THE ASYMMETRIC SYNTHESIS OF β-LACTAMS

A Thesis

by

Sheila Muriel Tenneson

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Department of Chemistry

McGill University

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To

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Sheila Muriel Tenneson

Department of Chemistry
McGill University
Montréal, Canada

ABSTRACT

The total synthesis of the biologically active, dextrorotatory enantiomer of 3-methyl-7β-phenylacetamido-Δ^3-*O*-2-isocephem-4-carboxylic acid was accomplished. The key step involved the asymmetric cycloaddition of azidoacetyl chloride to the cinnamylidene Schiff base of protected D-threonine to generate the desired monocyclic *cis* β-lactam diastereomer (9:1). The absolute configuration of the final product was confirmed by comparing its antimicrobial activity with that of the corresponding racemate[1].

The influence of a) the β-chiral center in the starting α-amino acid, b) the bulk of the carboxylic acid and c) the distribution of bulk throughout the imine on the stereochemical outcome of the reaction was studied. The absence of racemization during the cycloaddition was demonstrated by the use of deuterated precursors. The great potential of D-glucosamine derivatives as chiral templates in this reaction was clearly illustrated.

LA SYNTHÈSE ASYMÉTRIQUE DE β-LACTAMES

Sheila Muriel Tenneson

Département de chimie
Université McGill
Montréal, Québec

RÉSUMÉ

La synthèse totale de l'énantiomère dextrogyre biologiquement actif de l'acide méthyl-3-phénylacétamido-7β-*O*-isocéphème-2-carboxylique-4 fut réalisée. L'étape principale consiste en la cycloaddition asymétrique du chlorure d'azidoacétyle sur la base de Schiff dérivée de le cinnamaldéhyde et de la D-thréonine protégée, ce qui engendra le diastéréoisomère désiré (9:1) d'une β-lactame monocyclique *cis*. La configuration absolue du produit final fut confirmée par comparaison de son activité antimicrobienne avec celle du mélange racémique correspondant.

L'influence a) du centre asymétrique en *beta* de l'acide α-aminé de départ, b) de l'encombrement stérique généré par le groupement protecteur de l'acide carboxylique ainsi que c) de l'encombrement stérique général à travers l'imine, sur la stéréochimie de la cycloaddition, fut étudiée. L'absence de racémisation pendant la réaction de cycloaddition fut démontrée par l'utilisation de produits de départ deutérés. Le potentiel évident des dérivés de la D-glucosamine en tant que générateurs d'asymétrie dans ce type de réaction fut clairement illustré.

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GLOSSARY OF ABBREVIATIONS

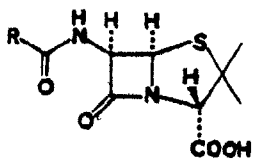
Ac	acetyl
Ala	alanine
anal.	analysis
anh.	anhydrous
app.	apparent
approx.	approximately
Ar	aryl
b	broad
Bn	benzyl
BOC	*tert*-butyloxycarbonyl
BOC-ON	2-(BOC-oxyimino)-2-phenylacetonitrile
Bu	butyl
Bu^t	*tert*-butyl
calcd.	calculated
cat.	catalyst
Cbz	benzyloxycarbonyl
CI/IB	chemical ionization/isobutane
cm	centimeter(s)
d	doublet
DBU	1,8-diazabicyclo-[5,4,0]undec-7-ene
DCC	*N,N'*-dicyclohexyl-carbodiimide
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
EEDQ	*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
EI	electron impact
Et	ethyl
expl.	experimental
F.T.	Fourier transform
g	gram(s)
HMPT	hexamethylphosphoric triamide
^{1}H.m.r.	proton magnetic resonance
HPLC	high pressure liquid chromatography
hr.	hour(s)
Hz	Hertz
i	iso
i.r.	infrared
m	multiplet
max	maximum
Me	methyl
mg	milligram(s)
MHz	megaHertz

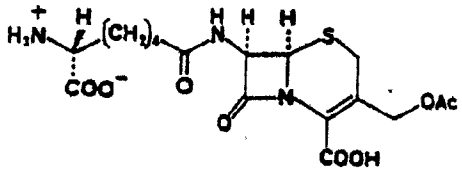
MIC	minimum inhibitory concentration
min.	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mmol	millimole(s)
m.p.	melting point
Ms	methanesulfonyl (mesyl)
n	normal
nm	nanometer(s)
nuc	nucleophile
p	para
p	quintet
PCC	pyridinium chlorochromate
p.e.	petroleum ether (30° - 60° C)
Ph	phenyl
Phe	phenylalanine
PNB	*para*-nitrobenzyl
poss.	possible
ppm	parts per million
Pr	propyl
p.s.i.	pounds per square inch
pyr	pyridine
q	quartet
quant.	quantitative
R.T.	room temperature
s	secondary
s	singlet
sat.	saturated
sec.	second(s)
Ser	serine
sh	shoulder
t	triplet
tert	tertiary
THF	tetrahydrofuran
Thr	threonine
Ts	toluenesulfonyl (tosyl)
unsat.	unsaturated
U.S.P.	United States Pharmacopeia
UV	ultraviolet

INTRODUCTION

Those antibiotics which are identified by a β-lactam (2-azetidinone) ring are probably the least toxic of all the antibacterial agents[2,3]. For this reason, these structures have attracted the interest of organic and medicinal chemists throughout the world. Once it was known that the activity of the naturally occurring ring system could be enhanced by certain structural alterations, a great number of analogues were, and still are being prepared and examined for biological effectiveness[4]. Some of this effort has taken place within this chemistry department over the past several years[5-8].

When the only known members of this family of antibiotics were the penicillins (*1* and *2*) and cephalosporins (*3*), the

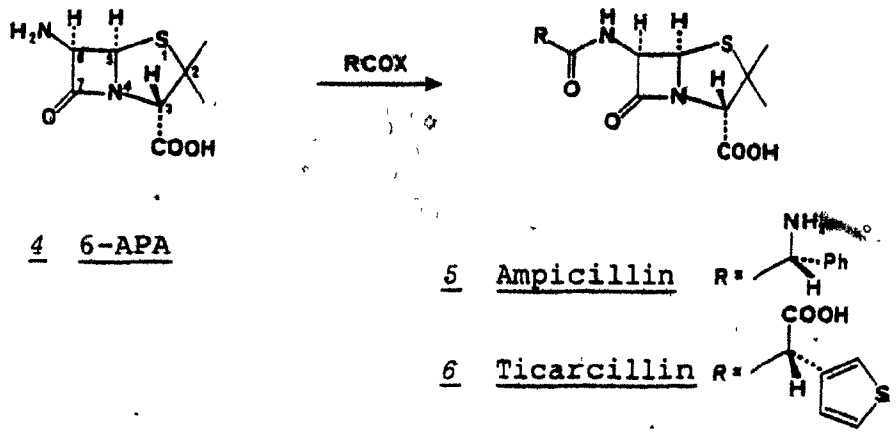




1 Penicillin G R = CH_2Ph
2 Penicillin V R = CH_2OPh

3 Cephalosporin C

main challenge was to make them available to the general public. Since these complex molecules are difficult to synthesize economically, it was the fermentation chemists and the microbiologists who first achieved this goal. They succeeded extremely well[9]. Penicillin G (*1*) and Penicillin V (*2*) are now widely available in large quantity, as is the product of *N*-deacylation, 6-amino-penicillanic acid (6-APA, *4*). With a price



4 6-APA

5 Ampicillin R = NH, Ph, H

6 Ticarcillin R = COOH, H, S

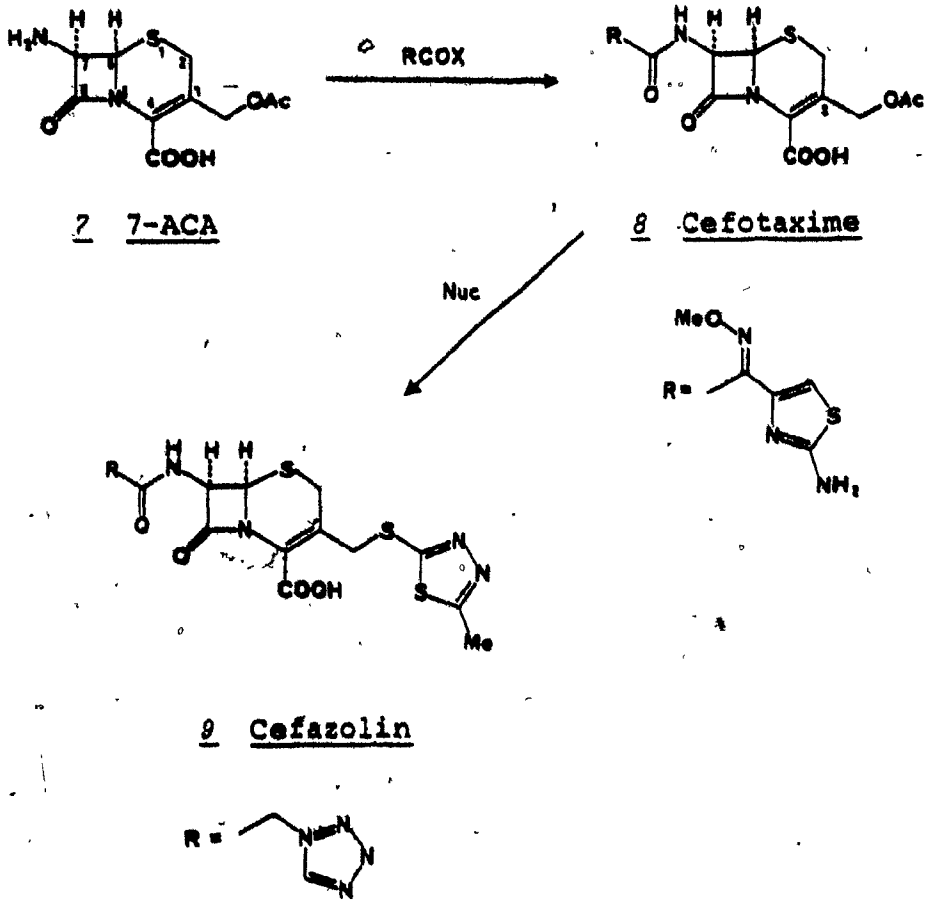
Fig. I

of approximately $50 per kilogram*, the latter provides an inexpensive starting material for more active, semi-synthetic penicillins such as Ampicillin (*5*) and Ticarcillin (*6*).

Separation of Cephalosporin C (*3*) from its fermentation

*Prices quoted are for bulk sales to industry.

medium is difficult[10]. The corresponding *N*-deacylated 7-amino-cephalosporanic acid (7-ACA, *7*), therefore costs about twice as much as 6-APA to produce. Nevertheless, 7-ACA is still the



7 7-ACA

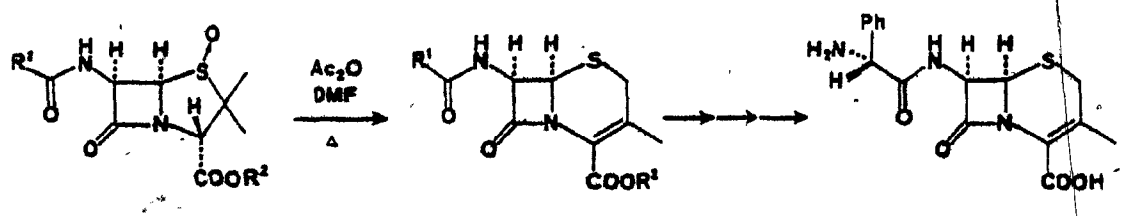
8 Cefotaxime

9 Cefazolin

Fig. II

best starting material for a cephalosporin containing a C-3 substituted methylene, as in Cefotaxime (*8*)[11] which retains the acetoxy group of the natural Cephalosporin C (*5*), or as in

Cefazolin (*9*)[12] in which the acetoxy group has been replaced by nucleophilic attack. Orally active deacetoxy-cephalosporins, such as Cephalexin (*12*)[13], can be produced from Penicillin G (*1*) or V (*2*) by Morin rearrangement[14] of the penicillin sul-



10 $R^1 = CH_2Ph$ or CH_2OPh *11* *12* Cephalexin

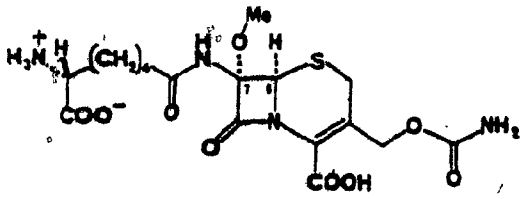
Fig. III

foxide ester *10*. Although this requires six or seven steps[13], the reactions are easy enough to make these compounds economically competitive with other semi-synthetic cephalosporins.

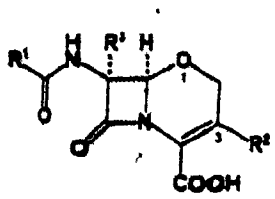
Penicillins and cephalosporins each account for about thirty per cent of the world market for antimicrobial agents[15]. Despite their higher cost, cephalosporins hold a favourable position because they tend to be less allergenic and less sensitive to β-lactamases. They have also provided analogues with greater activity against a broader spectrum of gram-negative bacteria. Cefotaxime (*8*) (fig. II) is one example of

the newest generation of cephalosporins. (The same side chain on the penicillin nucleus results in disappointing activity[15].)

Isolation of the natural cephamycins, for example *13*, in the early 1970's[17] showed that the resistance of cephalosporins to β-lactamases could be increased substantially by the presence of a 7α-methoxy group. Furthermore, the results from



13 Cephamycin C

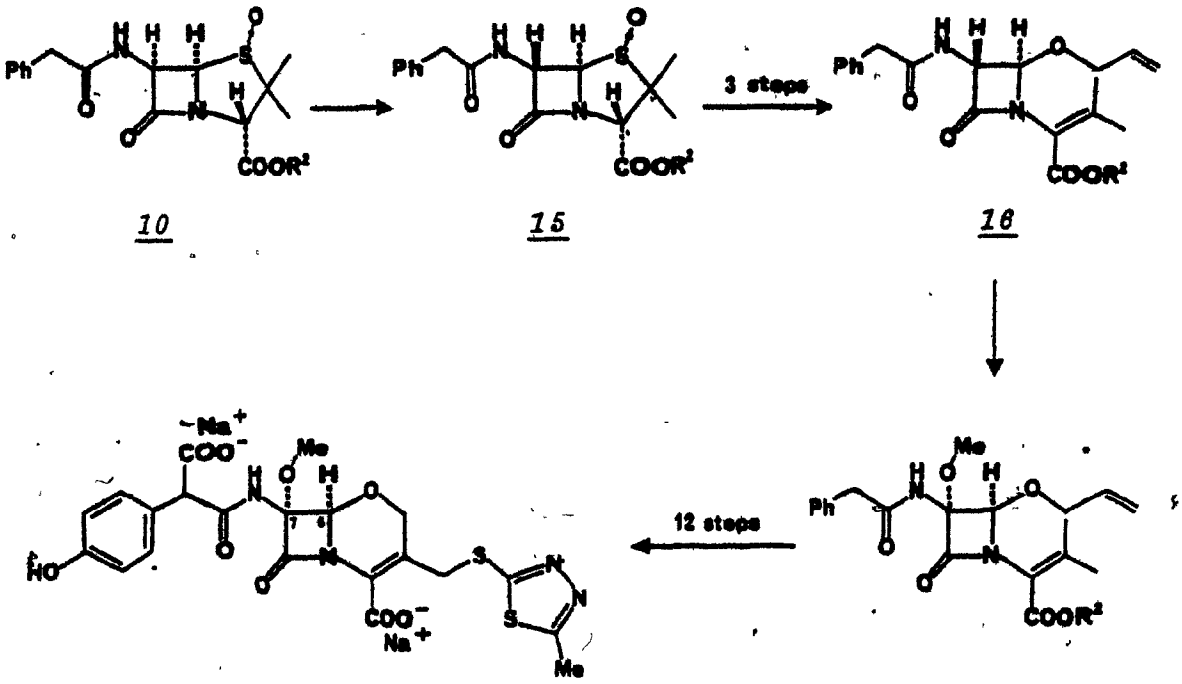


14 1-Oxacephem

synthetic programs at several pharmaceutical companies have shown that replacement of the group at the C-3 position by methoxy[18,19], chlorine[19] or hydrogen[16,20]; and/or the sulfur by oxygen[21-24] (giving 1-oxacephem *14*) can lead to more active compounds. Introduction of several of these features into one molecule, however, requires considerable effort.

The first 1-oxacephem to be chosen for clinical trials was Moxalactam (*18*)[25] (fig. IV). The starting material for the synthesis of this compound is the inexpensive Penicillin G (*1*), but in order to introduce the *cis* 7α-methoxy (relative to the C-6 proton), a *trans* secopenicillin *16* must be prepared from

the epipenicillin ester *15*. Once the correct stereochemistry has been established (*17*), twelve additional steps[26] (bringing



10 *15* *16*

17

18 Moxalactam

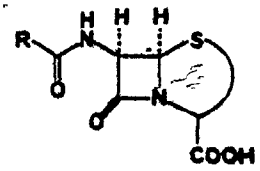
Fig. IV

the total to eighteen) are required to prepare Moxalactam (*18*).* Such long syntheses must be very efficient, and the final products highly efficacious in order to compete in the marketplace.

When a large number of reactions are necessary to produce a given set of alterations in a natural antibiotic, it becomes

*A better synthesis (~20% from Pen G) required only 14 steps [186].

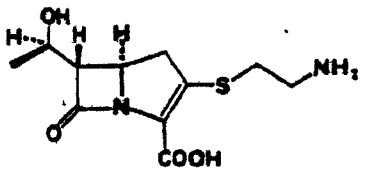
worthwhile to consider total synthesis as an alternative approach. For this to be a viable alternative, an efficient method of forming only the bioactive enantiomer must be devised. For the classical antibiotics, this enantiomer is a *cis* β-lactam



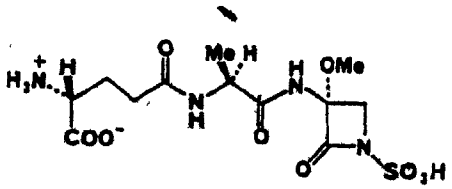
19

with the 7(6)-R, 6(5)-R* configuration (19).

The structures of recently isolated, highly active, antibiotics, such as Thienamycin (20)[27] and Isosulfazecin (21)[28], demonstrate that biological activity does not always depend on



20 Thienamycin



21 Isosulfazecin

*The number in parentheses refers to the penicillin nucleus when both cephalosporins and penicillins are mentioned.

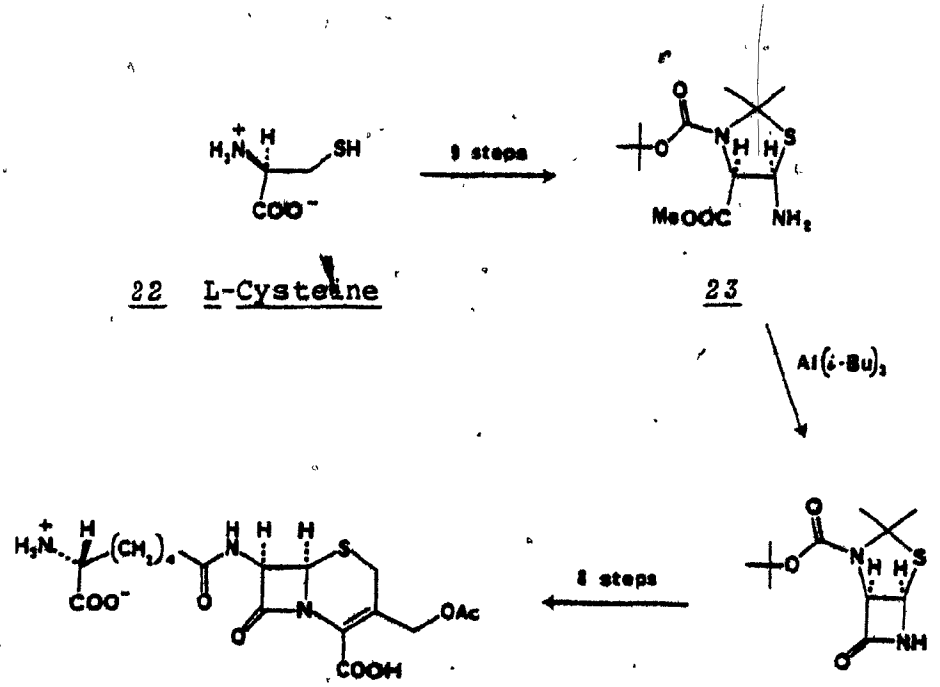
the 1-thio-bicyclic *cis* β-lactam system with an aminoacyl chain (*19*), as had originally been thought. The absence of some of these features in *20* and *21* is compensated by other structural parameters, such as a carbapenem (*20*) or a 1-*N*-sulfonic acid (*21*). There are, nevertheless, some features which still appear to be basic. All the active bicyclic systems found so far, have R stereochemistry* at the ring junction; and all the active β-lactams containing an aminoacyl side chain have the same spatial arrangement* (monocyclic = S, bicyclic = R) at the *N*-substituted carbon. Any stereospecific synthesis must take these stereochemical factors into account.

Chiral Precursors

In most stereospecific syntheses of β-lactam antibiotics, at least one of the chiral centers present in the selected starting material was retained throughout the synthesis to the final product. In his classical synthesis

*Because of the priority rules used to assign the R or S designation to a chiral center, changes in substituents may change the terminology. For example, the same spatial arrangement of groups at the ring junction of *O*-2-isocephems (*70*, p. 23) is designated S and not R.

of Cephalosporin C (*3*), Woodward[29] obtained the required *cis* β-lactam *24* by cyclizing the chiral β-amino ester *23*, prepared



22 L-Cysteine *23*

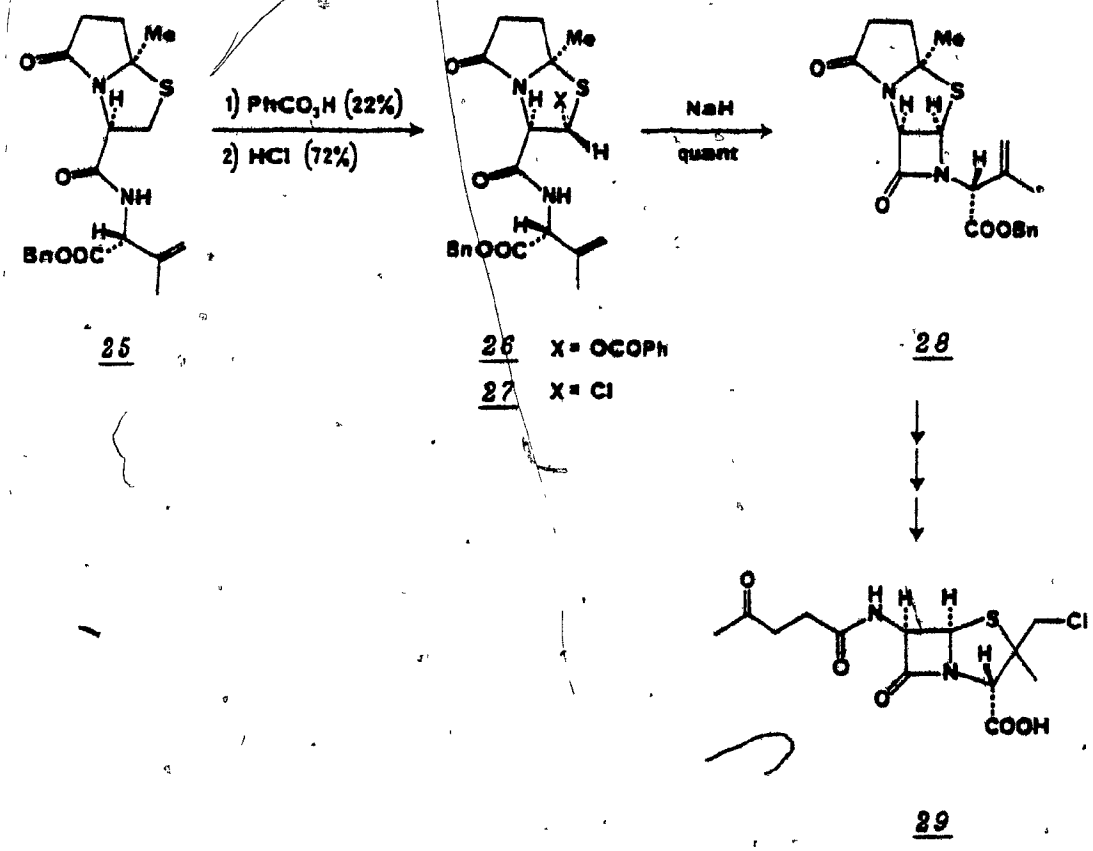
3 Cephalosporin C *24*

Fig. V

in nine steps from L-cysteine (*22*). Similar cyclizations using Grignard[30,31] and phosphorus[32,33] reagents have also led to chiral β-lactams with no troublesome epimerizations[34] during the process.

A somewhat different chiral precursor (*27*) (fig. VI)

prepared from L-cysteine (*22*), was described by Baldwin in his short synthesis of penicillin *29*[35]. The stereochemically correct *cis* β-lactam intermediate *28* was created by intramolecular



25 *26* X = OCOPh *28*

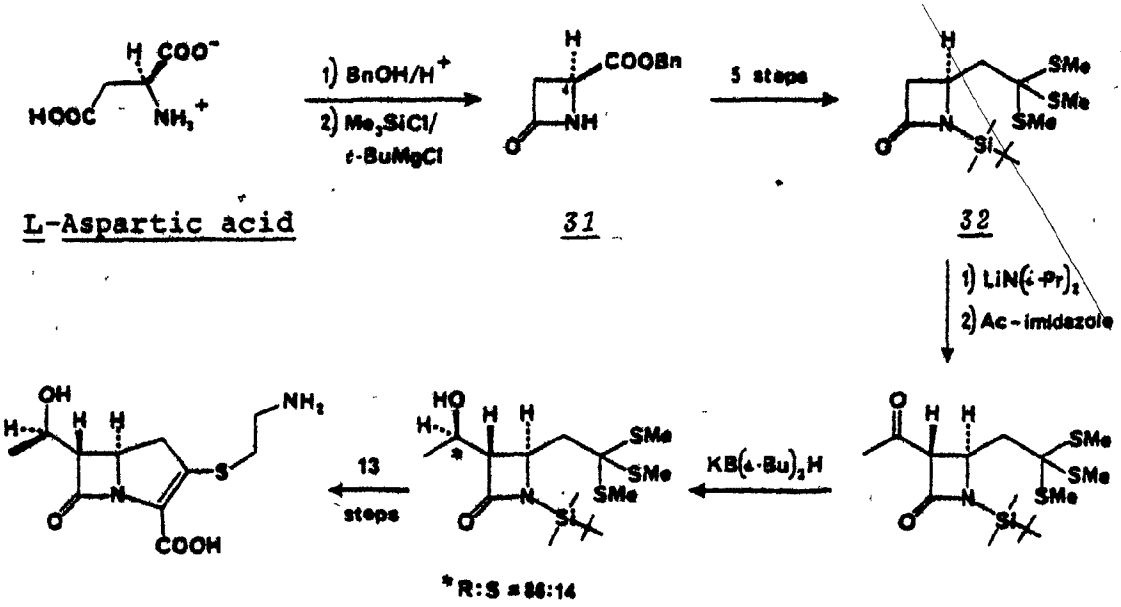
27 X = Cl

29

Fig. VI

displacement of the chloride in *27* by an amide anion. This approach was later exploited by Koppel *et al*[36] in the synthesis of Nocardicin A (*64*, p. 20).

In their recent stereospecific synthesis of Thienamycin (*20*)[37], Christensen and his coworkers at the Merck, Sharpe and Dohme Laboratories, used the β-lactam itself as the chiral template. Grignard cyclization[30] of the diester of L-aspartic acid gave the basic β-lactam *31* with a chiral center at C-4.



30 L-Aspartic acid *31* *32*

20 Thienamycin *34* *33*

Fig. VII

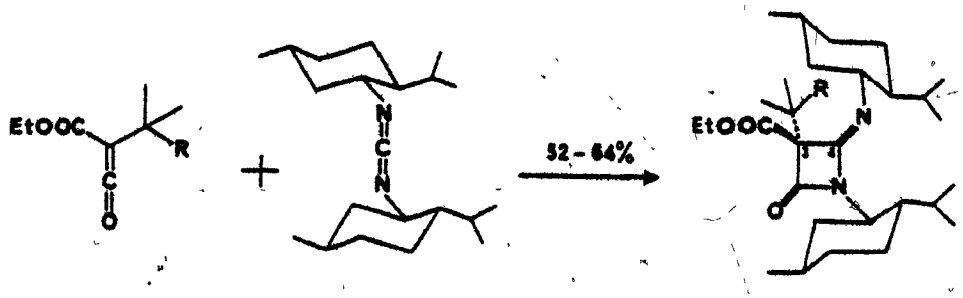
After several transformations designed to obtain *32* (which is stable to base), the second chiral center was introduced by reacting the lithium enolate of the latter with acetyl imid-

azole. Reduction of *33* with the sterically hindered K-Selectride yielded mainly the R-hydroxyethyl diastereomer *34* containing the requisite stereochemistry for transformation to Thienamycin (*20*).

Asymmetric Induction

The preparation of chiral precursors can be a lengthy process (Woodward, p. 9, and Christensen, p. 11), and/or an inefficient one (Baldwin, p. 10). More practical syntheses have been devised[38,39] but these usually led to racemic mixtures which required resolution, thus reducing the efficiency of the synthesis by at least fifty per cent. If the correctly substituted 2-azetidinone could be produced in one highly stereospecific and high yield step, a major hurdle in the synthesis of β-lactam antibiotics would be overcome. Ideally this reaction should also have the potential for general application to a wide variety of substrates. Only a few examples of asymmetric induction during β-lactam formation have appeared in the literature, all of them over the last ten years. Some of these reactions have been achieved with high stereospecificity, but as yet none of them have met all of the above criteria.

Bełżecki and his coworkers have reported two highly stereospecific syntheses of β-lactams. In their first approach[40], the

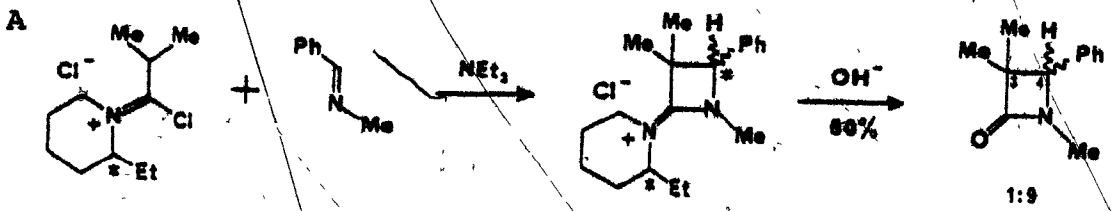


35a R = Me
b R = Ph

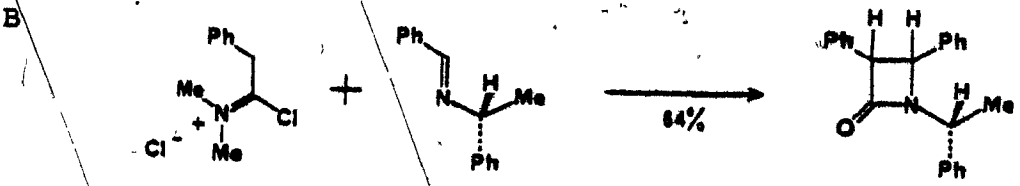
36

37a R = Me
b R = Ph

Fig. VIII



38 39 40



41 42 43

Fig. IX

symmetrical, chiral carbodiimide *36* (fig. VIII) was added to a bulky, unsymmetrical ketene *35* to give 4-imino-2-azetidinone *37* possessing a chiral center at C-3 with the R configuration exclusively[41]. Any decrease in the bulk of either one of the starting materials decreased the stereospecificity of the reaction.

Their second approach[42] involved treatment of the chiral α-chloro-iminium chloride *38* (fig. IX-A) with imine *39* to obtain a 1:9 mixture (80% induction*) of the C-4 enantiomers of β-lactams *40*. The absolute configuration of the major enantiomer was not reported. The authors also examined the possibility of extending this reaction to β-lactams with two chiral centers by using an unsymmetrical iminium chloride *41* and a chiral imine *42* (fig. IX-B). However, this resulted in all four diastereomeric β-lactams *43* with little stereoselectivity.

Several Japanese groups have found β-lactams useful as intermediates in the preparation of chiral amino acids. Among them was the first group to report asymmetric induction during 2-azetidinone formation. Okawara and Harada[43], were interested

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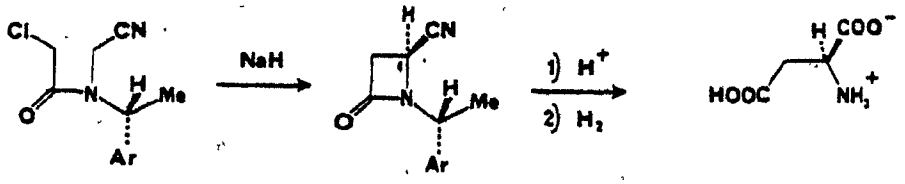
*% induction = major stereoisomer - minor/total × 100. Equivalent to % o.p.** when only two diastereomers are formed.

**% optical purity = $[\alpha]_D$ mixture/$[\alpha]_D$ pure enantiomer × 100.

in the synthesis of optically active aspartic acid (*30*). A product in which the S enantiomer predominated, was prepared in 54 to 96% yield by cyclization of the chiral *N*-acylaminoacetonitrile *44* with sodium hydride, acid hydrolysis of the crude



44 *45* *30* L-Aspartic Acid

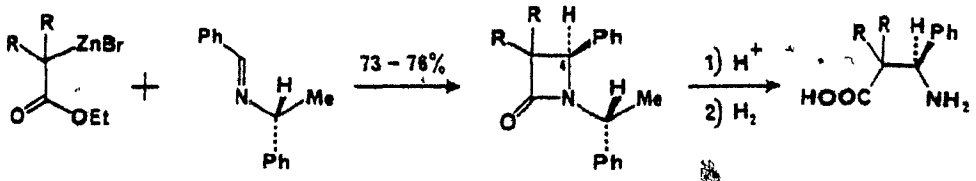
a Ar = 1-naphthyl
b Ar = Ph

Fig. X

intermediate β-lactam *45* and finally, hydrogenolysis of the (R)-1-arylethyl group on the amine. The optical purity (see footnote, p. 14) of the product depended on the size of the aryl group in *44*. The 1-naphthyl group led to higher asymmetric induction (54 to 67%) than did the phenyl group (41 to 49%). Equilibration studies demonstrated that the asymmetric induction was occurring during β-lactam formation. No example

of a 3,4-disubstituted β-lactam was included.

Furukawa and his colleagues[44] were interested in novel β-amino acids such as *48*, which they obtained by the method of Okawara and Harada from the chiral 2-azetidinones *47*. In order

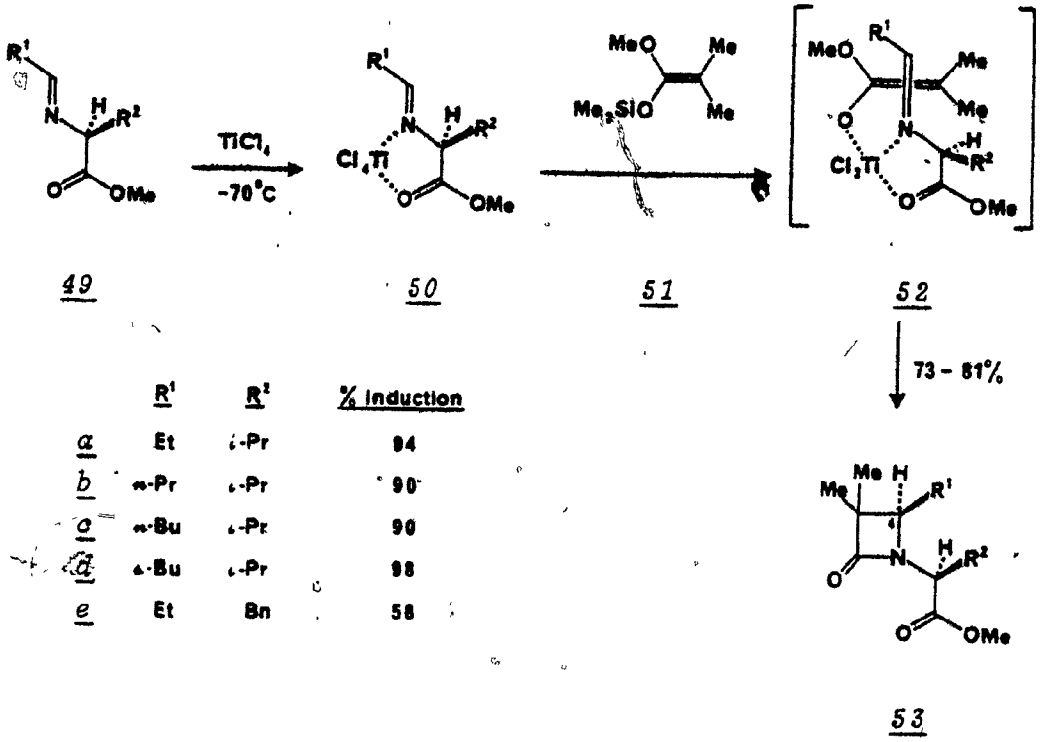


						O.P.
46a	R = Me	*42*	*47a*	R = Me	*48a*	R = Me 36%
b	R = H		*b*	R = H	*b*	R = H 28%

Fig. XI

to produce the α,α-dimethyl-β-amino acid *48a*, however, the β-lactam intermediate *47a* was prepared in another manner, by the reaction of chiral imine *42* with a Reformatsky reagent (*46*). This treatment resulted in an optically active product, but with only 36% asymmetric induction.

In 1980, Ojima and Inaba[45] reported very high stereospecificity (90 to 98%) in the synthesis of β-lactams from a variety of Schiff bases of valine methyl ester (*49a* to *d*) (fig. XII). By treating these imines with titanium tetra-



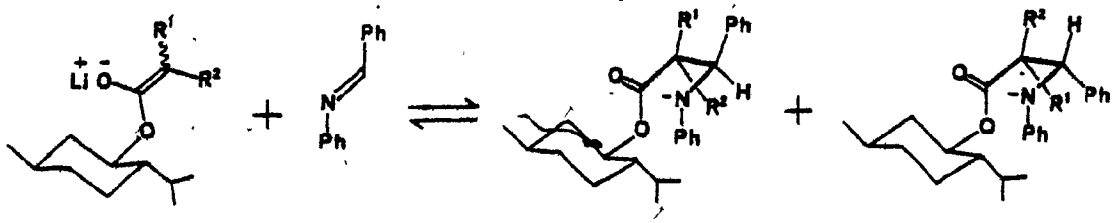
	R¹	R²	% Induction
a	Et	*i*-Pr	94
b	*n*-Pr	*i*-Pr	90
c	*n*-Bu	*i*-Pr	90
d	*s*-Bu	*i*-Pr	98
e	Et	Bn	58

Fig. XII

chloride at -78°C, the kinetically favoured E-titanium chelate *50* was formed. (Above -20°C, Z-*50* also appeared.) Treatment with dimethylketene methyl trimethylsilyl acetal *51* then gave the tridentate enolate intermediate *52* in which attack of the imine occurred from the less hindered side. The resulting β-lactam *53* had mainly the R configuration at C-4 (99:1 when R^1 = *s*-Bu). This stereospecificity was sensitive to the bulk of the aldimine[46], and to the bulk around the β-carbon in the

starting amino ester[45] which must be disubstituted for the induction of high asymmetry (phenylalanine derivative *49e* gave only 58% induction).

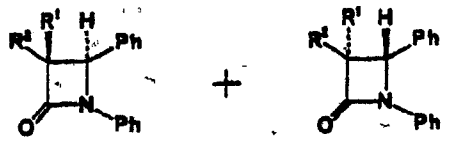
A similar approach which resulted in β-lactams with two chiral centers was reported at about the same time by Bergbreiter, Newcomb and coworkers[47]. In contrast, however, the observed asymmetry appeared to be thermodynamically, rather than kinetically controlled. *Trans* β-lactams *58* and *59* could



54 (X = Li) *55* *56* *57*

74 - 85%

	R^1	R^2	% Induction
a	Me	Ph	60
b	O^-X^+	Ph	14
c	H	NHCOPh	4



58 *59*

(X = H)

Fig. XIII

be prepared by the reaction of an unsymmetrical lithium enolate ester with an *N*-aryl-arylaldimine. When the alcohol moiety of the starting ester was chiral as in *54*, one of the enantiomers could be obtained in excess (up to 60%) but the respective configurations were not determined. From their studies, the authors concluded that only the first step in the reaction was reversible, giving the more reactive enolates *56* and *57* less chance to equilibrate to the more stable stereoisomer.

Several limitations to this reaction were mentioned. Only *N*-aryl-arylaldimines such as *55* could be used, possibly because of the need for an electrophilic, unhindered imine. *N*-Alkyl-imines were apparently deprotonated to give azaallyl anions. Also mono- or unsubstituted lithium enolates reacted poorly or not at all.

The first synthesis of a β-lactam antibiotic by asymmetric induction was described in 1978 by Kamiya and his colleagues at Fujisawa Pharmaceuticals[48]. Nocardicin A (*64*), one of the new class of monobactams, was synthesized using a variation of the popular imine - acyl chloride[49] cycloaddition reaction (fig. XIV). Fortunately, the R stereochemistry of the arylglycine residue in *60* (and *61*) led to a 3:1 excess of the desired β-lactam *63* with S configuration at C-3. By substituting the larger 1-naphthyl group for the protected *p*-hydroxyphenyl, they were able to increase the ratio of diastereomers from 3:1 to 10:1.

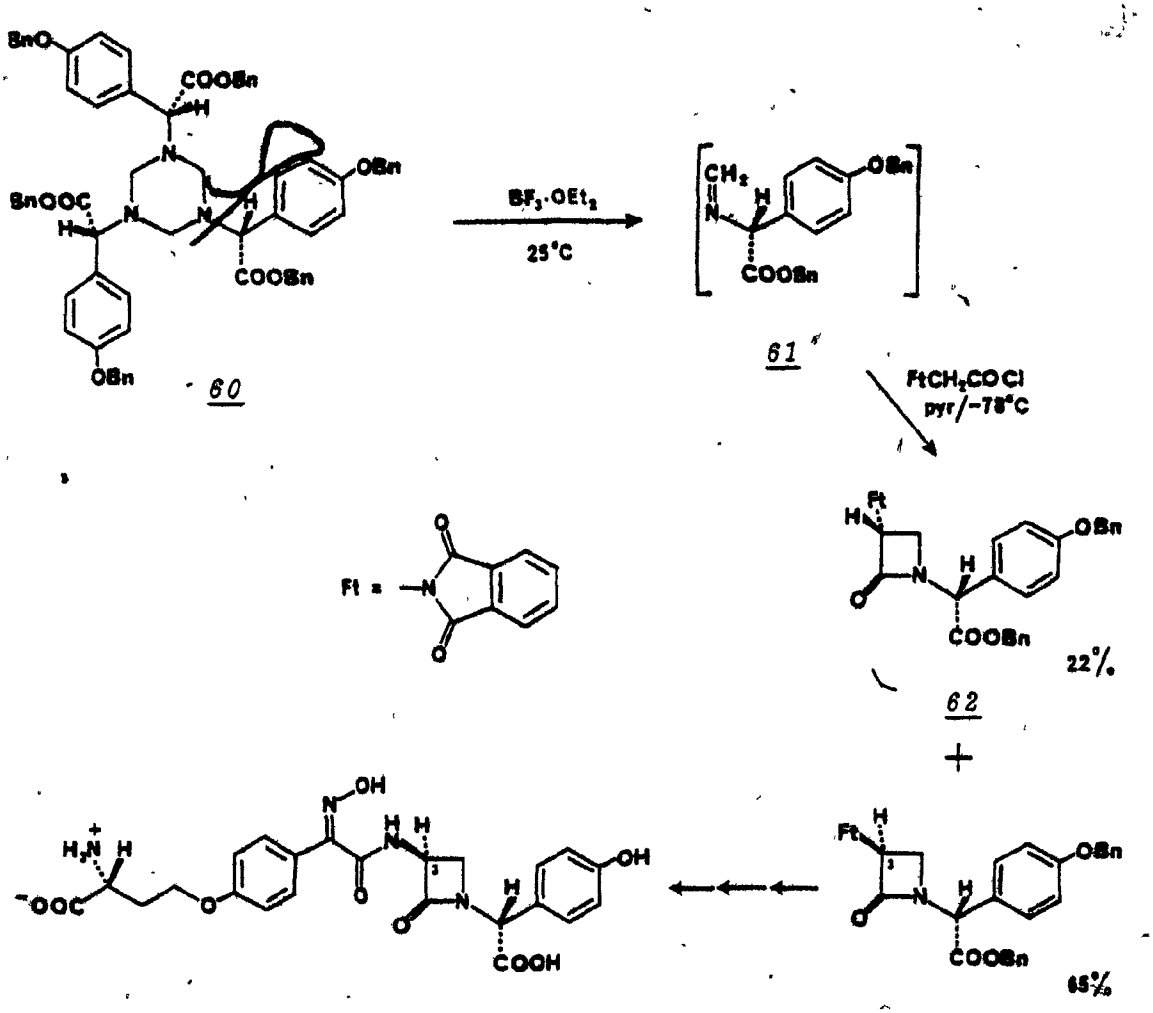


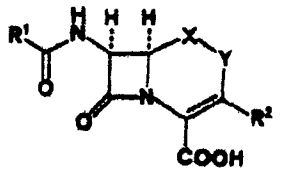
Fig. XIV

Some of these methods give products with high optical purity in good yield, but few of them can be generalized to structures which could lead to a bicyclic β-lactam antibiotic. The two most promising approaches seemed to be the imine - ketene

acetal method of Ojima and Inaba (p. 16), and the imine – acyl chloride method used by Kamiya *et al* (p. 19). In both cases, only one asymmetric center on the β-lactam was generated. Factors leading to the stereoselective formation of two centers were not investigated.

