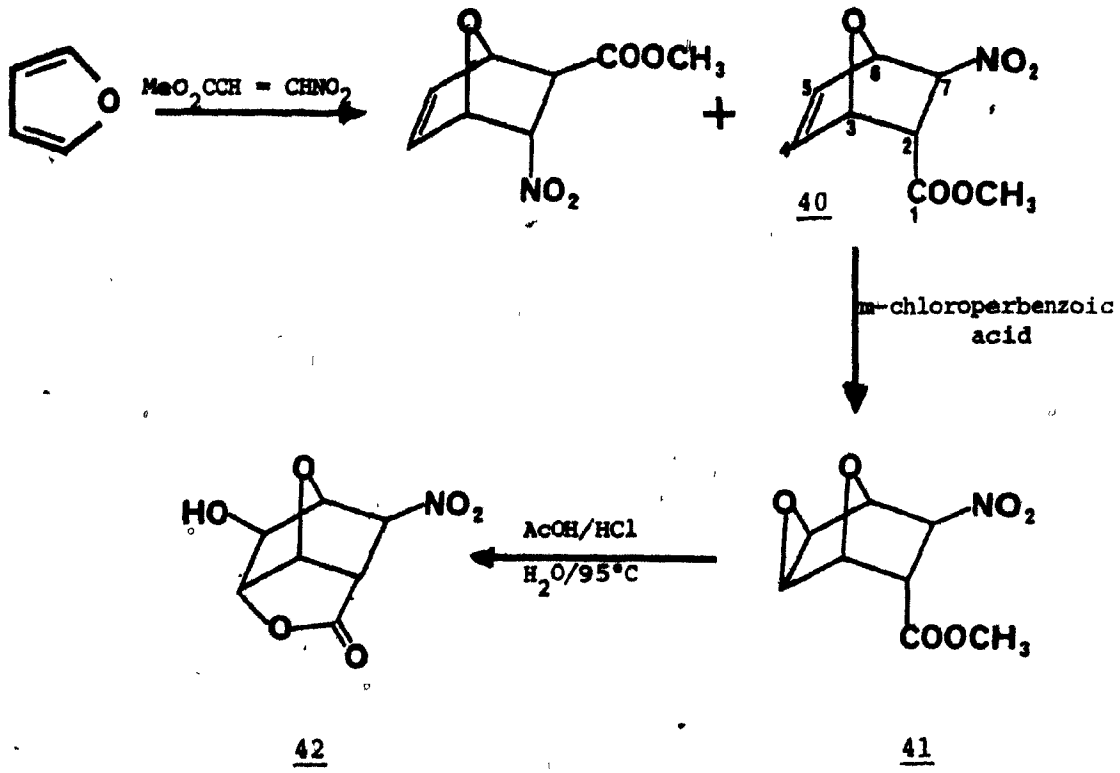
*In accordance to the aim of this project, only compounds whose structures were ambiguous, due to lack of good spectral data, and only the experimental procedures of reactions which were improved, will be described.

DISCUSSION

Chapter 3

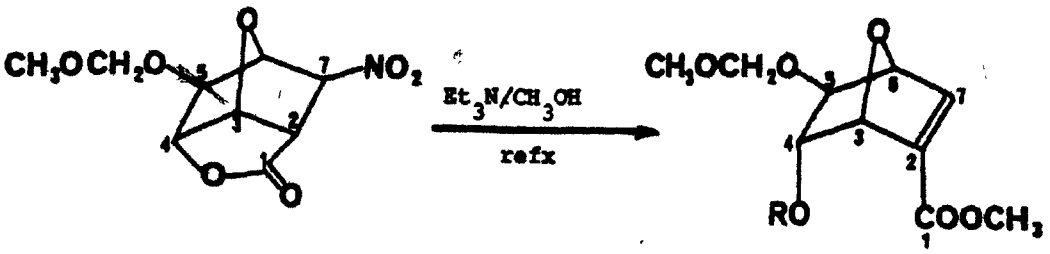
Synthesis of D,L-2'-epishowdomycin

The synthetic scheme for the total synthesis of D,L-2'-EPI-SHOWDOMYCIN (III) has been studied in the past, in our laboratory by Dr. Mu-Ill Lim[35]. Due to lack of satisfactory spectral data of some products and poor and/or irreproducible yields of a few reactions we decided to further study this synthetic pathway. Compounds 40 to 43* were prepared easily and in good yields as described by Lim[35].