The *O*-2-Isocephem Story

Between 1977 and 1980, Doyle, Belleau and their coworkers at Bristol Laboratories of Canada published a series of efficient and stereoselective syntheses of cephalosporin nuclear analogues *65*[50-54] . Their strategy was first to prepare the

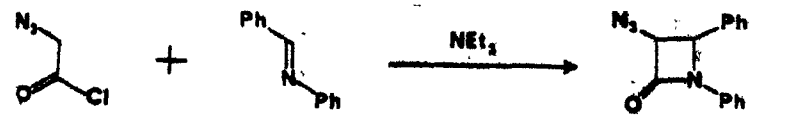


X = CH_2, CHCl, $CHMe_2$, CO
Y = O, S, SO, SO_2, NCO_2Et, NMe, CH_2, CO, CHOR, CHBr

65

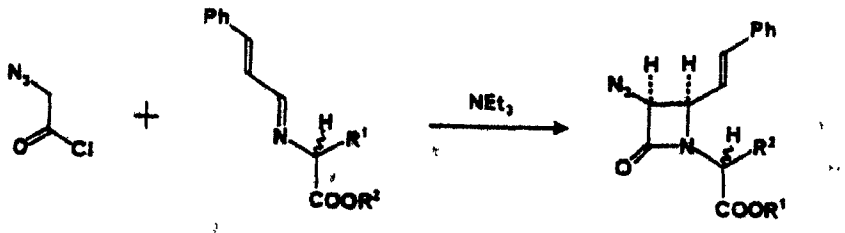
monocyclic β-lactam, and then to elaborate the second ring. By modifying the imine – azidoacetyl chloride method of Bose[55] (fig. XV-A), Doyle *et al* were able to obtain only *cis* β-lactams *69* (fig. XV-B) instead of *cis/trans* mixtures (*67*). Their modi-

A **Bose:**



66 *55* *67* 3:1 cis/trans

B **Doyle:**



66 (±)-*68* (±)-*69* 100% cis

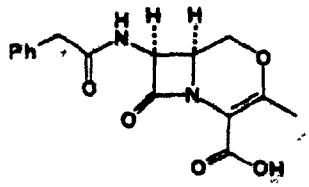
Fig. XV

fication was in the structure of the imine *68*, prepared from an α-amino ester and an α,β-unsaturated aldehyde, preferably *trans* cinnamaldehyde.

The most interesting of the analogues proved to be the *O*-2-isocephems (*65*; X = CH_2, Y = O), whose effectiveness against certain pathogenic bacteria[54] compared favourably with cephalosporins currently in use. As with all β-lactam antibiotics, biological activity was associated with only one stereochemical

configuration. For the *O*-2-isocephems, this proved to be the *cis* dextrorotatory enantiomer[52]. Preliminary experiments at Bristol Laboratories had indicated that a levorotatory *O*-2-isocephem could be obtained from the amino acid L-threonine[56]. It was our objective to study this phenomenon in greater detail. This work will, therefore, describe the following:

a) the use of an imine derived from D-threonine as a chiral template during β-lactam formation for the successful total synthesis of the bioactive enantiomer of 3-methyl-7β-phenylacetamido-Δ^3-*O*-2-isocephem-4-carboxylic acid (*70*) (chapter 1)[1];



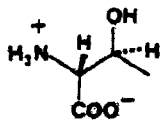
70

b) a study of some of the stereochemical requirements for asymmetric induction, for example, the influence of the β-chiral center in the starting amino acid and the importance of bulk in the carboxylic acid protecting group; as well as the use of deuterated precursors to demonstrate the absence of any detectable level of racemization at the α-carbon during the cycloaddition reaction (chapter 2);

c) the finding that amino sugars, in particular D-glucosamine derivatives, offer great potential as chiral templates, with the added advantage of producing crystalline intermediates in high yield (chapter 3).

CHAPTER 1

Since biologically active *O*-2-isocephems are dextrorotatory and since an *O*-2-isocephem with a net negative optical rotation could be obtained from (-)-L-threonine[56] by the stereoselective cycloaddition of Doyle *et al*[50], the obvious choice of starting material for our studies was (+)-D-threonine (D-Thr, *71*). Fortunately, this amino acid is one of the few



71 D-Threonine

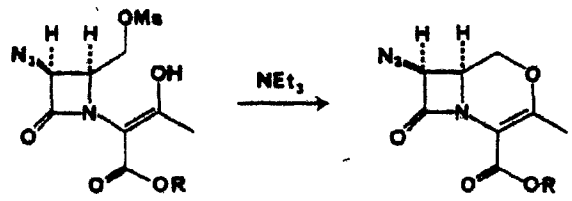
D-amino acids which are readily available*.

Our first priority was to determine whether asymmetric induction was occurring during formation of the β-lactam ring, and if so, to what extent. Thus it was desirable to choose protecting groups for (+)-D-threonine which would survive not

*Sigma Chemical Company, St. Louis, Missouri.

only the cycloaddition reaction itself, but also the extensive chromatography which might be necessary to separate and identify the diastereomers formed.

These constraints eliminated such alcohol protecting groups as the labile trimethylsilyl ether, and possibly the acid-sensitive tetrahydropyranyl ether which would also have contributed an additional, highly undesirable, chiral carbon. Should one major stereoisomer be formed, completion of the synthesis *via* Doyle's enol 72[51] was contemplated. The chosen



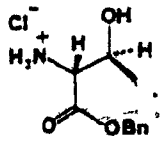
72 73

Fig. XVI

alcohol protecting group should then be selectively removable in the presence of the acid protecting group and the eventual leaving group at C-5 (mesylate) under conditions which would leave the β-lactam ring intact. Protection of the carboxylic acid as an ester should be compatible with all of these transformations. The benzyl ester was chosen because Doyle *et al*

had already demonstrated that it could be removed with ease from an *O*-2-isocephem without destructive side reactions[51].

Two approaches to the esterification of (+)-D-threonine were possible: direct esterification under acid conditions, or protection of the amine first, followed by esterification and deprotection of the amine. Several direct methods for preparing amino acid benzyl esters were reported in the literature. The most recent is by Patel and Price[57] who used thionyl chloride as a dehydrating agent and a source of mineral acid to produce the hydrochloride salts directly in good yield from amino acids containing no other unprotected functional groups. With (+)-D-threonine (*71*), however, this method yielded a maximum of 50% (+)-D-threonine benzyl ester hydrochloride



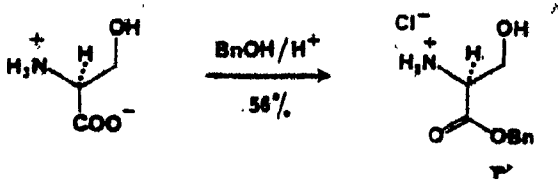
(+)-*74*

(D-Thr-OBn.HCl, *74*)* only when benzyl alcohol was present in

*A foldable flow chart is available in Appendix I, which illustrates the relevant reactions and compounds of this chapter.

great excess, making large scale reactions impractical.

The treatise of Greenstein and Winitz[58] on amino acid chemistry outlines two older, direct methods for the preparation of amino acid benzyl esters. The first method made use of polyphosphoric acid and benzyl alcohol to esterify unprotected L-cysteine (*22*, p. 9) in unspecified yield[59]. We were unable to obtain greater than 30% yield of ester *74* by this procedure. The second method (the last direct procedure tried) involved treatment with *p*-toluenesulfonic acid, and azeotropic distillation of the water produced[60]. Fölsch[61] had previously employed this method for the preparation of L-serine benzyl ester hydrochloride (L-Ser-OBn.HCl, *76*) in 56% yield from L-serine (*75*) without protection of the hydroxyl group. We



75 *76*

Fig. XVII

found that even with a dehydrating agent in the Dean-Stark trap, reflux times of up to five or six days were sometimes

necessary to obtain D-Thr-OBn hydrochloride (*74*) in a maximum yield of 62%. A less direct approach, invqlving protection of the amino group, then appeared more practical for future preparations of *74*.

The well-known *tert*-butyloxycarbonyl (BOC) group[62] was chosen to protect the amine since it could be removed easily by ethereal hydrogen chloride followed by precipitation of the ester as its hydrochloride salt[63]. Several ways to esterify BOC-Thr[64,183] have been reported. All of these use a benzyl

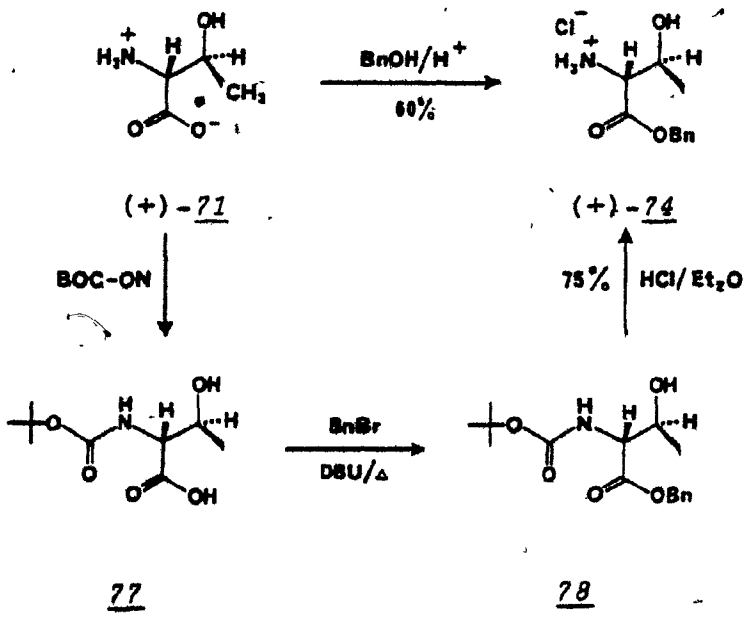
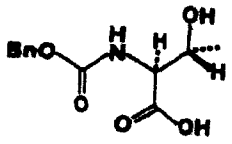


Fig. XVIII

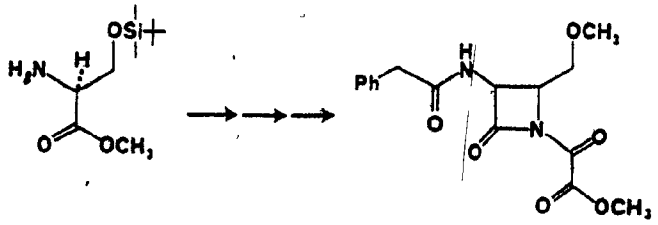
halide and a base with long reaction times[65-68] and/or high boiling solvents such as *N*,*N*-dimethylformamide (DMF)[63,66]. In 1978, Ono *et al*[69] described a method for synthesizing a wide variety of esters, including the benzyl ester of *N*-benzyloxycarbonyl-L-threonine (Cbz-D-Thr, *79*). He capitalized on the ambi-



79

dent (charge delocalization) properties of 1,8-diazabicyclo-[5,4,0]undec-7-ene (DBU) to obtain greater solvation of the carboxylate anion and thus shorter reaction times. This allowed the use of less polar solvents. Using DBU, we found that esterification of BOC-D-Thr[64,183] (*77*) with benzyl bromide was complete after three hours in refluxing benzene, as opposed to sixteen hours in refluxing ethyl acetate with triethylamine as the base. In spite of the three steps that were required, this process consistently provided ester (+)-*74* in higher overall yield (~75%) in a shorter time than did the method of azeotropic distillation. The melting point and optical rotation of D-Thr-OBn hydrochloride (*74*) agreed well with those reported for the L-derivative by Schnabel, Klostermeyer and Berndt[63].

The alcohol protecting group which seemed to meet all of the conditions described previously, was the *tert*-butyldimethylsilyl ether. First introduced by Corey[70], and used extensively since for the protection a variety of alcohols[71-74] and in particular, for nucleosides by Ogilvie and his coworkers[75], we assumed that it could be introduced without too much difficulty, and later removed by one of several mild methods[76-79]. It was also expected to be stable to chromatography[80]. Moreover, about the time we began this work, Just and Liak[81] published the synthesis of monocyclic β-lactam *81* from the *O*-*tert*-butyl-

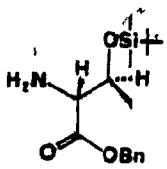


80 *81*

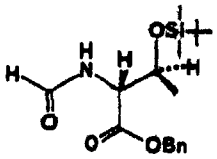
Fig. XIX

dimethylsilyl ether of L-serine methyl ester *80* under conditions very similar to those we hoped to employ.

Use of the recommended conditions (treatment with *tert*-butyldimethylsilyl chloride and imidazole in *N*-*N*-dimethylfor-



(+)-*82*



(-)-*83*

mamide at room temperature for eighteen hours[70,77]) for the silylation of ester *74*, however, resulted in substantial quantities of *N*-formyl silyl ether (-)-*83* (34 to 64%) as well as

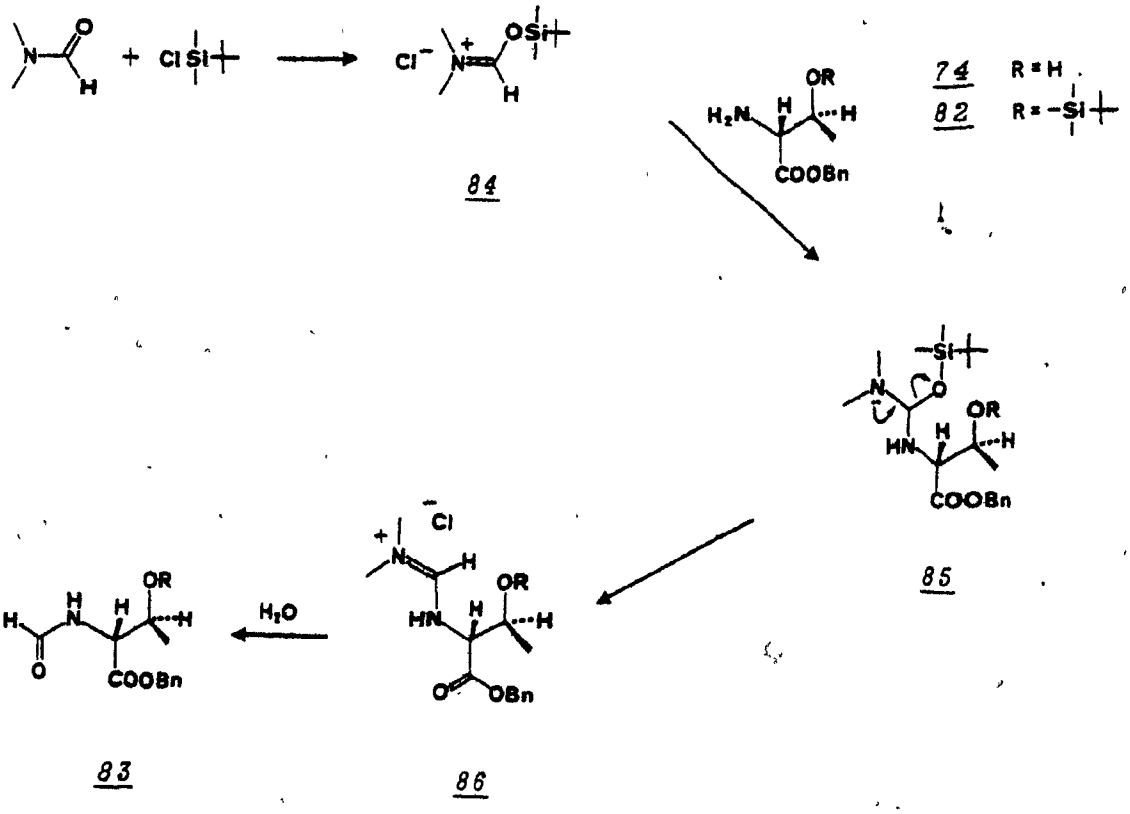


Fig. XX

the desired amino silyl ether (+)-*82* (30 to 44%).

A possible explanation for the occurrence of this side reaction could be the formation of a Vilsmeier type[82-84] iminium salt *84* (fig. XX) from the reaction of *N*,*N*-dimethylformamide with *tert*-butyldimethylsilyl chloride. An analogous iminium salt *87* was described by Ikawa *et al*[85] as arising from the reaction of ethyl chloroformate with DMF. These salts are

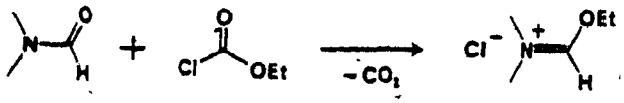
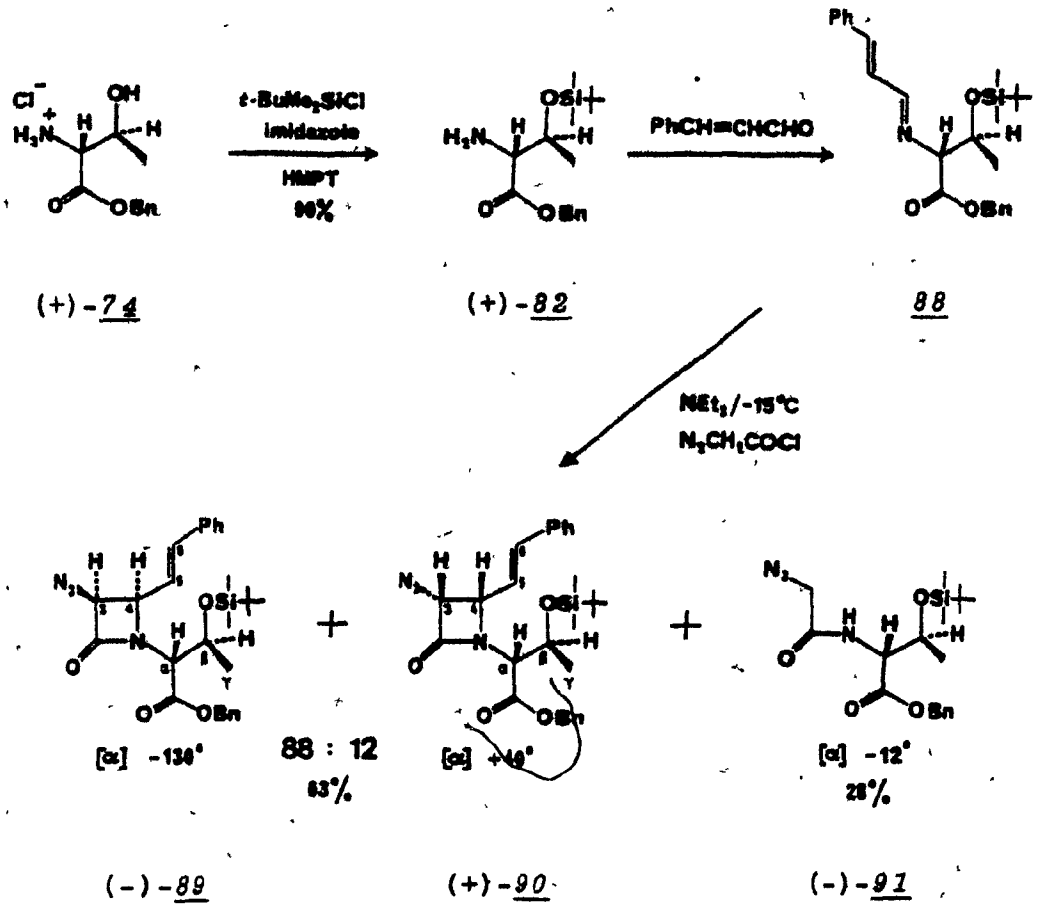


Fig. XXI

known electrophilic species[83] and so would be susceptible to attack from the amino group of either the starting material or the silyl ether *82*. Elimination of the siloxy group in *85*, followed by hydrolysis of the resulting iminium salt *86* (during work-up) would then lead to formamide *83*. Since the substitution of hexamethylphosphoric triamide (HMPT) for DMF gave the silyl ether (+)-*82* cleanly and in very high yield, the factors affecting this reaction were not studied any further.

There remained only the cinnamylidene Schiff base *88* to synthesize before the cycloaddition reaction could be studied.



(+)-74 (+)-82 88

(-)-89 (+)-90 (-)-91

Fig. XXII

It was prepared without difficulty by the procedure of Doyle *et al*[50], and was always used immediately without further purification. Since Doyle *et al* had established the particular conditions for cycloaddition which lead exclusively to *cis* β-lactams, that is, addition of azidoacetyl chloride to a cold (-15°C) solution of the α,β-unsaturated Schiff base and triethylamine in

dichloromethane[50,51], we adhered to those conditions with only minor modifications.

The orange oil which resulted from this procedure contained two major components (as indicated by t.l.c.). Separation by flash chromatography[86], allowed the identification of the more polar constituent as amide (-)-*91* (25 to 30%), and of the other constituent as a mixture of β-lactams *89* and *90* (60 to 65%). These yields are similar to those reported for cycloadditions with the cinnamylidene Schiff bases of L-serine (Just and Liak[81]) and D-threonine (Bose *et al*[87]) derivatives.

When isolating the products of an asymmetric synthesis, it is important to ensure that the process of isolation does not change the relative ratios of the diastereomers. Therefore, mixed fractions from the chromatography column containing any trace of β-lactam material were combined with the pure β-lactam fractions.

The first time this reaction was carried out, other impurities were also identified. These proved to be mainly cinnamaldehyde and the silyl ether *82* which may arise from cleavage of Schiff base *88*, either during the course of the reaction (accounting for amide (-)-*91*) or during work-up. Longer reaction times and slightly higher temperatures (-10° to 0°C) did not improve the yield of β-lactams. Their isolation was simplified by modifying the work-up procedure to hydrolyze any unreacted Schiff base by treatment with aqueous acid and then

to extract most of the resulting cinnamaldehyde as the bisulfite adduct.

Analysis of the mixture of β-lactams by high pressure liquid chromatography (HPLC) on a micro-silica analytical column revealed the presence of two major components in a ratio of 88:12 (major, less polar to minor, more polar) (see fig. XXII, p. 34). Enough of each component was separated for the purposes of identification. Both proved to be β-lactams, the major isomer *89* possessing negative optical rotation {$[\alpha]_D^{23}$ -130° (c 3, $CHCl_3$) and -150° (c 2.5, hexane)} and the minor isomer *90* positive optical rotation {$[\alpha]_D^{23}$ +40° (c 2, $CHCl_3$) and +80° (c 1.5, hexane)}. (A minor component of about 1% which appeared just before the major peak was not identified.)

The two main components were distinguishable by their respective ^{1}H.m.r. spectra. The 60MHz spectra were not sufficiently resolved to support our assumption that both were *cis* β-lactams but they did provide some useful information. The spectrum of the major β-lactam (-)-*89* was characterized by a prominent band appearing as an AB quartet (δ = 5.16 ppm) which was assigned to the benzyl ester methylene, while that of the minor β-lactam (+)-*90* contained a singlet (δ = 5.07 ppm) for the same group. Such an AB quartet is typical of a methylene in an area of the molecule in which there is restricted rotation[88].* The silylated compounds *82*, *88* and *91* (p. 34) and *83* (p. 32) also displayed AB quartets, with ratios ($\nu_A - \nu_B/J$) of

*or a consequence of "intrinsic diastereomerism" which is fortuitously cancelled in the minor isomer[187].

0.5 instead of 1.0 as for major β-lactam (-)-*89*. It is not surprising that introduction of the bulky *tert*-butyldimethylsilyl group creates some steric hindrance in these D-threonine derivatives. Apparently, the overall stereochemical properties of the major β-lactam accentuate those factors which restrict rotation about the ester function, while those of the minor isomer eliminate them.

Another indicator of strain in (-)-*89* was the upfield shift of the silyl group signals from their relative positions in all of the other silylated derivatives. Both silyl methyls absorbed above internal tetramethylsilane (TMS) (-0.11 and -0.04 ppm) rather than one on either side, while the *tert*-butyl signal shifted to 0.76 ppm from 0.85 ppm. Experience would show that these silyl peaks could be used as markers when examining the 60MHz ^{1}H.m.r. spectra of mixtures of β-lactam diastereomers. The *tert*-butyl signal was the most useful in this respect, since one of the methyl silyl peaks of the minor isomer was often hidden under those of the major. It was thus possible to obtain a rough estimate of the ratio of major to minor diastereomer.

Later, when 200MHz ^{1}H.m.r. spectra became available, it was possible to distinguish every single proton in each of the separated diastereomers. The coupling constants for the H-3 and H-4 protons of (-)-*89* and (+)-*90* (5.3 and 5.2Hz, respectively) confirmed that both β-lactams were *cis* isomers[89].

Once we had established that asymmetric induction was

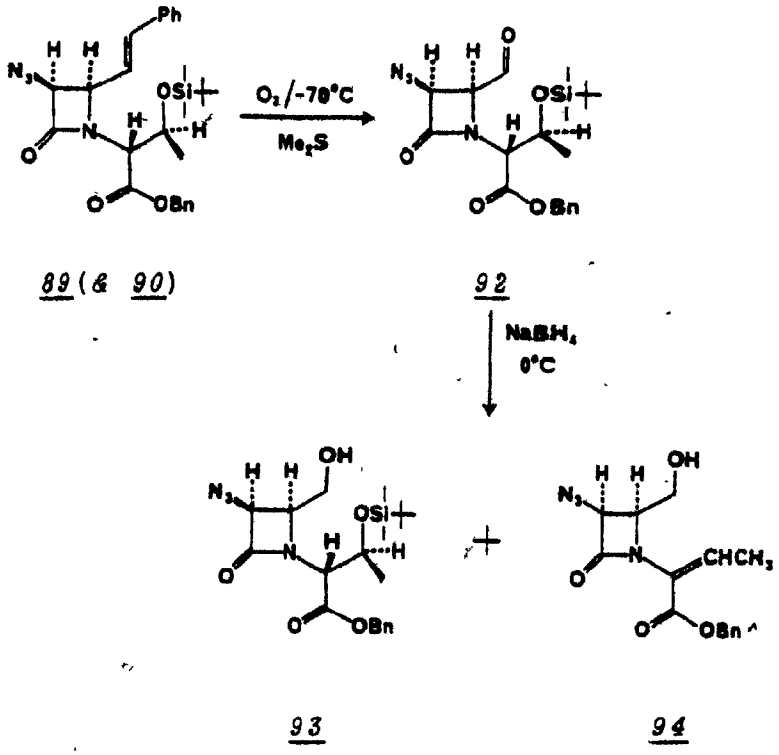
indeed occurring during the cycloaddition, it was necessary to determine whether (-)-*89*, the major β-lactam, had the correct stereochemistry for eventual biological activity. In drawing the structures of (-)-*89* and (+)-*90* (fig. XXII, p. 34), we had assumed that the major stereoisomer possessed the correct configuration, although this had not yet been proven. The simplest reliable way to establish the absolute configuration was actually to produce a stereochemically pure *O*-2-isocephem and test it for antimicrobial activity. To do this required the separation of a relatively large amount of pure (-)-*89* from the mixture of β-lactams.

Although a Waters 500 preparative HPLC was made available to us at Bristol Laboratories of Canada, the column was unfortunately not efficient enough to separate (-)-*89* from (+)-*90*. None of the fractions from this column (checked on an analytical micro-silica column) were free of the minor isomer (+)-*90*. A more efficient, semi-preparative column was needed. We were also able to establish that silica gel was better than reverse phase adsorbents for this separation[90] .

A semi-preparative column (150 mm × 10 mm) was, therefore, packed with Spherisorb S-10W (a spherical particle, micro-silica gel, Si 100, 10 μm) and the separation carried out on an Altex 300 preparative liquid chromatograph. Initially, the mobile phase employed consisted of ethyl acetate/hexanes (7:93) but after about fifteen injections, the column's resolution began to

deteriorate. Change of the mobile phase to methanol/ethyl acetate/hexanes (0.1:5:95), accompanied by pre-equilibration of the column for about twenty minutes, solved the problem. As only a few milligrams of pure material could be separated *per* injection, several weeks of arduous work were required to collect enough pure β-lactam (-)-*89* (three grams) for a completion of the synthesis. These three precious grams were submitted to further reactions only after the precise conditions had been established using the mixture of *89* + *90*.

The first attempt to prepare primary alcohol *93* involved

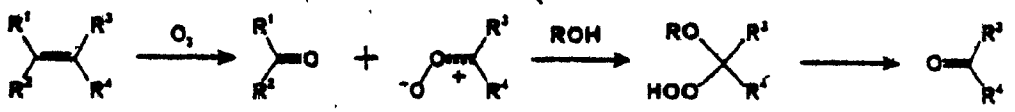


89 (& *90*) → *92* → *93* + *94*

Fig. XXIII

ozonolysis (at -70°C) of *89* + *90* in dichloromethane, followed by reduction of the ozonide with dimethyl sulfide to aldehyde *92*. This aldehyde was assumed to be unstable[51,53] and was immediately treated with sodium borohydride in tetrahydrofuran (THF) at 0°C. Primary alcohol *93* was produced by this method but so was the α,β-unsaturated ester *94*, resulting from elimination of the *tert*-butyldimethylsiloxy group. In an effort to avoid this side reaction, we elected to reduce the product of ozonolysis directly to the alcohol[51,91].

Chinn[92], in his review of ozonolysis conditions, states that "solvents which form hydroperoxides....often give better yields of products after reduction than do inert solvents." In protic solvents, the zwitterionic intermediate *95* (fig. XXIV) has no chance to rearrange before being protonated. For this



95

Fig. XXIV

reason, a small amount of methanol (15%) was added to the ozonolysis reaction mixture. The ozonide was reduced with

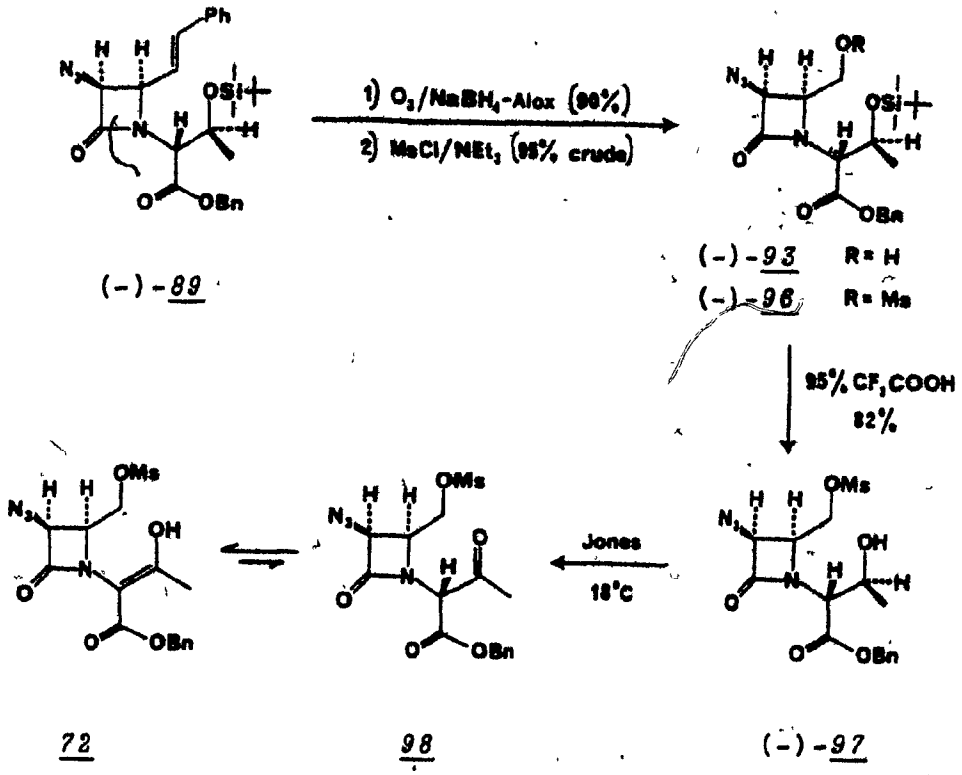


Fig. XXV

sodium borohydride adsorbed on alumina ($NaBH_4$-Alox)[93] rather than by sodium borohydride in solution. Under these conditions, no undesirable side reactions were encountered.

Treatment of the pure major β-lactam (-)-*89* as above gave primary alcohol (-)-*93* in 90% yield after chromatography. The 60MHz ^{1}H.m.r. spectrum still contained an AB quartet (δ = 5.10 ppm) for the benzyl ester methylene but the silyl absorptions had shifted downfield to positions more comparable with those

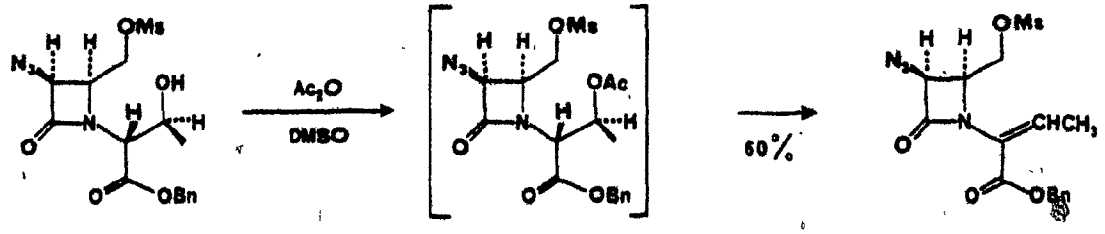
for the acyclic D-threonine derivatives *82*, *88* and *91* (p. 34), indicating that the styryl group was partially responsible for the steric strain previously noted for (-)-*89*. This time the doublet corresponding to H-3 appeared at δ = 4.73 ppm with a coupling constant (J = 5Hz) typical of *cis* β-lactams[89].

Mesylation[94] of alcohol (-)-*93* to give mesylate (-)-*96* and subsequent removal of the *tert*-butyldimethylsilyl protecting group with 95% trifluoroacetic acid[95] produced the first crystalline intermediate in this series, secondary alcohol (-)-*97* (fig. XXV). In its ^{1}H.m.r. spectrum, the AB quartet, so characteristic of the previous intermediates, was now absent. Removal of the bulky silyl group had relieved the rotational restrictions about the benzyl ester.

Oxidation of secondary alcohol (-)-*97* to the β-keto ester *98*, with concomitant tautomerization to enol *72* (fig. XXV), proved to be rather challenging. The expected enol would likely lead to closure of the six-membered ring under basic conditions, and we wished to avoid this initially. The first method attempted was oxidation with Jones reagent[96]. Its use for the formation of enolizable ketones from alcohols, without epimerization of the adjacent asymmetric center, has been reported[97,98]. Although the reaction was fast, and the enol definitely formed, a considerable amount of decomposition was also observed. Apparently, some hydrolysis of the benzyl ester was taking place. In fact, when the reaction was carried out

at -10° or 0°C, formation of benzoic acid, probably by oxidation of benzyl alcohol, was a major occurrence.

A variety of other oxidizing agents was evaluated. Other chromium reagents (sodium dichromate dihydrate/H_2SO_4[99], tetra-*N*-butylammonium chromate[100] and chromium trioxide/ether[101]) gave no reaction at all. Neither did Pfitzner-Moffatt oxidation (dimethylsulfoxide/dicyclohexylcarbodiimide/H_3PO_4)[102,103]. The acetic anhydride variation of the Pfitzner-Moffatt method[102,104] did produce some enol (detected by t.l.c.) but the main product (60%) proved to be the α,β-unsaturated ester *99*. Presumably *99* arises from acetylation of the secondary alcohol (-)-*97* with subsequent elimination of acetic acid. The spectral data from *99* agreed well with those of *94*, the α,β-unsaturated ester formed during sodium borohydride reduction of aldehyde *92* (p. 39). The differences corresponded to those expected between an alcohol and its mesylate.



(-)-*97* *99*

Fig. XXVI

Three other oxidation methods were attempted. Fetizon's reagent (silver carbonate/celite)[105] and activated manganese dioxide[106] both appeared to promote side reactions rather than the desired oxidation. Pyridinium chlorochromate (PCC)[107], buffered by sodium acetate, also caused decomposition. When used alone, however, PCC did produce enol *72* (identified by t.l.c.) but at a very slow rate (about 50% after two days). Work-up after four days gave an unacceptably low yield of the enol even though t.l.c. showed very little of the decomposition products observed in the previous experiments. Considerable amounts of polar material were present, however. Since the desired enol *72* is known to be unstable[51], we decided not to pursue this approach but to concentrate on optimizing the oxidation under the Jones conditions[96].

This oxidation was first studied with a model compound, alcohol *100* obtained by the desilylation of amide (-)-*91* in 95% trifluoroacetic acid[95] (fig. XXVII, p. 45). Decomposition of the product (enol *101*) predominated under the following conditions:

a) leaving the reaction mixture at 0°C after addition of the reagent,

b) warming up to room temperature (25°C), and

c) using excess reagent.

When stoichiometric amounts of Jones reagent were used, the optimum temperature for conversion of alcohol *100* to enol

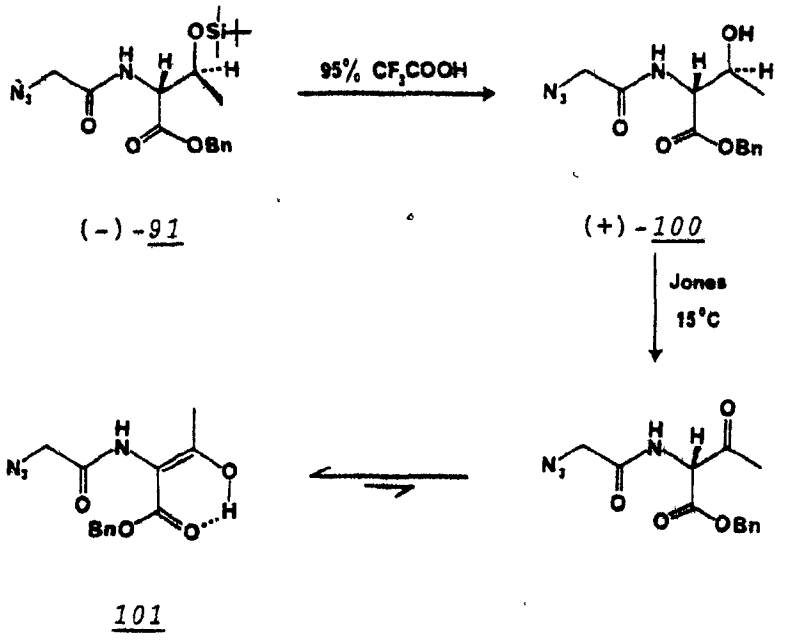


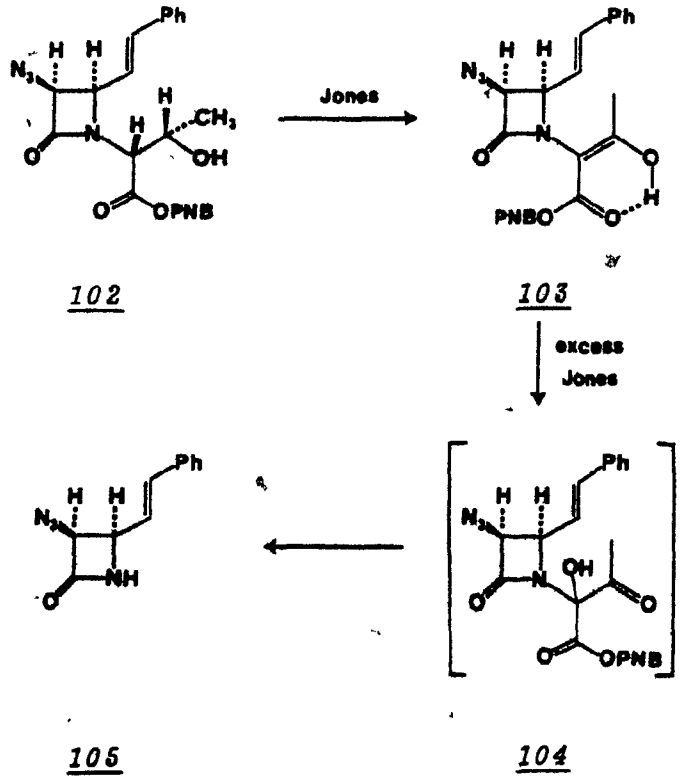
Fig. XXVII

101 was about 15°C. For the β-lactam-containing secondary alcohol (-)-97, the optimum temperature was a little higher (about 18°C). Even under these conditions, neither oxidation reaction could be completed without decomposition, although acyclic 101 seemed to withstand long reaction times better than β-lactam-containing enol 72.

One way around this problem was to stop the reaction before significant amounts of decomposition had occurred (approximately 50% oxidation) and separate the product from unreacted alcohol which could then be recycled. Our experience with

the dimethylsulfoxide/acetic anhydride oxidation (p. 43 and 128) had shown that chromatography was not a feasible method of separation for enol *72* and alcohol (-)-*97* because enol *72* was not eluted from the column even with a very polar solvent. We decided to extract *72* as the enolate salt, followed immediately by neutralization with ice-cold 10% hydrochloric acid.

Extraction with ice-cold solutions of 1% sodium hydroxide or 10% sodium carbonate caused some undesirable side reactions,



102 *103* *104* *105*

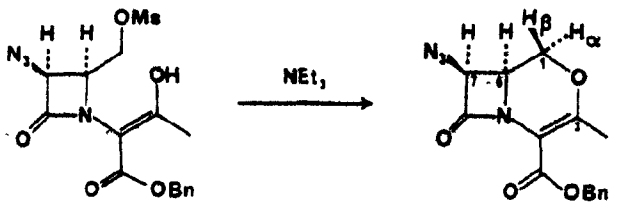
Fig. XXVIII

while saturated sodium bicarbonate did not extract any *72* (or *101*). An acceptable compromise was found by using 4% sodium carbonate. This procedure typically allowed the recovery of enol *72* in 55% yield and unreacted alcohol (-)-*97* in 35% yield. Enol *72*, obtained in this way had spectral characteristics similar to those reported by Doyle *et al*[51] for the same compound.

After this work was completed, Bose, Manhas and their colleagues[87] reported the Jones oxidation of alcohol *102* to enol *103* (fig. XXVIII, p. 46) in 77% yield "under carefully controlled conditions" (not specified). The known resistance to acid of *p*-nitrobenzyl esters in comparison with benzyl esters[108] may have contributed to the improved stability of *102* and *103* during the reaction. When they used an excess of Jones reagent, the *N*-unsubstituted β-lactam *105* was isolated. Formation of this species was attributed to over-oxidation of enol *103* to the α-hydroxy-ester *104* and subsequent cleavage. The analogous *N*-unsubstituted β-lactam was not isolated from the reaction of (-)-*97* (p. 41). However, something more than ester hydrolysis was obviously occurring, and the observations of Bose *et al* offer a reasonable explanation for the increased decomposition that we observed at room temperature. It would appear that the resistance of enolizable ketones to Jones reagent[97] depends on the ease with which the enol is formed.

The cyclization of enol *72* to azido-*O*-2-isocephem *73* as

described by Doyle *et al*[51] was always carried out as soon as possible after extraction of the enol. With the recycling of recovered starting material, 73% of (-)-*73* was obtained from



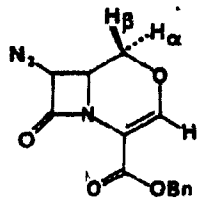
72 (-)-*73*

Fig. XXIX

secondary alcohol (-)-*97* (p. 42) after chromatography. Comparison of the physical and spectral characteristics of (-)-*73* with those reported by Doyle *et al*[51] showed that these molecules were very similar but not identical. The azido-*O*-2-isocephem described in the literature was a crystalline racemate whereas (-)-*73* proved to be an oil consisting of a single enantiomer; $[\alpha]_D^{23}$ -22° (c 2, $CHCl_3$).

In $CDCl_3$, the ^{1}H.m.r. spectrum of (-)-*73* showed a complex pattern for H-1α, H-1β, H-6 and H-7 describable as an AMXY pattern with only the Y absorption (H-6) being unclear. The pattern in C_6D_6 was almost first order (its overall appearance might be described as an AMNX pattern). The changes in chem-

ical shift from $CDCl_3$ to C_6D_6[109] (Table 1) for all four protons concerned are in the same direction and are of about the same magnitude as those already published for the *O*-2-isocephem *106*[51]. The coupling constants are also comparable. The assignments for the proton peaks in (-)-*73* are, therefore, identical to those for (±)-*106*.



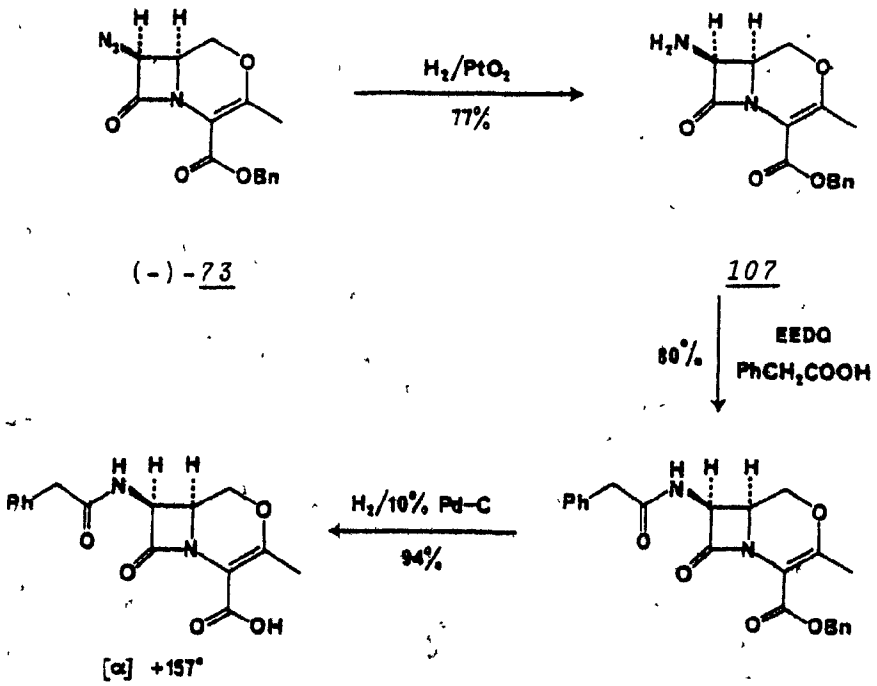
(±)-*106*

proton	$\delta(CDCl_3)$*	$\delta(C_6D_6)$*	Δ(*73*)†	Δ(*106*)[51]
H-1β	3.98	3.39	0.59	0.55
H-1α	4.56	3.91	0.65	0.66
H-6	3.7	2.93	0.8	0.78
H-7	5.16	4.30	0.86	0.8

*in ppm. †$\Delta = \delta(CDCl_3) - \delta(C_6D_6)$.

TABLE 1: 60MHz ^{1}H.m.r. aromatic solvent induced shift in (-)-*73*

There now remained only the synthesis of a relevant *O*-2-isocephem carboxylic acid with a suitable amide side chain for the correlation of bioactivity and configuration by comparison with an identical compound in the racemic series. We accomplished this by previously published procedures[51]. Hydrogenolysis over platinum of the azido group of (-)-*73* yielded 77% of the corresponding amine *107* with spectral characteristics which agreed with those reported for the racemate[51]. As for (-)-*73*



(-)-*73*

107

(+)-*70*

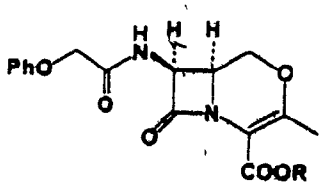
(+)-*108*

Fig. XXX

the complex pattern observed in the 60MHz ^{1}H.m.r. spectrum for H-1, H-6 and H-7 could be analysed in terms of individual protons (this time in $CDCl_3$) even though the spectrum could not be precisely classified as first order.

Since the phenylacetamido-racemate (BC-L30)[54] had earlier been prepared and tested at Bristol Laboratories, we coupled the readily available phenylacetic acid to amine *107* with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[51,110] to obtain an 80% yield of amide-ester (+)-*108*, $[\alpha]_D^{23}$ +154° (c 0.6, $CHCl_3$). Hydrogenolysis (10% Pd/C) of the benzyl ester group[51] finally resulted in 94% of the dextrorotatory *O*-2-isocephem (+)-*70* as a glassy solid, which was recrystallized to give a white powder, m.p. 95° - 105°C (dec.), $[\alpha]_D^{23}$ +157° (c 1, acetone).

No spectral data for either the ester *108* or its carboxylic acid *70* have been reported, but the data that we obtained are consistent with those published for the phenoxyacetyl analogues *109* and *110*[51], thus confirming the structural assignment. The melting point of the racemate is much higher (197° - 198°C[54]) than that of the pure enantiomer (+)-*70* (95° - 105°C).



109 R = Bn
110 R = H

Biological Activity and Absolute Configuration

The respective *in vitro* antimicrobial activities, expressed as minimum inhibitory concentration (MIC), of (+)-*70* and its corresponding racemate (BC-L30[54]) against five typical strains of bacteria are listed in Table 2. These results establish that the absolute configuration of (+)-3-methyl-7β-phenylacetamido-*O*-2-isocephem-4-carboxylic acid (*70*) (and of its precursors) is identical to that of the natural penicillins and cephalosporins.

Micro-organism	MIC(μg/ml) (+)-*70*	BC-L30
Streptococcus pneumoniae	0.25	0.5
Streptococcus pyogenes	0.5	0.5
Staphylococcus aureus – Smith	0.5	1.0
Escherichia coli	32	125
Proteus mirabilis	8	16

TABLE 2: Antimicrobial activity of (+)-*70* *versus* BC-L30.

CHAPTER 2

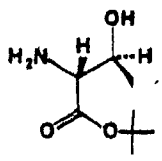
At the time this work was undertaken, we became aware of the results of Just and Liak[5] on the cycloadditon of the L-serine derivative *111* with azidoacetyl chloride by the method

111 (±)-*112*

Fig. XXXI

of Doyle *et al*[50,51]. They reported that the product, *cis* β-lactam *112*, was racemic. When our own results showed that 76% asymmetric induction could be obtained from the D-threonine derived Schiff base *88* (p. 34), it seemed logical to attribute the difference to the effect of increased steric hindrance caused by the β-methyl group of D-threonine and/or the larger

carboxyl protecting group of *88 versus 111*. We thought that by manipulating the size of the ester functional group, better optical yields might be obtained. One of the most widely used groups for the introduction of steric bulk is the *tert*-butyl group. Accordingly, we set about the preparation of the required *tert*-butyl D-threonate (D-Thr-OBu^t, *113*)* in order to

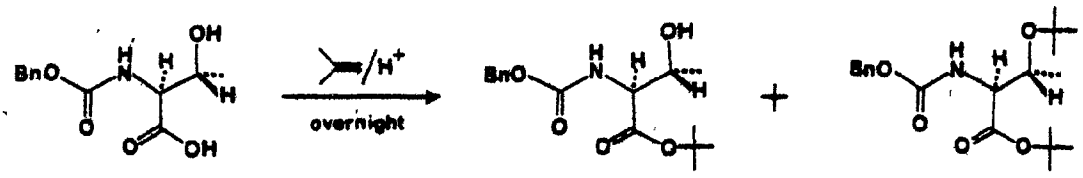


113

test our hypothesis.

Several methods[111-116] for the formation of the *tert*-butyl esters of amino acids are known. Two of them have been used to prepare derivatives of *tert*-butyl D-threonate. The first was described by Callahan and Zimmerman in a U.S.A.

*A foldable flow chart is available in Appendix II which illustrates most of the reactions and compounds described in this chapter.



79 *114* *115*

Fig. XXXII

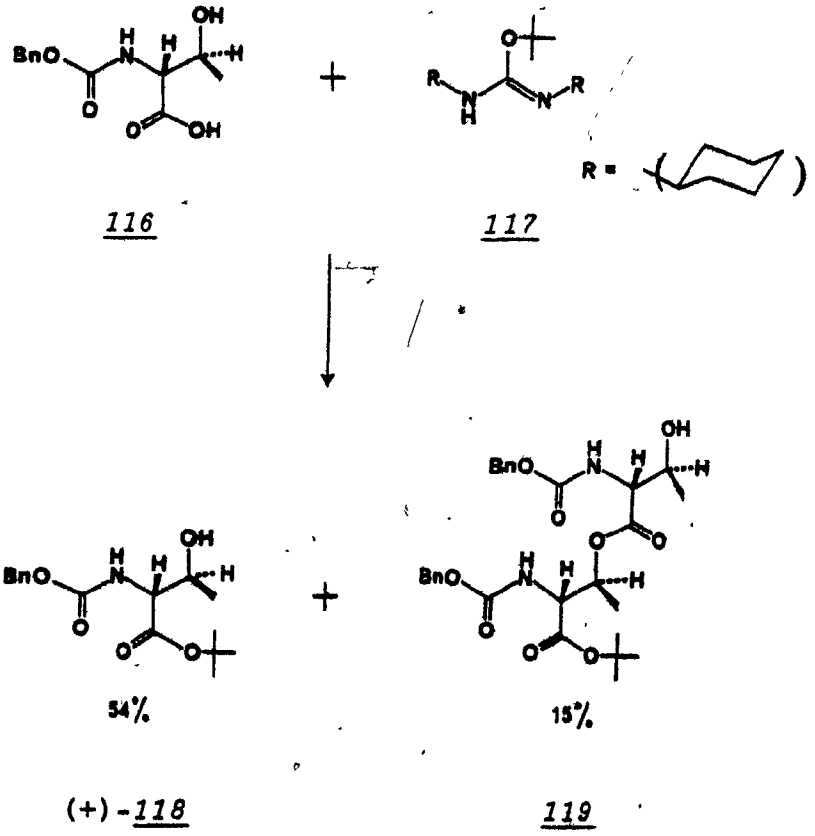
patent[111]. From the reaction of Cbz-L-Thr (*79*) with isobutylene and sulfuric acid, they obtained both the *tert*-butyl ester *114* and the di-*tert*-butyl ether-ester *115*, the latter apparently predominating. Longer reaction times[117] might have yielded only di-*tert*-butyl ether-ester *115* but our comparative studies required the use of the *tert*-butyldimethylsilyl ether as the β-hydroxyl protecting group.

Moore and Szelke[112] had obtained BOC-L-Thr-OBu^t in 51% yield by reacting BOC-L-Thr with *N,N'*-dicyclohexyl-*O*-*tert*-butylisourea (*117*) (fig. XXXIII, p. 56). Identical yields were reported for other hydroxy-amino acid derivatives. These rather low yields can be attributed mainly to the instability of the *tert*-butylisourea[118]. On the other hand, etherification should not be a problem with this reaction since aliphatic alcohols are essentially unreactive towards isoureas[118].

Other methods (such as transesterification with *tert*-butyl

acetate/$HClO_4$[113,114] or reaction of the silver carboxylate with *tert*-butyl iodide[115]), previously applied to different amino acids, were expected to give troublesome side reactions involving the unprotected β-hydroxyl group. Furthermore, the published yields were not encouraging.

In order to carry out the esterification with isourea, it was necessary to choose an amino protecting group compatible with the *tert*-butyl ester. Although selective cleavage of the



116 *117*

(+)-*118* *119*

Fig. XXXIII

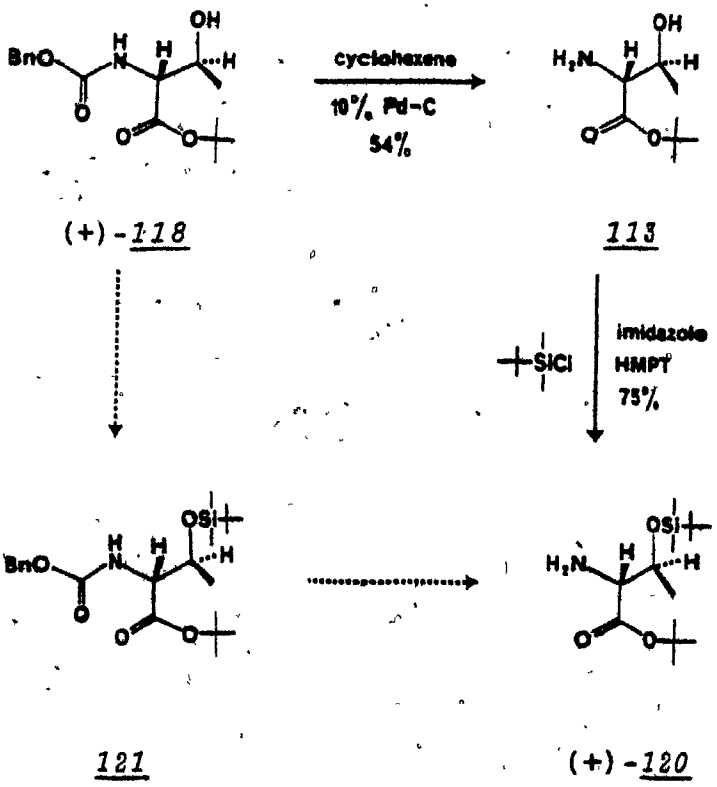
BOC group in the presence of a *tert*-butyl ester has been described[119,120], the Cbz group was chosen because it can easily be hydrogenolyzed[121-123] under conditions which should leave the *tert*-butyl ester intact. In this way, the problem of selectivity would not arise.

The desired Cbz-D-Thr-OBu^t {(+)-*118*, $[\alpha]_D^{23}$ +20° (c 0.6, ethanol)} was synthesized in 54% yield after chromatography, from Cbz-D-Thr (*116*)[121] and *N,N'*-dicyclohexyl-*O*-*tert*-butylisourea (*117*)[124], prepared *in situ* from *tert*-butanol and *N,N'*-dicyclohexylcarbodiimide (DCC) (fig. XXXIII, p. 56). The major impurity from this reaction (15% after chromatography) proved to be the dimer *119* (see experimental section, p. 137). This could only have arisen from attack on unreacted DCC by the secondary alcohol of product *118* (or of starting material *116*) thus yielding an isourea with which a molecule of starting material *116* could react to produce dimer *119*. A pure isourea, prepared separately rather than generated *in situ*, might improve the yield of *tert*-butyl ester. Unfortunately, the only reported isolation of an *O*-*tert*-butylisourea (from *N,N'*-diisopropylcarbodiimide) occurred after a reaction time of twenty-eight days[125].

After this work had been completed, we became aware of the work of Kinoshita *et al*[126] who prepared Cbz-L-Thr-OBu^t {$[\alpha]_D^{26}$ -20.6° (c 1.07, ethanol)} by acetoacetylation of the β-hydroxyl group in Cbz-L-Thr with diketene, esterification with isobutylene and then deacylation with hydrazine. The spectral and

physical properties of our Cbz-D-Thr-OBu^t (*118*) were in good agreement with their data.

Transfer hydrogenation from cyclohexene, a new procedure for the removal of Cbz groups from amino acid and peptide derivatives[127], was selected for the deprotection of (+)-*118*. The reaction appeared to work well on a small scale, giving a good yield (78%) of a crude product, the i.r. and ^{1}H.m.r. spectra of which agreed with the expected structure. However, the yield of *113* after chromatography (54%) was disappointing. This loss may



(+)-*118* *113*

121 (+)-*120*

Fig. XXXIV

have been due to some cleavage of the *tert*-butyl ester as well as poor mobility of this rather polar compound on the column. The behaviour of the isolated *113* left the impression that it was unstable. It is possible to envision the breakdown of *113* by elimination of isobutylene to regenerate the amino acid,

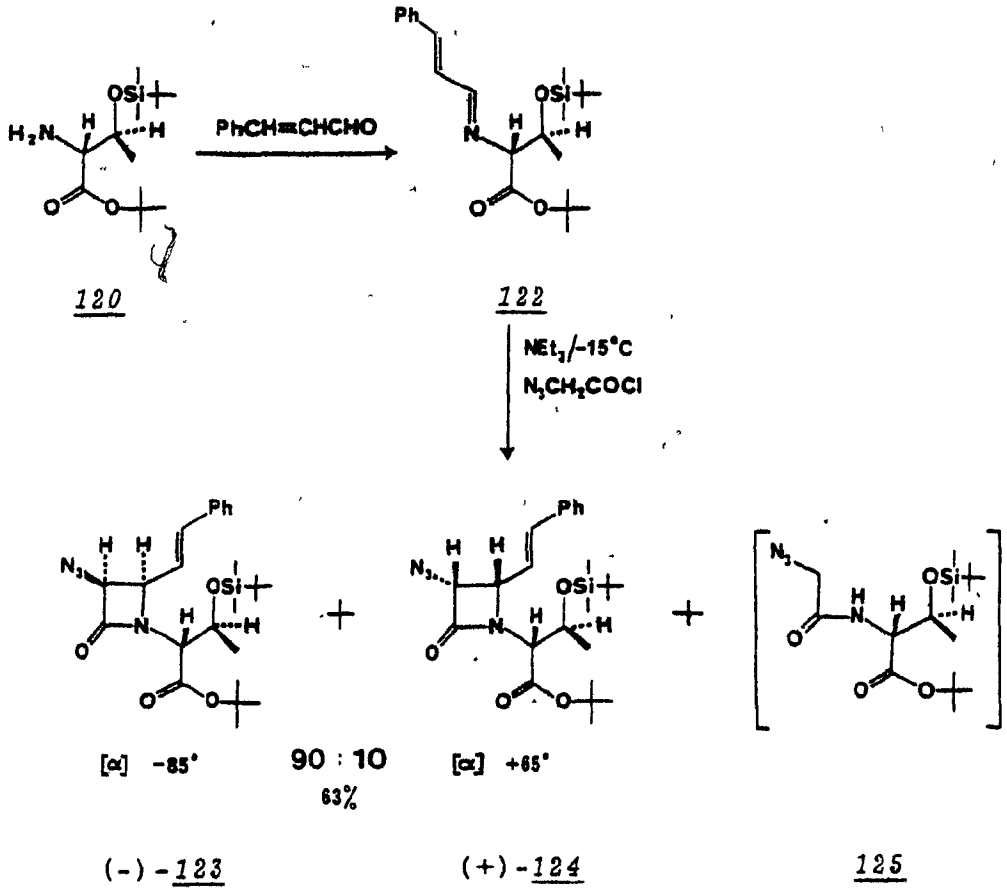
113 *71*

Fig. XXXV

D-threonine, as in fig. XXXV. However this process need not be intramolecular. The *tert*-butyldimethylsilyl ether (+)-*120* (prepared in 75% yield as described in chapter 1, p. 33) appeared to be more stable than *113* although it too seemed to decompose at room temperature. In view of their relative instability, both *113* and *120* were used as soon as possible after formation.

This problem might be circumvented by silylation of the *N*-protected ester *118* first to give *121*, followed by hydrogenolysis of the Cbz group at 25°C with 1,4-cyclohexadiene[128] to yield the relatively more stable (+)-*120*.

Synthesis of Schiff base *122* and cycloaddition to give a



120 122

(-)-123 (+)-124 125

Fig. XXXVI

mixture of β-lactams 123 + 124 (63% from 120) by the method described in chapter 1 (p. 34) went as expected. A polar impurity produced during the reaction was assumed to be the corresponding amide 125 (not isolated). With the knowledge gained through the synthesis of 89 + 90 (p.35-7), we were able to use the silyl absorptions in the ^{1}H.m.r spectrum, especially the *tert*-butyl silyl signal, as markers for the two expected dia-

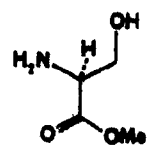
stereomers. Again the minor isomer gave absorption bands downfield from the major one, both the two silyl methyl singlets being discernible in this case. Integration of these peaks indicated the presence in the mixture of about 11 or 12% of the minor isomer. Further evidence was provided by the observation of a small singlet about 5Hz upfield from the *tert*-butyl ester signal. These conclusions were substantiated by HPLC analysis which revealed a ratio of 90:10 for the major, less polar isomer *123* to the minor, more polar isomer *124*.

Isolation and characterization of these diastereomers essentially supported the assigned structures. As for the benzyl ester derivatives, the minor isomer (+)-*124* was the more polar isomer with a positive optical rotation {$[\alpha]_D^{23}$ +65° (c 0.6, $CHCl_3$)}. Individual absorptions for all protons were visible in the 200MHz ^{1}H.m.r. spectrum. A coupling constant of 5.2Hz between H-3 and H-4 confirmed the *cis* configuration for the β-lactam. However, the 200MHz ^{1}H.m.r. spectrum of the major, less polar isomer (-)-*123* {$[\alpha]_D^{23}$ -85° (c 1.5, $CHCl_3$)} did not immediately allow measurement of the analogous coupling constant. Inspection of the ^{1}H.m.r. spectra of (-)-*89*, (+)-*90* (p. 34) and (+)-*124* (p. 60) would lead one to expect a pattern of six lines (a doublet for H-3 and a doublet of doublets for H-4), instead of the four line pattern observed for (-)-*123*. Using the coupling constant ($J_{4,5}$ = 8.4Hz) measured from the H-5 signal, and assuming that the H-4 doublet of doublets is upfield, one can

estimate that the upfield and downfield peaks for H-3 and H-4 respectively should coincide at δ = 4.84 ppm (the most intense peak). The coupling constant between H-3 and H-4 would then equal the separation of the two upfield signals in the pattern, that is, 5.4Hz. This implies that (-)-*123* also has the *cis* configuration. In any event a drastic change in mechanism between the cycloaddition of imine *88* (p. 34) and of *122* is unlikely.

In fact, the 90:10 ratio for the two diastereomers was not significantly different from that obtained in the case of *88* (88:12). It appeared, therefore, that increased steric hindrance did not have as much an effect as we expected by comparison of the report of Just and Liak[81] with our earlier work.

In the light of these results, it became of interest to evaluate the effect of the second chiral center of the starting amino acid on the stereochemistry of the cycloaddition reaction. Since the amino acid, L-serine (*75*, p. 63), lacks such a center, its benzyl ester (*76*) is a more appropriate substrate for comparison with our D-threonine benzyl ester (*74*) than the L-serine methyl ester (*126*) used by Just and Liak[81].



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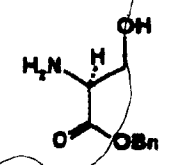

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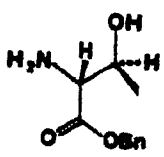

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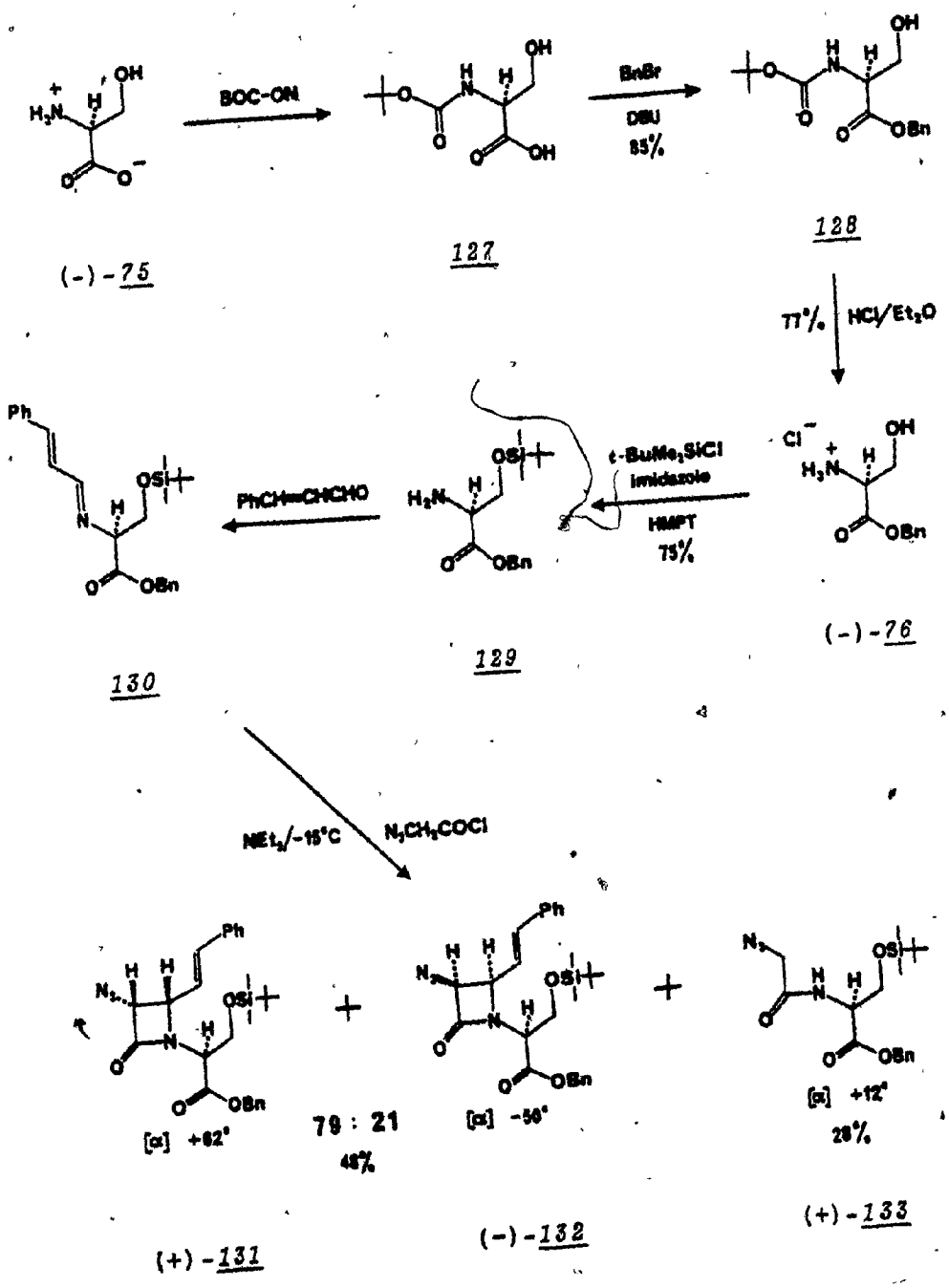


(-)-*75* *127* *128*

(-)-*76* *129* *130*

(+)-*131* (-)-*132* (+)-*133*

Fig. XXXVII

The same reactions which had been developed for the preparation of the threonine benzyl ester derivatives were applied to the synthesis of the serine benzyl ester derivatives (fig. XXXVII). Because it was more readily available in larger quantity and at a lower cost than D-serine, the L-enantiomer (*75*) was used as starting material. Esterification of BOC-L-Ser[64,183] (*127*) by Ono's method[69] to give BOC-L-Ser-OBn (*128*), followed by cleavage of the BOC protecting group[63] gave the ester hydrochloride *76* which had a slightly higher rotation {$[\alpha]_D^{23}$ -6.1° (c 4.5, methanol)} than that reported by either Fölsch[61] {$[\alpha]_D$ -4.1 ± 0.5° (c 4.4, methanol)} or Losse and Augustin[129] {$[\alpha]_D$ -4.19° (c 4.53, methanol)}. Silylation[70] to give *129*, followed by reaction with *trans*-cinnamaldehyde[50] yielded imine *130*. Cycloaddition as usual resulted in a mixture of β-lactams *131* + *132* (48% from *129*) plus the amide (+)-*133* (28%) which this time was isolated and characterized.

It was immediately obvious from the ^{1}H.m.r. spectrum of *131* + *132* that an asymmetric cycloaddition had occurred, although with less stereospecificity than for the D-threonine derivatives. The marker silyl peaks in the 60MHz ^{1}H.m.r. spectrum indicated that approximately 25 to 30% of a minor isomer was present. Integration of these peaks was less accurate since the differences in chemical shift were smaller. The signs of steric strain were also reduced. First, the silyl methyl absorptions appeared as one, broadened singlet and not as two

distinct peaks. Secondly, the AB quartet absorption for the benzyl ester methylene of the major isomer was characterized by a $\nu_A-\nu_B/J$ of 0.5, similar to the acyclic silylated D-Thr compounds *82*, *88* and *91* (p. 34). (All of the other silylated derivatives in this series displayed singlets rather than AB quartets.) Finally, as mentioned above, the chemical shift differences were smaller than in the D-threonine series.

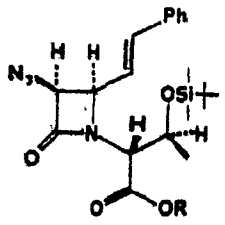
Analysis and separation of these stereoisomers by HPLC proved to be quite difficult. When a Waters 440 apparatus became available, it was possible to recycle the samples through the column. Even so, about forty-five minutes was required for each separation. Eventually the ratio of stereoisomers for this reaction was determined to be 79:21, and enough of each isomer was obtained to allow the identification of both as optically active *cis* β-lactams.

The optical rotations of these β-lactams, and also their configurations, reflected the L-stereochemistry of the starting material but their other properties were as expected. The major, less polar β-lactam (+)-*131* exhibited a positive optical rotation $\{[\alpha]_D^{23}$ +62° (c 1.4, $CHCl_3)\}$ while that of the minor, more polar β-lactam (-)-*132* was negative $\{[\alpha]_D^{23}$ -50° (c 0.6, $CHCl_3)\}$. The rotation of (-)-*132* is only an approximation since there was still about 1 to 2% (measured by HPLC) of *131* in the sample. The coupling constant for the H-3 and H-4 absorptions in the 200MHz ^{1}H.m.r. spectra of both stereoisomers was 5.1Hz showing

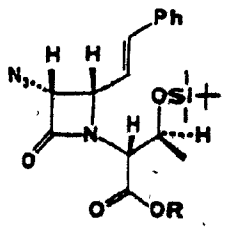
them to be *cis* isomers. The results of this investigation demonstrated that the β-methyl group of D-threonine (and the chiral center to which it is attached) was of importance for the extent of asymmetric induction, but not for its occurrence.

Because of the discrepancy between our results with L-Ser-OBn and those of Just and Liak with L-Ser-OMe where the possibility of racemization was mentioned[81], it became necessary to estimate quantitatively the involvement of any racemization during the cycloaddition reaction.

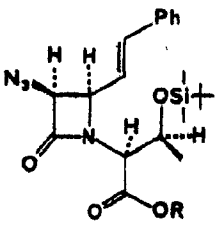
Synthesis of the biologically active *O*-2-isocephem (+)-*70* (chapter 1) had established that the absolute stereochemistry of the *cis* β-lactam in the major diastereomer formed during the cycloaddition of D-threonine derivatives, was that shown for (-)-*89* and (-)-*123* in fig. XXXVIII. Since the two chiral centers in the D-threonine moiety of (-)-*89* were eventually destroyed during this synthesis, depicting their stereochemistry



(-)-*89* R = Bn
(-)-*123* R = t-Bu



(+)-*90* R = Bn
(+)-*124* R = t-Bu



134 R = Bn
135 R = t-Bu

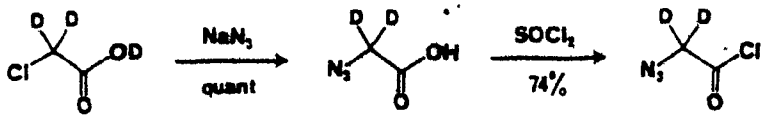
Fig. XXXVIII

as in fig. XXXVIII required the assumption that they remained unchanged during the cycloaddition. This is a reasonable assumption for the β-carbon. However, since the α-carbon of *N*-substituted α-amino esters can be racemized under relatively mild conditions[62, 130], it was necessary to verify that such α-protons were not removed under conditions leading to β-lactam formation.

Furthermore, the data so far obtained for the minor isomers (+)-*90* and (+)-*124* (including their positive optical rotations) do not prove that the absolute stereochemistry of their *cis* β-lactam rings is the mirror image of the "natural" configuration (see fig. XXXVIII). It is not impossible that the cycloaddition may be giving 100% asymmetric induction with about 10% racemization; rather than 76 to 80% asymmetric induction as we have been assuming. The minor isomers would in that case have the stereochemistry shown for *134* and *135* in fig. XXXVIII.

Under dry conditions, the only source of protons in the cycloaddition reaction is the reagent azidoacetyl chloride. We decided to test the possibility of racemization by using the corresponding deuterated reagent. If racemization were to occur, then one would expect to find deuterium incorporation at the α-carbon of at least one, if not both, stereoisomers. Because the 200MHz ^{1}H.m.r. spectra of all the styryl β-lactams that we had prepared showed the α-protons to be clearly separated from every other proton, it would be relatively easy to estimate the extent of deuterium incorporation at the α-position.

Therefore, chloroacetic acid-d_3 (*136*) (supplied by Merck, Sharpe and Dohme) was converted to azidoacetyl chloride-d_2 (*138*) *via* azidoacetic-d_2 acid (*137*) essentially as described

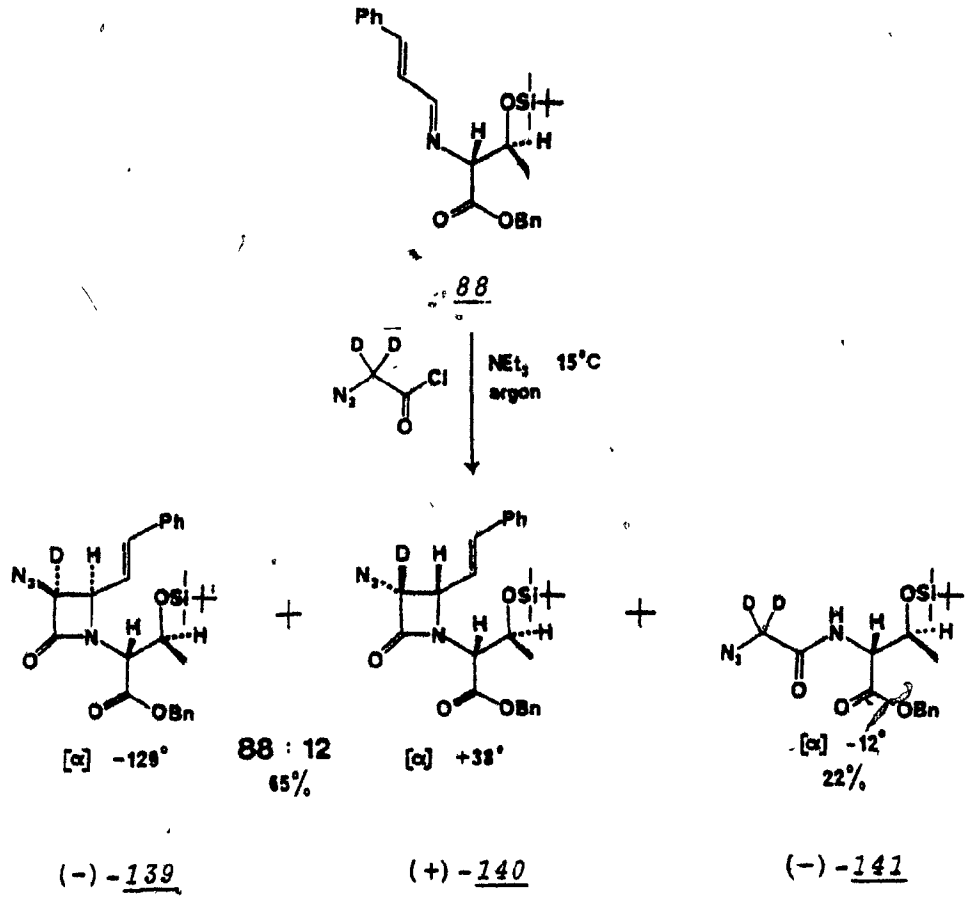


136 *137* *138*

Fig. XXXIX

for the protonated compound by Wieland and Hennig[131]. The azidoacetyl chloride-d_2 (*138*) showed no absorption in the C-H stretch region of the infrared spectrum.

The cycloadditions of Schiff bases *88* (fig. XL, p. 69) and *130* (fig. XLI, p. 70) with this deuterated reagent were carried out in the usual manner, except that anhydrous magnesium sulfate was present and an atmosphere of argon was used. The special care with which moisture was excluded from these reactions seemed to have lowered the amount of amides *141* and *144* formed (to 22 and 16% respectively from 28%), otherwise the yields were essentially the same as before. Both amides were isolated and characterized as well as the two pairs of diastereomers, *139* and *140* from D-Thr-OBn, and *142* and *143* from



(-)-*139* (+)-*140* (-)-*141*

Fig. XL

L-Ser-OBn. The 200MHz ^{1}H.m.r. spectra of these compounds gave no suggestion of any deuterium incorporation at the α-carbons (within the limits of accuracy of the integration, ±10%).

The mass spectra of all of these compounds also indicated that no more than one deuteron was incorporated *per* β-lactam product and no more than two *per* amide. These mass spectra were all taken at different times under different conditions from the

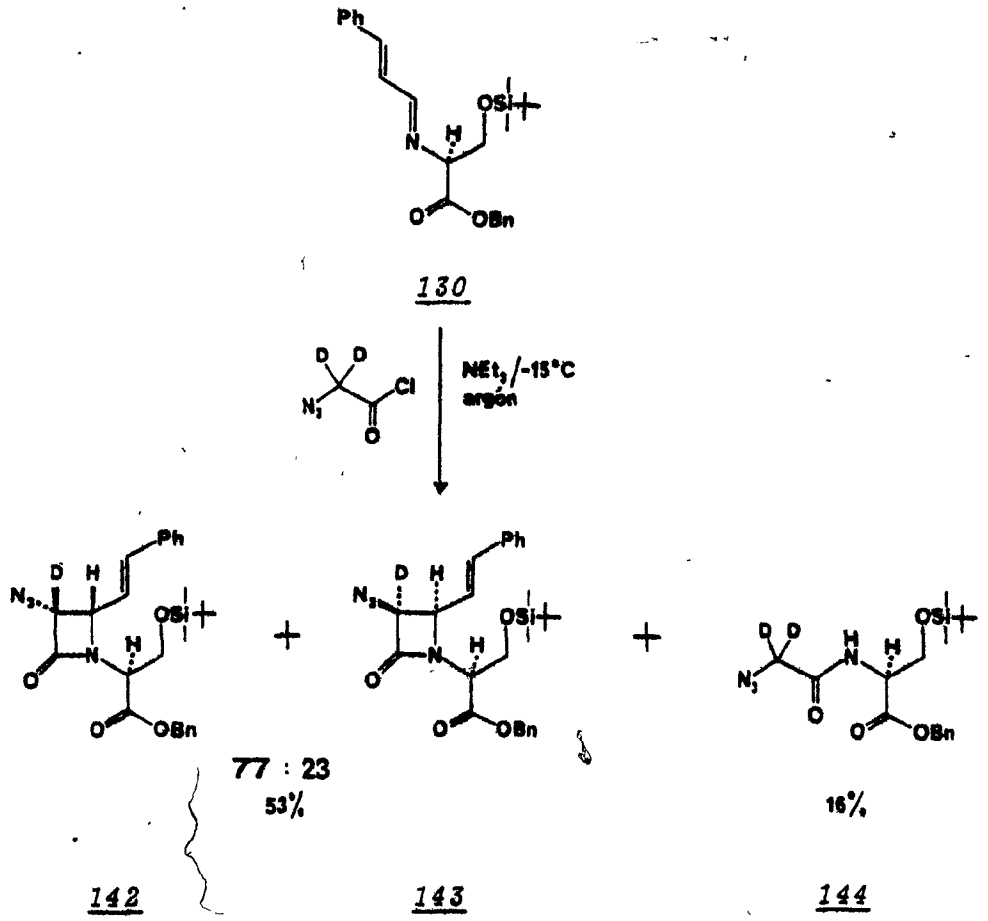
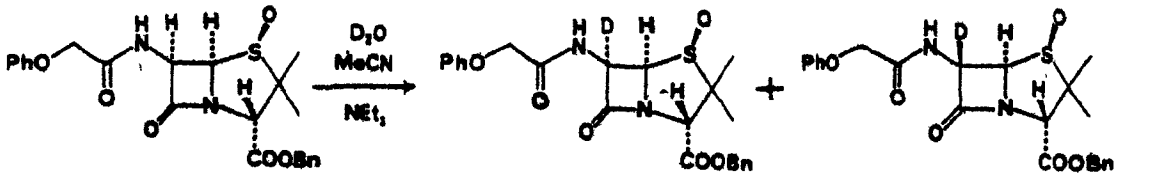


Fig. XLI

non-deuterated analogues. Since the D-Thr-OBn β-lactam derivatives were the more easily purified, the 16eV fragmentation mass spectra of the two deuterated analogues *139* and *140* were run at the same time, under the same conditions as their protonated counterparts (-)-*89* and (+)-*90* (p. 34). Only the differences expected for mono-deuterated compounds could be observed. In

addition, the specific rotations in chloroform, of β-lactams (-)-*139* (-129°) and (+)-*140* (+38°), as well as of the dideuterated amide (-)-*141* (-12°), were consistent with those of the corresponding protonated β-lactams (-)-*89* (-130°) and (+)-*90* (+40°), and amide (-)-*91* (-12°).

Some exchange of deuterium by hydrogen (about 20 to 40%) at the deuterated positions of all the deuterated analogues (figs. XL and XLI) was evident in their respective ^{1}H.m.r. (and mass) spectra. Most likely this exchange involved the reagent either before, or during the cycloaddition process, through traces of water in the reaction medium. Exchange at the position in penicillins (C-6) equivalent to the C-3 of these monocyclic β-lactams is usually accompanied by epimerization[132], a process not detected in our case. Exchange without epimerization has recently been reported by Claes *et al*[133] in the S-sulfoxide of Pen-



	145	*146*
1 hr	34%	4%
12 hr	70%	23%

Fig. XLII

icillin V benzyl ester (*10*), but under more strenuous conditions relative to those of our cycloaddition reaction.

The lack of deuterium incorporation at the α-carbon of both the D-Thr-OBn and the L-Ser-OBn derived β-lactams indicated that racemization was not occurring during the cycloaddition. What then was happening during the cycloaddition with the L-Ser-OMe derivative reported by Just and Liak?