*Since each of the described compounds has a different proper numbering system according to the I.U.P.A.C. rules, and in order to be consistent and simplify matters, the arbitrary numbering given in compound 40 will be used through out the discussion.

Several attempts to prepare compound 44 from 43 using DBU[35] in boiling CH_2Cl_2:CH_3OH (1:1) gave products of a different nature in a reproducible manner, instead of the expected 44. Compound 44 was finally prepared in a 40% yield using triethyl amine in refluxing methanol.



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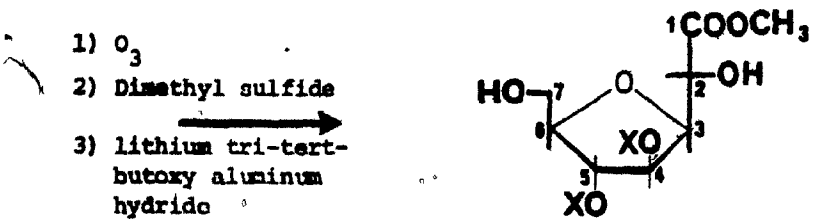
44 R = H

45 R = CH_2OCH_3

The structure and stereochemistry of compound 44 follows from its mode of formation and p.m.r. spectrum, which shows that H_4 is split by H_3 (2 Hz), whereas the coupling constant H_4-H_5 is close to 0 Hz indicating an exo-configuration of the 5-substituent.

Alcohol 44 was then protected with a methoxy-methyl group and the product (45) was ozonolyzed and reduced to the aldehyde-ketoester by dimethyl sulfide followed by lithium tri-tert-butoxy-aluminum hydride to get diol 46.