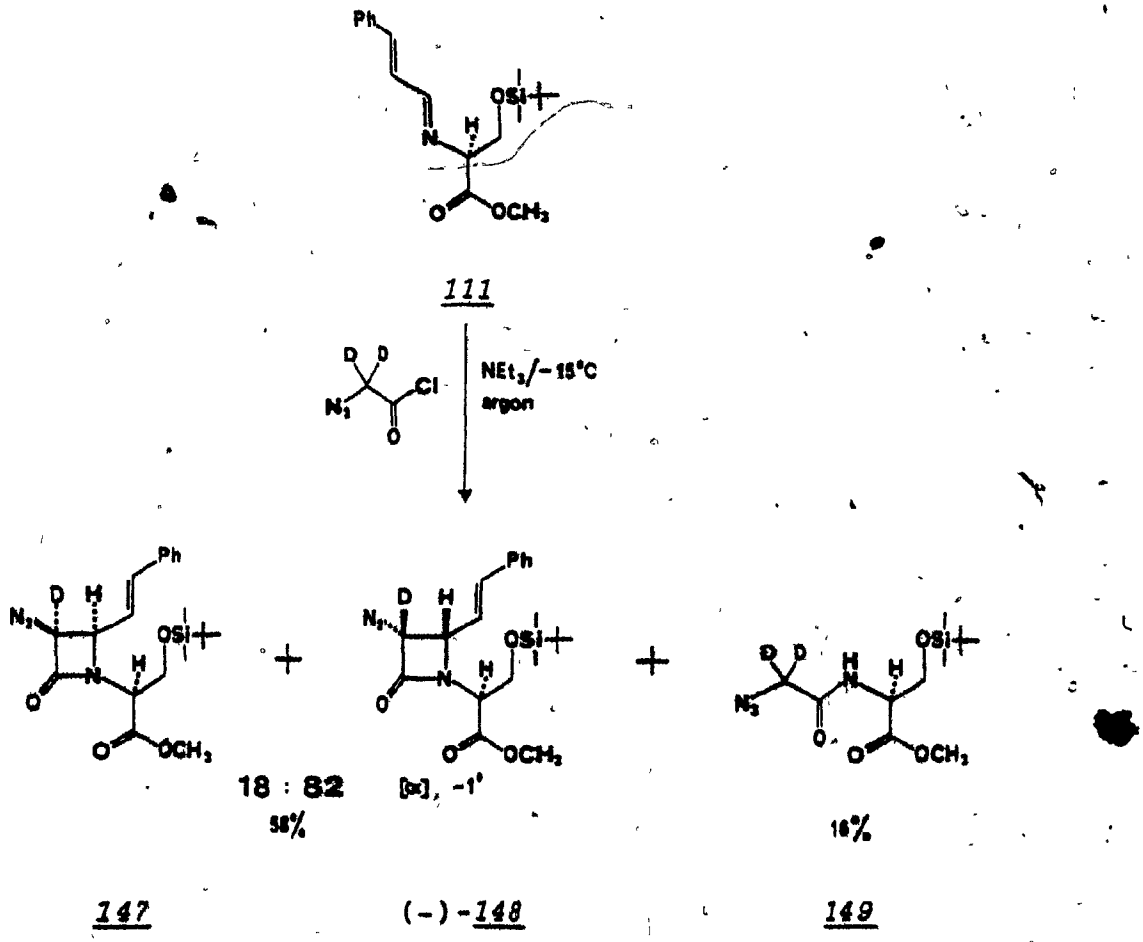


Fig. XLIII

To find an answer to this question, we prepared their Schiff base *111* (fig, XLIII) and reacted it with azidoacetyl chloride-d_2 (*138*) in the usual manner. The resulting solid was separated by flash chromatography[86] into amide *149* and a solid β-lactam fraction. With the experience gained from our previous work, we examined the 60MHz ^{1}H.m.r. spectrum of the β-lactam fraction for evidence of two diastereomers. Three extra, small singlets appeared in this spectrum, two of them downfield from the marker silyl peaks, methyl and *tert*-butyl, and one of them upfield from the methyl ester signal. This suggested that the reaction did indeed proceed asymmetrically. As for the L-Ser-OBn β-lactams (p. 65), separation of the two diastereomers by HPLC was an arduous process. After a retention time of about one hour, we observed an isomer ratio of 18:82. In this case, the major isomer *148* was more polar than the minor isomer *147*, in contrast with all the other pairs of diastereomers. This ratio cannot be considered significantly different from those obtained for the L-Ser-OBn series because of the difficulty of separating the amide from the mixture. Although great care was exercised, the inclusion of a small amount of β-lactam material with the amide fraction (or with other, less polar impurities) could easily skew the ratio in favour of one or the other isomer.

A small amount of the major isomer *148* was isolated by HPLC and characterized. Since this material was a solid, it was also possible to crystallize major isomer *148* directly out of the mix-

ture of β-lactams. The mother liquor contained a ratio of about 40:60 of minor to major isomer, making it easier to isolate the minor isomer *147* by HPLC. The 200MHz ^{1}H.m.r. spectra of both diastereomers again showed no sign of deuterium incorporation at the α-carbons. Although a reasonable quantity of recrystallized major isomer *148* was now available, it was not possible to detect a significant, reliable rotation for this compound. We have recorded a value of $[\alpha]_D^{23}$ -1° (c 2.5, $CHCl_3$) only because the results of measurements using two polarimeters consistently gave very small negative values. It would seem that the separate contributions of the three chiral centers in this molecule cancel each other. Any product with such a small optical rotation could easily be mistaken for a racemate. Because the separation proved to be so difficult, no attempt was make to collect enough material to determine the optical rotation of the minor isomer *147*.

That L-Ser-OMe also resulted in two diastereomers with no deuterium incorporation at the α-carbon demonstrated that racemization did not occur in this reaction either. The ratio of β-lactam diastereomers resulting from cycloaddition with azidoacetyl chloride is, therefore, a measure of the amount of asymmetric induction, and the amino acid residue in each diastereomer retains the original stereochemistry of the starting α-amino acid. When that starting material was an R-amino acid, the stereochemistry of the *cis* β-lactam ring in the major stereoisomer is the same as that of the natural penicillins and cephalo-

sporins. That of the minor β-lactam isomer has the opposite configuration, as depicted throughout this work.

After the completion of these studies, some publications have appeared which contribute to our overall understanding of the factors influencing the stereochemistry of the azidoacetyl chloride cycloaddition. Table 3 (p. 76) contains a list of these reactions (items 5 to 8) in comparison with our own results (items 1 to 4). Only *cis* β-lactams were reported, probably because all the reactions, including ours, were carried out with *N*-alkyl rather than *N*-aryl substituted Schiff bases[184]. The diastereomeric ratios obtained for these *cis* β-lactams suggest that they are sensitive to the distribution of steric factors in the whole molecule. The effect of the following structural alterations, with respect to the D-Thr-OBn derived Schiff base *88* (Table 3, item 2), are illustrated:

a) the absence of a bulky alcohol protecting group,

b) the absence of substituents at the β-carbon of the starting amino acid, and

c) substitution of the α-carboxylate ester.

The results of these reactions also demonstrate that a chiral center is a necessary, but not a sufficient condition for asymmetric induction.

This last statement, and the effect of not having a bulky alcohol protecting group, were amply illustrated by the results of Bose, Manhas and their coworkers[87]. They described the

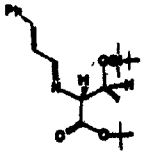
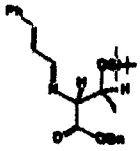
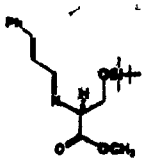
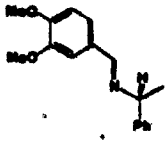
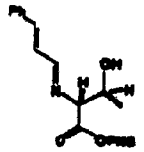
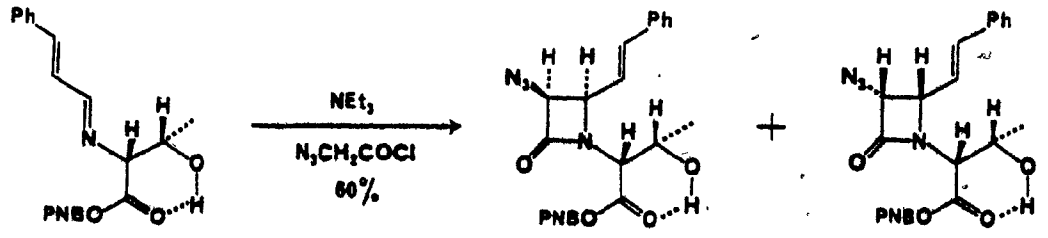
Item	Imine	No.	*cis* β-Lactams Major	Minor	Reference
1		*122*	90 ±2	10	ch. 2, p. 60
2		*88*	88 ±2	12	ch. 1, p. 34
3		*111*	82 ±2	18	ch. 2, p. 73
4		*130*	79 ±2	21	ch. 2, p. 64
5		*159*	70	30	137
6		*151*	54	46	134
7		*154*	51	49	135
8		*149*	50	50	87

Table 3: Comparison of imine structure and *cis* β-lactam ratios.

cycloaddition of the *p*-nitrobenzyl ester of *N*-cinnamylidene D-threonine (*149*), the alcohol group of which was not protected in the usual way. They speculated, however, that the hydrogen bond formed between the ester and the alcohol group was strong enough to afford protection under the mild conditions used.

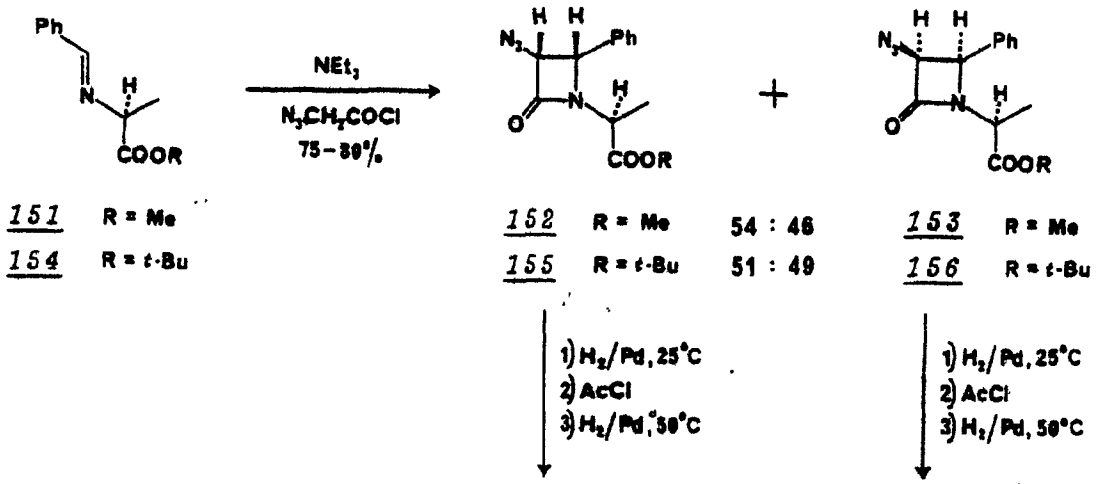


149 *102* *150*

Fig. XLIV

Even though two chiral centers were present in the starting material, the two diastereomers *102* and *150* were formed in about equal quantities (Table 3, item 8). This is in complete contrast to our results (an isomer ratio of 88:12) obtained using the large *tert*-butyldimethylsilyl alcohol protecting group (Table 3, item 2).

The importance of β-carbon substituents in the starting amino ester on the course of the reaction is demonstrated by the work of Ojima *et al*[134-136]. This group was interested in the



N-Ac-D-Phe-L-Ala-OMe *N*-Ac-L-Phe-L-Ala-OMe

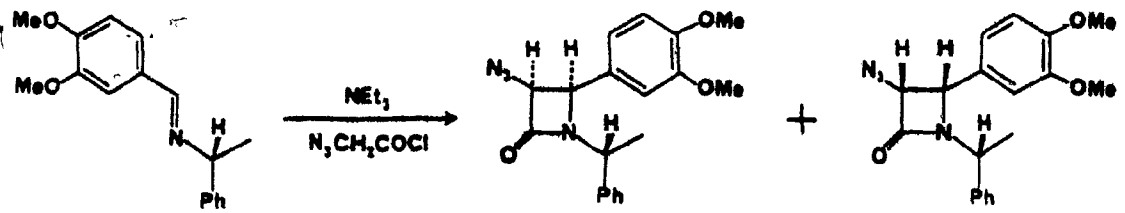
157 *158*

Fig. XLV

preparation of oligopeptides by the hydrogenolysis of 4-aryl-2-azetidinones (such as *152* and *153*). The diastereomeric ratios of the β-lactams were only reported for the reactions involving the methyl[134] and *tert*-butyl[135] esters of *N*-benzylidene-L-alanine, *151* and *154* respectively. These Schiff bases contained no β-carbon substituent, and on cycloaddition with azidoacetyl chloride, yielded ratios of stereoisomers (representing relative isolated yields) equal to 54:46 and 51:49 respectively (Table 3, items 6 and 7). These ratios are virtually 1:1, indicating that no asymmetric induction took place. The respective stereochemistries were assigned by chemically relating the

β-lactams *152* and *153* to the known dipeptides *157* and *158* as shown in fig. XLV.

Subsequent work by Ojima[137] has shown that the lack of β-carbon substituents can be offset to a certain extent by substituting the carboxylate ester with a phenyl group. The reaction of the aryl Schiff base of (+)-R-phenethylamine *159* yielded a ratio of diastereomers equal to 70:30 (Table 3, item 5). The



159 *160* *161*

Fig. XLVI

stereochemistry of the β-lactam ring in the major isomer *160* (determined as for the L-alanine derivatives above) proved to be that of the natural penicillins and cephalosporins. On the basis that the carbon *alpha* to the amine in *159* has the R-configuration, we would have predicted the same stereochemistry. To what extent the 3,4-dimethoxy groups of the aromatic aldehyde moiety in *159* also contributed to the asymmetry of the reaction,

cannot be determined because the reaction with a benzaldehyde moiety has not been reported.

Two further results described by Bose, Manhas *et al*[87] indicated that the bulk of the acyl chloride reagent itself can contribute to the stereochemical outcome of the reaction. In

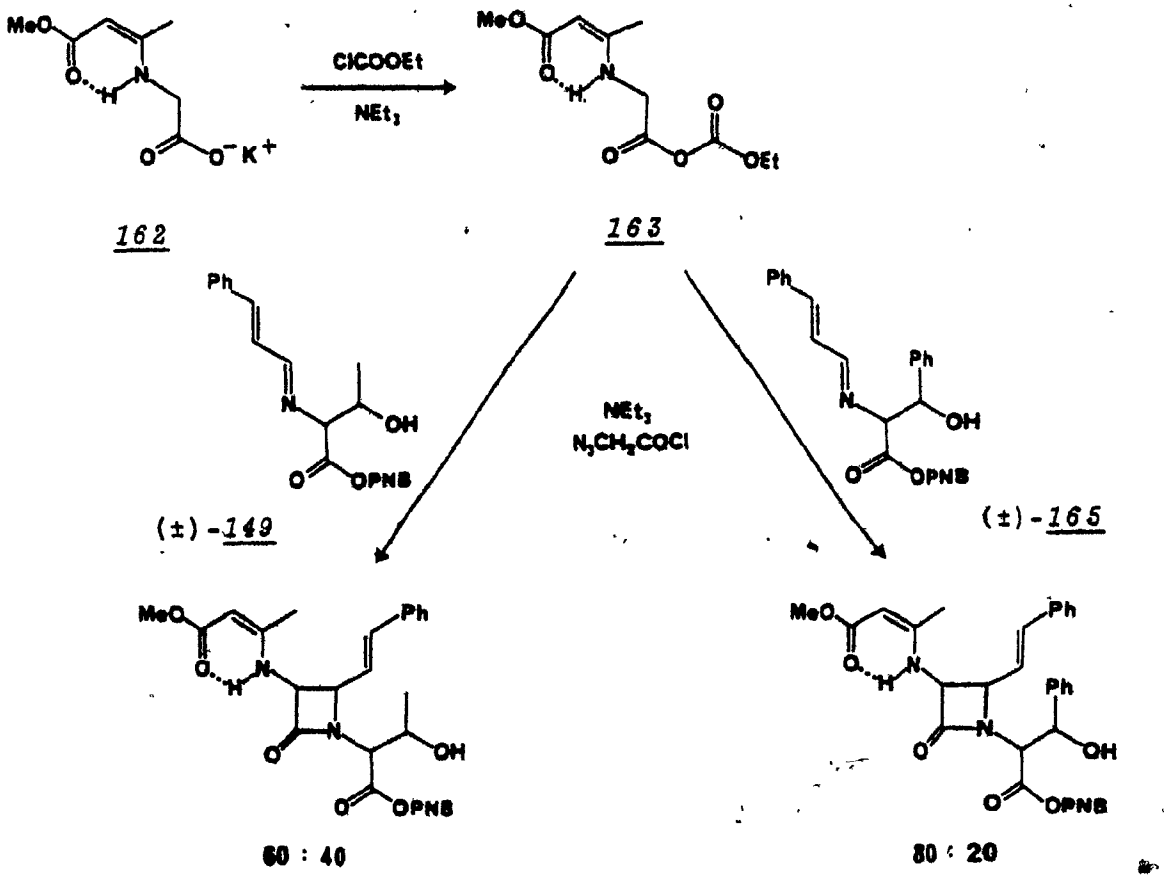


Fig. XLVII

an effort to find an alternative to the hazardous azido reagents[138], they found that "Dane salt" *162*[139], when suitably activated, could also lead to *cis* β-lactams[87, 140]. When the active ester *163* was reacted with the DL-threonine derivative *149* (again unprotected at the alcohol), a diastereomeric ratio of 60:40 was obtained for the β-lactam mixture *164*[87]. This represents a minor improvement over the 1:1 ratio obtained using azidoacetyl chloride. A further improvement, reflected in a diastereomeric ratio of 80:20 for the β-lactams *166*, was achieved with the DL-phenylserine derivative *165*[87] (fig. XLVII), in which the bulkier phenyl group replaced the β-methyl group of threonine. It would be interesting to see whether or not a further increase in asymmetric induction could be obtained if the alcohol group of either *149* or *165* was protected.

Conclusions

From these studies, we can draw the following conclusions about the formation of an excess of one *cis* β-lactam diastereomer (asymmetric induction) using the Doyle modification[50, 51] of the imine – azidoacetyl chloride method of Bose[56].

1) The imine must contain both a chiral center *alpha* to the nitrogen and a bulky substituent either on the α- or the β-carbon.

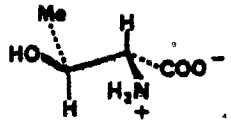
2) When the α-chiral center has the R configuration, the stereochemistry of the major β-lactam diastereomer is the same as that of the natural penicillins and cephalosporins.
3) Racemization of the α-chiral center does not occur during the cycloaddition.
4) When the imine is derived from an α-amino acid, the size of the carboxyl protecting group has little or no effect on the stereochemical outcome of the reaction.
5) Increased asymmetric induction is obtained when the β-carbon is di-substituted with at least one bulky substituent.
6) Increased asymmetric induction can be achieved by substituting the azido group of the acyl chloride reagent by a bulkier group, provided it also promotes *cis* stereoselectivity.
7) Bulkier α,β-unsaturated imines as such, or in combination with other bulky groups on the *N*-substituent, may also lead to increased asymmetric induction.

CHAPTER 3

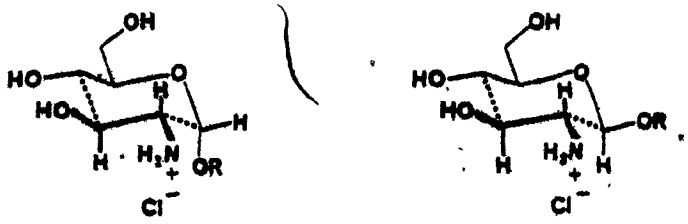
The studies carried out so far have shown how a 3-amino-β-lactam with the stereochemistry of the natural penicillin and cephalosporin antibiotics may be obtained. Because of the predominance of non-crystalline intermediates and the necessity to purify by chromatographic methods, the synthesis outlined in chapter 1 is not readily applicable to economical industrial processes. Furthermore, the D-amino acids are relatively expensive starting materials. Ideally, we required an inexpensive material with stereochemistry structurally related to that of D-threonine, functional groups suitably situated for the eventual elaboration of the *O*-2-isocephem ring system and the potential for producing readily purified crystalline β-lactams in high yield.

Inexpensive carbohydrates are finding more and more applications as chiral templates[141,142] . Since their chemistry is well-documented, we thought that a starting material which would fulfil our requirements might be found among them. The most readily available amino carbohydrate is D-glucosamine hydrochloride (*167*, R = H, fig. XLVIII, p. 84). The structure

and the stereochemistry about carbons 2 and 3 of *167* match those of the α- and β-carbons of D-threonine (*71*). There is also an



71 D-Threonine



167A *167B*

167 D-Glucosamine (R = H)

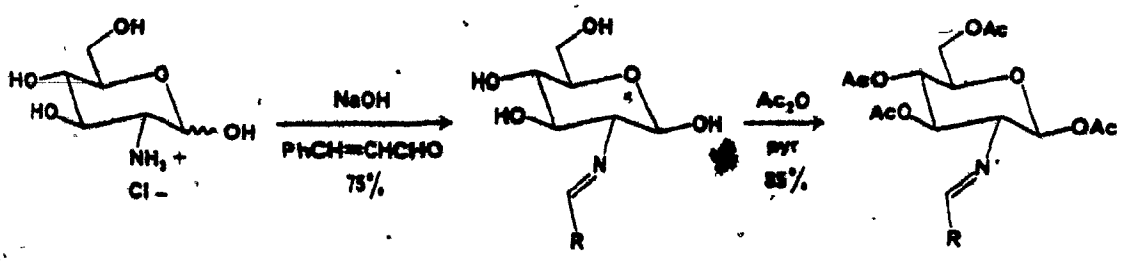
Fig. XLVIII

anomeric center (a hemi-acetal) which may later be converted to an ester or a carboxylic acid. This anomeric center, as is well-known, allows the existence of two configurations, an axial α- (*167A*) and an equatorial β-anomer (*167B*). An additional stereochemical factor is thereby made available for a study of its influence on the cycloaddition reaction.

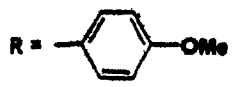
Methods exist for the formation and separation of various α- and β-glycosides of glucosamine[143, 144], but, for our pur-

poses, it was considered more desirable to synthesize each anomer by specific methods. A search of the literature indicated that such a goal would be readily attainable for the α- and β-anomeric acetates. This in turn made the choice of protecting groups an easy one, since many methods for the cleavage of acetyl groups are known[145-148]. Because the β-acetate appeared to be the more easily obtainable anomer, it was synthesized first.

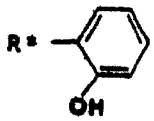
In 1931, when Bergmann and Zervas first prepared the anisylidene Schiff base of D-glucosamine (*168*)[149], they deduced by chemical means, that the tetra-acetylated imine *169* consisted of the β-anomer exclusively. It was not until 1973, however,



167

168 R = 

169

170 R = 

171

172 R = 

173

Fig. XLIX

that Panov *et al*[150] showed by ^{1}H.m.r. studies in DMSO-d_6 that the unprotected imine *168* (and also the salicylidene analogue *170*) was itself formed exclusively as the β-anomer. In the region of the 100MHz ^{1}H.m.r. spectrum (δ = 5.5 to 7 ppm) where the anomeric hydroxyls were expected to absorb, there was only one proton signal which was exchangeable in D_2O. For both imines *168* and *170*, this was a doublet (J = 6.5Hz) at δ = 6.75 ppm[150]. This corresponded well with the data obtained by Casu *et al*[151] for the β-anomers of a variety of monosaccharides (δ = 6.3 to 6.6 ppm, J = 6.0 to 7.0Hz).

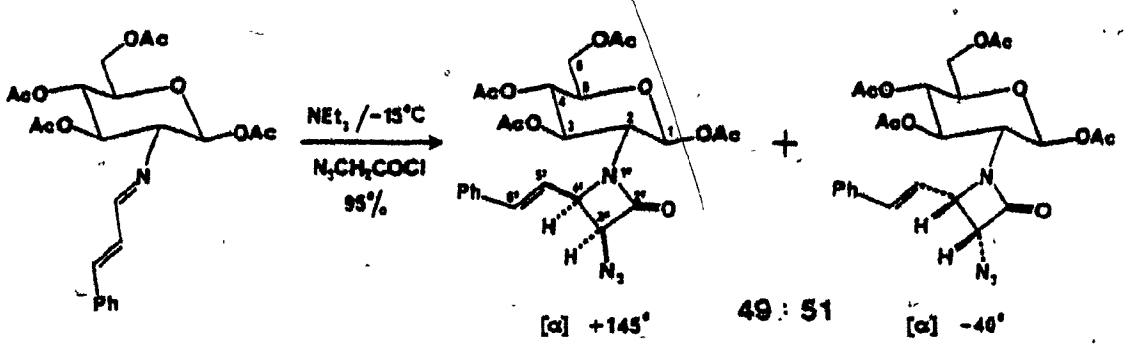
Similar results were obtained for the crystalline *N*-cinnamylidene Schiff base of D-glucosamine (*172*)* (fig. XLIX) which we prepared in an aqueous medium by the method by Bergmann and Zervas[149]. The only signal exchangeable in D_2O appearing in the relevant region of the 200MHz ^{1}H.m.r. spectrum of *172* was a doublet (J = 7Hz) at δ = 6.60 ppm.

Peracetylation of *172* with acetic anhydride/pyridine[152] afforded the crystalline β-acetate *173*, the 200MHz ^{1}H.m.r. spectrum of which was consistent with the 100MHz ^{1}H.m.r. spectra published for *169* and *171*[150]. Our proton assignments were made in accordance with these spectra. The anomeric proton appeared

*A foldable flow chart is available in Appendix III, which illustrates the relevant reactions and compounds of this chapter.

as a doublet at δ = 5.95 ppm (J = 8.5Hz). This is typical of β-acetates in which shielding is increased and larger coupling constants are observed in comparison with α-acetates[153]. No absorption, characteristic of an α-acetate, was observed.

The cycloaddition of *173* with azidoacetyl chloride (in contrast to the amino acid derivatives) gave a 95% yield of crystalline β-lactam material. Unfortunately, analysis by HPLC showed that the two diastereomers *174* and *175* were present in



173 (+)-*174* (−)-*175*

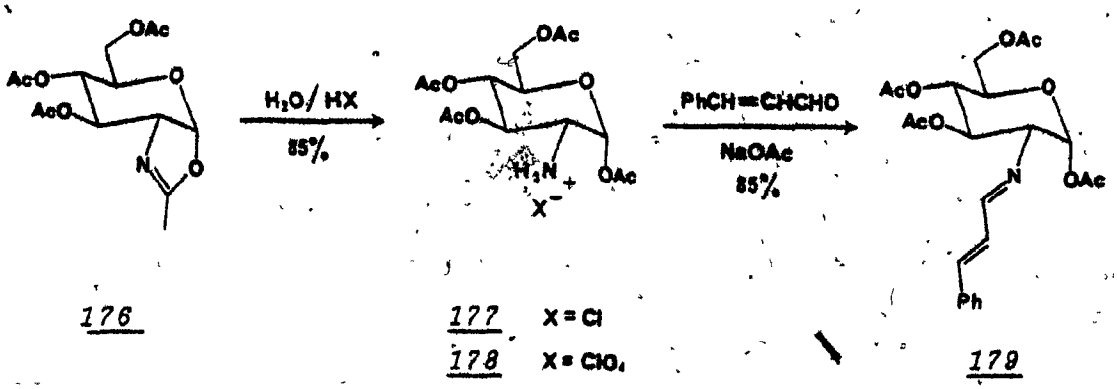
Fig. L

equal amounts. Chromatography proved to be unnecessary, however, for the separation of these two stereoisomers. A sufficient quantity of each diastereomer was obtained in a pure state by the Pasteur method[154]. When the mixture of β-lactams was crystallized, two distinctly different types of crystals

were produced...needles and rhombohedra. Large rhombohedral crystals were easily separated from the mixture by hand. Then those needles, still obviously part of radiating clusters, were collected. The needles were identified by HPLC as the less polar isomer (+)-*174* {$[\alpha]_D^{23}$ +145° (c 1.5, $CHCl_3$)}, and the rhombohedra as the more polar isomer (-)-*175* {$[\alpha]_D^{23}$ -40° (c 2, $CHCl_3$)}. The 200MHz ^{1}H.m.r. spectra clearly showed both diastereomers to be *cis* β-lactams ($J_{3',4'}$ = 5.1Hz). The different stereochemistries shown in fig. L were assigned later when the data could be compared with those obtained from the corresponding α-anomers.

Since we now know (chapter 2, p. 75) that the presence of chiral centers *per se* is not necessarily enough to induce asymmetry during the cycloaddition, examination of a molecular model of *173* can explain the above result. The groups which would be expected to have the largest effect on a reaction at the imine are the C-1 and C-3 acetoxy groups. In *173*, these groups are disposed "symmetrically" relative to the reaction center. In the α-anomer, there is spatial "dyssymmetry" about this center. It was, therefore, of interest to determine the behaviour of the α-anomer during the same reaction.

The preparative method selected for this anomer was inspired by the report of Sinaÿ *et al*[155] who described the formation of the ammonium perchlorate salt *178* (fig. LI, p. 89) by addition of perchloric acid to the known 2-methyl-oxazoline



176

177 X = Cl
178 X = ClO_4

179

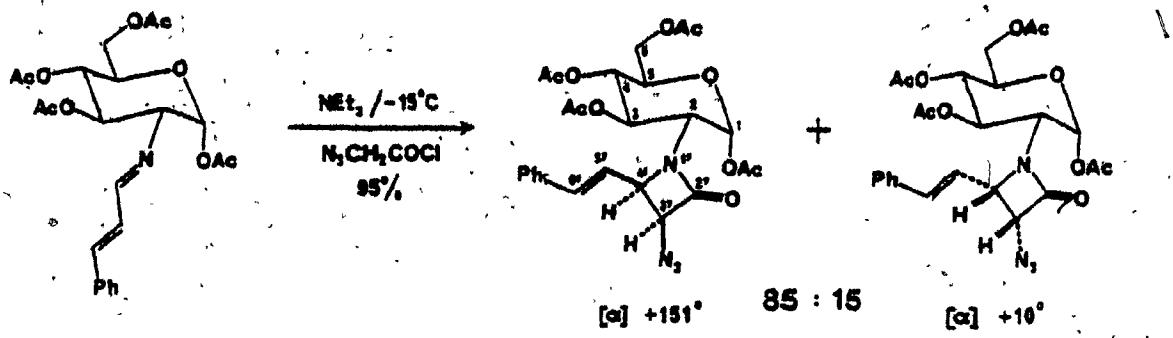
Fig. LI

176[156, 157]. The ^{1}H.m.r. spectrum (in DMSO-d_6) of *178* displayed a doublet at δ = 6.24 ppm with J = 4Hz, indicating that the acetoxy group at C-1 was in the α-configuration. When we treated 2-methyl-oxazoline *176* with 1N hydrochloric acid, the ^{1}H.m.r. spectrum (DMSO-d_6) of the resulting salt *177* also displayed a doublet at δ = 6.25 ppm with J = 3.5Hz. In methanol-d_4, this doublet occurred at δ = 6.35 ppm (J = 4Hz). Since this spectrum showed better resolution, it was the one reported in detail. The melting point and infrared spectrum of *177* were consistent with those of the ammonium chloride salt prepared by Micheel *et al*[158], although we recorded a higher optical rotation {$[\alpha]_D^{23}$ +146° (c 2, water) *versus* +130° (c 1, water)}.

Formation of the *N*-cinnamylidene Schiff base *179* required initial neutralization of the salt *177*. In order to avoid the

possibility of transacylation within the basic amine intermediate, the salt was added to a cold mixture of cinnamaldehyde and sodium acetate. This resulted in an almost quantitative yield of crystalline Schiff base *179*. Later, it was found that the addition of sodium acetate to a mixture of the other two reagents (a more convenient procedure) had no adverse affect on the reaction. No *N*-acylated by-products could be detected.

Treatment of Schiff base *179* with azidoacetyl chloride, again gave a 95% yield of solid β-lactam material. This time, analysis of the crude product by HPLC showed that the two diastereomers *180* and *181* had formed in a ratio of 85:15, with the major isomer *180* being the less polar one. This isomer was very easily isolated in about 75% yield by taking advantage of solubility differences. Once the polar impurities were removed

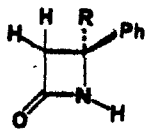


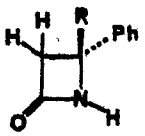
179 (+)-*180* (+)-*181*

Fig. LII

from the crude product by filtration through silica gel, trituration with anhydrous ether, filtration and recrystalization readily yielded the pure major isomer (+)-*180* {$[\alpha]_D^{23}$ +151° (c 2, $CHCl_3$), $J_{3',4'}$ = 5.2Hz}. The amorphous solid remaining could unfortunately not be crystallized. Nevertheless, flash chromatography[86] allowed the separation in pure form of enough minor isomer (+)-*181* {$[\alpha]_D^{23}$ +10° (c 4, $CHCl_3$), $J_{3',4'}$ = 5.1Hz} for characterization.

In order to obtain some idea of the β-lactam stereochemistries of these two isomers, qualitative optical rotatory dispersion (O.R.D.) curves of dioxane solutions were obtained and compared with that of the β-lactam (-)-*89*, derived from D-Thr-OBn (chapter 1, p. 34), the stereochemistry of which has already been established unequivocably. The styryl group was the most useful chromophore here, as the β-lactam absorption was swamped by all the esters present in





182a R = H *182b*
183a R = Me *183b*

these molecules. Rehling and Jensen[159] reported a λ_{max} of 262nm for the two 4-phenylazetidinones *182* and *183*. Since

cinnamic acid has a λ_{max} of 273 nm[160], the styryl group would be expected to have a λ_{max} somewhere between 260 and 270 nm. Indeed both (+)-*180* and (+)-*181* showed Cotton effects in that region. The major diastereomer (+)-*180* exhibited a negative Cotton effect with a trough at 272 nm and a peak at 244 nm, while the minor diastereomer (+)-*181* gave a positive effect with a peak at 272 nm and a trough at 244 nm. Both appear to give a λ_{max} at approximately 260 nm. The O.R.D. curve of the D-Thr-OBn derivative (-)-*89* proved to be somewhat complicated. It consisted of a trough at 270 nm, a peak at 265 nm, a trough at 255 nm and a peak at 245 nm indicating a negative Cotton effect.. Therefore, the β-lactam ring in (+)-*180* was assigned the stereochemistry already established for (-)-*89* (chapter 1), that of the natural penicillins and cephalosporins.

With the data now available, the stereochemistry of the

isomer	$[\alpha]_D^{23}$	retention time	page
174	+145°	20 min.	87
180	+151°	21 min.	90
175	-40°	25 min.	87
181	+10°	32 min.	90

TABLE 4: *N*-(2-Azetidinonyl)-D-glucosamine stereoisomers.

proton	(non-polar)		(polar)	
	174(β)	*180*(α)	*175*(β)	*181*(α)
A (CH=*CH*Ph)	6.83	6.73	6.77	6.68
B (CH*CH*=CH)	6.02	6.03	6.22	6.18
X (H-4')	4.55	4.53	4.31	4.39
Y (H-3')	4.73	4.66	4.76	4.77

TABLE 5: Comparison of the 200MHz ^{1}H.m.r. shifts of the β-lactam and olefinic protons of *174*, *175*, *180* and *181*.

β-anomers *174* and *175* could be assigned by comparison with the α-anomers *180* and *181*. Both of the less polar isomers *174* and *180* had very similar, positive optical rotations (Table 4, p. 92) which were markedly different from those of the two polar isomers *175* and *181*. On this basis the β-lactam ring in *174* was assigned the stereochemistry of *180*, and *175* that of *181*. The 200MHz ^{1}H.m.r. spectra of the azetidinonyl and olefinic protons in each of the four steroisomers supported this correlation. The coupling constants among these protons varied very little from one stereoisomer to another. The chemical shifts (Table 5) did vary, however, creating patterns which could be divided into two pairs, *174* + *180* and *175* + *181*. The spectra of the carbohydrate moieties were as expected for 2-acylamino-glucopyranoses,

H-1, H-3 and H-5 being deshielded in the α-anomers in comparison with the β-anomers[153, 161].

All of the evidence for the stereochemistry of the major diastereomer (+)-*180* suggests that we are dealing with the stereoisomer related to the biologically active β-lactams. Final proof would be provided by its transformation into an appropriate *O*-2-isocephem. Also, in view of the economy and ease with which a large amount of the β-lactam intermediate with the tentatively correct configuration could be prepared, suitable alteration of the carbohydrate portion would be attractive from the industrial standpoint.

An approach analogous to that outlined in chapter 1 was envisioned (see fig. LIII, p. 95); that is, formation of mesylate *189*, deprotection of its carbohydrate portion to give the polyol *184* and, finally, transformation of the carbohydrate into a species *185* resembling enol *72* (p. 41), perhaps by periodate cleavage. The preparation of mesylate *189*, however, was not as trivial a matter as it had originally appeared.

Our first attempts were directed towards obtaining the primary alcohol *188* (fig. LIII) directly from the styryl β-lactam (+)-*180* (as in chapter 1, p. 41). Treatment of (+)-*180* with ozone in 2-propanol/dichloromethane, and then with sodium borohydride adsorbed on alumina ($NaBH_4$-Alox)[93] for two hours gave an amorphous solid which exhibited no

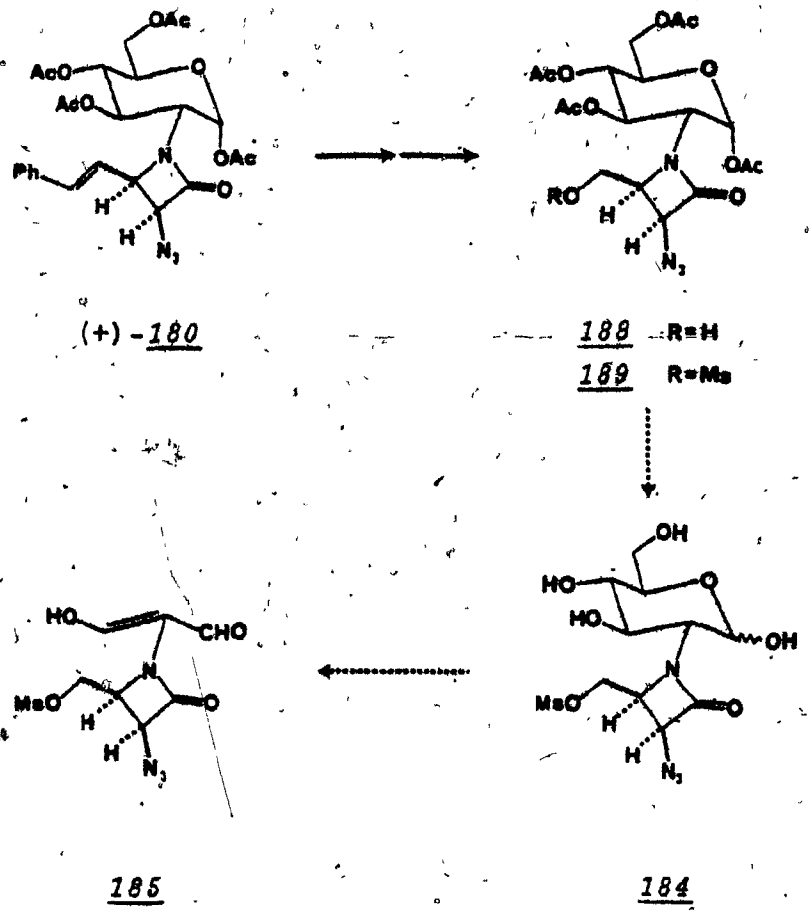
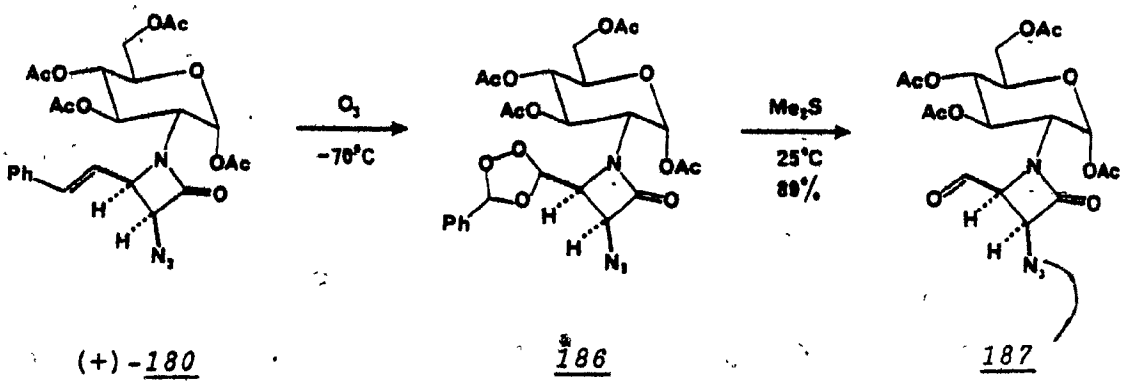


Fig. LIII

olefinic protons, a small aldehyde absorption and an equivalent amount of aromatic protons in its ^{1}H.m.r. spectrum. Even after chromatography, however, the aromatic protons were still present. So was the small aldehyde absorption. Because the aldehyde, if present, may have been hydrated, the product was heated under reflux for one hour in benzene using a Dean-

Stark trap. No significant increase in the ^{1}H.m.r. aldehyde signal occurred after this treatment. Furthermore, t.l.c. indicated that this material broke down slowly to give a more polar compound, and a non-polar one with the same Rf as benzaldehyde. This information, coupled with the reports of Just and Liak[81], and Oh[162], led us to conclude that the product was an unusually stable ozonide *186*. This was confirmed by isolation



(+)-*180* *186* *187*

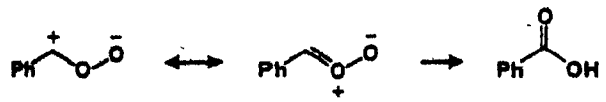
Fig. LIV

of the same material immediately after ozonolysis of *180* in dichloromethane in the absence of any alcohol or reducing agent.

Reduction of ozonide *186* with $NaBH_4$-Alox[93] overnight gave several, more polar products which, at that time, were ascribed to deacetylation. Reaction of *186* with dimethyl sulfide for twenty-four hours at room temperature, however, gave only one product, the aldehyde *187*. This crude material was about 20%

hydrated, as shown by the 60MHz ^{1}H.m.r. spectrum. The H-1 and H-3 absorptions of the carbohydrate residue in the hydrate (δ = 6.3 and 5.8 ppm, respectively) were clearly separated from those of the aldehyde (δ = 6.18 and 5.3 ppm, respectively). These signals disappeared after an hour of heating under reflux in benzene using a Dean-Stark trap, and a corresponding increase in the aldehyde signal was observed. The doublet of doublets at δ = 5.8 ppm was assigned to H-3 of the hydrate by comparing its spectrum with that of alcohol *188* produced later (p. 100). Drying the crude aldehyde *187* *in vacuo* over phosphorous pentoxide for eighteen hours followed by recrystallization, yielded a product which appeared to be resistant to rehydration.

A large scale version of this reaction yielded 65% benzoic acid along with 89% of the crystalline product. Since dry dichloromethane had been used, it would seem that most of the aldehyde was not produced by reduction at all, but by decomposition of the ozonide to give the zwitterion *190* which subsequently rearranged to benzoic acid[163]. Some benzaldehyde



190

Fig. LV

was formed, however. This may be the result of either slow reduction by dimethyl sulfide or reaction of the ozonide with traces of water to release hydroperoxide[92,163,164]. If the latter path is followed, then the presence of some kind of peroxide scavenger is a necessity.