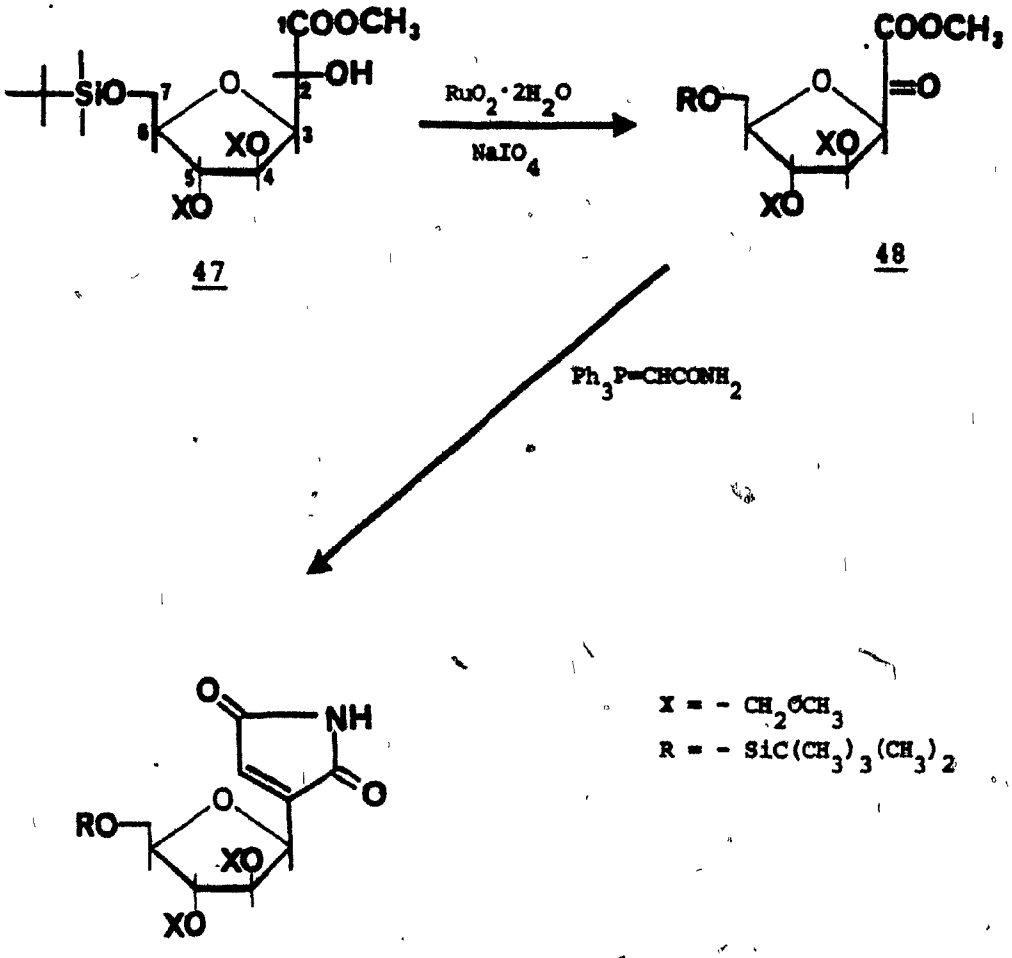


46

X = CH_2OCH_3

Selective silylation with t-butyldimethylsilyl chloride gave 47, which seemed to consist of one diastereoisomer only. Assignment of most p.m.r. spectral signals was possible upon addition of $Eu(fod)_3$ to the deuterobenzene solution of 47. The lowest field signal (1H, C_2) appeared as a doublet (J = 8 Hz). The C_3-proton appeared at higher field as a doublet of doublets ($J_{2,3}$ = 8 Hz, $J_{3,4}$ = 4 Hz). This last value of J = 4 is a good indication of a cis relation between H_3 and H_4. H_5 proton showed as 4 lines where H_6 consisted of a complex signal which did not permit first order interpretation.

Oxidation of 44 using ruthenium dioxide dihydrate/sodium periodate in a pH controlled medium gave 48 as a white, slightly unstable solid. 48 was used directly in the next step where, upon addition of carbamoylmethylenetriphenylphosphorane in freshly distilled chloroform, 49 was obtained.



The p.m.r. spectrum of 49 (Fig. 13) clearly shows the disappearance of the methyl ester group, the presence of a single NH proton at δ 8.08 and a vinyl proton at δ 6.39. The I.R. shows characteristic absorptions at 1790 and 1740 cm^{-1} for the carbonyl function and at 1655 cm^{-1} for the olefinic bond. In the mass spectrum, the molecular ion was found at m/e 431 and other major peaks were found at m/e 400 (M^+-OCH_3) and 374 ($M^+-C(CH_3)_3$). The U.V. spectrum revealed

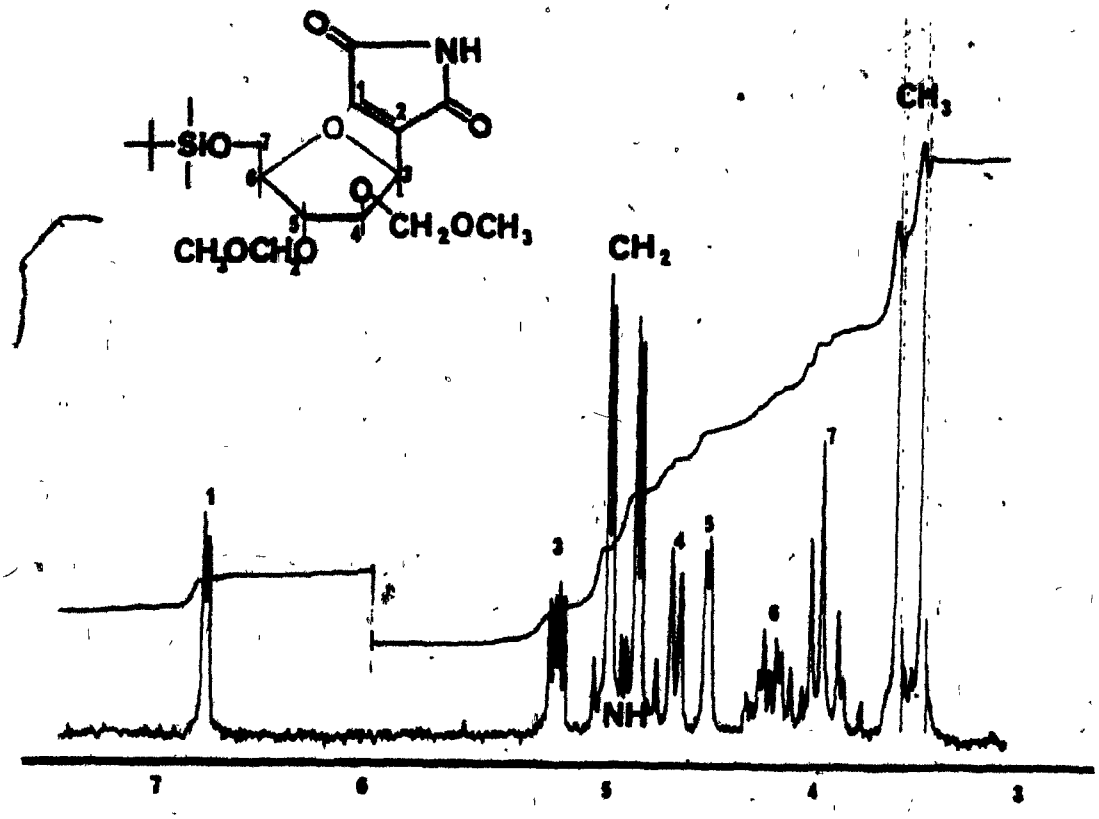


Figure 13: P.m.r. spectrum of 49 in $CDCl_3$ (90 MHz).

the typical maleimide chromophore having absorption maximum at 222 nm. Treatment of 49 with 80% aqueous trifluoroacetic acid completed the synthesis of 2'-epi-showdomycin (III). The elemental analysis, I.R., UV and mass spectral data were consistent with the structure of (III). The mass spectrum, which is quite typical of other C-nucleosides, shows a minor molecular ion at m/e 229 and a strong peak at m/e 211 corresponding to loss of water from M^+. Comparison of the major peaks of III to those of showdomycin revealed considerable similarities in the fragmentation pattern. The p.m.r. spectra (Fig. 14) shows clearly H_1 as a doublet coupled to H_3 ($J_{1,3}$ = 2 Hz), and H_3 as a doublet of doublets ($J_{3,1}$ = 2 Hz, $J_{3,4}$ = 4 Hz). As before, a coupling constant of 4 Hz between H_3 and H_4 indicated cis related protons. Comparison of spectra[36,37] of C-nucleosides and sugars having a β-configuration at the anomeric center to related compounds having an α-configuration shows that the chief difference is that the coupling constant corresponding to $J_{3,4}$ in the α-anomers, having otherwise identical stereochemistry, is close to 0 Hz. Therefore we are quite convinced that the stereochemistry of III is as indicated.

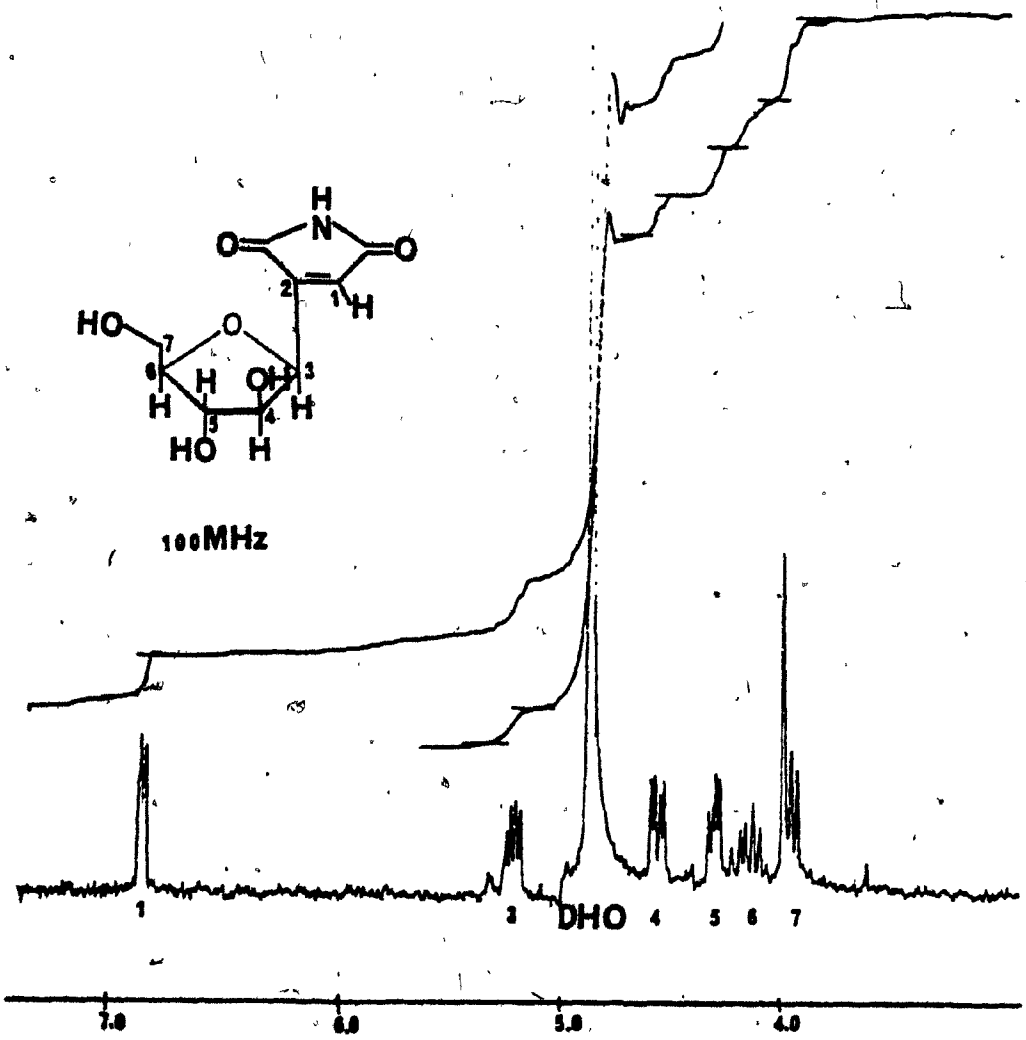
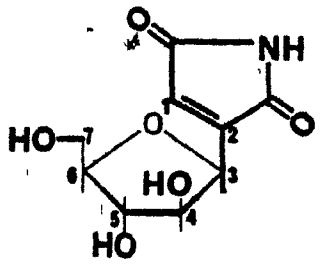