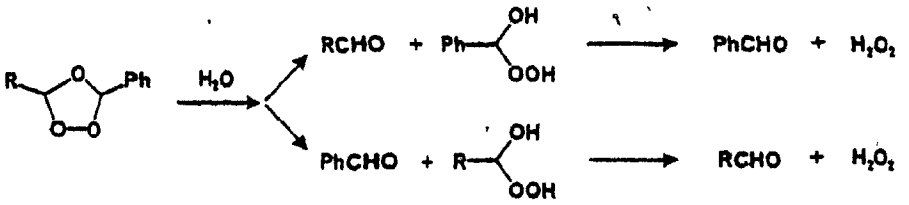


Fig. LVI

Initial attempts to reduce aldehyde *187* to alcohol *188* (fig. LVII, p. 100) with lithium tri-*tert*-butoxyaluminohydride ($LiAl(OBu^t)_3H$) in tetrahydrofuran gave no apparent alcohol formation even after stirring overnight at room temperature. Heating under reflux for an hour after the addition of excess reagent also caused no change. Only after more than thirty-six hours at room temperature did any alcohol product appear to be formed. By this time, polar material was also building up, most likely as a result of azide reduction and/or β-lactam ring opening. Since aldehydes are normally reduced easily by $LiAl(OBu^t)_3H$[165], this resistance of our aldehyde may have been caused by a combination of two highly hindered species,

the reagent itself and aldehyde *187*. Further evidence of the hindered nature of the aldehyde environment was obtained when a small quantity of ozonide *186* (fig. LIV, p. 96) was treated at 0°C for three hours with $LiAl(OBu^t)_3H$. Less than 50% reaction occurred (t.l.c.). After warming to room temperature, it took another three hours before all of the ozonide had been reduced to aldehyde. These reaction times are much longer than those usually reported for such reductions. Ozonide reduction by sodium borohydride, a reducing agent of similar strength[166], is generally complete after two hours at low temperatures (-70° to 0°C)[50,51,95]. Aldehyde reduction by $LiAl(OBu^t)_3H$ can be accomplished within three hours at 0°C[81], and ketone reduction within thirty minutes at 50°C[167].

The borohydrides are among the least hindered reducing agents, so a second attempt was made to synthesize alcohol *188* from aldehyde *187* with sodium borohydride adsorbed on alumina ($NaBH_4$-Alox)[93]. In an effort to eliminate any possible deacetylation, a 2:1 mixture of $NaBH_4$-Alox and anhydrous magnesium sulfate[168] was used in an aprotic medium. Thin layer chromatography showed that a polar product was being produced and then transformed to another compound just slightly less polar than the starting aldehyde. (This was no doubt also occurring during ozonide reduction with this reagent.) These two products were isolated and identified. The more polar product proved

to be the expected primary alcohol *188*. The less polar product contained no H-1 absorption characteristic of an anomeric acetate in its ^{1}H.m.r. spectrum, but it still had four acetyl groups. Azide and β-lactam absorptions were also present in its infrared spectrum. A study of a molecular model of alcohol *188*

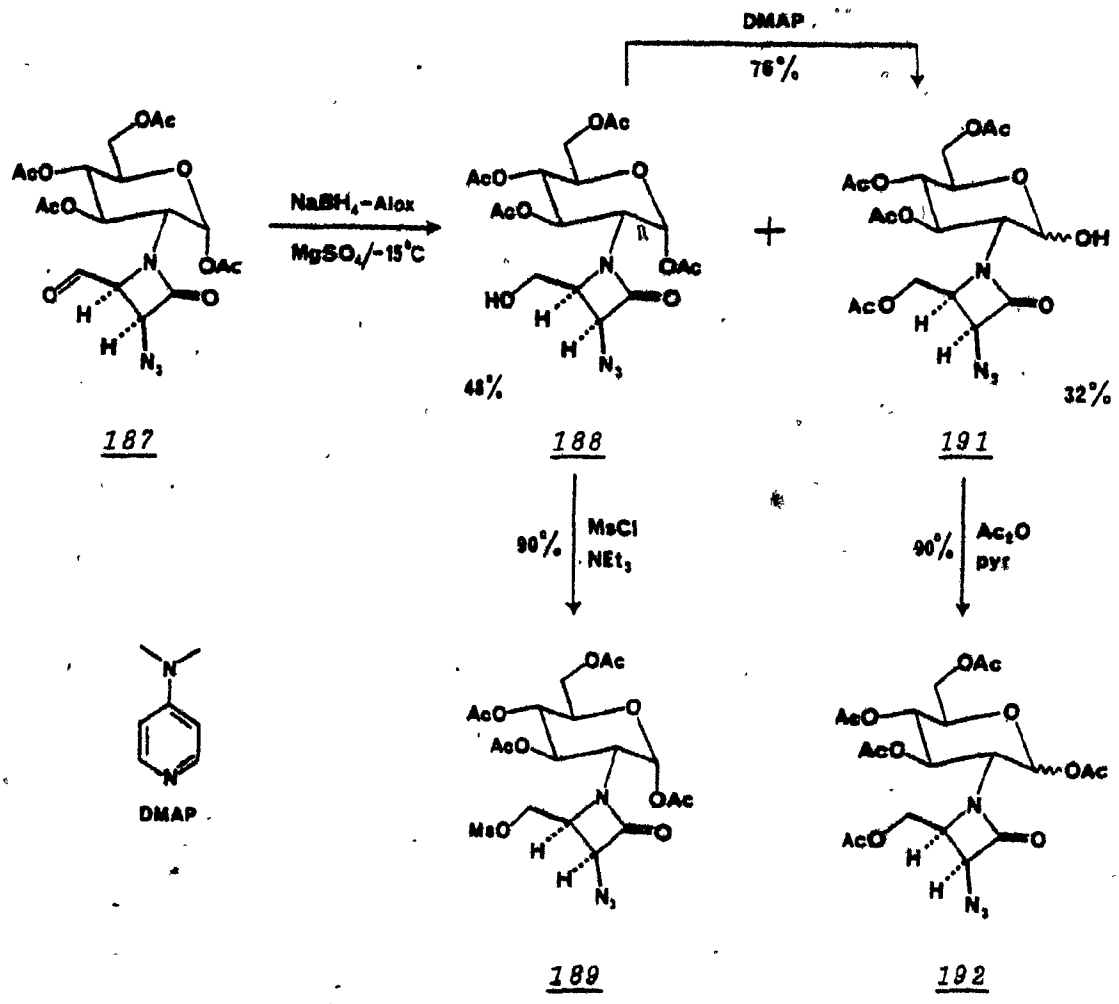
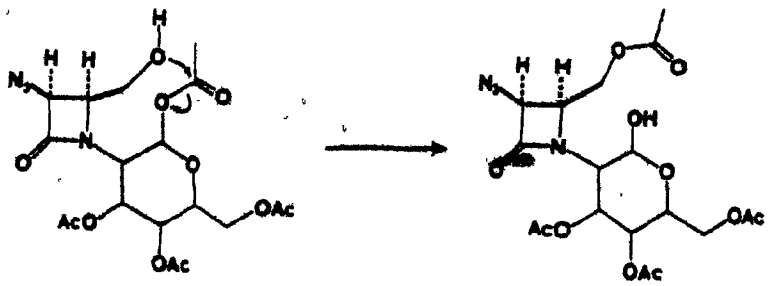


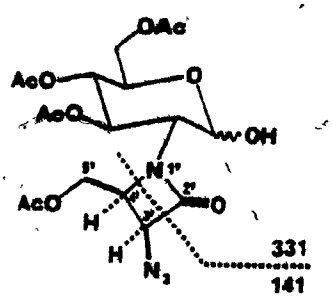
Fig. LVII



188 *191*

Fig. XLVIII

showed that, although intramolecular attack by the primary hydroxyl group on the anomeric acetate would involve an eight membered cyclic intermediate, the constraints introduced by the two rings already present can easily bring the two groups within reach for interaction without requiring distortion. The product of this transesterification would be the hemi-acetal-acetate *191*. The fragmentation mass spectrum of this side product did not contain a parent peak, but a peak corresponding to the loss of nitrogen (N_2) from the azide group (472 - 28 = 444) was present (2%). The most abundant peaks were those associated with acetate fragments (m/e 43, 60 and 86) except for m/e 113 (43%), which could correspond to the loss of nitrogen from the smaller fragment (m/e 141) resulting from β-lactam

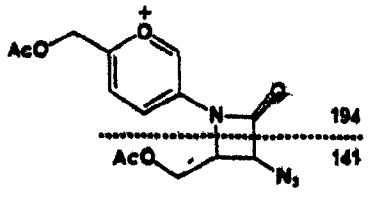


191

cleavage at the $C_{2'} - C_{3'}$ and $N_{1'} - C_{4'}$ bonds (141 - 28 = 113)[169]. Peaks corresponding to the loss of two (m/e 211) and three (m/e 151) molecules of acetic acid from the other fragment (m/e 331) were also present (10% each).

Acetylation of this side product at 25°C yielded a crystalline compound *192* (Fig. LVII, p. 100) with a 200MHz ^{1}H.m.r. spectrum displaying five singlets, indicating the presence of five acetyl groups, and a doublet at δ = 6.26 ppm ($J_{1,2}$ = 3.4Hz) for an α-anomeric acetate. A small doublet at δ = 5.82 ppm ($J_{1,2}$ = 9Hz) and broad triplet at δ = 5.4 ppm ($J_{2,3} = J_{3,4}$ = 10Hz) indicated that about 20 to 25% of the product consisted of the β-anomer. These values correspond to those for the H-1 and H-3 protons of the β-anomeric styryl β-lactams *174* and *175* (p. 87 and 158). The electron impact (EI) mass spectrum of this compound was not especially helpful. Aside from the most abundant peak at m/e 86, only small ones were present. It did, however, have a peak at m/e 335

(9%), which could correspond to the species *193*. Similar species have been described by Budzikiewicz, Djerassi and

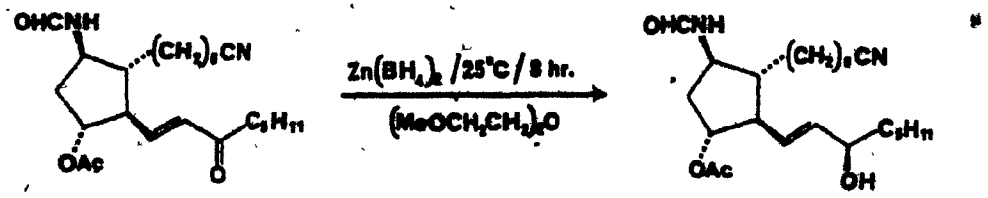


193

Williams[170] as being part of one pathway for the fragmentation of peracetylated monosaccharides. Azetidinone cleavage would then result in a fragment with m/e 194 (8%).

All attempts to prevent the formation of hemi-acetal-acetate *191* (p. 100) were unsuccessful. Changing the solvent to a more polar one (tetrahydrofuran) or carrying out the reaction at room temperature served only to speed up the undesired transesterification and/or deacetylations. Use of a less polar solvent (carbon tetrachloride/dichloromethane, 3:1) slowed down the reduction (even at 25°C) allowing mainly deacetylation to occur. The best yield of alcohol *188* (48%) was obtained when the aldehyde *187* was added slowly to a cold (-15°C) suspension of the 2:1 mixture of $NaBH_4$-Alox/magnesium sulfate[168] (p. 99) in dichloromethane. This also resulted in a 32% yield of the hemi-acetal-acetate *191*.

Zinc borohydride is reputed to be neutral rather than basic[171] and has been used to reduce the α,β-unsaturated ketone *194* with no ill effect on the acetate group after

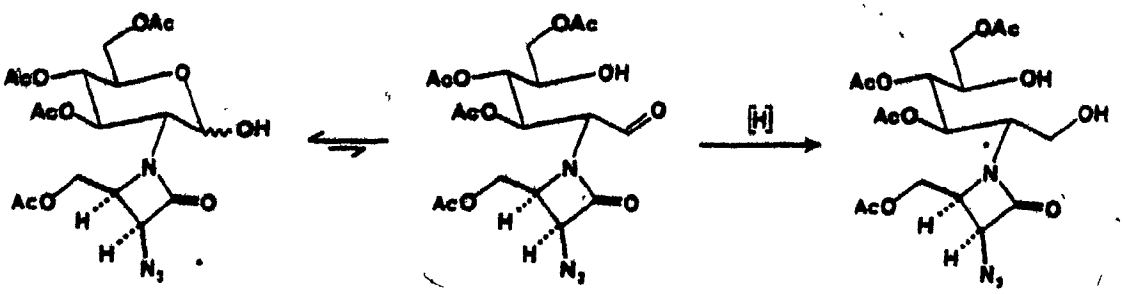


194 *195*

Fig. LIX

eight hours in diglyme at room temperature[172]. Addition of this reagent to aldehyde *187* in tetrahydrofuran at 25°C resulted in very rapid disappearance of the starting material, but only a small amount of the alcohol formed. At 0°C, only a trace of alcohol (t.l.c.) was obtained accompanied by a substantial precipitate containing many polar compounds. One of the possible destructive side reactions may be coordination of the zinc with the β-lactam nitrogen (or carbonyl) thus promoting the opening of the four-membered ring. Metal coordination in penicillins and cephalosporins is known to increase considerably the rate of β-lactam ring hydrolysis[173]. Conversely, intramolecular transacetylation may be inherently

favoured, and accelerated by zinc catalysis. Once formed, hemi-acetal-acetate *191* could undergo carbohydrate ring opening followed by reduction of the aldehyde so-formed to give diol *196*. Transacetylation and deacylation in diol *196* would lead to many polar side-products.



191 *196*

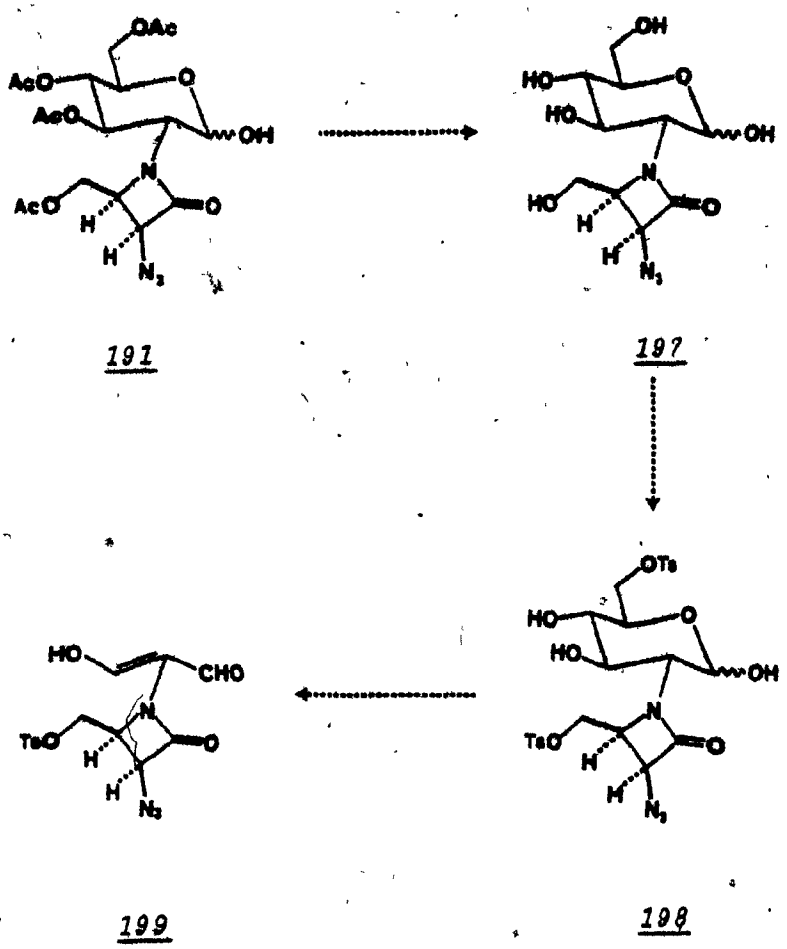
Fig. LX

Considering the difficulties encountered in our attempts to prevent transacetylation in primary alcohol *188*, similar problems were expected during the preparation of mesylate *189* (fig. LVII, p. 100). Normally[94], methanesulfonyl (mesyl) chloride is added to a cold (-15°C) solution of an alcohol in the presence of triethylamine. We reversed this procedure by adding the triethylamine slowly to a mixture of methanesulfonyl chloride and alcohol *188*. The reaction of the primary hydroxyl group with the intermediate sulfene[174] must be faster than

transesterification because mesylate *189* was obtained in 90% yield (after chromatography).

None of the product of transesterification, hemi-acetal-acetate *191*, was detected by spectroscopic means. Since both *189* and *191* had the same mobility on silica gel, the presence of small amounts of *191*, subsequently removed by recrystallization, cannot be completely discounted. Mesylation of any *191* present would give an anomeric mesylate which is not expected to be particularly stable. When the mesylation was carried out with alcohol *188* still contaminated with *191*, there was produced a more polar compound which seemed to decompose on standing at room temperature as well as on thin layer chromatography plates. This behaviour was ascribed to the labile anomeric mesylate (or chloride[185]). That this kind of decomposition was not noticeable in the crude mesylate produced from pure alcohol *188* is further evidence against transesterification during mesylation.

Although the target intermediate, mesylate *189*, could be synthesized successfully, the fact that one of the steps gave a low yield after a difficult chromatographic separation, prompted us to look at alternative pathways. One immediate possibility was to transesterify alcohol *188* completely to the hemi-acetal-acetate *191*. Theoretically, *191* could then be transformed into the required enol (for example, *199*, fig. LXI, p. 107) by deacetylation, selective tosylation of the



191 *197* *198* *199*

Fig. LXI

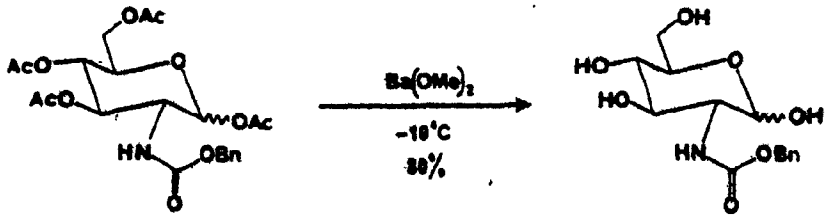
primary hydroxyl groups and finally periodate cleavage of the carbohydrate moiety.

Attempts to obtain an increased amount of the hemi-acetal acetate *191* from the $NaBH_4$-Alox reduction of aldehyde *187* (fig. LVII, p.100) always led to substantial amounts of more polar products when the reaction was forced towards completion. A

different approach was, therefore, investigated. A dilute dichloromethane solution of primary alcohol *188* was stirred at room temperature with a selection of nitrogen bases. With imidazole, the reaction never seemed to produce more than 50% product, even after refluxing for three hours. Triethylamine gave better results but also produced a considerable quantity of polar material. 1,8-Diazabicyclo[5,4,0]undec-7-ene (DBU) destroyed the alcohol completely. 4-*N*,*N*-Dimethylaminopyridine (DMAP) gave the best results, 76% of recrystallized *191* after stirring twenty-four hours at room temperature. Interestingly, when this transesterification with DMAP was tried using THF as solvent, the reaction was much slower and produced more polar material. This may reflect a difference in the conformation of *191* when dissolved in ether solvents than when dissolved in chlorinated hydrocarbons, making intermolecular attack more favourable.

Before this synthesis could be further extended, it was necessary to deacetylate one of the intermediates. Traditional methods[145] for deacetylation of carbohydrates include treatmeth with methanolic ammonia and catalytic amounts of barium or sodium methoxide in methanol. A potential condidate for deacetylation was the mesylate *189* (p. 100). Mesylates have been known to survive sodium methoxide catalyzed deacetylation[175], but it was also important to know if the β-lactam would survive under these conditions. In order to simplify

matters, studies were carried out using styryl β-lactam *180* (p. 90) and, principally, hemi-acetal-acetate *191*. Treatment of *191* with ammonia, even at -10°C, produced a mixture of polar compounds with no β-lactam absorption in the infrared spectrum. Deacetylation catalyzed by barium methoxide[176], was first tried on 1,3,4,6-tetra-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-D-glucopyranose (*200*). The product, *N*-Cbz-D-glucosamine (*201*) was produced in about 80% yield after two days,



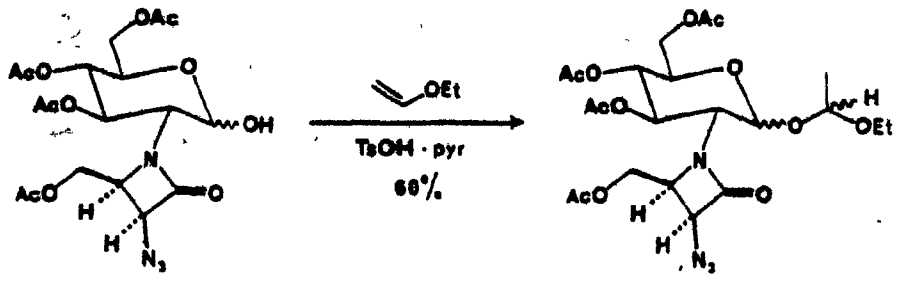
200 *201*

Fig. LXII

by gradual crystallization from the reaction medium at -10°C. This crystallization process seems to be essential to prevent attack on the newly formed hemi-acetal (aldehyde) at the anomeric center. Neither *191*, nor the styryl β-lactam *180*, behaved similarly towards barium or sodium methoxide. In particular, *180* was completely decomposed by this process. A milder method, lithium hydroxide in THF/water (3:1)[177, 146], was tried

on *191* but this did not lead to one distinct product either. There are several factors involved in this deacetylation. Under catalytic conditions, the acetates may be cleaved faster than the β-lactam, but the length of time required for complete deacetylation is long enough for the azetidinone to be cleaved as well. Moreover, it is possible that intramolecular attack on the azetidinone by one of the newly formed hydroxyl groups can occur.

One process which could be controlled during the deacetylation of *191* was attack on the anomeric center. The ethoxyethyl protecting group can be introduced and removed under very mild conditions[178]. Accordingly, hemi-acetal-acetate *191* was treated with ethyl vinyl ether and a catalytic amount of pyridinium tosylate to give a 60% yield of the ethoxyethyl glycoside *202* as a gum incorporating at least four stereoisomers.



191 *202*

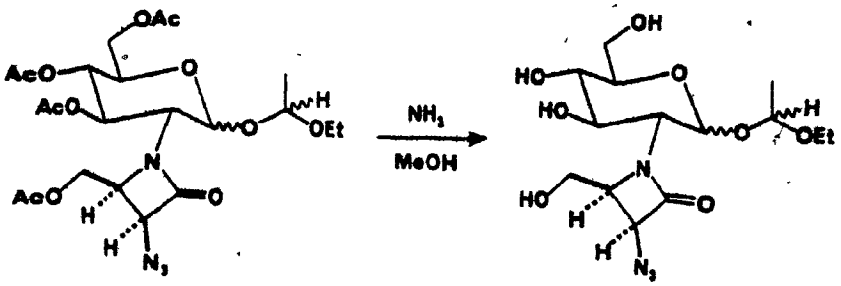
Fig. LXIII

The reaction of *202*, instead of taking two hours as reported for a furanose[178], was still incomplete after four days. Fifteen per cent of the starting material was recovered untouched.

It was immediately obvious, on reaction of *202*, that one of the problems associated with deacetylation had been solved. All of the deacetylation methods attempted on *202* gave a major product which was slightly more polar (t.l.c.) than *N*-Cbz-D-glucosamine (*201*, p. 109). Treatment with a catalytic amount of barium methoxide, or four equivalents of potassium cyanide in 95% ethanol at room temperature[179], however, decomposed this product almost as fast as it formed. The milder conditions of potassium carbonate (0.5M) in THF, under which the benzyl ester of a monocyclic β-lactam had been cleaved successfully[180], were also evaluated. This reaction proved to be very slow with a considerable amount of starting material still remaining after five days. The product also seemed to decompose in the reaction medium, although at a slower rate than in the above two deacetylation procedures.

The method which gave the least decomposition involved treatment with 50% methanolic ammonia. On a small scale and after two days at room temperature, the starting material had completely disappeared. Some spots on the t.l.c. plates, possibly corresponding to incompletely deacetylated compounds, were still present. Preparative thin layer chromatography allowed the isolation, in a somewhat impure state, of the major

product in a maximum yield of 50%. The infrared spectrum showed azide (2110 cm^{-1}), β-lactam (1760 cm^{-1}) and very strong hydroxyl (3400 cm^{-1}) absorptions plus some acetate (1740 cm^{-1}) bands. The 60MHz ^{1}H.m.r. spectrum was not of much help except to show that the ethoxyethyl protecting group was still present and that most, but not all, of the acetate absorptions had disappeared. This provided circumstantial evidence that most of the impure material isolated was the desired polyol *203*.



202 *203*

Fig. LXIV

At this point we took stock of the difficulties which had been accumulating as our work progressed. The problem of intramolecular transacetylation could be resolved in a reasonably satisfactory manner. But the necessity for protection, and thus eventual deprotection, of the anomeric hydroxyl, and the tendency for the β-lactam to react under the conditions

required for complete deacetylation, convinced us that, if D-glucosamine was to be the starting material, protecting groups other than acetates should be chosen. At least the C-3 and C-4 hydroxyls should be protected by groups removable under neutral or mildly acidic conditions in order to study the key step of 3,4-diol cleavage of the carbohydrate ring. A cyclic acetal, such as an isopropylidene, would be compatible with these conditions. Some very recent work by Ojima and his coworkers[135,181] indicates that such a protecting group would indeed be advantageous but for quite another reason.

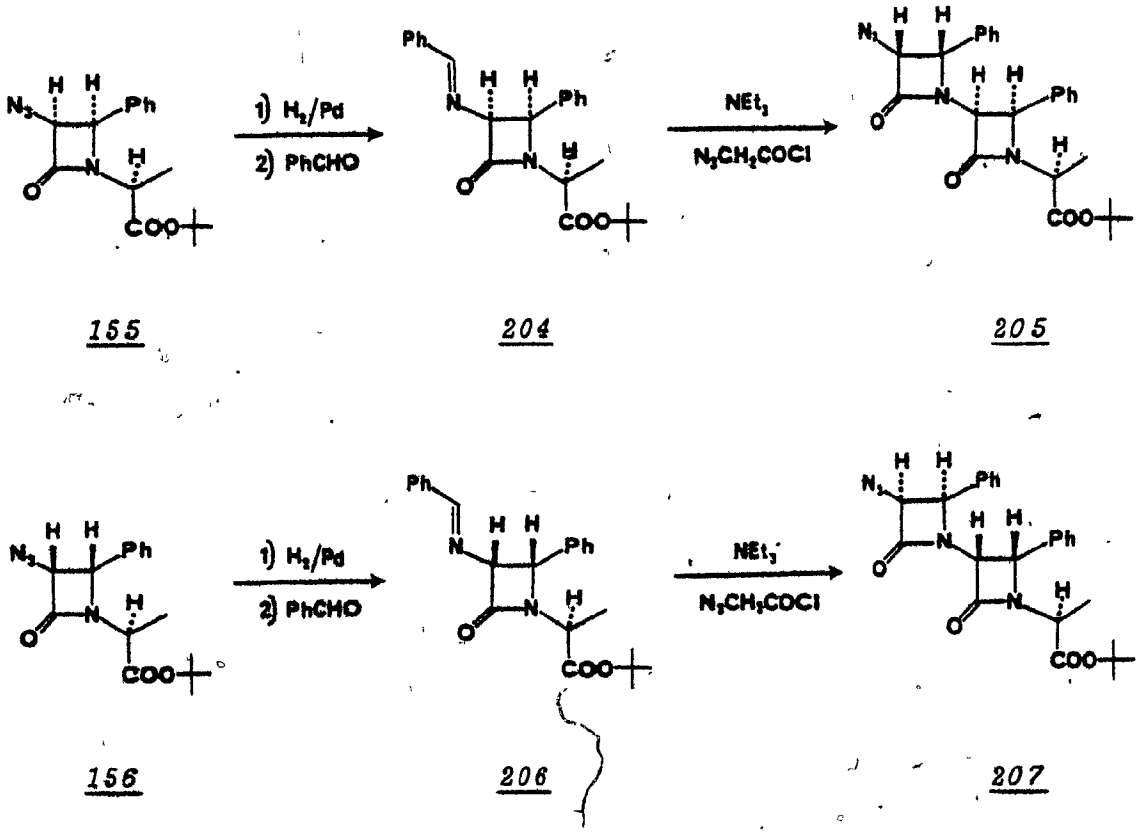


Fig. LXV

In the course of their work on the use of β-lactams as oligopeptide precurors, Ojima *et al* had occasion to synthesize the bis-β-lactams *205* and *207*[135] (fig. LXV, p. 113). They transformed the separated monocyclic β-lactams *155* and *156*, prepared from *tert*-butyl *N*-benzylidene-L-alaninate (*154*, p.) into the 3-*N*-benzylideneamino-2-azetidinones *204* and *206*, which were then reacted with azidoacetyl chloride. In each case, the bis-azetidinone produced consisted of only one diastereomer (*205* and *207* respectively). In effect, a rigid cyclic system (a monocyclic β-lactam) served as a chiral template to achieve 100% asymmetric induction. This suggests that a cyclic acetal protecting group on D-glucosamine, a more rigid system than the tetra-acetylated derivative used in this study, might also lead to 100% asymmetric induction.

In this context, it might be worthwhile investigating the possible use of pentose synthons since they contain one hydroxyl group less to protect and deprotect. Among the most readily available pentoses, three have the correct stereochemistry at C-3, D-xylose, L-arabinose and D-lyxose. Although the 2-amino-pentoses would have to be synthesized, the additional steps at the beginning may still be worth the trouble as long as only one diastereomer is obtained.

Contributions to Knowledge

1) This work represents the first asymmetric synthesis of a bioactive bicyclic β-lactam antibiotic.

2) It has been established that asymmetric induction occurs during *cis*, monocyclic β-lactam formation, generating two chiral centers simultaneously.

3) The stereochemistry of the major β-lactam diastereomer produced by cycloaddition with azidoacetyl chloride was determined.

4) Some of the factors affecting the stereochemical outcome of the cycloaddition were studied.

5) It has been demonstrated that racemization of the carbon *alpha* to the nitrogen in the starting imine does not occur during cycloaddition.

6) The potential of amino sugars, in particular of D-glucosamine derivatives, as chiral templates for this cycloaddition was illustrated.

General Experimental

The azidoacetic acid used to prepare azidoacetic chloride was generously supplied by Bristol Laboratories of Canada. $NaBH_4$-Alox[93] was prepared using neutral alumina, Brockman activity 1 from Fisher Scientific Ltd, Montreal.

Reagent grade solvents were used throughout unless otherwise specified. Dry tetrahydrofuran (THF) was achieved by refluxing in the presence of sodium and benzophenone, and dry methanol (MeOH) by refluxing over magnesium. Other dry solvents were obtained either by standing over molecular sieves[182] (acetonitrile, benzene, dichloromethane, *N*,*N*-dimethylformamide, hexanes, hexamethylphosphoric triamide and pyridine) or commercially (anhydrous ether from Mallinckrodt Chemicals, Montreal, and ethanol from Consolidated Alcohols, Toronto). Drying of organic solutions during work-up was accomplished over anhydrous magnesium sulfate unless otherwise stated.

Solvent evaporation was carried out under reduced pressure (water aspirator) with a bath temperature of 20° to 30°C.

Ozone was generated by a Welsbach Ozonator.

Analytical thin layer chromatography was carried out on aluminum-backed sheets, precoated with Kieselgel $60F_{254}$, 0.2 mm thick (Merck Co. Ltd., Darmstadt). Column chromatography was performed by the "flash chromatography" technique as described by Still *et al*[86] on 32 to 63μ (400 to 230 mesh) silica gel

(British Drug Houses, Toronto). Semi-preparative, high pressure liquid chromatography separations were accomplished on a column (150 × 10 mm) packed with Spherisorb S-10W (Technical Marketing Associates, Montreal) fitted to either an Altex 300 Liquid Chromatograph with a preparative UV detector, or a Waters Associates model 440 chromatograph with both UV and refractive index detectors.

Melting points (m.p.) were determined in open capillary tubes on a Büchi SMP-20 and are uncorrected. Optical rotations were obtained on Perkin-Elmer 110 polarimeters (sodium vapour lamp, D line) in spectrograde solvents, infrared (i.r.) spectra on a Perkin-Elmer 297 spectrophotometer, and mass spectra (m.s.) on HP 5984 or LKB 9000 mass spectrometers. Proton magnetic resonance (^{1}H.m.r.) spectra were recorded on Varian T-60, T-60A or XL-200 spectrometers, using tetramethylsilane (TMS) as internal standard. Chemical shifts are reported on the δ scale in parts per million (ppm).

In the ^{1}H.m.r. spectra, all apparently simple multiplets are described as they appeared, and are given chemical shifts equal to their center position. For more complex coupling patterns (AB, ABX, etc.), each individual peak is listed, while undefined multiplets (m) are described by their range of absorption.

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

EXPERIMENTAL

CHAPTER 1

Benzyl D-Threonate hydrochloride [(+)-*74*][69, 63]

Benzyl bromide (1.3 ml, 1.9 g, 11 mmol) in 5 ml benzene was added dropwise to a mixture of BOC-D-Thr[64, 183] (*77*, 2.2 g, 10 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU, 1.6 ml, 1.6 g, 11 mmol) in 45 ml dry benzene. The mixture was refluxed for 3 hours, cooled and diluted with ether (200 ml). After successive washings with water, 5% citric acid, sat. $NaHCO_3$ and brine, the solvent was dried and evaporated to give 3.0 g of a pale yellow oil. Flash chromatography using 2000 ml of 27% EtOAc/hexanes yielded 2.8 g of BOC-D-Thr-OBn (*78*) (90% yield) as a colourless oil (lit.[64]: m.p. 40° - 41°C): $[\alpha]_D^{23}$ +12° (c 3, $CHCl_3$) and +19° (c 8, MeOH) {lit. for BOC-L-Thr-OBn[64]: $[\alpha]_D$ -19.6° (c 1, MeOH)}; i.r. (film) ν_{max}: 3440 (OH and NH), 1740 (ester), 1720, 1690, 1500 (urethane) and 1390, 1365 (*tert*-butyl) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 1.22 (d, 3H, $J_{\beta,\gamma}$ = 6.5Hz, $CHCH_3$), 1.44 (s, 9H, OBu^t), 2.93 (bd, 1H, $J_{O,\beta}$ = 5Hz, exchangeable, *OH*), 4.1 - 4.6 (m, 2H, N*CHCH*Me), 5.18 (s, 2H, OCH_2Ph), 5.52 (bd, 1H, $J_{N,\alpha}$ = 9Hz, exchangeable, *NH*) and 7.33 (s, 5H, *Ph*) ppm; m.s. (EI, 70eV, 65°C) m/e: 265 (0.3, $M^{+\cdot}$-44), 91 (79) and 57 (100).

BOC-D-Thr-OBn (*78*, 2.8 g, 9.1 mmol) was dissolved in 10 ml of ether (anh.) and added to 10 ml of ether (anh.) saturated with hydrogen chloride gas. Within 1 min. a white precipitate began to form. The mixture was left at 25°C overnight, then filtered to give 1.9 g (75% from BOC-D-Thr) of (+)-D-Thr-OBn.HCl (*74*). Recrystallization from acetonitrile/THF gave white needles: m.p. 126.5° - 127.5°C (lit. for L-Thr-OBn.HCl[63]: 125° - 126°C); $[\alpha]_D^{23}$ +10° (c 2, EtOH) {lit. for L-Thr-OBn.HCl[63]: $[\alpha]_D$ -11.1° (c 1, EtOH)}; i.r. (KBr) ν_{max}: 3380 (hydroxyl), 3200 - 2500, including 3040, 2850, 2580 ($^+NH_3$) and 1740 (ester) cm^{-1}; ^{1}H.m.r. (DMSO-d_6) δ: 1.26 (d, 3H, $J_{\beta,\gamma}$ = 6Hz, $CHCH_3$), 3.9 - 4.4 (m, 2H, $NCHCH$Me), 5.23 (s, 2H, OCH_2Ph), 5.78 (bs, *OH*), 7.3 - 7.5 (m, 5H, includes s at 7.40, *Ph*) and 8.61 (bs, $^+NH_3$) ppm.

Benzyl *O-tert*-Butyldimethylsilyl-D-threonate [(+)-*82*][70]

Method A

Benzyl D-threonate hydrochloride (*74*, 0.25 g, 1.0 mmol) dissolved in 0.5 ml of dry HMPT was added to a mixture of imidazole (0.26 g, 3.8 mmol) and *tert*-butyldimethylsilyl chloride (0.28 g, 1.9 mmol) in 0.5 ml of dry HMPT under nitrogen. After stirring overnight at 25°C, the mixture was poured into water (30 ml) and extracted with hexanes (3 × 50 ml). The combined organic phases were washed with brine (5 × 30 ml), dried and evaporated to give 0.32g (quant.) of pale yellow oil which was

in future used without further purification. However, for the purposes of characterization, the oil was, in this case, purified by chromatography using 50% EtOAc/p.e. to give 0.29 g (90%) (+)-*82* as a colourless oil; $[\alpha]_D^{23}$ +12° (c 8, $CHCl_3$); i.r. (film) ν_{max}: 3400 and 3330 (NH_2), 1745 (ester), 1600 (amine) and 1260, 840 (silyl ether) cm^{-1}; ^{1}H.m.r. (CCl_4) δ: -0.04, 0.04 (2s, 6H, Si*Me*$_2$), 0.84 (s, 9H, Si*Bu*^{t}), 1.21 (d, 3H, $J_{\beta,\gamma}$ = 6Hz, CH*CH*$_3$), 1.42 (bs, 2H, exchangeable, *NH*$_2$), 3.15 (d, 1H, $J_{\alpha,\beta}$ = 3Hz, NH$_2$*CH*(COOR)CH), 4.21 (d × q, 1H, $J_{\alpha,\beta}$ = 3Hz, $J_{\beta,\gamma}$ = 6Hz, CH*CH*(OSi)Me), 4.79, 4.99, 5.02, 5.22 (ABq, 2H, J_{AB} = 12Hz, O*CH*$_2$Ph) and 7.22 (s, 5H, *Ph*) ppm; m.s. (EI, 70eV, 20°C) m/e: 324 (0.3, $M^{+\cdot}$ + 1), 266 (14), 159 (36), 91 (100) and 73 (56).

Method B

This method differs from A in the use of 1.3 mmol of *tert*-butyldimethylsilyl chloride in DMF which had been dried over 4A molecular sieves for about 4 hours. Work-up gave 0.31 g yellow oil which contained two major components (t.l.c. $CHCl_3$). Chromatography with 40% EtOAc/p.e. gave 0.12 g (34%) of *N*-formyl ester *83* and 0.15 g (45%) of the desired *82*.

Repetition of this reaction using 1.8 mmol of *tert*-butyldimethylsilyl chloride in DMF dried over 4A molecular sieves for 18 hours yielded 64% of *83* and 30% of *82*. *N*-formyl ester *83* (p. 32): $[\alpha]_D^{23}$ -3° (c 6, $CHCl_3$); i.r. (film) ν_{max}: 3440 and 3340 (NH), 1750 (ester) and 1690, 1500 (amide) cm^{-1}:

^{1}H.m.r. ($CDCl_3$) δ: -0.03, 0.03 (2s, 6H, $SiMe_2$), 0.84 (s, 9H, $SiBu^t$), 1.15 (d, 3H, $J_{\beta,\gamma}$ = 6.5Hz, $CHCH_3$), 4.28 - 4.78 [m, 2H, including 4.45 (poss. d × q, $J_{\alpha,\beta}$ = 2Hz, $J_{\beta,\gamma}$ = 6.5Hz, $CHCH$(OSi)Me) and 4.68 (d × q, $J_{\alpha,\beta}$ = 2Hz, $J_{N,\alpha}$ = 9Hz, becomes bs on exchange, $NHCH$(COOR)CH)], 4.89, 5.09, 5.11, 5.31 (ABq, 2H, J_{AB} = 12 Hz, OCH_2Ph), 6.65 (d, 1H, $J_{N,\alpha}$ = 9Hz, exchangeable, *NH*), 7.33 (s, 5H, *Ph*) and 8.26 (bs, 1H, *CHO*) ppm; m.s. (EI, 70eV, 25°C) m/e: 352 (0.2, $M^{+\cdot}$ + 1), 307 (4, $M^{+\cdot}$ - 44), 294 (38), 159 (75) and 73 (100).

Benzyl *O-tert*-Butyldimethylsilyl-*N*-cinnamylidene-D-threonate (*88*)[50]

The silylated amino-ester (+)-*82* (3.2 g, 10 mmol) and distilled *trans*-cinnamaldehyde (1.4 ml, 1.4 g, 11 mmol) were dissolved in 30 ml of dry CH_2Cl_2. Using a modified Dean-Stark apparatus filled with 3A molecular sieves, the mixture was refluxed for 1 hour, then stirred in the presence of $MgSO_4$ (anh.) at 25°C for another hour. Filtration and evaporation of the solvent gave Schiff base *88* as a yellow oil: i.r. (CH_2Cl_2) ν_{max}: 1745 (ester), 1680 (excess aldehyde), 1635 (α,β-unsaturated imine) and 1615 (sh, conjugated C=C) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: -0.03, 0.03 (2s, 6H, $SiMe_2$), 0.81 (s, 9H, $SiBu^t$), 1.15 (d, 3H, $J_{\beta,\gamma}$ = 6.5Hz, $CHCH_3$), 3.76 (d, 1H, $J_{\alpha,\beta}$ = 8Hz, C=NCH(COOR)CH), 4.21 (d × q, 1H, $J_{\alpha,\beta}$ = 8Hz, $J_{\beta,\gamma}$ = 6.5Hz, $CHCH$(OSi)Me), 4.99, 5.19, 5.21, 5.41 (ABq, 2H, J_{AB} = 12Hz, OCH_2Ph), 6.96 (app. d, 2H,

J = 4.5Hz, CH*CH*=*CH*Ph), 7.3 - 7.6 (m, 10H, including s at 7.33, 2× *Ph*) and 7.95 (app. t, 1H, J = 4.5Hz, N=*CH*CH=CH) ppm.

β-Lactams (-)-*89* and (+)-*90*[50,51]

Schiff base *88* (10 mmol) was dissolved in 40 ml of dry CH_2Cl_2 and cooled under nitrogen in an ice-salt bath (-15°C). Distilled NEt_3 (1.8 ml, 1.3 g, 13 mmol) was added, followed by the dropwise addition, over 60 minutes, of a solution of azidoacetyl chloride[131] (1.1 ml, 1.4 g, 12 mmol) in 10 ml of dry CH_2Cl_2. Stirring was continued at ice-salt bath temperature (-15° to -10°C) another 0.5 hr. The mixture was diluted with hexanes (100 ml), filtered, then washed with brine, 5% HCl (2 × 50 ml), sat. $NaHSO_3$, sat. $NaHCO_3$ and once more with brine. Drying and evaporation gave 4.8 g of orange oil which contained two major components as determined by t.l.c. (25% EtOAc/p.e.).