Figure 14: P.m.r. spectrum of III in D_2O



III

This synthetic route seems capable of producing D,L,2'-epi-showdomycin in better yields than before[35] and appears to offer an interesting route to analogues and homologues of showdomycin.

EXPERIMENTAL

Chapter 3

α,β-unsaturated ester 44

A solution of 43 (1.2 g, 4.89 mmole) and triethylamine (510 mg, 3 mmole) in dry methanol (60 ml) was refluxed for 8 hrs. Following the solution was filtered to remove small amounts of precipitate and the filtrate was evaporated to dryness. The residue was dissolved in chloroform, washed twice with water, dried over sodium sulfate and evaporated. The oil obtained was chromatographed on a column of alumina using 3:1 chloroform to ether and slowly increasing the polarity to pure ethyl ether. Compound 44 was obtained as a colourless oil in 40% yield (500 mg). I.R. ($CHCl_3$) 3500 (alcohol), 1730 (ketoester), 1625 (olefin) cm^{-1}. P.m.r. ($CDCl_3$-d_6); δ 3.36 (s, 3H, $CH_2O\underline{CH_3}$), 3.42 (s. 3H, $CH_2O\underline{CH_3}$), 3.66 (d, 1H, $CH_3OCH_2O\underline{CH}$, 0.8 Hz), 3.76 (s, 3H, OCH_3), 4.20 (q, 1H, $CH_3OCH_2O\underline{CH}$, 0.8 Hz and 4 Hz), 4.66 (q, 2H, OCH_2O, J_1=3 Hz and J_2=6 Hz), 4.76 (s, 2H, $O\underline{CH_2}O)_4$, 4.99 (d, 1H, J= 2 Hz, C=C$H\underline{CH}$), 5.12 (d, 1H, J=4 Hz, CH=C-CH), 7.15 (d, 1H, J=2 Hz, C=CH) p.p.m. Anal: Calcd for $C_{10}H_{14}O_6$: C, 52.17, H, 6.13. Found: C, 52.08, H, 6.28.