This crude oil was divided into two portions. Flash chromatography of each portion, and then of the resulting mixed fractions using 1000 ml of 20% EtOAc/p.e., followed by 1000 ml of 25% EtOAc/p.e., yielded 3.3 g (63% from *74*) of mixed β-lactams *89* and *90* and 1.1 g (27%) of amide *91*. Analysis of the mixed β-lactams by HPLC (Waters 440, micro-silica analytical column, MeOH/EtOAc/hexanes (0.1:5:95), 3.0 ml/min) showed a ratio of major, less polar β-lactam (-)-*89* to minor, more polar β-lactam (+)-*90* of 88:12, with retention times of 5.5 and 6.5 min. respectively. Separation on a semi-preparative scale

(Altex, 23 × 25 p.s.i., ≈ 11 ml/min.) gave the following two diastereomers with retention times of 4.4 and 5.6 min. respectively: major β-lactam (-)-*89*: $[\alpha]_D^{23}$ -150° (c 2.5, hexane) and -130° (c 3, $CHCl_3$); i.r. (film) ν_{max}: 2100 (azide), 1775 (β-lactam), 1740 (ester) and 1650 (ArC=C) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: -0.11, -0.04 (2s, 6H, Si*Me*$_2$), 0.76 (s, 9H, Si*Bu*^{t}), 1.25 (d, 3H, $J_{\beta,\gamma}$ = 6.3Hz, CH*CH*$_3$), 4.42 (d, 1H, $J_{\alpha,\beta}$ = 3.9Hz, N*CH*(COOR)CH), 4.52 (d × q, 1H, $J_{\alpha,\beta}$ = 3.9Hz, $J_{\beta,\gamma}$ = 6.3Hz, CH*CH*(OSi)Me), 4.84, 4.87 (app. d, Y of a poss. ABXY pattern, 1H, J_{XY} = 5.3Hz, J_{BY} < 1Hz, H-3), 4.89, 4.92, 4.93, 4.96 (app. d × d, X of ABXY, 1H, J_{BX} = 8.8Hz, J_{XY} = 5.3Hz, H-4), 5.02, 5.08, 5.24, 5.30 (ABq, 2H, J_{AB} = 12Hz, O*CH*$_2$Ph), 6.17, 6.21, 6.25, 6.29 (app. d × d, B of ABXY, 1H, J_{AB} = 16Hz, J_{BX} = 8.8Hz, CH*CH*=CH), 6.56, 6.64 (app. d, A of ABXY, J_{AB} = 16Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.25 - 7.45 (m, 10H, 2× *Ph*) ppm; m.s. (EI, 16eV, 87°C) m/e: 492 (5, $M^{+\cdot}$ - 28), 438 (2), 437 (11), 436 (28), 435 (89) and 102 (100); Anal. calcd. for $C_{28}H_{36}N_4O_4Si$: C 64.59, H 6.97, N 10,76; found: C 64.37, H 7.07, N 10.65;

minor β-lactam (+)-*90*: $[\alpha]_D^{23}$ +80° (c 1.5, hexane) and +40° (c 2, $CHCl_3$); i.r. (film) ν_{max}: 2100 (azide), 1770 (β-lactam), 1750 (b sh., ester) and 1650 (ArC=C) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: -0.07, 0.07 (2s, 6H, Si*Me*$_2$), 0.85 (s, 9H, Si*Bu*^{t}), 1.21 (d, 3H, $J_{\beta,\gamma}$ = 6.4Hz, CH*CH*$_3$), 4.44 (d, 1H, $J_{\alpha,\beta}$ = 3.4Hz, N*CH*(COOR)CH), 4.62 (d × q, 1H, $J_{\alpha,\beta}$ = 3.4Hz, $J_{\beta,\gamma}$ = 6.4Hz, CH*CH*(OSi)Me), 4.77, 4.79, 4.81, 4.83 (app. d × d, X of a poss. ABXY pattern, 1H, J_{BX} = 8.8Hz, J_{XY} = 5.2Hz, H-4), 4.88, 4.91

(app. d, Y of ABXY, 1H, J_{XY} = 5.2Hz, J_{BY} < 1Hz, H-3), 5.07 (s, 2H, OCH_2Ph), 6.25, 6.30, 6.33, 6.38 (app. d × d, B of ABXY, 1H, J_{AB} = 16Hz, J_{BX} = 8.8Hz, CH*CH*=CH), 6.54, 6.62 (app. d, A of ABXY, 1H, J_{AB} = 16Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.2 - 7.6 (m, 10H, 2× *Ph*) ppm; m.s. (EI, 16eV, 75°C) m/e: 492 (5, $M^{+\cdot}$ - 28), 438 (2), 437 (8), 436 (25), 435 (76) and 91 (100);

Amide (-)-*91* was further purified by chromatography with 25% EtOAc/hexanes to give a colourless oil: $[\alpha]_D^{23}$ -12° (c 2.3, $CHCl_3$); i.r. (film) ν_{max}: 3420 and 3320 (NH), 2110 (azide), 1750 (ester) and 1690 and 1520 (amide) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: -0.03, 0.05 (2s, 6H, $SiMe_2$), 0.87 (s, 9H, $SiBu^t$), 1.19 (d, 3H, $J_{\beta,\gamma}$ = 6.5Hz $CHCH_3$), 4.03 (s, 2H, N_3CH_2CO), 4.3 - 4.7 (m, 2H, NH*CHCH*Me), 4.96, 5.16, 5.17, 5.37 (ABq, 2H, J_{AB} = 12Hz, OCH_2Ph), 6.99 (bd, 1H, $J_{N,\alpha}$ = 9Hz, exchangeable, *NH*) and 7.35 (s, 5H, *Ph*) ppm; m.s. (EI, 70eV, 69°C) m/e: 363 (0.2, $M^{+\cdot}$ - 15 - 28), 349 (2, $M^{+\cdot}$ - 57), 159 (20) and 91 (100); Anal. calcd. for $C_{19}H_{30}N_4O_4Si$: C 56.13, H 7.44, N 13.78; found: C 56.18, H 7.68, N 14.00.

Aldehyde *92* and α,β-Unsaturated Ester *94*

Ozone (∿7 mmol/hr.) was bubbled for 20 min. through a solution of mixed β-lactams *89* + *90* (0.52 g, 1.0 mmol) in 50 ml of dry CH_2Cl_2, previously cooled under nitrogen to -70°C. Excess O_3 was removed by bubbling nitrogen for 5 min. Dimethyl sulfide (1 ml) was then added, the mixture warmed to R.T. (about

2 hr.), concentrated, taken up in p.e. (50 ml) and washed with brine (5 × 20 ml). Drying and solvent evaporation gave 0.56 g of reddish oil the ^{1}H.m.r. spectrum ($CDCl_3$) of which contained no olefinic protons (δ = 6 - 7 ppm) and indicated the presence of benzaldehyde, plus the desired aldehyde *92*, δ = 9.6 ppm (d, 1H, J = 4Hz).

Because this aldehyde was presumed to be unstable[51,53], it was immediately treated with $NaBH_4$ (0.2 g, 0.6 mmol) in dry THF (10 ml) at 0°C for 1.5 hr. This solution was acidified with 5% acetic acid, diluted with 50% EtOAc/p.e. and washed with sat. $NaHCO_3$ and brine. Drying and solvent evaporation yielded 0.45 g of red oil. Chromatography (900 ml of 30% EtOAc/p.e. followed by 300 ml of 50% EtOAc/p.e.) yielded 0.13 g (28%) of primary alcohol *93* (see next procedure p. 126) and 0.10 g (32%) of the α,β-unsaturated ester *94* which was further purified by preparative t.l.c. with 40% EtOAc/p.e.: i.r. (film) ν_{max}: 3460 (OH), 2110 (azide), 1770 (β-lactam), 1720 (α,β-unsat. ester) and 1650 (C=C) cm^{-1}; ^{1}H.m.r. (CCl_4) δ: 1.83 (d, 3H, $J_{\beta,\gamma}$ = 7Hz, C=C*CH*$_3$), 2.93 (bs, 1H, exchangeable, *OH*), 3.68 (bd, 2H, $J_{4,5}$ = 5Hz, CH*CH*$_2$OH), 4.28 (app. q, 1H, $J_{3,4} = J_{4,5}$ = 5Hz, H-4), 4.69 (d, 1H, $J_{3,4}$ = 5Hz, H-3), 5.11 (s, 2H, O*CH*$_2$Ph), 6.83 (q, 1H, $J_{\beta,\gamma}$ = 7Hz, C=*CH*Me) and 7.27 (s, 5H, *Ph*) ppm.

Primary Alcohol (-)-*93*[91,93]

A solution of the major β-lactam (-)-*89* (3.0 g, 5.7 mmol) in 5:1 CH_2Cl_2/MeOH (60 ml) was cooled under nitrogen in a dry-ice/acetone bath (-70°C). Ozone (~36 mmol/hr.) was bubbled through the solution until the characteristic blue colour appeared (25 min.), and the excess removed by bubbling nitrogen for 5 min. $NaBH_4$-Alox[93] (4.0 g) was added and the suspension allowed to warm to R.T. under nitrogen with stirring (about 2.5 hr.), when it was poured into pH = 4 buffer (25 ml) and separated. The organic phase was washed twice more with buffer, filtered, then concentrated and the resulting yellow oil taken up in p.e. (100 ml) and extracted with water (10 × 20 ml) to remove the benzyl alcohol. Drying, solvent evaporation and flash chromatography (33% EtOAc/hexanes) yielded 2.3 g (90%) of (-)-*93* as a colourless oil: $[\alpha]_D^{23}$ -158° (c 4, $CHCl_3$); i.r. (film) ν_{max}: 3460 (hydroxyl), 2115 (azide), 1770 (β-lactam) and 1740 (ester); ^{1}H.m.r. ($CDCl_3$) δ: 0.02, 0.08 (2s, 6H, $SiMe_2$), 0.85 (s, 9H, $SiBu^t$), 1.30 (d, 3H, $J_{\beta,\gamma}$ = 6Hz, $CHCH_3$), 2.90 (t, 1H, $J_{5,6}$ = 7Hz, exchangeable, *OH*), 3.93 (d × d, 2H, $J_{4,5}$ = 4Hz, $J_{5,6}$ = 7Hz, becomes bd, $J_{4,5}$ = 4Hz, on exchange, $CHCH_2OH$), 4.2 - 4.6 (m, 3H, N*CHCH*Me and H-4), 4.73 (d, 1H, $J_{3,4}$ = 5Hz, H-3), 4.85, 5.05, 5.15, 5.35 (ABq, 2H, J_{AB} = 12Hz, OCH_2Ph) and 7.32 (s, 5H, *Ph*) ppm; m.s. (CI/IB, 58°C) m/e: 449 (100, $(M + 1)^+$), 421 (30), 391 (40), 357 (41) and 109 (75); Anal. calcd. for $C_{21}H_{32}N_4O_5Si$: C 56.23, H 7.19, N 12.49; found: C 56.96,

H 6.94, N 12.52.

Mesylate (-)-*96*[94]

To a cooled (-15°C) solution of primary alcohol (-)-*93* (2.0 g, 4.5 mmol) and NEt_3 (0.8 ml, 0.6 g, 6 mmol) in dry CH_2Cl_2 (20 ml) under nitrogen, methanesulfonyl chloride (0.42 ml, 0.62 g, 5.4 mmol) was added dropwise over 15 min. The solution was stirred at -15° to -10°C for 3 hr. when t.l.c. (25% EtOAc/p.e.) showed no more starting material. Dilution with hexanes (100 ml), washing with sat. $NaHCO_3$, 5% HCl and brine (twice), drying and evaporation of solvent gave 2.3 g of (-)-*96* as a pale yellow oil (95% crude). For characterization purposes, a small portion was further purified by chromatography with 33% EtOAc/p.e.: $[\alpha]_D^{23}$ -113° (c 2.6, $CHCl_3$); i.r. (film) ν_{max}: 2110 (azide), 1780 (β-lactam), 1740 (ester) and 1360, 1175 (sulfonate) cm^{-1}; ^1H.m.r. ($CDCl_3$) δ: 0.03, 0.10 (2s, 6H, $SiMe_2$), 0.87 (s, 9H, $SiBu^t$), 1.20 (d, 3H, $J_{\beta,\gamma}$ = 6Hz, $CHCH_3$), 3.03 (s, 3H, OSO_2CH_3), 4.2 - 5.37 [m, 8H, including 4.87, 5.07, 5.17, 5.37 (ABq, J_{AB} = 12Hz, OCH_2Ph), $NCHCHMe$, H-3, H-4, H-5 and OCH_2Ph] and 7.32 (s, 5H, *Ph*) ppm; m.s. (EI, 15eV, 70°C) m/e: 511 (0.2, $M^{+\cdot}$ - 15), 469 (8), 441 (4), 159 (29) and 91 (100); CI/IB, 25° to 110°C) m/e: 527 (31, $(M + 1)^+$) and 403 (100); Anal. calcd. for $C_{22}H_{34}N_4O_7SSi$: C 50.17, H 6.51, N 10.64; found: C 49.71, H 6.46, N 10.35.

Secondary Alcohol (-)-*97*

Mesylate (-)-*96* (2.0 g, 3.8 mmol) was dissolved in 4.0 ml of 95% trifluoracetic acid and stirred at 25°C for 45 min. when 5:1 ether/CH_2Cl_2 (30 ml) and ice were added. The aqueous layer was washed once with the above solvent, and the combined organic phases washed carefully with cold 4% $NaHCO_3$ until the washes remained basic, then washed once more with brine, dried and evaporated yielding 1.4 g (90% crude) of an off-white solid. Crystallization from CH_2Cl_2/CCl_4 gave 1.3 g (82%) of (-)-*97* as a white crystalline solid: m.p. 99.5° - 100.5°C; $[\alpha]_D^{23}$ -112° (c 2, $CHCl_3$); i.r. (CH_2Cl_2) ν_{max}: 3600, 3400 (OH), 2110 (azide), 1760 (β-lactam), 1740 (ester) and 1360, 1175 (sulfonate) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 1.22 (d, 3H, $J_{\beta,\gamma}$ = 6.5Hz, $CHCH_3$), 2.92 (s, 3H, OSO_2CH_3), 3.38 (bd, 1H, $J_{O,\beta}$ = 7Hz, exchangeable, *OH*), 4.1 - 4.6 (m, 5H, N*CHCH*Me, H-3, H-4), 4.87 (d, 1H, $J_{3,4}$ = 4.5Hz, H-3), 5.19 (s, 2H, OCH_2Ph) and 7.32 (s, 5H, *Ph*) ppm; m.s. (EI, 16eV, 72°C) m/e: 368 (1, $M^{+\cdot}$ - 44), 288 (2), 149 (13) and 91 (100); (CI/IB, 35° - 133°C) m/e: 289 (56) and 220 (100); Anal. calcd. for $C_{16}H_{20}N_4O_7S$: C 46.60, H 4.89, N 13.58; found: C 46.71, H 4.50, N 13.07.

α,β-Unsaturated Ester *99*

Secondary alcohol (-)-*97* (0.89 g, 0.22 mmol) was added to 2.0 ml of 3:1 DMSO/acetic anhydride (both freshly distilled) and stirred for 16 hours at room temperature. Three products were

detected by t.l.c. (50% EtOAc/p.e.), all less polar than the starting material which was absent. The least polar component was not identified. The most polar component corresponded, on t.l.c., to the product of Jones oxidation, enol *72* (see procedure p. 130) but none of it was recovered after chromatography with 50% EtOAc/p.e. followed by EtOAc and then, MeOH. The major component of intermediate polarity was isolated in 59% yield (50 mg) and identified as the α,β-unsaturated ester *99*: i.r. (film) ν_{max}: 2110 (azide), 1770 (β-lactam), 1720 (α,β-unsaturated ester, 1650 (conjugated olefin) and 1360, 1175 (sulfonate) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 2.00 (d, 3H, $J_{\beta,\gamma}$ = 6.5Hz, C=CH*CH*$_3$), 2.99 (s, 3H, OSO_2CH_3), 4.3 - 4.8 (m, 3H, H-4 and H-5), 4.98 (d, 1H, $J_{3,4}$ = 5Hz, H-3), 5.23 (s, 2H, OCH_2Ph), 6.82 (q, 1H, $J_{\beta,\gamma}$ = 6.5Hz, C=*CH*CH$_3$) and 7.37 (s, 5H, *Ph*) ppm.

Secondary Alcohol (+)-*100*

Amide (−)-*91* (0.21 g, 0.50 mmol) was dissolved in 2 ml of 95% trifluoroacetic acid and stirred at room temperature for 15 min. when t.l.c. (60% EtOAc/p.e.) indicated that the reaction was complete. The mixture was diluted with ether and washed once with brine, three times with sat. $NaHCO_3$ (until extracts remained basic) and once more with brine. The combined aqueous fractions were back-extracted with ether (twice), and the combined ether extracts dried and concentrated *in vacuo* to a beige solid. Recrystallization from CH_2Cl_2/p.e. yielded 0.11 g (75%)

of (+)-*100* as a white crystalline solid: m.p. 99° - 100°C; $[\alpha]_D^{23}$ +5.5° (c 1, $CHCl_3$); i.r. (KBr) ν_{max}: 3450 (H-bonded OH), 3300 (NH), 2100 (azide), 1710 (H-bonded ester) and 1655, 1550 (amide) cm^{-1}: ^{1}H.m.r. ($CDCl_3$) δ: 1.21 (d, 3H, $J_{\beta,\gamma}$ = 6.5Hz, $CHCH_3$), 2.28 (bs, 1H, exchangeable, *OH*), 4.03 (s, 2H, N_3CH_2CO), 4.2 - 4.8 [m, 2H, including 4.40 (poss. d × q, $J_{\alpha,\beta}$ = 3Hz, $J_{\beta,\gamma}$ = 6.5Hz, *CH*CH(OH)Me) and 4.64 (d × d, $J_{N,\alpha}$ = 9Hz, $J_{\alpha,\beta}$ = 3Hz, NH*CH*(COOR)CH], 5.22 (s, 2H, OCH_2Ph), 7.01 (bd, 1H, $J_{N,\alpha}$ = 9H, exchangeable, *NH*) and 7.38 (s, 5H, *Ph*) ppm; m.s. (EI, 15eV, 49°C) m/e: 292 (0.6, $M^{+\cdot}$), 248 (16), 148 (100) and 91 (61).

Enol *72*[51]

Jones reagent[96] (0.21 ml, 0.56 mmol) was added dropwise over 10 min. to a cold (-15°C), stirred suspension of secondary alcohol (-)-*97* (0.21 g, 0.51 mmol) in 3 ml of acetone (spectrograde) and celite (0.2 g). The low temperature bath was then changed for one which was kept at 17° to 19°C throughout the remainder of the reaction. After a total of 45 min., t.l.c. indicated that the reaction was approximately 50% complete. Ether (20 ml) was then added, stirring continued for 10 min. and the ether decanted. This procedure was repeated twice for 5 min. each time and the last extract filtered.

The combined ether fractions were washed with brine. The enol was then extracted into ice-cold 4% Na_2CO_3 (2 × 10ml + 5 ml). (T.l.c. later indicated that all the enol had been ex-

tracted in the first fraction.) Each basic fraction was IMMEDIATELY poured into enough ice-cold 10% HCl (7 ml or 3.5 ml) to neutralize the base, and these combined aqueous fractions were extracted with CH_2Cl_2 (4 × 75 ml). Washing of the combined CH_2Cl_2 fractions with brine, drying and evaporation gave 0.12 g (56%) of *72* as a clear, very pale yellow oil which was always used for the next reaction as soon as possible: i.r. (film) ν_{max}: 3350 (weak, OH), 2110 (azide), 1780 (β-lactam), 1660 (enol form, β-keto-ester) and 1620 (enolic olefin) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 2.08 (s, 2.6H, C=C*CH*$_3$, enol form) and 2.28 (d, 0.4H, J = 2Hz, CHCO*CH*$_3$, keto form), 2.91 (s, 3H, OSO_2*CH*$_3$), 4.1 - 4.5 (m, 3H, H-4, H-5), 4.85 (d, 1H, $J_{3,4}$ = 4.5Hz, H-3), 5.24, 5.26 (2s, 2H, O*CH*$_2$Ph), 7.38 (s, 5H, *Ph*) and 12.3 (bs, < 1H, enol *OH*) ppm.

The combined ether fractions were washed with brine, dried and evaporated to give 75 mg (35% recovery) of the starting alcohol (-)-*97* which could then be recycled.

[6S-(6R*,7R*)]-Benzyl 7β-Azido-3-methyl-Δ^3-*O*-2-isocephem-4-carboxylate [(-)-*73*][51]

Enol *72* (0.12 g, 0.29 mmol) and NEt_3 (50 µl, 0.35 g, 0.35 mmol) in 15 ml dry CH_2Cl_2 were refluxed for 3 hours. After cooling, the reaction mixture was washed with brine (2 × 5 ml), 4% HCl (2 × 5 ml) and brine, dried and evaporated to give 80 mg (89% crude) of the azido-*O*-2-isocephem (-)-*73* (50% from secon-

dary alcohol (-)-*97*). A total amount of 0.26 g (73%) of crude (-)-*73* was eventually obtained from 1.1 mmol of (-)-*97* by recycling the recovered starting material. Purification by chromatography (24% EtOAc/hexanes) gave a clear syrup (0.22 g, 63%): $[\alpha]_D^{23}$ -22° (c 2.4, $CHCl_3$); i.r. (film) ν_{max}: 2110 (azide), 1780 (β-lactam), 1715 (α,β-unsaturated ester) and 1615 (vinyl ether) cm^{-1}: ^{1}H.m.r. (C_6D_6) δ: 2.18 (s, 3H, *CH*$_3$), 2.93 (d × d × d, 1H, $J_{1\alpha,6}$ = 4Hz, $J_{1\beta,6}$ = 9Hz, $J_{6,7}$ = 5Hz, H-6), 3.39 (d × d, 1H, $J_{1\alpha,1\beta}$ = 10Hz, $J_{1\beta,6}$ = 9Hz, H-1β), 3.91 (d × d, 1H, $J_{1\alpha,1\beta}$ = 10Hz, $J_{1\alpha,6}$ = 4Hz, H-1α), 4.30 (d, 1H, $J_{6,7}$ = 5Hz, H-7), the multiplets between 2.78 and 4.34 have the overall appearance of an AMNX pattern, 5.19 (s, 2H, O*CH*$_2$Ph) and 7.1 - 7.5 (m, 5H, *Ph*) ppm; For comparison with Doyle *et al*[51]: ^{1}H.m.r. ($CDCl_3$) δ: 2.23 (s, 3H, *CH*$_3$), 3.4 - 4.2 [m, 2H, including 3.98 (app. t, $J_{1\alpha,1\beta}$ = $J_{1\beta,6}$ = 9Hz, H-1β), H-1β, H-6], 4.56 (d × d, 1H, $J_{1\alpha,1\beta}$ = 9Hz, $J_{1\alpha,6}$ = 3Hz, H-1α), 5.16 (d, 1H, $J_{6,7}$ = 4.5Hz, H-7), the multiplets between 3.38 and 5.20 appear to be an AMXY pattern, 5.28 (s, 2H, O*CH*$_2$Ph) and 7.2 - 7.4 (m, 5H, *Ph*) ppm; m.s. (CI/IB, 70°C), m/e: 315 (100, $(M + 1)^+$) and 289 (50); Anal. calcd. for $C_{15}H_{14}N_4O_4$: C 57.32, H 4.49, N 17,83; found: C 56.85, H 4.54, N 17.61.

[6S-(6R*,7R*)]-Benzyl 7β-Amino-3-methyl-Δ^3-*O*-2-isocephem-4-carboxylate (*107*)[51]

A suspension of azido-*O*-2-isocephem (-)-*73* (0.19 g, 0.60

mmol) and PtO_2 (92 mg) in 32 ml absolute ethanol was hydrogenolysed at atmospheric pressure for 20 min. Filtration through celite and evaporation gave 0.17 g of dark brown resin which was taken up in ether (30 ml) and extracted into cold 10% HCl (3 × 10 ml). The combined aqueous phases were make basic with cold dilute NH_4OH. Re-extraction into CH_2Cl_2 (5 × 50 ml), washing once with brine (10 ml), drying and evaporation yielded 0.13 g (77%) of *107* as a light yellow resin: i.r. ($CHCl_3$) ν_{max}: 3400, 3350 (NH_2), 1770 (β-lactam), 1710 (α,β-unsaturated ester) and 1615 (vinyl ether) cm^{-1}: ^{1}H.m.r. ($CDCl_3$) δ: 1.70 (bs, 2H, exchangeable, *NH*$_2$), 2.27 (s, 3H, *CH*$_3$), 3.4 - 4.7 [poss. ABXY pattern, 4H, including 3.58 (d × d × d, $J_{1\alpha,6}$ = 3.5Hz, $J_{1\beta,6}$ = 10Hz, $J_{6,7}$ = 4.5Hz, H-6), 3.93 (app. t, $J_{1\alpha,1\beta} = J_{1\beta,6}$ = 10Hz, H-1β), 4.45 (d × d, $J_{1\alpha,1\beta}$ = 10Hz, $J_{1\alpha,6}$ = 3.5Hz, H-1α) and 4.69 (d, $J_{6,7}$ = 4.5Hz, H-7)], 5.25 (s, 2H, O*CH*$_2$Ph) and 7.2 - 7.6 (m, 5H, *Ph*) ppm.

[6S-(6R*,7R*)]-Benzyl 3-Methyl-7β-phenylacetamido-Δ^3-*O*-2-isocephem-4-carboxylate [(+)-*108*][51,54]

Amino-*O*-2-isocephem *107* (0.13 g, 0.46 mmol), phenylacetic acid (69 mg, 0.51 mmol) and EEDQ (0.14 g, 0.56 mmol) were dissolved in dry CH_2Cl_2 (20 ml) and stirred at 25°C overnight. The mixture was worked-up by washing with 1% $NaHCO_3$ (2 × 10 ml), 4% HCl (2 × 10 ml) and brine, dried and concentrated *in vacuo*. Chromatography (50% EtOAc/p.e.) yielded 0.15 g (80%) of amido-

O-2-isocephem (+)-*108* as a white, amorphous solid: m.p. (sealed tube) 52° - 53°C (partial sublimation ∿ 42°C); $[\alpha]_D^{23}$ +154° (c 0.6, $CHCl_3$); i.r. ($CHCl_3$) ν_{max}: 3320 (NH), 1760 (β-lactam), 1710 (α,β-unsaturated ester), 1680, 1520 (amide) and 1610 (vinyl ether) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 2.25 (s, 3H, CH_3), 3.48 (s, 2H, PhCH_2CO), 3.6 - 4.7 (m, 3H, H-1, H-6), 5.21 (s, 2H, OCH_2Ph), 5.44 (d × d, 1H, $J_{6,7}$ = 4Hz, $J_{7,9}$ = 7Hz, becomes d, $J_{6,7}$ = 4Hz, on exchange, H-7) and 7.3 - 7.5 (m, 11H, including 2s at 7.23 and 7.38, 2× *Ph* and *NH*) ppm; m.s. (CI/IB, 36° - 135°C) m/e: 407 (10, $(M + 1)^+$) and 232 (100).

[6S-(6R*,7R*)]-3-Methyl-7β-phenylacetamido-Δ^3-*O*-2-isocephem-4-carboxylic acid [(+)-*70*][51, 54]

To a mixture of amido-*O*-2-isocephem (+)-*108* (0.13 g, 0.32 mmol) in 7 ml of dry THF and 10 ml of absolute EtOH was carefully added 10% Pd-C (100 mg). Hydrogenolysis at atmospheric pressure for 15 min., filtration through celite and solvent evaporation resulted in a yellow mixture of solid and syrup which was dissolved in about 1 ml of CH_2Cl_2. Petroleum ether was added slowly, with scratching, until a solid appeared. Addition of p.e. was continued (total of 50 ml) until t.l.c. (EtOAc) indicated that no more product was present in solution. The organic layer was removed and the process repeated with hexanes to give 96 mg (94%) of an off-white solid. Recrystallization of this product from acetone/ether/hexane gave the *O*-2-isocephem

(+)-*70* as a white powder: m.p. 95° - 105°C (dec.); $[\alpha]_D^{23}$ +157° (c 1.1, acetone); i.r. (KBr) ν_{max}: 3600 - 2500 (COO-H), 1755 (β-lactam), 1685 (sh, conjugated carboxylic acid), 1660, 1530 (amide) and 1610 (vinyl ether) cm^{-1}; 200MHz ^{1}H.m.r. (DMSO-d_6) δ: 2.15 (s, 3H, CH_3), 3.46 (s, 2H, PhCH_2CO), 3.75 (d × d × d, 1H, $J_{1\alpha,6}$ = 3.8Hz, $J_{1\beta,6}$ = 10.0Hz, $J_{6,7}$ = 4.9Hz, H-6), 3.92 (d × d, 1H, $J_{1\alpha,1\beta}$ = 10.4Hz, $J_{1\beta,6}$ = 10.0Hz, H-1β), 4.41 (d × d, 1H, $J_{1\alpha,1\beta}$ = 10.4Hz, $J_{1\alpha,6}$ = 3.8Hz, H-1α), 5.52 (d × d, 1H, $J_{6,7}$ = 4.9Hz, $J_{7,9}$ = 8.5Hz, H-7), 7.1 - 7.4 (m, 5H, including s at 7.26, *Ph*) and 8.82 (d, 1H, $J_{7,9}$ = 8.5Hz, *NH*) ppm; m.s. (CI/IB, 105°C) m/e: 317 (4, $(M + 1)^+$), 273 (12), 176 (67) and 142 (100); Anal. calcd. for $C_{16}H_{16}N_2O_5$: C 60.76, H 5.10, N 8.86; found: C 60.66, H 5.30, N 8.96.

EXPERIMENTAL

CHAPTER 2

tert-Butyl *N*-Benzyloxycarbonyl-D-threonate [(+)-*118*][112,124]

A suspension of distilled *tert*-butanol (1.0 g, 13 mmol), DCC (2.7 g, 13 mmol) and cuprous chloride (40 mg) was stirred under nitrogen at 26°C for 5 days. The volume of the mixture gradually reduced until, by the last day, it was too viscous to stir. An infrared spectrum of this mixture showed an intense peak at 1660 cm^{-1} (isourea and/or urea) while there was only a very weak peak remaining at 2110 cm^{-1} (N=C=N).

Dry CH_2Cl_2 (10 ml) and HMPT (2 ml) were added, the mixture was cooled in ice and then Cbz-D-Thr[121] (*116*, 0.96 g, 3.8 mmol) in 10 ml of CH_2Cl_2 was added dropwise over 60 minutes. The mixture was allowed to warm to room temperature and stirred for 16 hours. Filtration through celite, washing with 5% citric acid, sat. $NaHCO_3$ and brine, drying and concentration *in vacuo* gave 1.1 g of a yellow oil which contained two components (t.l.c., 25% EtOAc/hexanes). The major component was the less polar one.

Chromatography of the oil, using 1000 ml of 35% EtOAc/hexanes followed by 1000 ml of 40% EtOAc/hexanes, and rerunning the mixed fractions on a smaller column, yielded 0.64 g (54%) of (+)-*118* as a white solid and 0.16 g (15%) of an oil later

identified as dimer *119*. A small portion of (+)-*118* was recrystallized from ether/p.e. to give colourless needles: m.p. 65.5° - 66°C (lit.[126] : m.p. 66° - 67°C); $[\alpha]_D^{23}$ +7.5° (c 2, $CHCl_3$) and +20° (c 0.6, EtOH) {lit.[126] : $[\alpha]_D^{26}$ -20.6° (c 1.07, EtOH)}; i.r. (film) ν_{max}: 3440, 3360 (OH and NH), 1740 (sh, ester), 1730, 1710, 1530, 1520 (urethane) and 1390, 1365 (*tert*-butyl) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 1.16 (d, 3H, $J_{\beta,\gamma}$ = 6.5Hz, $CHCH_3$), 1.41 (s, 9H, $COOBu^t$), 2.85 (bs, 1H, exchangeable, *OH*), 4.0 - 4.3 (bd, 2H, J = 8Hz, becomes m on exchange, NH*CHCH*Me), 5.09 (s, 2H, OCH_2Ph), 5.56 (bd, 1H, J = 8Hz, exchangeable, *NH*) and 7.37 (s, 5H, *Ph*) ppm; m.s. (EI, 70eV, 32°C) m/e: 265 (3, $M^{+\cdot}$ - 44), 209 (13), 91 (100) and 57 (35); Anal. calcd. for $C_{16}H_{23}NO_5$: C 62.12, H 7.49, N 4.53; found: C 62.06, H 7.75, N 4.70.

The oil impurity was further purified by chromatography with 30% EtOAc/hexanes and found to have spectral characteristics which indicated it to be dimer *119* (p. 56): i.r. (film) ν_{max}: 3430, 3340 (NH and OH), 1750 - 1690 (ester and urethane), 1520 (urethane) and 1390, 1365 (*tert*-butyl) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 1.16 - 1.32 [2d, 6H, J = 6.5Hz (δ = 1.21 ppm) and J = 7Hz (δ = 1.26 ppm), $CH(OH)CH_3$ and $CH(OCOR)CH_3$], 1.44 (s, 9H, $COOBu^t$), 2.86 (bs, 1H, exchangeable, *OH*), 4.0 - 4.6 (m, 3H, 2× NH*CH*CH, CH*CH*(OH)Me), 5.13 (s, 4H, 2× OCH_2Ph), 5.3 - 5.8 [m, 3H, including 5.44 (d × q, J = 3Hz and 7 Hz, CH*CH*(OCOR)Me) and 5.65, 5.75 (2d, each J = 9Hz, 2× *NH*CH)] and 7.33, 7.35 (2s, 10H, 2× *Ph*) ppm; m.s. (EI, 70eV, 150°C) m/e: 444 (3, $M^{+\cdot}$ - 57 - 43),

399 (2), 309 (1), 247 (8), 91 (100) and 57 (28).

tert-Butyl D-Threonate (*113*)[127]

Cbz-D-Thr-OBu^t [(+)-*118*, 0.50 g, 1.6 mmol) was dissolved in 6 ml cyclohexene and 10 ml absolute EtOH, 10% Pd-C (0.13 g) was added and the mixture refluxed for 2 hours. Filtration of the cooled solution through celite, and removal of the solvent *in vacuo* yielded 0.22 g (78% crude) of brown oil. Purification by chromatography using 200 ml of 90% EtOAc/hexanes, then 100 ml of 5% MeOH/EtOAc gave 0.15 g (54%) of *113* as an oil: i.r. (film) ν_{max}: 3370, 3300 (OH and NH_2), 1730 (ester), 1590 (amine) and 1390, 1365 (*tert*-butyl) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 1.24 (d, 3H, $J_{\beta,\gamma}$ = 6Hz, $CHCH_3$), 1.51 (s, 9H, $COOBu^t$), 2.24 (bs, 3H, exchangeable, NH_2 and OH), 3.14 (bs, 1H, NH_2CH(COOR)CH) and 3.80 (app. bp, 1H, $J_{\alpha,\beta} = J_{\beta,\gamma}$ = 6Hz, CHCH(OSi)Me) ppm.

tert-Butyl *O*-*tert*-Butyldimethylsilyl-D-threonate [(+)-*120*][70]

tert-Butyl D-threonate (*113*, 0.23 g, 1.3 mmol) in 2 ml of dry HMPT was added to a mixture of imidazole (0.29 g, 4.2 mmol) and *tert*-butyldimethylsilyl chloride (0.30 g, 2.0 mmol) in 0.5 ml of dry HMPT under nitrogen, and stirred at room temperature for 18 hours. The resulting orange solution was poured into 30 ml of brine and extracted with 1:1 ether/hexanes (4 × 50 ml) which were then concentrated and partitioned between a total of 100 ml hexanes and 30 ml water. The two phases were separated

and the organic phase washed thoroughly with brine (10 × 10 ml), dried and evaporated to give 0.38 g of an oil. Chromatography with 55% EtOAc/hexanes yielded 0.29 g (75%) of pure (+)-*120*: $[\alpha]_D^{23}$ +16° (c 3, $CHCl_3$); i.r. (film) ν_{max}: 3380, 3310 (NH_2), 1730 (ester), 1590 (amine), 1390, 1365 (*tert*-butyl) and 1255, 835 (silyl ether) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 0.01 (s, 6H, Si*Me*$_2$), 0.82 (s, 9H, Si*Bu*^{t}), 1.19 (d, 3H, $J_{\beta,\gamma}$ = 6Hz, CH*CH*$_3$), 1.43 (s, 9H, COO*Bu*^{t}), 1.89 (bs, 2H, exchangeable, *NH*$_2$), 3.10 (bd, 1H, $J_{\alpha,\beta}$ = 2.5Hz, NH$_2$*CH*(COOR)CH) and 4.17 (d × q, 1H, $J_{\alpha,\beta}$ = 2.5Hz, $J_{\beta,\gamma}$ = 6Hz, CH*CH*(OSi)Me) ppm.

tert-Butyl *O*-*tert*-Butyldimethylsilyl-*N*-cinnamylidene-D-threonate (*122*)[50]

tert-Butyl *O*-*tert*-butyldimethylsilyl-D-threonate [(+)-*120*, 0.29 g, 1.0 mmol) and *trans*-cinnamaldehyde (0.13 ml, 0.14 g, 1.0 mmol) in 5 ml of dry CH_2Cl_2 were refluxed for 1.5 hours using a modified Dean-Stark trap filled with 3Å molecular sieves. After stirring at room temperature in the presence of anhydrous $MgSO_4$ for another 0.5 hours, the mixture was filtered and the solvent evaporated to give 0.39 g of *122* as a yellow oil: i.r. (film) ν_{max}: 1735 (ester), 1685 (excess aldehyde), 1635 (α,β-unsaturated imine) and 1615 (sh, conjugated C=C) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: -0.07, 0.00 (2s, 6H, Si*Me*$_2$), 0.80 (s, 9H, Si*Bu*^{t}), 1.22 (d, 3H, $J_{\beta,\gamma}$ = 6Hz, CH*CH*$_3$), 1.46 (s, 9H, COO*Bu*^{t}), 3.56 (app. d, 1H, $J_{\alpha,\beta}$ = 8Hz, N*CH*(COOR)CH), 4.16 (app. d × q, 1H,

$J_{\alpha,\beta}$ = 8Hz, $J_{\beta,\gamma}$ = 6Hz, CH*CH*(OSi)Me), 6.99 (app. d, 2H, J = 4.5Hz, N=CH*CH*=*CH*Ph), 7.2 - 7.7 (m, 5H, *Ph*) and 7.99 (app. t, J = 4.5Hz, N=*CH*CH=CH) ppm.

β-Lactams (-)-*123* and (+)-*124*[50,51]

Azidoacetyl chloride[131] (0.11 ml, 0.14 g, 1.2 mmol) in 2 ml of dry CH_2Cl_2 was added dropwise over 30 minutes to a cold (-15°C) suspension of Schiff base *122* (0.39 g, see p. 139), NEt_3 (0.18 ml, 0.13 g, 1.3 mmol) and $MgSO_4$ (anh.) in 5 ml of dry CH_2Cl_2 under nitrogen. Stirring at ice-salt bath temperature (-18° to -10°C) was continued for another hour. Work-up of the reaction was accomplished by dilution with hexanes (100 ml), washing successively with brine, 4% HCl, sat. $NaHSO_3$ (twice), sat. $NaHCO_3$ and brine, drying and solvent evaporation to give 0.37 g of an orange oil. T.l.c. (30% EtOAc/hexanes) showed two components. (The minor, more polar component was assumed to be the amide *125* (p. 60) but it was not isolated.) When 15% EtOAc/hexanes was used as the t.l.c. solvent, the major, less polar component could be seen to consist of one large, less polar and one very small, more polar component.

Flash chromatography with 15% EtOAc/hexanes, and recycling the mixed fractions with 200 ml of 13% EtOAC/hexanes followed by 200 ml of 20% EtOAc/hexanes, gave 0.31 g (63% from *120*) of mixed β-lactams *123* and *124* as a clear, pale yellow oil. HPLC (Waters 440, MeOH/EtOAc/hexanes (0.1:5:95), 5.0 ml/min.) showed

the ratio of major (less polar) to minor (more polar) diastereomer to be 90:10 with retention times of 10.4 and 14.4 minutes respectively. Separation using these conditions yielded 2 β-lactams with the following characteristics:

major β-lactam (-)-*123*: $[\alpha]_D^{23}$ -85° (c 1.5, $CHCl_3$); i.r. (film) ν_{max}: 2100 (azide), 1770 (β-lactam), 1735 (ester), 1650 (ArC=C) and 1390, 1365 (*tert*-butyl) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: -0.05, 0.00 (2s, 6H, $SiMe_2$), 0.80 (s, 9H, $SiBu^t$), 1.24 (d, 3H, $J_{\beta,\gamma}$ = 6.3Hz, $CHCH_3$), 1.49 (s, 9H, $COOBu^t$), 4.14 (d, 1H, $J_{\alpha,\beta}$ = 4.5Hz, NCH(COOR)CH), 4.47 (d × q, 1H, $J_{\alpha,\beta}$ = 4.5Hz, $J_{\beta,\gamma}$ = 6.3Hz, CHCH(OSi)Me), 4.78, 4.80, 4.84, 4.87 (m, XY of a poss. ABXY pattern, 2H, H-3 and H-4), 6.19, 6.23, 6.27, 6.31 (app. d × d, B of ABXY, 1H, J_{AB} = 15.8Hz, J_{BX} = 8.4Hz, CHCH=CH), 6.63, 6.71 (app. d, A of ABXY, 1H, J_{AB} = 15.8Hz, J_{AX} < 1Hz, CH=CHPh) and 7.2 - 7.4 (m, 5H, *Ph*) ppm; m.s. (EI, 70eV, 88°C) m/e: 476 (0.1) 429 ($M^{+\cdot}$ - 57), 345 (52), 75 (58), 73 (92) and 57 (100);

minor β-lactam (+)-*124*: $[\alpha]_D^{23}$ +65° (c 0.6, $CHCl_3$); i.r. (film) ν_{max}: 2100 (azide), 1770 (β-lactam), 1730 (ester), 1650 (ArC=C) and 1390, 1365 (*tert*-butyl) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: 0.01, 0.12 (2s, 6H, $SiMe_2$), 0.90 (s, 9H, $SiBu^t$), 1.20 (d, 3H, $J_{\beta,\gamma}$ = 6.3Hz, $CHCH_3$), 1.39 (s, 9H, $COOBu^t$), 4.23 (d, 1H, $J_{\alpha,\beta}$ = 3.7Hz, NCH(COOR)CH), 4.60 (d × q, 1H, $J_{\alpha,\beta}$ = 3.7Hz, $J_{\beta,\gamma}$ = 6.3Hz, CHCH(OSi)Me), 4.75, 4.78, 4.80, 4.83 (app. d × d, X of a poss. ABXY pattern, 1H, J_{BX} = 8.8Hz, J_{XY} = 5.2Hz, H-4) 4.87, 4.89 (app. d, Y of ABXY, 1H, J_{XY} = 5.2Hz, J_{BY} < 1Hz, H-3), 6.27,

6.32, 6.35, 6.40 (app. d × d, B of ABXY, 2H, J_{AB} = 16Hz, J_{BX} = 8.8Hz, CH*CH*=CH), 6.60, 6.68 (app. d, A of ABXY, 1H, J_{AB} = 16Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.2 - 7.4 (m, 5H, *Ph*) ppm; m.s. (EI, 70eV, 94°C) m/e: 458 (0.1, $M^{+\cdot}$ - 28), 430 (1, $M^{+\cdot}$ - 56), 345 (81), 73 (91) and 57 (100).

Benzyl L-Serinate Hydrochloride [(-)-*76*][69,63]

The preparation of benzyl L-serinate hydrochloride [(-)-*76*] from 10 mmol of BOC-L-Ser[64,183] (*127*) was identical to that of benzyl D-threonate hydrochloride [(+)-*74*, p. 118]. BOC-L-Ser-OBn (*128*) was obtained as an oil in 85% yield after chromatography with 1300 ml of 40% EtOAc/hexanes. (In a subsequent reaction starting from BOC-D-Ser, BOC-D-Ser-OBn was obtained as a white solid with m.p. 70° - 71°C.) BOC-L-Ser-OBn: $[\alpha]_D^{23}$ -3° (c 4, $CHCl_3$); i.r. (film) ν_{max}: 3430 (NH), 1740 (ester), and 1705, 1500 (urethane) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 1.45 (s, 9H, OBu^t), 2.97 (bt, 1H, $J_{O,\beta}$ = 5Hz, exchangeable, *OH*), 3.6 - 4.6 (m, 3H, NH*CHCH*$_2$OH), 5.22 (s, 2H, O*CH*$_2$Ph), 5.61 (d, 1H, $J_{N,\alpha}$ = 7Hz, exchangeable, *NH*) and 7.33 (s, 5H, *Ph*) ppm; m.s. (EI, 70eV, 68°C) m/e: 265 (0.4, $M^{+\cdot}$ - 30), 91 (73), 60 (47) and 57 (100).