Formation of the methoxymethyl ether 45

To a stirred solution of 44 (1.3 g, 5.6 mmole) in dry chloroform (50 ml) were added methylal (10 ml) and approximately 3 g of phosphorous pentoxide. After 2 hrs at room temperature, the mixture was poured into 5% sodium bicarbonate solution. The organic layer was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was purified on a column of silicic acid using chloroform: hexane: ethyl ether (5:3:.5), giving 1.3 g (84%) of 45 as a homogeneous syrup. I.R. ($CHCl_3$) 1730 (Ketoester), 1625 cm^{-1} (olefin); p.m.r. ($CDCl_3$) δ 3.36, (s, 3H), 3.42 (s, 3H), 3.66 (s, 1H), 3.76 (s, 3H), 4.20 (d, 1H, J=4 Hz), 4.66 (q, 2H, J=3 Hz), 4.76 (s, 2H), 4.99 (d, 1H, J=1 Hz), 5.12 (d, 1H, J=4 Hz), 7.15 (d, 1H, J=2 Hz); m.s. (70 eV): m/e 259 (M^+-CH_3), 243 (M^+-OCH_3), 213 ($M^+-CH_3OCH_2O$), 148. Anal. Calcd. for $C_{12}H_{18}O_7$: C, 52.55; H, 6.62. Found: C, 52.38; H, 6.53.

Diol 46

To a solution of 45 (327 mg, 1.19 mmole) in dry methylene chloride at -78° was bubbled ozone until a faintly blue color was observed. Excess ozone was flushed with dry nitrogen while the system was kept at -78° and dimethyl sulfide (0.5 ml) was added. The mixture was allowed to rise to room temperature over a period of 5 hr. The solution was then evaporated to dryness. To a pre-cooled solution of the residue in freshly distilled tetrahydrofuran (50 ml) at 0° was added lithium tri-tert-butoxy-aluminum hydride (0.91 g, 3.57 mmole). The resulting clear solution was then allowed to warm to room temperature and stirred overnight under dry nitrogen. A solution of ammonium sulfate (2 g) in water (2 ml) and celite (1 g) was added to the reaction at 0°. The mixture was stirred for 30 min and finally filtered over a layer of celite. Following evaporation of the solvent the residue was dissolved in ethyl acetate, washed with water, dried ($MgSO_4$), and evaporated. The crude clear oil was crystallized from chloroform-hexane giving 222 mg (60%) of 46 as white crystals m.p 70-71°C. I.R. ($CHCl_3$) 3500 (alcohol), 1745 cm^{-1} (keto-ester); p.m.r. ($CDCl_3$): δ 3.33 (s, 3H), 3.36 (s, 3H), 3.67-3.96 (m, 6H), 4.06-4.56 (m, 5H), 4.56-4.83 (m, 5H) p.p.m. Anal. Calcd. for $C_{12}H_{22}O_9$: C, 46.45; H 7.15. Found C, 46.28; H, 7.28.

Monosilyl ether 47

The mixture of 46 (169 mg, 0.54 mmole), dimethyl-tert-butylsilyl chloride (82 mg, 0.54 mmole) and imidazole (92 mg, 1.35 mmole) in dimethylformamide (5 ml) was stirred at room temperature for 18 hr. Following the reaction mixture was added to 60 ml of ethyl ether and washed three times with water, dried, and evaporated, leaving a syrup. The latter was chromatographed on a column of silicic acid eluting with ethyl ether-hexane (2:1) giving 197 mg (85%) of 47 as an oil which crystallizes below room temperature. I.R. ($CHCl_3$) 3500 (alcohol), 1745 cm^{-1} (ketoester); p.m.r. ($CDCl_3$) δ 0.10 (s, 6H), 0.96 (s, 9H), 3.32 (s, 3H), 3.36 (s, 3H), 3.48 (m, 1H), 3.60-4.00 (m, 6H), 4.08-4.43 (m, 4H), 4.62 (b.s. 4H) p.p.m. Anal. Calcd for $C_{18}H_{36}O_9Si$: C, 50.94; H, 8.94. Found: C, 50.68; H, 8.37.

Oxidation to form ketoester 48

To a solution of 47 (424 mg, 1 mmole) in carbon tetrachloride (40 ml) were added ruthenium dioxide dihydrate (20 mg) and a solution of sodium periodate (856 mg, 4 mmole) in water (40 ml). The pH of the reaction mixture was controlled between 6 and 7 by the addition of a 5% sodium bicarbonate solution. After 6 hr stirring at room temperature or until a yellow color persisted, the reaction was terminated by adding a few drops of isopropyl alcohol. After collection of the black precipitated solid (RuO_2) on Celite, the organic phase was washed with water and brine, dried over magnesium sulfate, and evaporated, leaving 359 mg of crude 48 as a low m.p. white solid (less than 25°). Attempted purification by chromatography on silicic acid led to partial decomposition and accordingly the material was used directly in the next step. I.R. (neat) 1750, 1775 cm^{-1} (keton, ketoester); p.m.r. ($CDCl_3$) δ 0.08 (s, 6H), 0.86 (s, 9H), 3.13 (s, 3H), 3.25 (s, 3H), 3.36-3.58 (m, 2H), 3.72 (s, 3H), 3.76 (m, 1H), 4.13 (b.s, 1H), 4.30 (m, 1H), 4.40-4.70 (m, 4H), 5.06 (d, 1H, J=5 Hz) p.p.m.

Maleimide 49

A solution of carbamoylmethylenetriphenylphosphorane (400 mg, 1.2 mmole) and the crude keto ester 48 (450 mg, 1.07 mmole) in freshly distilled chloroform (20 ml) was stirred at room temperature for 2 hr. The solvent was then evaporated and the residue was purified by chromatography on silica gel column using petroleum ether - ethyl acetate (3:1), giving 216 mg (47%) of solid 49 m.p. 49°-50°C. I.R. ($CHCl_3$) 3460, 3250 (alcohol, amine), 1740, 1790 (maleimide), 1655 cm^{-1} (olefin); p.m.r. ($CDCl_3$, D_2O), 100 MHz: δ 0.10 (s, 6H, $Si(CH_3)_2$), 0.82 (s, 9H, $C(CH_3)_3$), 3.20 (s, 3H, OCH_3), 3.26 (s, 3H, OCH_3), 3.53-3.70 (m, 2H, $SiO\underline{CH_2}CH$), 3.70-4.10 (m, 1H, $SiOCH_2\underline{CH}$), 4.30 (d, 1H, $OCH_2O\underline{CH}$, J=2 Hz), 4.50 (d, 1H, $OCH_2O\underline{CH}$, J=4 Hz), 4.65 (q, 2H, $CH_3O\underline{CH_2}$), 4.80 (q, 2H, CH_3OCH_2), 5.0 (q, 1H, anomeric proton, J_1=4 Hz, J_2=3 Hz); 6.39 (d, 1H, C=CH, J=2 Hz); m.s. (70 eV): m/e 431 (M^+), 400 (M^+-OCH_3), 374 ($M^+-C(CH_3)_3$), 328. Anal. Calcd. for $C_{19}H_{33}NO_8Si$: C, 52.90; H, 7.66; N, 1.62. Found: C, 52.68; H, 7.76; N, 1.49.

D,L-2'-EPI-Showdomycin (III)

A solution of 49 (212 mg, 0.5 mmole) in a mixture of trifluoroacetic acid-water-tetrahydrofuran (4:1:1) (12 ml) was stirred at room temperature for 4 hr. After evaporation to dryness in vacuo the residue was crystallized from acetone-hexane to give 68 mg (60%) of 49 with m.p. 170-171°. I.R. (KBr) 3470, 3110, (alcohol, amine), 1775, 1720 (maleimide), 1625 cm^{-1} (olefin); u.v. (λ_{max}^{EtOH}) 222 nm (log ε 4.36); p.m.r. (D_2O), 100 MHz; δ 3.8-3.9 (m, 2H, $HO\underline{CH_2}$-CH), 4.1-4.25 (m, 1H, $HOCH_2\underline{CH}O$), 4.28 (q, 1H, J_1=2 Hz, J_2=3 Hz), 4.55 (q, 1H, J_1=2 Hz, J_2=4 Hz), 5.1 (q, 1H, anomeric H, J_1=2 Hz, J_2=4 Hz) 6.85 (d, 1H, C=$\underline{CH}$, J=2 Hz); m.s. (70 eV): m/e 229 (M^+), 211 (M^+-H_2O), 180, 140 ($M^+-HOCHCH_2B$), 127 (B+31), 126 (B+30), 110, 87, 85, 69, 57, 55, 45, 44, 43, 32, 31, 28 (B is maleimide). Anal. Calcd. for $C_9H_{11}NO_6$: C, 47.16; H, 4.80; N, 6.15. Found: C, 47.05; H, 4.75, N, 6.08.

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