Treatment of BOC-L-Ser-OBn (*128*) with ether saturated with hydrogen chloride gas[63] gave 1.8 g (77%) of L-Ser-OBn.HCl [(-)-*76*] which was recrystallized twice from *sec*-propanol/ether to give fine white needles: m.p. 172.5° - 174.5°C (lit.[61]:

172° - 174°C); $[\alpha]_D^{23}$ -6.1° (c 4.5, MeOH) (lit.: $[\alpha]_D$ -4.19° (c 4.53, MeOH)[129] and $[\alpha]_D$ -4.1 ± 0.5° (c 4.4, MeOH)[61]); i.r. (KBr) ν_{max}: 3360 (hydroxyl), 3150 - 2500 including 3030, 2900, 2750, 2620 ($^+NH_3$) and 1750 (ester) cm^{-1}; ^{1}H.m.r. (DMSO-d_6) δ: 3.91 (bd, 2H, $J_{\alpha,\beta}$ = 3Hz, $CHCH_2OH$), 4.16 (bt, 1H, $J_{\alpha,\beta}$ = 3Hz, $H_3N^+CHCH_2$), 5.23 (s, 2H, OCH_2Ph), 5.67 (bs, *OH*), 7.2 - 7.5 (m, 5H, including s at 7.34, *Ph*) and 8.53 (bs, $^+NH_3$) ppm.

Benzyl *O-tert*-Butyldimethylsilyl-L-serinate [(-)-*129*][70]

Benzyl L-serinate hydrochloride [(-)-*76*, 0.23 g, 1.0 mmol] was added to a mixture of imidazole (0.25 g, 3.6 mmol) and *tert*-butyldimethylsilyl chloride (0.22 g, 1.5 mmol) in 2 ml of dry HMPT and stirred for 18 hours at room temperature under nitrogen. This mixture was then poured into water (20 ml) and extracted with 1:1 ether/p.e. (5 × 50 ml). The combined organic phases were concentrated, taken up in p.e. (100 ml), extracted with brine (10 × 20 ml) to remove the HMPT, dried and concentrated *in vacuo* to 0.26 g of oil (85% crude). Flash chromatography using U.S.P. ether yielded 0.24 g (75%) of (-)-*129*: $[\alpha]_D^{23}$ -10° (c 3, $CHCl_3$); i.r. (film) ν_{max}: 3380, 3320 (NH_2), 1740 (ester), 1600 (amine) and 1260, 840 (silyl ether) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 0.00 (s, 6H, $SiMe_2$), 0.83 (s, 9H, $SiBu^t$), 2.08 (bs, 2H, exchangeable, NH_2), 3.4 - 4.1 (m, possibly an ABX pattern, 3H, NH_2CHCH_2OSi), 5.13 (s, 2H, OCH_2Ph) and 7.29 (s, 5H, *Ph*) ppm; m.s. (EI, 15eV, 41°C) m/e: 310 (4, $M^{+\cdot}$ + 1), 294 (1), 252 (51),

174 (35) and 91 (100).

Benzyl *O-tert*-Butyldimethylsilyl-*N*-cinnamylidene-L-serinate (*130*)[50]

A mixture of benzyl *O-tert*-butyldimethylsilyl-L-serinate [(-)-*129*, 0.14 g, 0.45 mmol] and *trans*-cinnamaldehyde (0.06 ml, 0.5 mmol) in 5 ml of dry CH_2Cl_2 was refluxed for 1 hour using a modified Dean-Stark apparatus filled with 3A molecular sieves. After stirring in the presence of $MgSO_4$ (anh.) at room temperature for another hour, the mixture was filtered and evaporated to give Schiff base *130* as a yellow oil; i.r. (film) ν_{max}: 1740 (ester), 1635 (α,β-unsaturated imine) and 1615 (sh, ArC=C) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 0.00 (s, 6H, $SiMe_2$), 0.83 (s, 9H, $SiBu^t$), 3.7 -4.3 (m, 3H, $NCHCH_2OSi$), 5.26 (s, 2H, OCH_2Ph), 7.00 (app. d, 2H, J = 4.5Hz, N=*CH*CH=CH), 7.2 - 7.5 (m, 10Hz, including s at 7.37, 2× *Ph*) and 8.05 (app. t, 1H, J = 4.5Hz, N=CH*CH*=*CH*Ph) ppm.

β-Lactams (+)-*131* and (-)-*132*[50,51]

Schiff base *130* (0.45 mmol, see above) and distilled NEt_3 (80 µl, 0.58 mmol) in 4 ml of dry CH_2Cl_2 were cooled in an ice-salt bath (-15°C) under nitrogen. A solution of azidoacetyl chloride[131] (50 µl, 0.55 mmol) in 1 ml of dry CH_2Cl_2 was added dropwise over 30 minutes, and stirring continued at -15° to -10°C for another hour. Dilution with hexanes (50 ml), successive washing with brine, 10% HCl (twice), sat. $NaHSO_3$ (twice), sat. $NaHCO_3$ and brine, then drying and solvent evaporation

yielded 0.19 g of yellow oil. The major, non-polar component of this oil was separated from the minor, polar component (as detected by t.l.c., 20% EtOAc/p.e.) by flash chromatography with 200 ml 19% EtOAc/hexanes followed by 100 ml 25% EtOAc/hexanes. The non-polar fraction proved to be 0.11g (48% from *129*) of mixed β-lactams *131* and *132*, and the polar fraction 0.05 g (28%) of amide (+)-*133* (p. 63).

HPLC (Waters 440, MeOH/EtOAc/hexanes (0.1:5:95), 5.0 ml/min., 1 recycle) of the mixed β-lactams showed that the two stereoisomers were present in the ratio of major:minor = 79:21 with retention times (before recycling) of 32 and 34.8 min. respectively. Separation on a semi-preparative scale was accomplished under slightly different conditions (MeOH/EtOAc/hexanes (0.1:8:92), 2 recycles) giving retention times (before recycling) of 10.8 and 12 min. respectively:

major β-lactam (+)-*131*: $[\alpha]_D^{23}$ +62° (c 1.4, $CHCl_3$); i.r. (film) ν_{max}: 2100 (azide), 1770 (β-lactam), 1740 (ester) and 1650 (ArC=C) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: -0.06, -0.04 (2s, 6H, $SiMe_2$), 0.84 (s, 9H, $SiBu^t$), 3.9 - 4.1 (m, AB part of a poss. ABX pattern, 2H, J_{AB} = 10Hz, $CHCH_2OSi$), 4.51, 4.53, 4.54, 4.56 (app. d × d, X of above ABX, 1H, J = 4.5Hz and 6.4Hz, $NCHCH_2OSi$), 4.71, 4.73, 4.75, 4.77 (app. d × d, X of a poss. ABXY pattern, 1H, J_{BX} = 8.7Hz, J_{XY} = 5.1Hz, H-4), 4.83, 4.86 (app. d, Y of ABXY, 1H, J_{XY} = 5.1Hz, J_{BY} < 1Hz, H-3), 5.08, 5.15, 5.21, 5.27 (ABq, 2H, J_{AB} = 12.1Hz, OCH_2Ph), 6.17, 6.21,

6.25, 6.29 (app. d × d, B of ABXY, 1H, J_{AB} = 15.9Hz, J_{BX} = 8.7Hz, CH*CH*=CH), 6.63, 6.71 (app. d, A of ABXY, 1H, J_{AB} = 15.9 Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.3 - 7.5 (m, 10H, including 2s at 7.34 and 7.37, 2× *Ph*) ppm; m.s. (EI, 70eV, 70°C) m/e: 478 (1, $M^{+\cdot}$ - 28), 463 (2), 424 (1.5), 423 (6), 422 (19), 421 (60) and 91 (100); Anal. calcd. for $C_{27}H_{34}N_4O_4Si$: C 64.00, H 6.76, N 11.06; found: C 63.71, H 6.79, N 10.67.

minor β-lactam (-)-*132*: (HPLC indicated ~ 1 - 2% of (+)-*131* still present) $[\alpha]_D^{23}$ -50° (0.6, $CHCl_3$); i.r. (film) ν_{max}: 2100 (azide), 1770 (β-lactam), 1750 (ester) and 1650 (weak, ArC=C) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: 0.06 (s, 6H, Si*Me*$_2$), 0.88 (s, 9H, Si*Bu*^{t}), 4.0 - 4.2 (m, poss. AB part of an ABX pattern, 2H, J_{AB} = 10.5Hz, CH*CH*$_2$OSi), 4.38, 4.41, 4.42, 4.45 (app. d × d, X of above ABX, 1H, J = 7.5Hz and 5.2Hz, N*CH*(COOR)CH), 4.55, 4.58, 4.60, 4.63 (app. d × d, X of a poss. ABXY pattern, 1H, J_{XY} = 5.1Hz, J_{BX} = 9.1Hz, H-4), 4.80, 4.83 (app. d, Y of ABXY, 1H, J_{XY} = 5.1Hz, J_{BY} < 1Hz, H-3), 5.12 (s, 2H, O*CH*$_2$Ph), 6.16, 6.21, 6.24, 6.29 (app. d × d, B of ABXY, 1H, J_{AB} = 15.9Hz, J_{BX} = 9.1Hz, CH*CH*=CH), 6.60, 6.68 (app. d, A of ABXY, 1H, J_{AB} = 15.9Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.2 - 7.4 (m, 10H, including 2s at 7.31 and 7.33, 2× *Ph*) ppm; m.s. (EI, 70eV, 76°C) m/e: 478 (1.5, $M^{+\cdot}$ - 28), 463 (2), 424 (1.4), 423 (6), 422 (19), 421 (56) and 91 (100).

Amide (+)-*133* was further purified by chromatography with 25% EtOAc/p.e.; $[\alpha]_D^{23}$ +12° (c 1, $CHCl_3$); i.r. (film) ν_{max}: 3420, 3320 (NH), 2110 (azide), 1745 (β-lactam) and 1685, 1520

(amide) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 0.00, 0.03 (2s, 6H, $SiMe_2$), 0.88 (s, 9H, $SiBu^t$), 3.73 - 4.25 [m, 4H, including 4.02 (s, N_3CH_2CO), 3.73, 3.79, 3.89, 3.95 (app. d × d, B of an ABXY pattern, J_{AB} = 10Hz, J_{BX} = 3.5Hz) and 4.04, 4.08, 4.21, 4.25 (app. d × d, A of ABXY, J_{AB} = 10Hz, J_{BX} = 2.5Hz) $CHCH_2OSi$], 4.60, 4.65, 4.70, 4.73, 4.78, 4.83 (app. d × t, X of ABXY, 1H, J = 3Hz, J_{XY} = 8Hz, $NHCH(COOR)CH_2$), 5.22 (s, 2H, OCH_2Ph), 7.11 (bd, Y of ABXY, 1H, J_{XY} = 8Hz, *NH*) and 7.37 (s, 5H, *Ph*) ppm; m.s. (EI, 18eV, 68°C) m/e: 364 (M^+ - 28), 335 (18), 308 (12), 307 (52) and 91 (100).

Azidoacetic-d_2 Acid (*137*)[131]

Chloroacetic acid-d_3* (*136*, 2.4 g, 25 mmol) and sodium azide (1.8 g, 27 mmol) were added successively to 10 ml of ice-cold 2.7N NaOH. Ether (10 ml) was layered onto the mixture and heated under reflux for 39 hours. The ether was evaporated and replaced with CH_2Cl_2 (20 ml). The mixture was then cooled in ice, acidified slowly with 14.5 ml of ice-cold 2N H_2SO_4 and allowed to warm to room temperature (about 1 hour) with stirring. Separation of the phases, extraction of the aqueous layer with CH_2Cl_2 (9 × 20 ml) and normal processing of the combined organic layers yielded 2.1 g of yellow oil (80% crude).

Another 0.5 g oil was obtained by continuous extraction of the aqueous phases with ether overnight to give a total of 2.6 g (quantitative) of crude azidoacetic-d_2 acid (*137*):

*Supplied by Merck, Sharpe and Dohme, Ltd, Montreal, P.Q.

i.r. (CH_2Cl_2) ν_{max}: 3500 - 2400 including 2660, 2530 (COO-H), 2180 (C-D), 2110 (azide) and 1730 (carboxyl) cm^{-1}; ^1H.m.r. ($CDCl_3$) δ: 11.2 (s, COO*H*) ppm; m.s. (EI, 70eV, 130°C) m/e: 103 ($M^{+\cdot}$), 73 (29), 44 (100), 31 (26), 30 (23), 29 (40) and 28 (84).

Azidoacetyl Chloride-d_2 (*138*)[131]

Distilled thionyl chloride (3.5 ml, 48 mmol) was added dropwise to an ice-cold solution of crude azidoacetic-d_2 acid (*137*, 2.6 g, 25 mmol) in 1.5 ml of dry CH_2Cl_2. Effluent gases were collected in a trap of cold (0°C) 5N NaOH. When addition was complete, the reaction mixture was heated in an oil bath (temperature monitored so as not to exceed 55°C) for 3 hours. Gas evolution appeared to stop after about 2.5 hours. After cooling, the reaction mixture was fractionally distilled *in vacuo* (water aspirator) at room temperature to remove solvent and residual thionyl chloride, and then the temperature was raised slowly until 2.2 g (74%) of azidoacetyl chloride-d_2 (*138*) as a clear liquid were collected at 38° - 39°C/10 Torr (lit. for N_3CH_2COCl[131]: 41°C/12 Torr): i.r. (film) ν_{max}: no absorption around 3000 cm^{-1}, 2180 (C-D), 2110 (azide) and 1820, 1790 (acyl halide) cm^{-1}.

3-Deutero-β-lactams (-)-*139* and (+)-*140* (from D-Thr-OBn)

Schiff base *88* (prepared from 1.0 mmol of the silylated

D-Thr-OBn (+)-*82* and 1.1 mmol of *trans*-cinnamaldehyde, p. 121) in 4 ml of dry CH_2Cl_2 was cooled to -15°C under argon. Treatment with NEt_3 (0.18 ml, 1.3 mmol) and a solution of azidoacetyl chloride-d_2 (*138*, 0.11 ml, 1.2 mmol) in 2 ml of dry CH_2Cl_2 (with anh. $MgSO_4$) was carried out as for the preparation of (-)-*89* and (+)-*90* (p. 122). Work-up and chromatography gave 0.34 g (65% from (+)-*82*) of mixed β-lactams (-)-*139* and (+)-*140* and 0.09 g of amide (-)-*141* (22%). The two stereoisomers (present in the ratio of major:minor = 88:12) were separated by HPLC (Altex 300): major 3-deutero-β-lactam (-)-*139* (compare with (-)-*89*, p. 123): $[\alpha]_D^{23}$ -129° (c 4.4, $CHCl_3$); i.r. (film): no significant differences; 200MHz ^{1}H.m.r. ($CDCl_3$) ABXY pattern became ABX, δ: 4.90, 4.94 (app. d, X of ABX, 1H, J_{BX} = 9.3Hz, J_{AX} < 1Hz, H-4), 6.16, 6.21, 6.24, 6.29 (app. d × d, B of ABX, 1H, J_{AB} = 15.9Hz, J_{BX} = 9.3Hz, CH*CH*=CH) and 6.56, 6.64 (app. d, A of ABX, 1H, J_{AB} = 15.9Hz, J_{AX} < 1Hz, CH=*CH*Ph) ppm; m.s. (EI, 16eV, 74°C) m/e: 493 (5, $M^{+\cdot}$ - 28), 438 (10), 437 (34), 436 (100), 435 (43), 159 (57) and 91 (73); minor 3-deutero-β-lactam (+)-*140* (compare with (+)-*90*, p. 123): $[\alpha]_D^{23}$ +38° (0.5, $CHCl_3$); F.T.i.r. (CCl_4) ν_{max}; 2109 (azide), 1774 (β-lactam) and 1750 (ester) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) ABXY pattern became ABX, δ: 4.78, 4.83 (app. d, X of ABX, 1H, J_{BX} = 9.1Hz, J_{AX} < 1Hz, H-4), 6.25, 6.30, 6.33, 6.38 (app. d × d, B of ABX, 1H, J_{AB} = 16Hz, J_{BX} = 9.1Hz, CH*CH*=CH) and 6.54, 6.62 (app. d, A of ABX, 1H, J_{AB} = 16Hz, J_{AX} < 1Hz, CH=*CH*Ph) ppm;

m.s. (EI, 16eV, 75°C) m/e: 493 (3, $M^{+\cdot}$ - 28), 438 (4), 437 (15), 436 (45), 435 (18), 159 (55) and 91 (100).

Note: Each of these diastereomers displays a small d (J = 5Hz) in its 200MHz ^{1}H.m.r. spectrum indicating 20 to 25% exchange of D for H at H-3; δ = 4.86 ppm in (-)-*139* and 4.90 ppm in (+)-*140*.

dideuterated amide (-)-*141* (compare with (-)-*91*, p. 124): $[\alpha]_D^{23}$ -12°C (c 0.5, $CHCl_3$); i.r. (film): no significant differences; ^{1}H.m.r. ($CDCl_3$): only a small s at δ = 4.03 ppm indicating about 25% exchange of D for H; m.s. (EI, 70eV, 69°C) m/e: 365 (0.1, $M^{+\cdot}$ - 15 - 28), 364 (0.3), 351 (1.4), 323 (1), 322 (1), 159 (19) and 91 (100).

3-Deutero-β-lactams *142* and *143* (from L-Ser-OBn)

Schiff base *130* (prepared from 0.30 mmol of silylated L-Ser-OBn (*129*) and 0.32 mmol of *trans*-cinnamaldehyde, p. 144) in 3 ml of dry CH_2Cl_2 was cooled to -15° under argon in the presence of $MgSO_4$ (anh.). This was followed by treatment with NEt_3 (50 µl, 0.36 mmol) and then a solution of azidoacetyl chloride-d_2 (*138*, 30 µl, 0.32 mmol) in 1 ml of dry CH_2Cl_2, as in the preparation of the protonated analogues (+)-*131* and (-)-*132*, p. 144). Identical work-up and chromatography yielded 82 mg (53% from *129*) of mixed β-lactams *142* and *143*, and 20 mg (16%) of amide *144* (p. 70). The two β-lactams (present in the ratio of major:minor = 77:23) were separated by HPLC (Waters 440):

major 3-deutero-β-lactam *142* (compare with (+)-*131*, p. 145):

i.r. (film): no significant differences; 200MHz ^{1}H.m.r. ($CDCl_3$) ABXY changed to ABX, δ: 4.71, 4.76 (app. d, X of ABX, 1H, J_{BX} = 8.8Hz, J_{AX} < 1Hz, H-4), 6.16, 6.21, 6.24, 6.29 (app. d × d, B of ABX, 1H, J_{AB} = 15.9Hz, J_{BX} = 8.8Hz, CH*CH*=CH) and 6.63, 6.71 (app. d, A of ABX, 1H, J_{AB} = 15.9Hz, CH=*CH*Ph) ppm; m.s. (EI, 70eV, 71°C) m/e: 479 (1, $M^{+\cdot}$ - 28), 464 (2), 424 (5), 423 (17), 422 (47), 421 (23) and 91 (100);

minor 3-deutero-β-lactam *143* (compare with (-)-*132*, p. 146): i.r. (film): no significant differences; 200MHz ^{1}H.m.r. ($CDCl_3$) ABXY pattern changed to ABX, δ: 4.57, 4.61 (app. d, X of ABX, 1H, J_{BX} = 9.1Hz, J_{AX} < 1Hz, H-4), 6.16, 6.21, 6.24, 6.29 (app. d × d, B of ABX, 1H, J_{AB} = 15.9Hz, J_{BX} = 9.1Hz, CH*CH*=CH) and 6.60, 6.68 (app. d, A of ABX, 1H, J_{AB} = 15.9Hz, J_{AX} < 1Hz, CH=*CH*Ph) ppm; m.s. (EI, 70eV, 59°C) m/e: 479 (1, $M^{+\cdot}$ - 28), 464 (1.4), 424 (4), 423 (13, 422 (37), 421 (19) and 91 (100);

Note: Both of these diastereomers display a small d (J = 5Hz) in their 200MHz ^{1}H.m.r. spectra indicating about 25% exchange of D for H at H-3; δ = 4.80 ppm for *142* and 4.92 ppm for *143*.

dideuterated amide *144* (compare with (+)-*133*, p. 146): i.r. (film): no significant differences; ^{1}H.m.r. ($CDCl_3$) no s for 2H at δ = 4.02 ppm; m.s. (EI, 70eV, 80°C) m/e: 337 (4, M^+ - 57), 309 (1), 308 (1) and 91 (100).

3-Deutero-β-lactams *147* and (-)-*148* (from L-Ser-OMe)

Methyl *O-tert*-butyldimethylsilyl-*N*-cinnamylidene-L-serinate (*111*, p. 53) was prepared as described by Just and Liak[81] from methyl *O-tert*-butyldimethylsilyl-L-serinate[81] (*80*, p. 31 , 0.42 g, 1.8 mmol) and distilled *trans*-cinnamaldehyde (0.23 ml, 0.24 g, 1.8 mmol).

A suspension of $MgSO_4$ (anh.), Schiff base *111* and distilled NEt_3 (0.32 ml, 0.23 g, 2.3 mmol) in 5 ml of dry CH_2Cl_2 was cooled in an ice-salt bath (-15°C) under argon. Azidoacetyl chloride-d_2 (*138*, 0.20 ml, 0.26 g, 2.1 mmol) in 1 ml of dry CH_2Cl_2 was then added dropwise over 20 minutes. The mixture was allowed to stir at -15° to -10°C for another 70 minutes, then filtered and diluted with 1:1 ether/hexanes (100 ml). Washing successively with brine, 4% HCl, sat. $NaHSO_3$ and brine, drying and concentration *in vacuo* gave 0.60 g of a yellow oil which still contained cinnamaldehyde as well as the expected β-lactam mixture and amide (t.l.c., 30% EtOAc/hexanes).

This oil was separated by chromatography with 200 ml of hexanes, 400 ml of 24% EtOAc/hexanes and finally 200 ml of 30% EtOAc/hexanes into 0.45 g (58% crude) of mixed β-lactams *147* and *148* as a yellow waxy solid {$[\alpha]_D^{23}$ +2 (c 5, $CHCl_3$)}, and 0.09 g (16%) of amide *149* as an oil. HPLC (Waters 440, MeOH/EtOAc/hexanes (0.1:5:95), 5.0 ml/min.) of the mixed β-lactams showed the presence of two stereoisomers in the ratio of minor, less polar to major, more polar isomer = 18:82 with retention

times of 55 and 58 minutes respectively.

These conditions were used to isolate from a small quantity of the mixture, pure (-)-*148*, the major diastereomer, as a white powder: major 3-deutero-β-lactam (-)-*148*: m.p. 80.5° - 81°C (lit.[81] : 74.5° - 75.5°C); $[\alpha]_D^{23}$ -1° (c 2.5, $CHCl_3$); i.r. (KBr) ν_{max}: 2120 (C-D), 2100 (azide), 1775 (β-lactam) and 1750, 1735 (ester) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: -0.08, -0.06 (2s, 6H, $SiMe_2$), 0.80 (s, 9H, $SiBu^t$), 3.71 (s, 3H, $COOCH_3$), 3.83, 3.85, 3.88, 3.90 (B of an ABX pattern) and 3.91, 3.94, 3.95, 3.98 (A of ABX, 2H, app. J_{AB} = 9.5Hz, J_{AX} = 4Hz, J_{BX} = 6.5Hz, $CHCH_2OSi$), 4.45, 4.47, 4.48, 4.50 (app. d × d, X of above ABX, 1H, J_{AX} = 6.5Hz, J_{BX} = 4Hz, $NCH(COOR)CH_2$), 4.74, 4.78 (app. d, X of a second ABX pattern, 1H, J_{BX} = 9.0Hz, J_{AX} < 1Hz, H-4), 6.15, 6.19, 6.23, 6.27 (app. d × d, B of 2nd ABX, 1H, J_{AB} = 15.9 Hz, J_{BX} = 9.0Hz, $CHCH$=CH), 6.66, 6.74 (app. d, A of 2nd ABX, 1H, J_{AB} = 15.9Hz, J_{AX} < 1Hz, CH=CHPh) and 7.2 - 7.3 (m, 5H, *Ph*) ppm; a small d (J = 5Hz) at δ = 4.85 ppm indicated about 25% exchange of D for H at H-3; m.s. (EI, 70eV, 50°C) m/e: 403 (2, $M^{+\cdot}$ - 28), 348 (11), 347 (36), 346 (86), 345 (28), 118 (100) and 89 (99).

HPLC was not as satisfactory a procedure for the isolation of pure minor isomer *147*. Therefore, the crude β-lactam mixture was recrystallized from hexane to give needle crystals with m.p. 79° - 80°C and identical spectral characteristics to (-)-*148* above. Pure *147* was then more easily obtained by HPLC separation from the mother liquor which now contained a ratio of minor to major isomer of about 40:60: minor 3-deutero-β-lactam *147*:

i.r. (film) ν_{max}: 2110 (C-D and azide), 1775 (β-lactam) and 1750 (ester) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: 0.04 (s, 6H, $SiMe_2$), 0.85 (s, 9H, $SiBu^t$), 3.65 (s, 3H, $COOCH_3$), 3.94, 3.97, 3.99, 4.02 (B of an ABX pattern) and 4.03, 4.07, 4.08, 4.12 (A of ABX, 2H, J_{AB} = 10.5Hz, J_{AX} = 5.1Hz, J_{BX} = 7.9Hz, $CHCH_2OSi$), 4.29, 4.32, 4.33, 4.36 (app. d × d, X of above ABX, 1H, J_{AX} = 7.9Hz, J_{BX} = 5.1Hz, $NCH(COOR)CH_2$), 4.54, 4.58 (app. d, X of a second ABX pattern, 1H, J_{BX} = 9.0Hz, J_{AX} < 1Hz, H-4), 6.19, 6.23, 6.27, 6.31 (app. d × d, B of 2nd ABX, 1H, J_{AB} = 15.9Hz, J_{BX} = 9.0Hz, $CHCH$=CH), 6.60, 6.67 (app. d, A of ABX, 1H, J_{AB} = 15.9 Hz, J_{AX} < 1Hz, $\tilde{C}H$=CHPh) and 7.2 - 7.4 (m, 5H, *Ph*) ppm; a small d (J = 5Hz) at δ = 4.81 ppm indicated about 25% exchange of D for H at H-3; m.s. (EI, 70eV, 59°C) m/e: 403 (4, $M^{+\cdot}$ - 28), 348 (12), 347 (41), 346 (100), 345 (32), 118 (57) and 115 (56).

<u>dideuterated amide</u> *149*: i.r. (film) ν_{max}: 3420, 3350 (NH), 2110 (C-D and azide), 1750 (ester and 1680, 1530 (amide) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 0.00 (s, 6H, $SiMe_2$), 0.83 (s, 9H, $SiBu^t$), 3.69 - 4.14 [m, 5H, including 3.69 (s, $COOCH_3$), 3.80, 3.86 (partial B of an ABXY pattern, J_{BX} = 3.5Hz) and 3.94, 3.98, 4.10, 4.14 (A of ABXY, J_{AB} = 9.5Hz, J_{AX} = 2.5Hz), $CHCH_2OSi$], 4.47, 4.52, 4.58, 4.62, 4.67, 4.73 (app. d × t, Y of ABXY, 1H, J_{AX} = 2.5Hz, J_{BX} = 3.5Hz, J_{XY} = 8.5Hz, $NHCH(COOR)CH_2$) and 6.97, 7.11 (bd, Y of ABXY, 1H, J_{XY} = 8.5Hz, *NH*) ppm.

EXPERIMENTAL

CHAPTER 3

2-Cinnamylideneamino-2-deoxy-β-D-glucopyranose (*172*)[149-151]

trans-Cinnamaldehyde (0.60 ml, 0.63g, 4.8 mmol) was added to a mixture of 2-amino-2-deoxy-D-glucopyranose hydrochloride (*167*, 1.0 g, 4.6 mmol) in 4.7 ml of 1N NaOH. This mixture was shaken at room temperature until it was a solid mass (about 20 min.), cooled to 0°C and filtered. The residue was rinsed twice with ice-water, once with cold EtOH/ether (1:1) and twice with anhydrous ether to give 1.03 g (75%) of *172* as a white powder which was pure enough for characterization purposes: m.p. 159° - 160°C; $[\alpha]_D^{23}$ +14° (c 2, pyridine); i.r. (KBr) ν_{max}: 3440 - 3200 (hydroxyls), 1625 (α,β-unsaturated imine) and 1610 (conjugated C=C) cm^{-1}; 200MHz ^{1}H.m.r. (DMSO-d_6) δ: 2.73 (bt, 1H, J = 8Hz, H-2), 3.1 - 3.8 (m, H-3, H-4, H-5, H-6), 4.5 - 4.7 [m, 2H, including 4.59 (t, J = 6Hz, exchangeable, (C-6)H_2OH) and 4.66 (t, J = 7Hz, becomes bd, J = 7Hz, on D_2O treatment, H-1)], 4.89 (d, 1H, J = 5Hz, exchangeable, (C-4 or C-3)H*OH*), 4.99 (d, 1H, J = 6Hz, exchangeable, (C-3 or C-4)H*OH*), 6.60 (d, 1H, J = 7Hz, exchangeable, (C-1)H*OH*), 6.85, 6.90, 6.93, 6.98 (app. d * d, B of an ABX pattern, 1H, J_{AB} = 16Hz, J_{BX} = 9 Hz, N=CH*CH*=CH), 7.10, 7.18 (app. d, A of ABX, 1H, J_{AB} = 16Hz,

J_{AX} < 1Hz, CH=*CH*Ph), 7.3 - 7.6 (m, 5H, *Ph*) and 7.96 (app. d, X of ABX, 1H, J_{BX} = 9Hz, J_{AX} < 1Hz, N=*CH*CH=CH) ppm; Anal. calcd. for $C_{15}H_{19}NO_5$: C 61.42, H 6.53, N 4.77; found: C 61.52, H 6.54, N 4.57.

1,3,4,6-tetra-*O*-Acetyl-2-cinnamylideneamino-2-deoxy-β-D-glucopyranose (*173*)[152]

Schiff base *172* (0.59 g, 2.0 mmol), suspended in 2 ml of dry pyridine and 1.0 ml of acetic anhydride (11 mmol), was stirred for 18 hours at 0°C. Solid $Na_2CO_3.H_2O$ (0.80 g, 13 mmol) was added and stirred for another 2 hours, then poured into ice-water. The resulting white precipitate was filtered, then taken up in $CHCl_3$, washed with brine (×3), dried (anh. K_2CO_3) and evaporated to an oil which was triturated with hexanes to give a flocculent precipitate. Filtration yielded 0.65 g of *173*. A further 0.13 g was obtained by similar treatment of the residue from filtrate evaporation for a total of 0.78 g (85%). A small quantity was recrystallized from EtOAc/hexanes for characterization purposes: m.p. 211.5° - 213°C (dec.); $[\alpha]_D^{23}$ +58° (c 3, $CHCl_3$); i.r. (KBr) ν_{max}: 1760, 1740 (acetates), 1640 (α,β-unsaturated imine), 1620 (conjugated C=C) and 1380, 1370 ($COCH_3$) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 2.13, 2.22, 2.24, 2.27 (4s, 12H, 4× OAc), 3.42 (d × d, 1H, $J_{1,2}$ = 8.5Hz, $J_{2,3}$ = 9Hz, H-2), 3.86 - 4.60 [m, 3H, including 4.00 (poss. d × d × d, $J_{4,5}$ = 9Hz, $J_{5,6a}$ = 4.5Hz, $J_{5,6e}$ = 2Hz, H-5), 4.14 (d × d, $J_{5,6e}$ = 2Hz,

$J_{6a,6e}$ = 12.5Hz, H-6e) and 4.46 (poss. d × d, $J_{5,6a}$ = 4.5Hz, $J_{6a,6e}$ = 12.5Hz, H-6a)], 5.01 - 5.61 [m, 2H, including 5.16 (app. t, $J_{3,4} = J_{4,5}$ = 9Hz, H-4) and 5.47 (app. d × d, $J_{2,3}$ = 8.5 Hz, $J_{3,4}$ = 9Hz, H-3)], 5.95 (d, 1H, $J_{1,2}$ = 8.5Hz, H-1), 6.9 - 7.1 (m, 2H, N=CH*CH*=*CH*Ph), 7.3 - 7.7 (m, 5H, *Ph*) and 8.06 (app. d × d, 1H, J = 2Hz and 6.5Hz, N=*CH*CH=CH) ppm; m.s. (CI/IB, 100°C) m/e: 418 (4, $(M + 1)^+ - 44$) and 282 (100); Anal. calcd. for $C_{23}H_{27}NO_9$: C 59.86, H 5.90, N 3.04; found: C 60.00, H 6.03, N 3.20.

β-Lactams (+)-*174* and (-)-*175*[50,51] (from β-anomer)

Acetylated Schiff base *173* (0.65 g, 1.4 mmol) in 5 ml of dry CH_2Cl_2 was cooled to -15°C. Distilled NEt_3 (0.25 ml, 1.8 mmol) was added, followed by the dropwise addition of azidoacetyl chloride[131] (0.15 ml, 0.20 g, 1.6 mmol) in 1 ml of dry CH_2Cl_2 over 15 minutes. The mixture was stirred another 75 minutes (for a total of 1.5 hours) at -15° to -10°C, then diluted with ether/hexanes (1:1) and filtered. After successive washings with brine, 4% HCl, sat. $NaHSO_3$, sat. $NaHCO_3$ and brine, the filtrate was dried and concentrated *in vacuo* to give an off-white solid. (T.l.c. in both 40% and 60% EtOAc/hexanes showed only one component.) This solid had a tendency to crystallize from EtOAc/hexanes, but since it was desirable to avoid fractional crystallization, it was purified by chromatography. The sample was applied to the column in CH_2Cl_2, then eluted with

39% EtOAc/hexanes (1000 ml) and 44% EtOAc/hexanes (1000 ml). The clear oil obtained was triturated with ether to give 0.73 g (95%) of mixed β-lactams *174* and *175* as a white powder: m.s. (CI/IB, 103°C) m/e: 517 (100, $(M + 1)^+ - 28$), 397 (100, $(M + 1)^+ - 28 - 60 - 60$) and 338 (73).

Examination of this product by HPLC revealed a ratio of less polar to more polar stereoisomer of 49:51 with retention times of 21 and 24.5 minutes respectively. Crystallization by addition of hexanes to 1:1 EtOAc/CH_2Cl_2 resulted in two types of crystals, needles and rhombohedra. A sufficient quantity for the characterization of each crystal type was obtained by the Pasteur method[154] using tweezers and a reading lens. Each was identified by its retention time on HPLC: less polar β-lactam (+)-*174* (needles): m.p. 153° - 156°C (dec.); $[\alpha]_D^{23}$ +145° (c 1.5, $CHCl_3$); i.r. (KBr) ν_{max}: 2100 (azide), 1775 (sh, β-lactam), 1760, 1735 (acetates) and 1365 ($COCH_3$) cm^{-1}; and ($CHCl_3$) ν_{max}: 2110 (azide), 1775 (β-lactam), 1750 (sh, acetates) and 1370 ($COCH_3$) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: 2.03, 2.06, 2.09, 2.17 (4s, 12H, 4× OAc), 3.72 (d × d × d, 1H, $J_{4,5}$ = 10Hz, $J_{5,6a}$ = 4.5Hz, $J_{5,6e}$ = 1.5Hz, H-5), 3.96 - 4.07 [m, 2H, 2 sets of d × d at 4.01 ($J_{1,2}$ = 8.9Hz, $J_{2,3}$ = 11Hz, H-2) and 4.04 ($J_{5,6e}$ = 1.5Hz, $J_{6a,6e}$ = 12.6Hz, H-6e)], 4.25 (d × d, 1H, $J_{5,6a}$ = 4.5Hz, $J_{6a,6e}$ = 12.6Hz, H-6a), 4.51, 4.54, 4.56, 4.59 (app. d × d, X of ABXY, 1H, J_{BX} = 9.5Hz, J_{XY} = 5.1Hz, H-4'), 4.72, 4.75 (app. d, Y of ABXY, 1H, J_{XY} = 5.1Hz, J_{BY} < 1Hz, H-3'), 5.07 (d × d, 1H,

$J_{3,4}$ = 9.2Hz, $J_{4,5}$ = 10Hz, H-4), 5.31 (d × d, $J_{2,3}$ = 11Hz, $J_{3,4}$ = 9.2Hz, H-3), 5.71 (d, 1H, $J_{1,2}$ = 8.9Hz, H-1), 5.96, 6.00, 6.04, 6.08 (app. d × d, B of ABXY, 1H, J_{AB} = 15.7Hz, J_{BX} = 9.5 Hz, CH*CH*=CH), 6.79, 6.87 (app. d, A of ABXY, 1H, J_{AB} = 15.7Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.3 - 7.5 (m, 5H, *Ph*) ppm; Anal. calcd. for $C_{25}H_{28}N_4O_{10}$: C 55.15, H 5.18, N 10.29; found: C 55.08, H 5.13, N 10.47; more polar β-lactam (-)-*175* (rhombohedra): m.p. 160° - 163°C (dec.); $[\alpha]_D^{23}$ -40° (c 2, $CHCl_3$); i.r. (KBr) ν_{max}: 2100 (azide), 1775 (sh, β-lactam), 1750 (acetates), 1650 (ArC=C) and 1365 ($COCH_3$) cm^{-1}; and ($CHCl_3$) ν_{max}: 2100 (azide), 1770 (β-lactam), 1750 (sh, acetates), 1650 (ArC=C) and 1365 ($COCH_3$) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: 2.00, 2.06, 2.12, 2.18 (4s, 12H, 4× OA*c*), 3.72 - 3.82 [m, 2H, including 3.77 (d × d, $J_{1,2}$ = 9.0Hz, $J_{2,3}$ = 10.5Hz, H-2) and 3.77 (app. d × d × d, $J_{4,5}$ = 10Hz, $J_{5,6a}$ = 4.4Hz, $J_{5,6e}$ = 2.2Hz, H-5)], 4.04 (d × d, 1H, $J_{5,6e}$ = 2.2Hz, $J_{6a,6e}$ = 12.6Hz, H-6e), 4.23, 4.25, 4.27, 4.30, 4.32, 4.34 [m, 2H, including 4.28 (app. d × d, $J_{5,6a}$ = 4.4Hz, $J_{6a,6e}$ = 12.6Hz, H-6a) and 4.31 (app. d × d, X of an ABXY, J_{BX} = 9.5Hz, J_{XY} = 5.1Hz, H-4')], 4.75, 4.77 (app. d, Y of ABXY, 1H, J_{XY} = 5.1Hz, J_{AX} < 1Hz, H-3'), 5.02 (app. t, 1H, J = 9.7Hz, H-4), 5.33 (d × d, 1H, $J_{2,3}$ = 10.5Hz, $J_{3,4}$ = 9.3Hz, H-3), 6.03 (d, 1H, $J_{1,2}$ = 9.0Hz, H-1), 6.16, 6.20, 6.24, 6.28 (app. d × d, B of ABXY, 1H, J_{AB} = 15.7Hz, J_{BX} = 9.5Hz, CH*CH*=CH), 6.73, 6.81 (app. d, A of ABXY, 1H, J_{AB} = 15.7Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.3 - 7.5 (m, 5H, *Ph*) ppm; Anal. calcd. for $C_{25}H_{28}N_4O_{10}$:

C 55.15, H 5.18, N 10.29; found: C 55.30, H 5.29, N 10.20.

1,3,4,6-tetra-*O*-Acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride (*177*)[155]

The known compound, 2-methyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyrano)-Δ^2-oxazoline[156,157] (*176*, 6.2 g, 19 mmol) was dissolved in 50 ml dioxane and treated with 19 ml of 1N HCl, added dropwise at 25°C. The solvent was concentrated *in vacuo* (water bath not exceeding 25°C) to 6.8 g of off-white solid (95% crude) which was purified by precipitation from MeOH by ether (anh.) to give *177* in 85% yield: m.p. 179° - 180°C (dec.) (lit.[158]: 180°C); $[\alpha]_D^{23}$ +146° (c 2, H_2O) {lit.[158]: $[\alpha]_D^{20}$ +130° (c 1, H_2O)}; i.r. (KBr) ν_{max}: 3100 - 2400, 2540, 2030 ($^+NH_3$), 1765, 1755, 1740 (acetates) and 1380, 1370 ($COCH_3$) cm^{-1}; ^{1}H.m.r. (CD_3OD) δ: 2.05, 2.12, 2.25 (1bs and 2s, 12H, 4× OAc), 3.90 (d × d, 1H, $J_{1,2}$ = 4Hz, $J_{2,3}$ = 10Hz, H-2), 4.1 - 4.5 (m, 3H, H-5, H-6), 5.00, 5.15, 5.32, 5.48, 5.63 (m, 2H, H-3, H-4) and 6.35 (d, 1H, $J_{1,2}$ = 4Hz, H-1) ppm; m.s. (CI/IB, 38° - 81°C) m/e: 185 (13), 168 (13), 114 (66), 72 (100) and 59 (83).

1,3,4,6-tetra-*O*-Acetyl-2-cinnamylideneamino-2-deoxy-α-D-glucopyranose (*179*)[152]

A solution of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride (*177*, 5.0 g, 13 mmol) in 90 ml of MeOH and 60 ml of CH_2Cl_2 was added dropwise with stirring to a

cold (0°C) solution of NaOAc (1.1 g, 13 mmol) and *trans*-cinnamaldehyde (1.7 ml, 1.8 g, 13 mmol) in 30 ml of MeOH. (NaOAc in MeOH can be added to *177* and aldehyde without adverse affect.) This mixture was then diluted with CH_2Cl_2 (120 ml) and washed with water (100 ml). The aqueous layer was back-extracted twice with CH_2Cl_2 (2 × 100 ml). The combined organic layers were then washed twice with sat. $NaHCO_3$ and once with brine, dried (anh. K_2CO_3) and evaporated to give 6.0 g (98% crude) of off-white solid. Precipitation from EtOAc/hexanes gave 5.2 g (85%) of *179* as a white agglomeration of fine particles. For characterization purposes, a small portion was recrystallized from absolute EtOH/hexanes to give white needles; m.p. 173° - 174°C (dec.); $[\alpha]_D^{23}$ +80° (c 2, $CHCl_3$); i.r. (KBr) ν_{max}: 1750 (acetates), 1640 (α,β-unsaturated imine), 1620 (conjugated C=C) and 1370 ($COCH_3$) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 1.96, 2.06, 2.12, 2.22 (4s, 12H, 4× OAc), 3.61 (d × d, 1H, $J_{1,2}$ = 3.5Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.9 - 4.5 (m, 3H, H-5 and H-6), 5.0 - 5.4 (m, 1H, H-4), 5.63 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5Hz, H-3), 6.20 (d, 1H, $J_{1,2}$ = 3.5Hz, H-1), 6.87, 6.95, 6.98 (m, 2H, N=CH*CH*=*CH*Ph), 7.2 - 7.6 (m, 5H, *Ph*) and 8.06 (app. d × d, 1H, J = 2Hz and 6Hz, N=*CH*CH=CH) ppm; m.s. (CI/IB, 93° - 175°C) m/e: 282 (100, $(M + 1)^+ - 3\times60$); Anal. calcd. for $C_{23}H_{27}NO_9$: C 59.86, H 5.90, N 3.04; found: C 59.79, H 5.90, N 3.05.

β-Lactams (+)-*180* and (+)-*181*[50,51] (from α-anomer)

As in the preparation of β-lactams (+)-*174* and (-)-*175* (p. 157), Schiff base *179* (5.1 g, 11 mmol) in 40 ml of dry CH_2Cl_2 was treated with NEt_3 (14 mmol) and azidoacetyl chloride[131] (13 mmol) in 5 ml of CH_2Cl_2. The mixture was diluted with ether (100 ml), then washed with brine, 10% HCl, sat. $NaHCO_3$ (twice) and once more with brine. Drying and solvent evaporation yielded 6.1 g (quantitative) of mixed β-lactams *180* and *181* as a beige solid. HPLC (Waters 440, MeOH/EtOAc/hexanes (0.1:20:80), 7.5 ml/min.) showed that this mixture consisted of 86:14 major, less polar to minor, more polar diastereomer with retention times of 20 and 31.5 minutes respectively. With a change in conditions (MeOH/EtOAc/hexanes (0.1:30:70), 5.0 ml/min.), these retention times were reduced to 11 and 14.5 minutes respectively.

The crude mixture of β-lactams was filtered through a short silica gel column in 60% EtOAc/hexanes, and the solvent evaporated. The residue was then triturated with anhydrous ether and filtered, yielding 4.7 g (76%) of pure major β-lactam (+)-*180*. A small quantity was recrystallized by adding hexanes to a solution in 1:1 EtOAc/CH_2Cl_2 giving white needles: major, less polar β-lactam (+)-*180*: m.p. 165° - 165.5°C (dec.); $[\alpha]_D^{23}$ +151° (c 2, $CHCl_3$); i.r. (KBr) ν_{max}: 2100 (azide), 1770 (sh, β-lactam), 1750, 1745, 1735 (acetates) and 1365 ($COCH_3$) cm^{-1}; and ($CHCl_3$) ν_{max}: 2110 (azide), 1775 (β-lactam), 1760 (broad sh,

acetates) and 1370 ($COCH_3$) cm^{-1}; 200MHz ^1H.m.r. ($CDCl_3$) δ: 1.96, 2.05, 2.07, 2.10 (4s, 12H, 4× *OAc*), 3.92 - 4.2 [m, 3H, including 3.96 (d × d, $J_{1,2}$ = 3.3Hz, $J_{2,3}$ = 11Hz, H-2) and 4.04 (d × d, $J_{5,6e}$ = 2.3Hz, $J_{6a,6e}$ = 12.4Hz, H-6e), H-2, H-5, H-6e], 4.27 (d × d, 1H, $J_{5,6a}$ = 3.9Hz, $J_{6a,6e}$ = 12.4Hz, H-6a), 4.50, 4.52, 4.54, 4.56 (app. d × d, X of a poss. ABXY pattern, 1H, J_{BX} = 8.8Hz, J_{XY} = 5.2Hz, H-4'), 4.65, 4.67 (app. d, Y of ABXY, 1H, J_{XY} = 5.2Hz, J_{BY} < 1Hz, H-3'), 5.10 (app. t, 1H, J = 9.8Hz, H-4), 5.76 (d × d, 1H, $J_{2,3}$ = 11Hz, $J_{3,4}$ = 9.2Hz, H-3), 5.97, 6.01, 6.05, 6.09 (app. d × d, B of ABXY, 1H, J_{AB} = 16Hz, J_{BX} = 8.8Hz, CH*CH*=CH), 6.27 (d, 1H, $J_{1,2}$ = 3.3Hz, H-1), 6.69, 6.77 (app. d, A of ABXY, 1H, J_{AB} = 16Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.3 - 7.4 (m, 5H, *Ph*) ppm; m.s. (EI, 70eV, 76°C) m/e: 520 (0.1), 516 (9, $M^{+\cdot}$ - 28), 456 (8, $M^{+\cdot}$ - 28 - 60), 143 (13) and 43 (100); Anal. calcd. for $C_{25}H_{28}N_4O_{10}$: C 55.15, H 5.18, N 10.29; found: C 54.94, H 5.43, N 10.50.

Evaporation of the ether filtrates gave 1.1 g (18%) of an amorphous yellow solid consisting mainly of minor β-lactam (+)-*181* plus some *180* and impurities (t.l.c., 40% EtOAc/hexanes). Although this could not be crystallized, treatment with charcoal followed by chromatography using 40% EtOAc/hexanes (1500 ml) then 50% EtOAc/hexanes (500 ml) gave 0.37 g (8%) of pure minor β-lactam (+)-*181* as a white amorphous solid: minor, more polar β-lactam (+)-*181*: $[\alpha]_D^{23}$ +10° (c 4, $CHCl_3$); i.r. (KBr) ν_{max}: 2100 (azide), 1775 (sh, β-lactam), 1760 - 1740 (acetates), 1650

(ArC=C) and 1365 ($COCH_3$) cm^{-1}; and ($CHCl_3$) ν_{max}: 2110 (azide), 1775 (β-lactam), 1765 - 1750 (broad sh, acetates) and 1370 ($COCH_3$) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: 2.02, 2.06, 2.08, 2.22, (4s, 12H, 4× OAc), 3.91 - 4.2 [m, 3H, including 3.95 (d × d, $J_{1,2}$ = 3.4Hz, $J_{2,3}$ = 11Hz, H-2), H-2, H-5, H-6e], 4.28 (d × d, 1H, $J_{5,6a}$ = 3.5Hz, $J_{6a,6e}$ = 12Hz, H-6a), 4.35, 4.37, 4.40, 4.42 (app. d × d, X of an ABXY pattern, 1H, J_{BX} = 9.4Hz, J_{XY} = 5.1Hz, H-4'), 4.75, 4.78 (app. d, Y of ABXY, 1H, J_{XY} = 5.1Hz, J_{BY} < 1Hz, H-3'), 5.06 (app. t, 1H, J = 9.7Hz, H-4), 5.73 (d × d, 1H, $J_{2,3}$ = 11Hz, $J_{3,4}$ = 9.2Hz, H-3), 6.11, 6.16, 6.19, 6.24 (app. d × d, B of ABXY, 1H, J_{AB} = 15.8Hz, J_{BX} = 9.4Hz, CH*CH*=CH), 6.29 (d, 1H, $J_{1,2}$ = 3.4Hz, H-1), 6.64, 6.72 (app. d, A of ABXY, 1H, J_{AB} = 15.8Hz, J_{AX} < 1Hz, CH=*CH*Ph) and 7.3 - 7.5 (m, 5H, *Ph*) ppm; m.s. (EI, 70eV, 74°C) m/e: 521 (0.1), 516 (4, $M^{+\cdot}$ - 28), 456 (5, $M^{+\cdot}$ - 28 - 60), 143 (26), 115 (30) and 43 (100); Anal. calcd. for $C_{25}H_{28}N_4O_{10}$: C 55.15, H 5.18, N 10.20; found: C 54.91, H 5.24, N 10.11.

Aldehyde *187*

The major β-lactam (+)-*180* (2.2 g, 4.0 mmol) in 50 ml of dry CH_2Cl_2 was cooled to -70°C under nitrogen, and treated with ozone (~36 ± 4 mmol/hr.) until the solution turned blue (15 min.). Nitrogen was bubbled through the reaction mixture for 5 minutes to remove excess ozone, dimethyl sulfide (1.5 ml, 20 mmol) was added and the solution allowed to warm to room temperature.

After stirring for 24 hours, the mixture was diluted with 1:1 ether/hexanes, washed with brine, sat. $NaHCO_3$ (twice) to remove the benzoic acid (see p. 97), and once more with brine, then dried and evaporated to yield 2.0 g of off-white solid.

Chromatography (60% EtOAc/hexanes) yielded a white amorphous solid which was dried over P_2O_5 *in vacuo* for 18 hours to give 1.65 g (89%) of aldehyde *187*. A small amount of the dried aldehyde was recrystallized from $CHCl_3$/ether (anh.): m.p. 140° - 143°C (dec.); $[\alpha]_D^{23}$ +211° (c 2, $CHCl_3$); i.r. (KBr) ν_{max}: 2105 (azide), 1775 (β-lactam), 1770, 1735 (acetates) and 1720 (sh, aldehyde) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 2.04, 2.08 (s + bs, 12H, 4× *OAc*), 3.7 - 4.53 [m, 5H, including 4.45 (d × d, $J_{3',4'}$ = 5.5 Hz, $J_{4',5'}$ = 3.5Hz, H-4'), H-2, H-5, H-6, H-4'], 4.84 (d, 1H, $J_{3',4'}$ = 5.5Hz, H-3'), 5.01, 5.16, 5.22, 5.26, 5.32, 5.37, 5.53 (m, 2H, H-3 and H-4), 6.18 (d, 1H, $J_{1,2}$ = 3.5Hz, H-1) and 9.46 (d, 1H, $J_{4',5'}$ = 3.5Hz, *CHO*) ppm; m.s. (EI, 70eV, 103°C), m/e: 463 (0.3), 448 (0.6), 442 (0.3, $M^{+\cdot}$ - 28), 291 (34) and 194 (100); Anal. calcd. for $C_{18}H_{22}N_4O_{11}$: C 45.96, H 4.71, N 11.91; found: C 45.69, H 4.75, N 11.91.

Primary Alcohol *188* and Hemi-acetal-acetate *191*[93,168]

In a flame-dried 3 neck flask fitted with septa and a dropping funnel, $NaBH_4$-Alox[93] (4.0 g) and anhydrous $MgSO_4$[168] (2 g) were suspended in 4 ml of dry CH_2Cl_2 and cooled under nitrogen in an ice-salt bath (-15°C). Aldehyde *187* (0.94 g, 2.0 mmol),

which had been refluxed 1 hour in CH_2Cl_2 using a modified Dean-Stark apparatus filled with 3A molecular sieves, was transferred in 5 ml of CH_2Cl_2 *via* syringe to the dropping funnel, rinsing with another 2 ml of CH_2Cl_2. This solution was added dropwise to the cold suspension over 20 minutes, and stirred a further 20 minutes at -15°C.

T.l.c. (60% EtOAc/hexanes) indicated that aldehyde *187* was reacting to give a more polar product, which was in turn, being transformed into a compound just slightly less polar than the starting material. Filtration of the reaction mixture through celite, and evaporation of the solvent yielded 0.82 g of pale yellow solid. Chromatography with 60% EtOAc/hexanes (1000 ml) followed by 70% EtOAc/hexanes (1000 ml) separated this solid into 0.45 g (48%) of the more polar, primary alcohol *188*, and 0.3 g (32%) of a compound whose spectral characteristics indicated it to be the product of transesterification, hemi-acetal-acetate *191*.

Primary alcohol *188* was purified by precipitation from EtOAc/hexanes, and then from CH_2Cl_2/ether (anh.) to give a white granular solid: m.p. 166° - 171°C (dec.); $[\alpha]_D^{23}$ +201° (c 2, $CHCl_3$); i.r. (KBr) ν_{max}: 3470 (hydroxyl), 2110 (azide), 1770 - 1735 (β-lactam and acetates) and 1385, 1370 ($COCH_3$) cm^{-1}; and ($CHCl_3$) ν_{max}: 2120 (azide), 1775 (β-lactam), 1760 - 1750 (acetates) and 1380 ($COCH_3$) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 2.04 2.07, 2.17 (bs and 2s, 12H, 4× OAc), 2.41 (bs, 1H, ex-

changeable, *OH*), 3.7 - 4.5 (m, 7H, H-2, H-5, H-6, H-4', H-5'), 4.61 (d, 1H, $J_{3',4'}$ = 5Hz, H-3'), 5.04 (bt, 1H, J = 9.5Hz, H-4), 5.78 (d × d, 1H, J = 9Hz and 11Hz, H-3) and 6.25 (d, 1H, $J_{1,2}$ = 4Hz, H-1) ppm; m.s. (EI, 70eV, 135°C) m/e: 415 (0.6), 293 (9), 194 (100) and 152 (42); Anal. calcd. for $C_{18}H_{24}N_4O_{11}$: C 45.76, H 5.12, N 11.86; found: C 45.59, H 5.10, N 11.69.

The hemi-acetal-acetate *191* was purified by recrystallization from EtOAc/hexanes twice to give a white powder: m.p. 133.5° - 134.5°C; $[\alpha]_D^{23}$ +94° (c 2.5, $CHCl_3$); i.r. (KBr) ν_{max}: 3380 (hydroxyl), 2120 (azide), 1780 - 1730 (β-lactam and acetates), 1710 (acetate) and 1380 ($COCH_3$) cm^{-1}; and ($CHCl_3$) ν_{max}: 2120 (azide), 1770 (β-lactam), 1750 (acetates) and 1380 ($COCH_3$) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: 2.04, 2.05, 2.10, 2.13 (4s, 12H, 4× *OAc*), 4.1 - 4.6 (m, 7H, H-2, H-5, H-6, H-4', H-5'), 4.62 (d, 1H, $J_{3',4'}$ = 5.3Hz, H-3'), 5.12 (bt, 1H, J = 8Hz, H-4), 5.26 (bs, 1H, H-1) and 5.45 (d × d, 1H, J = 9Hz and 11Hz, H-3) ppm; m.s. (EI, 15eV, 48°C) m/e: 446 (8), 444 (2, $M^{+\cdot}$ - 28), 211 (10), 180 (20), 113 (43) and 86 (100); Anal. calcd. for $C_{18}H_{24}N_4O_{11}$: C 45.76, H 5.12, N 11.86; found: C 45.55, H 5.26, N 12.06. (For preparation of *191* from *188* see p. 169 .)

Penta-acetate *192*

Hemi-acetal-acetate *191* (31 mg, 0.067 mmol) dissolved in 1.4 ml of acetic anhydride/pyridine (2:5) was stirred at 25°C for 18 hours[152]. Addition of solid $NaHCO_3$, followed by normal work-up and chromatography (55% EtOAc/hexanes) gave a syrup

which on trituration with ether gave 16 mg (46% yield) of needle crystals. Another 44% (15 mg) of *192* as a white powder was obtained by evaporation of the ether: penta-acetate *192*: m.p. 158.5° - 159°C; $[\alpha]_D^{23}$ +175° (c 0.7, $CHCl_3$); i.r. ($CHCl_3$) ν_{max}: 2120 (azide), 1780 (β-lactam), 1750 (acetates) and 1370 ($COCH_3$) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) indicated the presence of both the α- and the β-anomer, with the α-anomer in excess (α to β ≈ 75:25); α-anomer, δ: 2.04, 2.05, 2.09, 2.14, 2.21 (5s, 15H, 5× OAc), 3.91 - 4.4 [m, 7H, including 3.95 (d × d, $J_{1,2}$ = 3.4Hz, $J_{2,3}$ = 11Hz, H-2), H-2, H-5, H-6, H-4', H-5'], 4.66 (d, 1H, $J_{3',4'}$ = 5.3Hz, H-3'), 5.07 (app. t, 1H, J = 9.7Hz, H-4), 5.78 (d × d, 1H, $J_{2,3}$ = 11Hz, $J_{3,4}$ = 9.2Hz, H-3) and 6.26 (d, 1H, $J_{1,2}$ = 3.4Hz, H-1) ppm; the quantity of β-anomer present was inferred from the combined integration of the above plus the following; δ: 2.16, 2.18 (2s, 2× OAc), 4.71 (d, J = 5Hz, H-3'), 5.40 (bt, J = 10Hz, H-3) and 5.82 (d, J = 9Hz, H-1) ppm; m.s. (EI, 70eV, 108°C) m/e: 447 (0.1), 335 (1), 194 (1) and 86 (100).

Mesylate *189*[94]

Methanesulfonyl chloride (0.06 ml, 0.08 g, 0.7 mmol) was added to a cooled (-15°C) solution of primary alcohol *188* (0.33 g, 0.70 mmol) in 3 ml of dry CH_2Cl_2, then NEt_3 (0.14 ml, 0.10 g, 1.0 mmol) was added dropwise over 5 minutes. The mixture was stirred at -15°C for a total of 45 minutes, poured into ice-water and separated. The aqueous layer was extracted twice with CH_2Cl_2 and the combined organic phases washed with cold 10% HCl

(twice), sat. $NaHCO_3$ and brine. Drying and solvent evaporation gave 0.38 g (99% crude) of off-white waxy solid. Chromatography with 25% EtOAc/CH_2Cl_2 (400 ml) then 30% EtOAc/CH_2Cl_2 (200 ml) yielded 0.34 g (90%) of *189* as a white waxy solid which was then crystallized from spectrograde EtOAc to give fine needles: m.p. 179° - 180.5°C (dec.); $[\alpha]_D^{23}$ +163° (c 2, $CHCl_3$); i.r. (KBr) ν_{max}: 2120 (azide), 1765 (sh, β-lactam), 1740 (acetates), 1365 ($COCH_3$) and 1175 (sulfonate) cm^{-1}; and ($CHCl_3$) ν_{max}: 2120 (azide), 1780 (β-lactam), 1750 (acetates), 1370 ($COCH_3$) and 1180 (sulfonate) cm^{-1}; 200MHz ^{1}H.m.r. ($CDCl_3$) δ: 2.05, 2.07, 2.09, 2.21 (4s, 12H, 4× OAc), 3.13 (s, 3H, OSO_2CH_3), 3.93 (d × d, 1H, $J_{1,2}$ = 3.4Hz, $J_{2,3}$ = 11Hz, H-2), 4.0 - 4.4 (m, 6H, H-5, H-6, H-4', H-5'), 4.73 (d, 1H, $J_{3',4'}$ = 5.2Hz, H-3'), 5.07 (d, 1H, $J_{3,4}$ = 9.2Hz, H-3) and 6.26 (d, 1H, $J_{1,2}$ = 3.4Hz, H-1) ppm; m.s. (EI, 70eV, 78°C) m/e: 491 (0.1, $M^{+\cdot}$ - 59), 371 (2), 194 (13), 151 (10) and 43 (100); Anal. calcd. for $C_{19}H_{26}N_4O_{13}S$: C 41.46, H 4.76, N 10.18, S 5.82; found: C 41.48, H 4.76, N 10.20, S 5.61.

Hemi-acetal-acetate *191* (from Primary Alcohol *188*)

Primary alcohol *188* (0.24 g, 0.50 mmol) and 4-dimethylaminopyridine (DMAP, 0.6 g, 0.5 mmol) were dissolved in 25 ml of dry CH_2Cl_2 and stirred at room temperature for 20 hours. The reaction mixture was washed with brine, 4% HCl and brine, then dried and evaporated to give 0.21 g of crude (89%), off-white solid.

Crystallization from EtOAc/hexanes yielded 0.18 g (76%) of a white powder identical to the less polar product obtained from the reduction of aldehyde *187*, the hemi-acetal-acetate *191* (see p. 167).

Ethoxyethyl Glycoside *202*[178]

A catalytic amount of pyridinium tosylate (21 mg) was added to a mixture of hemi-acetal-acetate *191* (0.40 g, 0.84 mmol) and 0.5 ml of distilled ethyl vinyl ether (0.38 g, 5.2 mmol) in 5 ml of dry CH_2Cl_2. The mixture was allowed to stand at room temperature for 4 days. Even though t.l.c. (50% EtOAc/hexanes) still showed the presence of some starting material, the mixture was poured into cold sat. $NaHCO_3$, separated, washed with sat. $NaHCO_3$, then brine, dried (anh. K_2CO_3) and concentrated *in vacuo* to give 0.49 g of crude yellow oil. Chromatography (50% EtOAc/hexanes) allowed the recovery of 60 mg of starting material *191* (15%) as well as 0.31 g (67%) of *202* as an oil: i.r. (film) ν_{max}: 2105 (azide), 1770 (β-lactam), 1745 (acetates) and 1365 ($COCH_3$) cm^{-1}; ^{1}H.m.r. ($CDCl_3$) δ: 1.0 - 1.4 [m, 6H, including 1.19 (t, J = 7.5Hz, OCH_2CH_3), $OCH(OCH_2CH_3)CH_3$], 2.03, 2.08, 2.10, 2.11, 2.13 (5s of varying size, 12H, 4× OAc of more than one isomer) and 3.1 - 5.8 [m, 14H, including 3.80 (q, J = 7.5Hz, OCH_2Me)] ppm; m.s. (EI, 70eV, 140°C) m/e: 413 (0.1, $M^{+\cdot}$ - 89 - 42), 335 (16), 86 (70) and 73 (100).

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APPENDIX I

APPENDIX I - REAGENTS

a. BOC-ON, NEt_3

b. BnBr, DBU, benzene, Δ

c. HCl, anhydrous ether

d. *t*-$BuMe_2SiCl$, imidazole, HMPT

e. PhCH=CHCHO, CH_2Cl_2: 1) Δ, 1 hour

2) $MgSO_4$ (anh.), R.T., 1 hour

f. NEt_3, N_3CH_2COCl, -15°C

g. 1) O_3, -70°C

2) Me_2S, -70°C to 25°C

h. $NaBH_4$, 0°C

i. 1) O_3, -70°C

2) $NaBH_4$-Alox, -70° to 25°C

j. CH_3SO_2Cl, NEt_3, -15°C

k. 95% CF_3COOH

l. Ac_2O, DMSO

m. Jones reagent: 1) -15°C, 10 min.

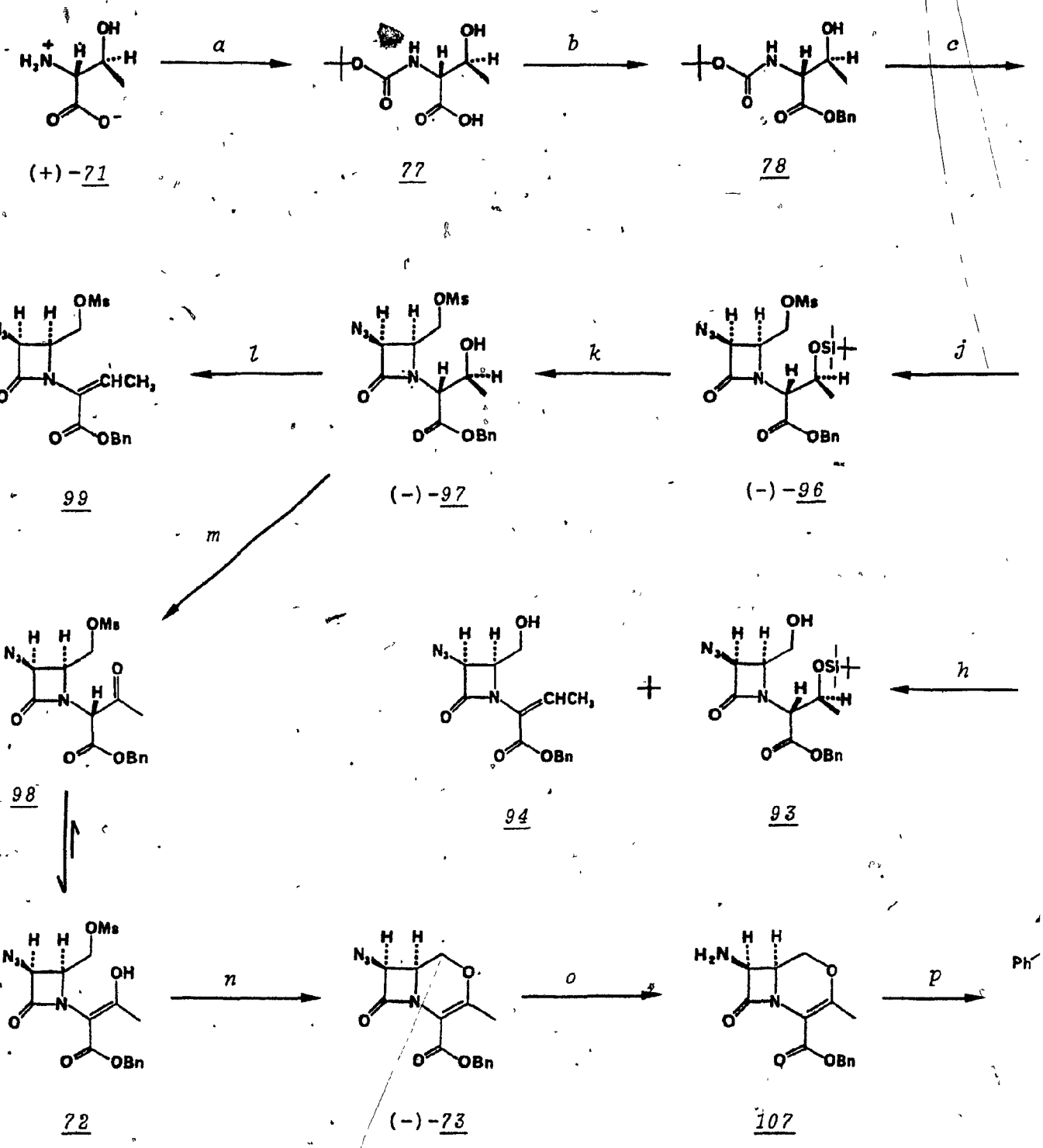
2) 19 ± 1°C, 35 min.

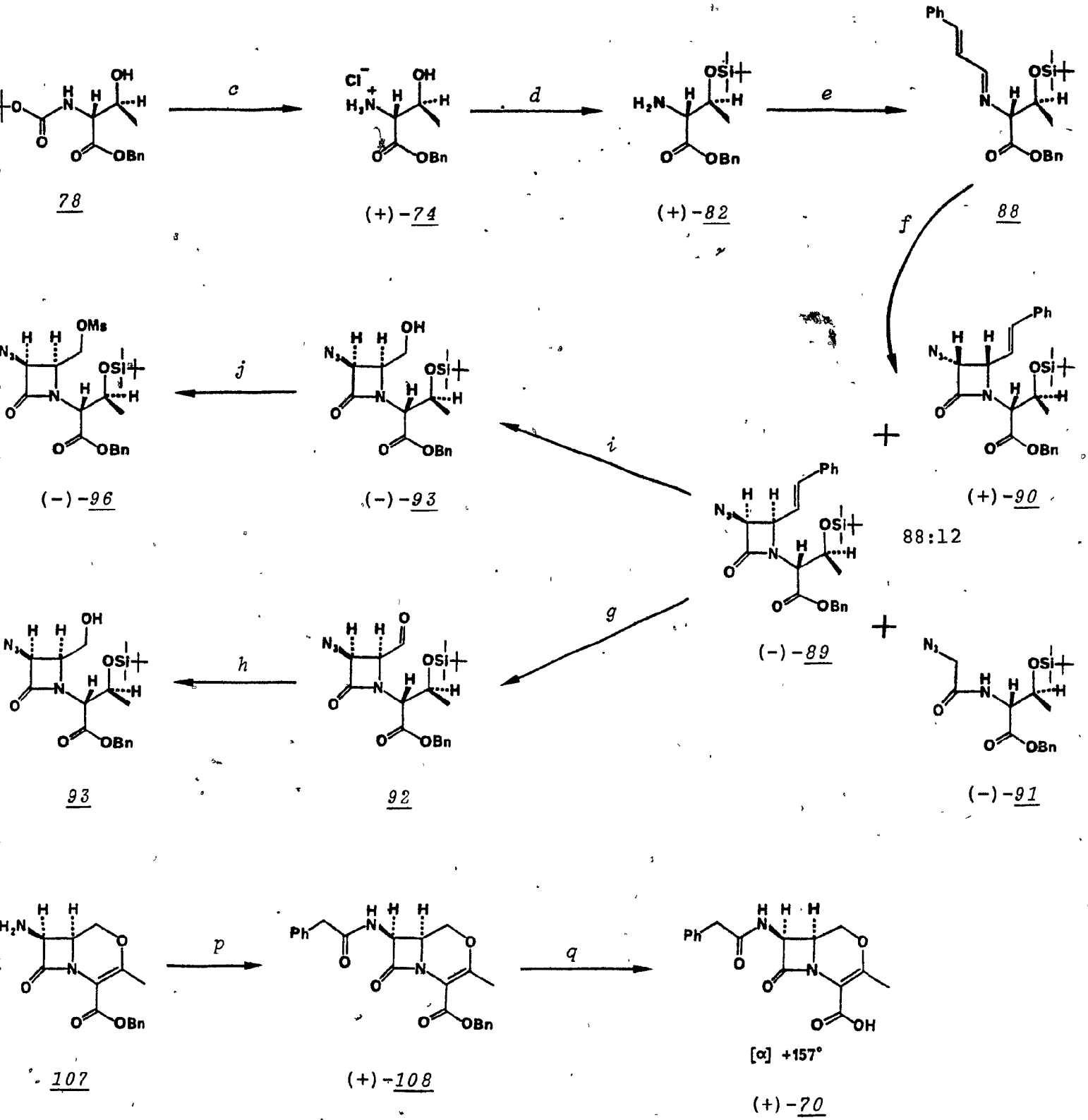
n. NEt_3, CH_2Cl_2, Δ

o. H_2, PtO_2

p. $PhCH_2COOH$, EEDQ

q. H_2, 10% Pd-C


a
b
c
(+)-71
77
78
T., 1 hour
l
k
j
99
(-)-97
(-)-96
m
h
98
94
93
n
o
p
72
(-)-73
107
Ph


78
(+)-74
(+)-82
88
(+)-90
88:12
(-)-89
(-)-91
(-)-93
(-)-96
92
93
107
(+)-108
[α] +157°
(+)-70
a
b
c
d
e
f
g
h
i
j
p
q

APPENDIX II

APPENDIX II - REAGENTS

a. *N,N'*-dicyclohexyl-*O*-*tert*-butylisourea

b. Cyclohexene, 10% Pd-C, EtOH, Δ

c. *t*-$BuMe_2SiCl$, imidazole, HMPT

d. PhCH=CHCHO, CH_2Cl_2: 1) Δ, 1.5 hours

2) $MgSO_4$ (anh.), R.T., 0.5 hour

e. NEt_3, N_3CH_2COCl, -15°C

f. NEt_3, N_3CD_2COCl, -15°C

g. NEt_3, N_3CD_2COCl, -15°C

h. BnBr, DBU, benzene, Δ

i. HCl, anhydrous ether

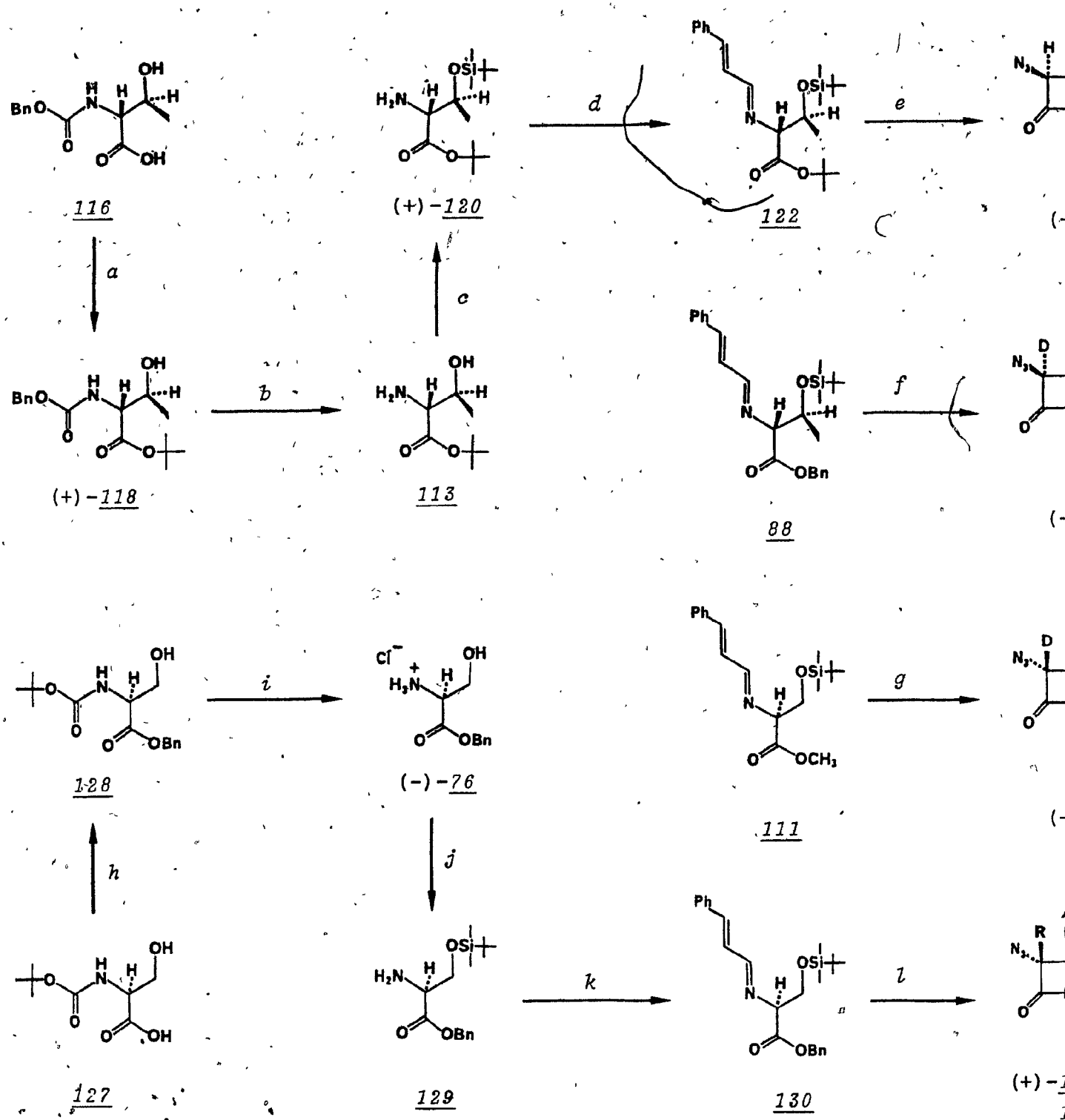
j. *t*-$BuMe_2SiCl$, imidazole, HMPT

k. PhCH=CHCHO, CH_2Cl_2: 1) Δ, 1 hour

2) $MgSO_4$ (anh.), R.T., 1 hour

l. *130* → *131* + *132* + *133*: NEt_3, N_3CH_2COCl, -15°C

130 → *142* + *143* + *144*: NEt_3, N_3CD_2COCl, -15°C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122
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c
d
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b
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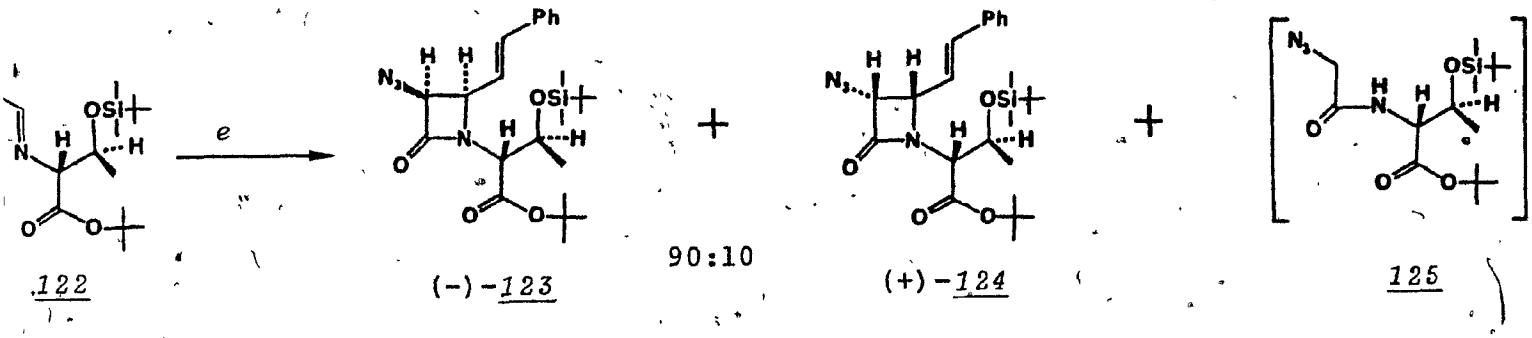
128
(-)-76
111
i
g
h
j
127
129
130
k
l



122 → (−)-123 + (+)-124 + 125 (90:10)

88 → (−)-139 + (+)-140 + (−)-141 (88:12)

111 → (−)-148 + 147 + 149 (82:18)

130 → (+)-131 R = H + (−)-132 R = H + (+)-133 R = H (79:21)

142 R = D + 143 R = D + 144 R = D (77:23)

APPENDIX III

APPENDIX III - REAGENTS

a. 1) 1N NaOH

2) PhCH=CHCHO

b. Ac_2O, pyridine, 0°C

c. NEt_3, N_3CH_2COCl, -15°C

d. 1N HCl, dioxane

e. PhCH=CHCHO, NaOAc, 0°C

f. NEt_3, N_3CH_2COCl, -15°C

g. O_3, -70°C

h. Me_2S, R.T., 24 hours

i. 2:1 $NaBH_4$-Alox/$MgSO_4$ (anh.), CH_2Cl_2, -15°C

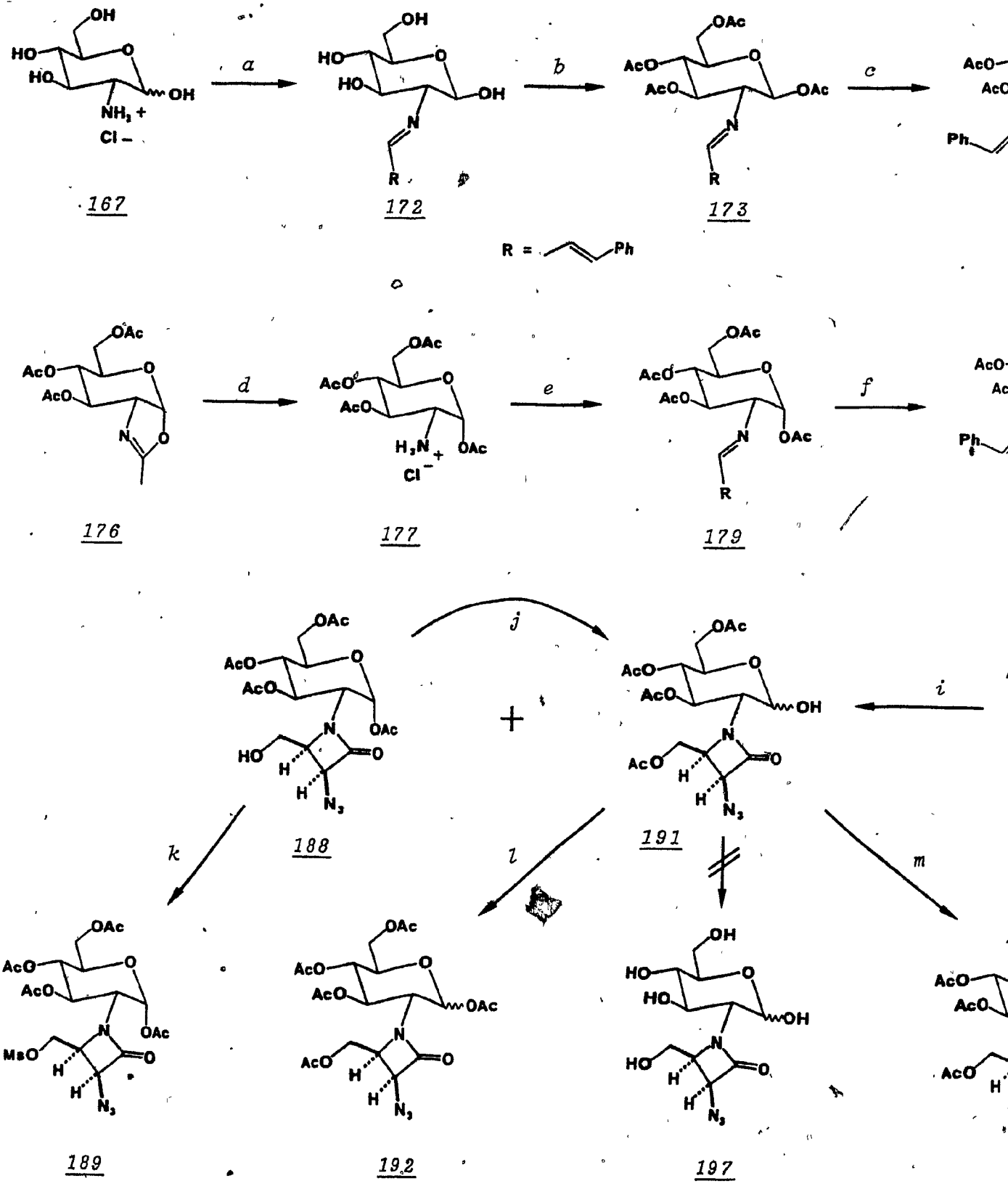
j. 4-Dimethylaminopyridine, CH_2Cl_2, 20 hours

k. CH_3SO_2Cl, NEt_3, -15°C

l. Ac_2O, pyridine

m. Ethyl vinyl ether, pyridinium tosylate, 4 days

n. 50% NH_3, MeOH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HO
HO
NH3 +
Cl –
OH
a
HO
HO
OH
N
R
b
OAc
AcO
AcO
OAc
N
R
c
AcO
AcO
Ph
R = Ph
167
172
173
OAc
AcO
AcO
N
O
d
OAc
AcO
AcO
H3N +
Cl –
OAc
e
OAc
AcO
AcO
N
OAc
R
f
AcO
Ac
Ph
176
177
179
OAc
AcO
AcO
N
OAc
HO
H
H
N3
j
+
OAc
AcO
AcO
OH
N
AcO
H
H
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i
188
191
k
l
m
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N
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H
N3
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H
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N3
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AcO
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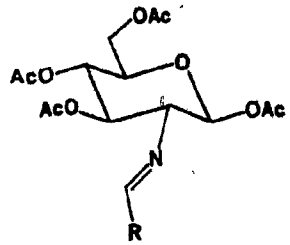
189
192
197

°C

s

4 days



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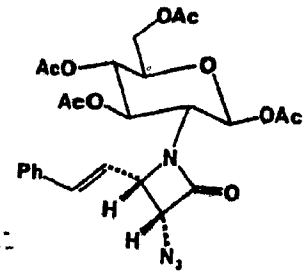

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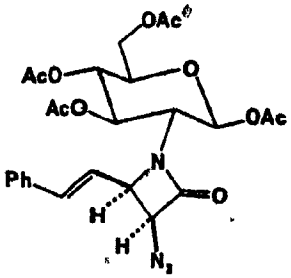

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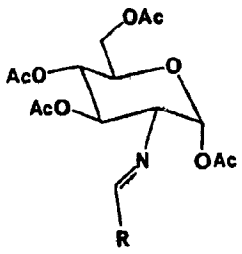

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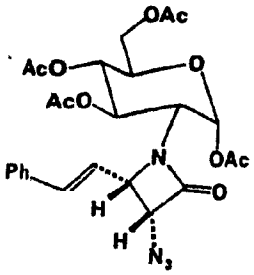

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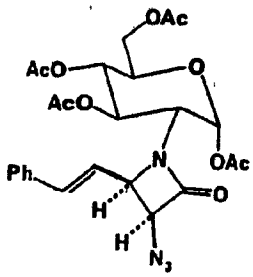

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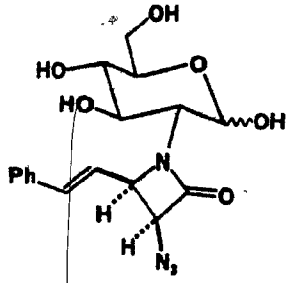

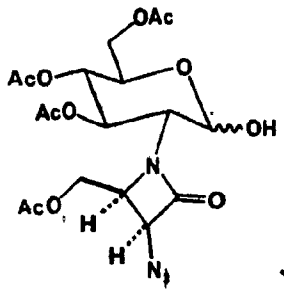

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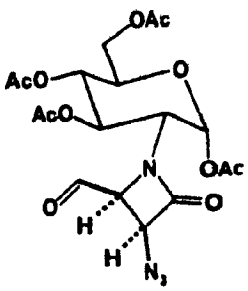

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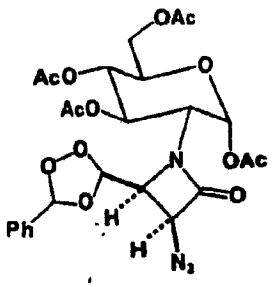

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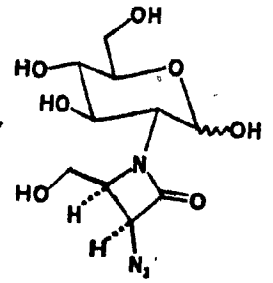

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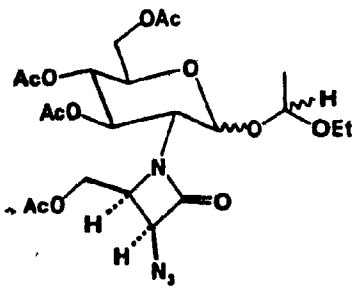

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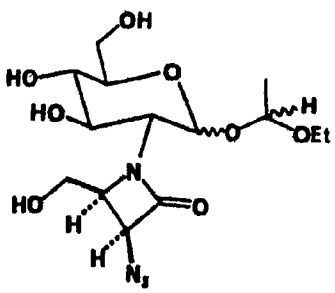

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